Construction of Potential miRNA-mRNA Regulatory Network in AMI Plasma by Bioinformatics Analysis

Fuyu Yang

Zunyi Medical University

Shihao Gao

Zunyi Medical University

Guoxin Meng

Zunyi Medical University

Wei Zhang

Zunyi Medical University

Tao Zhou (✉ chowtao@sina.com)

Guizhou Provincial People's Hospital

Research Article

Keywords: microRNAs, Acute myocardial infarction, Bioinformatics analysis, miRNA-mRNA regulatory network

Posted Date: January 17th, 2022

DOI: <https://doi.org/10.21203/rs.3.rs-1250640/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Background: Acute myocardial infarction (AMI) has become the main cause of incidence rate and mortality worldwide. More and more evidences indicate that abnormally expressed microRNAs (miRNAs) are involved in the pathogenesis of AMI. However, comprehensive studies on miRNA-mRNA regulatory networks in AMI plasma are still lacking.

Results: In the above three datasets, a total of 5 DEMs with downregulated expression were screened. Sp1 and EGR1 may regulate most down regulated DEM. 1074 downstream target genes were predicted. The predicted target genes of DEM were mainly enriched in PI3K/Akt signal pathway, mTOR signal pathway, Hippo signal pathway, AMPK signal pathway and HIF-1 signal pathway. By constructing DEM-hub gene network, it was found that most hub genes may be regulated by miR-142-3p, miR-375 and miR-190b. Among the top 10 hub genes, the expression of STAT3 and PTEN was consistent with that in GSE66360 dataset.

Conclusion: This study constructed a potential miRNA-mRNA regulatory network in the blood of AMI for the first time, which provides a new perspective for the Pathogenesis and treatment of AMI.

Background

Acute myocardial infarction (AMI) usually leads to myocardial injury and heart failure due to the sudden reduction of oxygen and blood supply [1]. AMI remains the main cause of incidence rate and mortality worldwide, increasing the economic burden of patients and the society [2, 3]. Early and successful myocardial reperfusion is the most effective strategy to reduce the size of acute myocardial infarction and improve clinical results. However, the process of restoring ischemic myocardial blood flow may lead to additional myocardial injury, which is called myocardial ischemia-reperfusion injury (MIRI) [4].

MicroRNA (miRNA) is a kind of noncoding RNA with a length of about 19-25 nucleotides encoded by endogenous genes, which can participate in the regulation of post transcriptional gene expression by binding to the 3'-untranslated region of target genes [5]. Many studies have shown that differentially expressed miRNAs are related to the occurrence and development of a variety of diseases, including AMI [6-8]. Since miRNAs can be stably expressed in body fluids, miRNAs (especially circulating miRNAs) may be an important part in the diagnosis and treatment of AMI in the future [9-11].

Ge et al [12] found that in the study of mouse myocardial infarction model, up regulating the expression level of miR-26b can target down regulating the expression of prostaglandin endoperoxide synthase 2 (PTGS2), inhibit the activation of mitogen activated protein kinase (MAPK) pathway and tumor necrosis factor- α (TNF- α) in mice after myocardial infarction and IL-6 expression decreased, while IL-10 expression increased, reducing inflammatory response and improving myocardial remodeling in myocardial infarction mice. Yang et al [13] found that inhibiting the expression of miR-148b by activating Wnt/ β -catenin signaling pathway can improve antioxidant capacity, inhibit cardiomyocyte apoptosis, promote cell survival, reduce myocardial infarction area and reduce MIRI. Shi et al [14] found that the down-

regulation of miR-126 promoted the expression of autophagy related protein beclin-1, over activated myocardial autophagy and aggravated myocardial injury. In addition, Martin et al reported that miRNA may play a role as a new biomarker of myocardial infarction [15].

Although many studies have been carried out on the expression and function of miRNA in AMI, so far, few people have studied the role of miRNA-mRNA regulatory network in AMI plasma. In this study, we compared DEMs in AMI plasma with normal plasma for the first time by analyzing three datasets in GEO database (GSE148153, GSE61741 and GSE31568). Then the upstream transcription factors, target genes, target gene function analysis and DEM-hub gene network were predicted. In addition, the plasma levels of hub genes were further verified in the GSE66360 dataset. Here, we aimed to construct a potential miRNA-mRNA regulatory network in the blood of AMI and provide promising targets for the diagnosis and treatment of AMI.

Results

Identification of DEMs in COPD Plasma

With an adjusted *P*value < 0.05 and $|\log FC| > 1.5$ condition screening, 175 DEMs (96 upregulated and 79 downregulated) were identified in GSE31568 dataset, 34 DEMs (9 upregulated and 25 downregulated) were identified in GSE61741, and 1702 DEMs (623 upregulated and 1079 downregulated) were identified in GSE148153. The heatmaps of DEMs in the above three datasets are shown in Figure 1A-C, and the details of DEMs are listed in Supplementary Table S1-3. There were a total of 11 overlapping DEMs in three datasets (GSE31568, GSE61741 and GSE148153) analyzed by Venn diagram, among which 5 downregulated DEMs (miR-578, miR-142-3p, miR-375, miR-190b and miR-609) continued to change in the three datasets(Figure 1D and E).

Prediction of Upstream Transcription Factors of DEMs

In this study, FunRich software was used to predict the upstream transcription factors of candidate DEMs. The upstream transcription factors of downregulated DEMs are shown in Figure 2. For the downregulated DEM, the transcription factors were BARX1, SRF, HOXC9, SP1, LHX3, EGR1, POU2F1 and NKX6-1.

Prediction of Downstream Target Genes of DEMs

A total of 1074 genes targeted by candidate DEMs were predicted in the miRNet database. For better visualization, the DEM-target gene network in Figure 3 shows the corresponding relationship between the downregulated DEMs and its target genes. In addition, the number of target genes per DEM is listed in Table 1, and all predicted target genes are listed in Supplementary Table S4.

TABLE 1 Potential Target Genes of the Significantly Downregulated DEMs

Downregulated DEMs	Number
hsa-miR-578	150
hsa-miR-142-3p	389
hsa-miR-375	477
hsa-miR-190b	62
hsa-miR-609	52
Total	1074

GO and KEGG Analysis of Target Genes

Then, we performed GO and KEGG analysis on 1074 target genes of DEMs. As shown in Figure 4A and B, biological process (BP) analysis showed that DEM target genes were particularly rich in muscle tissue development, dephosphorylation and striated muscle tissue development; cellular component (CC) analysis showed that DEM target genes were mainly enriched in cell-substrate junction, cell-substrate adherens junction and focal adhesion; molecular function (MF) analysis showed that DEM target genes were significantly enriched in GTP binding, purine ribonucleoside binding, purine nucleoside binding, ribonucleoside binding and guanyl ribonucleotide binding. In addition, KEGG pathway analysis showed that DEM target genes were significantly enriched in PI3K-Akt signaling pathway, human papillomavirus infection, Salmonella infection, viral carcinogenesis and proteoglycans in cancer (Figures 5A and B).

Construction of PPI and DEM-Hub Gene Network

The PPI network of genes targeted by downregulated DEMs was established the STRING database. Then, we uploaded the above PPI network to Cytoscape and used its cytohubba plugin to screen for hub genes. Figure 6 showed the top 30 hub genes for downregulated DEMs. The top 10 hub genes were CTNNB1, TP53, STAT3, MAPK3, ESR1, MYC, PTEN, CASP3, IGF1R and HSP90AA1 (Table 2).

In order to better study the molecular mechanism of DEMs in AMI plasma, DEM- hub gene network was constructed by Cytoscape software. The interactions between downregulated DEMs and hub genes were as follows: miR-142-3p interacted with two hub genes, including CTNNB1, ESR1; miR-375 interacted with 8 hub genes, including CTNNB1, TP53, STAT3, MAPK3, MYC, CASP3, IGF1R, HSP90AA1; and miR-190b interacted with PTEN (Figure 7).

TABLE 2 Top 10 Hub Genes of the Significantly Downregulated DEMs in the PPI Network Ranked by MCC

Gene Symbol	Score
CTNNB1	80,510,912,850,188
TP53	80,510,613,293,604
STAT3	80,507,020,030,922
MAPK3	80,500,373,410,997
ESR1	80,496,134,514,956
MYC	80,495,506,368,530
PTEN	80,334,559,846,470
CASP3	80,308,302,995,002
IGF1R	79,464,776,547,042
HSP90AA1	75,533,943,191,505

Validation of Hub Genes Expression

According to DEM-hub gene network, the plasma levels of the top 10 hub genes (CTNNB1, TP53, STAT3, MAPK3, ESR1, MYC, PTEN, CASP3, IGF1R, and HSP90AA1) were then identified using the GSE66360 dataset. According to the significant hub gene screening criteria, for the screened downregulated DEM, compared with normal plasma, only the expression of STAT3 and PTEN increased continuously in AMI plasma, while the expressions of ESR1, MYC, and TP53 were the opposite (Figure 8A-J). Therefore, miR-375-STAT3 and miR-190b-PTEN were identified as two potential regulatory pathways in AMI plasma.

Discussion

In the past few years, more and more research has been conducted on the diagnosis and treatment of AMI, but the prognosis for these patients remains poor, in part due to limited understanding of the mechanisms of AMI. Recently, using microarray technology, it is easier to reveal thousands of genetic changes in the progress of various diseases. In this study, data were extracted from three datasets, GSE148153, GSE61741 and GSE31568, to identify DEMs between AMI and normal plasma. Five downregulated DEMs (miR-578, miR-142-3p, miR-375, miR-190b and miR-609) were continuously changed in the three datasets and were identified as candidate DEMs for further analysis. Among them, miR-578 and miR-190b are key regulators of tumor development [16-18]. MiR-142-3p was downregulated in H₂O₂-stimulated cardiomyocytes in vitro and ischemia-reperfusion injured heart tissues in vivo. More importantly, miR-142-3p simulant can effectively reduce cardiomyocyte apoptosis and autophagy, so as to prevent cardiomyocyte death and myocardial infarction [19]. Recent studies suggested that plasma miR-375 can be used as an important clinical biomarker for the diagnosis of early AMI, and overexpression of miR-375 protected H9c2 cell apoptosis and cardiomyocyte injury induced by hypoxia/reoxygenation by regulating Nemo like kinase (NLK) pathway, which may play a preventive role

in hypoxia/reoxygenation injury and provide a new idea for clinical treatment of AMI patients [20]. It is noteworthy that some miRNAs have been reported in animal models of AMI, which in turn supports that these candidate DEMs play an important role in the pathogenesis and injury mechanism of AMI.

Recent studies have shown that miRNA expression can be regulated by transcription factors [21, 22], so we predict transcription factors that may regulate these candidate DEMs. Specific protein 1 (SP1) was the highest predictive upstream transcription factor. Sp1 was a transcription factor of SP/Kruppel like factor family. It regulated gene expression by binding to gene promoters and activating the transcription of many cellular genes [23, 24]. During myocardial ischemia/reperfusion, SP1 down-regulated myocardial sarco-endoplasmic reticulum Ca^{2+} ATPase 2a (SERCA2a), while luteolin pretreatment increased the expression of SERCA2a and reduced MIRI by up regulating SP1 [25]. Silencing SP1 inhibited autophagy by inhibiting the expression of poly ADP-ribose polymerase-1 (PARP1), protecting cardiomyocytes from MIRI and providing a promising therapeutic target for the treatment of myocardial reperfusion injury in the future [26]. Early growth response factor 1 (EGR1) expressed in various cell types is a zinc finger transcription factor [27]. Wang et al [28] found that EGR1 participated in myocardial injury induced by coronary microembolization through autophagy inhibition and apoptosis activation mediated by BIM/Beclin-1 pathway. Inhibiting EGR1 expression can improve cardiac function and reduce myocardial injury. EGR1/BIM/Beclin-1 pathway may be a potential therapeutic target of coronary microembolization. Similarly, in the AMI rat model, inhibition of EGR1 expression can reverse toll-like receptor 4/nuclear factor kappa-B Signal activation induced by AMI or hypoxia, which plays an important role in promoting cardiomyocyte apoptosis, inflammatory response and fibrosis [29]. However, Billah et al [30] believed that EGR1, as the main regulator of remote preprocessing, induced MIRI protection through IL-6-dependent JAK - STAT signal.

KEGG analysis showed that DEMs were mainly enriched in PI3K-Akt signal pathway, Hippo signal pathway and mTOR signal pathway. Chen et al [31] showed that poly (I: C) pretreatment had an important protective effect on MIRI by regulating TLR3 and downstream PI3K/Akt signaling, which may provide a potential pharmacological target for perioperative cardiac protection.

Tian et al [32] found that mammalian ste20-like protein kinases 1 (MST1)-Hippo pathway was activated in AMI and participates in the inflammatory response of cardiomyocytes by inhibiting HO-1 signaling pathway, providing a potential target for reversing cardiomyocyte viability and reducing the inflammatory response of AMI. Zhu et al [33] found that microRNA-2861 and microRNA-5115 could regulate MIRI by binding to GPR30 and activating GPR30/mTOR signal pathway. Moreover, the roles of AMPK signaling pathway and HIF-1 signaling pathway in AMI have been reported. Foglio et al [34] showed that high mobility group box-1 protein (HMGB1) may promote the survival of myocardial infarction cells by inhibiting apoptosis and AMPK dependent autophagy. Yu et al [35] found that the protective effect of astragaloside IV on myocardial infarction may involve upregulation of HIF-1 α , Notch1 and Jagged1 signals, alleviating myocardial injury in AMI rat model. It can be seen that the signal pathway analyzed by KEGG is involved in the development and protection mechanism of AMI, which may be a potential treatment strategy for AMI.

By constructing DEM-hub gene network, we found that most hub genes may be targeted by miR-142-3p, miR-375 and miR-190b. Among the top 10 hub genes, the expression of only two genes (STAT3 and PTEN) was consistent with that in the GSE66360 dataset, which may be due to different sources of samples. The transcription factor STAT3 is a key regulator of intercellular communication [36]. Through various interventions, including drug and non-drug treatment, STAT3 signal was activated to reduce endoplasmic reticulum stress [37], inhibit cardiomyocyte apoptosis [38], and reduce autophagy [39] and other pathways against myocardial ischemic injury. STAT3 activation plays a dual role in cardiac remodeling after MIRI. Moderate activation of STAT3 can resist cardiac remodeling, while continuous activation of STAT3 can aggravate cardiac remodeling [40]. PTEN is a non-secretory protein inherently expressed in cells, which is involved in regulating a variety of signal transduction pathways in cell physiological and pathological processes [41, 42]. Li et al [43] showed that the expression of miRNA-23a may regulate AMI in vivo and in vitro by targeting PTEN, and miRNA-23a-PTEN pathway may be a potential target for clinical treatment of AMI. Hu et al [44] showed that in the mouse AMI model, the down-regulation of MALAT1 can promote the expression of miR-320 and inhibit the expression of PTEN, so as to reduce cardiomyocyte apoptosis in vitro and in vivo, which provides a basis for the diagnosis and treatment of AMI. The activity of PTEN gene is significantly increased in patients with acute myocardial infarction, which can predict the deterioration of left ventricular systolic function, heart failure and death. It may be a new candidate diagnostic biomarker for AMI [45, 46].

Although we first studied the potential miRNA-mRNA regulatory network in AMI plasma by integrating multiple microarray datasets, our current study still has some limitations. First, we only focused on miRNAs and mRNAs between AMI and normal plasma. However, some of them may be different in different types of AMI and need to be further determined. Secondly, the sample size of each dataset used here was insufficient compared with the sample size usually required for biomarker analysis. Third, miRNA-mRNA interaction was only based on the prediction of public database, and further in vivo and in vitro experimental validation studies are needed to verify our analysis.

Conclusion

In conclusion, based on GEO database and bioinformatics analysis, we first constructed two potential miRNA-mRNA pathways (miR-375-STAT3, miR-190b-PTEN) in AMI plasma as potential biomarkers for diagnosis and treatment of AMI patients. We hope these findings will contribute to further research in the future and achieve the goal of improving the prognosis of AMI patients.

Methods

miRNA Microarray

According to the Gene Expression Omnibus (GEO) database (<https://www.ncbi.nlm.nih.gov/geo/>), the following words were used to screen miRNA datasets related to AMI plasma: "myocardial infarction" (study keywords), "non-coding RNA profiles by array" (study type), "homo sapiens" (organism) and

"plasma" (attribute name). Finally, three miRNA datasets (GSE148153, GSE61741 and GSE31568) were selected for subsequent analysis. The GSE61741 and GSE31568 datasets were based on GPL9040 platform (febit Homo Sapiens miRBase 13.0), and the GSE148153 datasets were based on GPL20712 (Agilent-070156 Human miRNA). The details of the three datasets are shown in Table 3, and the research design flow chart is shown in Figure 9.

TABLE 3 Details for GEO AMI Data

Accession	Platform	Sample	Normal	AMI	Gene/miRNA
GSE148153	GPL20712	Blood	7	5	miRNA
GSE61741	GPL9040	Blood	94	62	miRNA
GSE31568	GPL9040	Blood	70	20	miRNA
GSE66360	GPL570	Blood	50	49	gene

Screening of DEMs

GEO2R (<http://www.ncbi.nlm.nih.gov/geo/geo2r/>), a web application based on R language, helps analyze GEO data for comparing and screening DEMs between AMI and normal human plasma. The *P* value was adjusted by Benjamini and Hochberg false discovery rate (FDR) method to correct the occurrence of false positive results. Here, the adjusted *P* value is < 0.05 and |Log fold change (FC)|>1.5 are set as the threshold to identify DEM. The overlap of DEM in three datasets (GSE148153, GSE61741 and GSE31568) was analyzed by Venn diagram, and the continuously changing miRNA was selected as the candidate DEM. The heatmap of DEM and candidate DEM was generated by TBtools, which is a software integrating various biological data processing tools.

Prediction of Upstream Transcription Factors of DEMs

FunRich (<http://www.funrich.org/>) is an independent software tool, which is mainly used to analyze the functional enrichment and interaction network analysis of genes and proteins, and to predict the potential upstream transcription factors of candidate DEM. *P* value < 0.05 was considered statistically significant.

Prediction of Downstream Target Genes of DEMs

MiRNet (<https://www.mirnet.ca/>), a comprehensive toolkit containing miRNA target gene interaction information and displaying associations in visual networks, is used to predict potential downstream target genes of candidate DEMs.

GO and KEGG Analysis of Target Genes

In order to study the biological functions of DEM target genes, the online tools of the database for annotation, visualization, and integrated discovery (DAVID) were used for Gene Ontology (GO) and Kyoto

Encyclopedia of Genes and Genomes (KEGG) path analysis. Adjusted *P* value < 0.05 was statistically significant.

Construction of PPI Network and Screening of Hub Genes

The protein-protein interaction (PPI) network of DEM target genes was

Performed by the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) online database (<http://stringdb.org/>) and then visualized in Cytoscape software (version 3.8.2). Cytohubba was a plug-in of Cytoscape. It sorted nodes according to the characteristics of preloaded PPI network through several topology algorithms. Here, the Maximum Clique Centrality (MCC) method was used to select Hub genes as the top 30 nodes of PPI network. This method has better performance in predicting the accuracy of essential proteins. MCC's score on node *v* was defined as $MCC(v) = \sum C \hat{S}(v) (|C| - 1)!$, where *S*(*v*) was the set of the largest clique containing *v*, (*|C|* - 1)! was the product of all positive integers less than *|C|*.

Expression Analysis of Hub Genes by GSE66360 Dataset

Because there is no other data on mRNA expression in AMI plasma, GSE66360 downloaded from the GEO database was used to analyze the expression of hub genes. The dataset was based on the GPL570 platform (Affymetrix Human Genome U133 Plus 2.0 array), in which the peripheral blood of 49 AMI patients and 50 healthy people were selected. Student's *t*-test was used to determine differentially expressed genes (DEGs) between AMI and normal blood. Significant hub genes needed to meet the following two criteria: first, the target genes of upregulated DEM were downregulated or the target genes of downregulated DEM were upregulated; The second was that the *P* value should be less than 0.05.

Abbreviations

AMI: Acute myocardial infarction; MIRI: Myocardial ischemia-reperfusion injury; DEMs: Differentially expressed microRNAs; DEGs: differentially expressed genes; FC: Fold change; miRNAs: MicroRNAs; mRNA: Message RNA; DAVID : Database for annotation, visualization, and integrated discovery; GO: Gene Ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes; BP: Biological process; CC: Cellular component; MF: Molecular function; PPI: Protein-protein interaction; MCC: Maximal clique centrality; STRING: Search Tool for the Retrieval of Interacting Genes/Proteins; GEO: Gene Expression Omnibus; PTGS2: Prostaglandin endoperoxide synthase 2; MAPK: Mitogen activated protein kinase; TNF- α : Tumor necrosis factor- α ; NLK: Nemo like kinase; SP1: Specific protein 1; SERCA2a: Sarco-endoplasmic reticulum Ca^{2+} ATPase 2a; PARP1: Poly ADP-ribose polymerase-1; EGR1: Early growth response factor 1; MST1: Mammalian ste20-like protein kinases 1; HMGB1: High mobility group box-1 protein; FDR: False discovery rate;

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and analysed during the current study are available in the GEO database [<https://www.ncbi.nlm.nih.gov/geo/>].

Competing interests

The authors declare that they have no competing interests.

Funding

This work was supported by National Natural Science Foundation of China (82160056, 81960050); Guizhou Provincial Natural Science Foundation ([2020]1Y293); Science and Technology Foundation of Guizhou Provincial Health and Family Planning Commission (gzwkj2021-193).

Authors' contributions

All authors read and approved the final manuscript.

Acknowledgements

Not applicable.

Authors' information (optional)

¹ Zunyi Medical University, Zunyi 563000, Guizhou, China; ²Department of Cardiac Surgery, Guizhou Provincial People's Hospital, Guiyang 550002, Guizhou, China.

References

1. Davidson SM, Ferdinandy P, Andreadou I, et al. Multitarget Strategies to Reduce Myocardial Ischemia/Reperfusion Injury: JACC Review Topic of the Week. *J Am Coll Cardiol*. 2019;73(1):89-99.
2. Reed GW, Rossi JE, Cannon CP. Acute myocardial infarction. *Lancet*. 2017;389(10065): 197-210.
3. Li J, Li X, Wang Q, et al. ST-segment elevation myocardial infarction in China from 2001 to 2011 (the China PEACE-Retrospective Acute Myocardial Infarction Study): a retrospective analysis of hospital data. *Lancet*. 2015;385(9966):441-451.
4. Yellon DM, Hausenloy DJ. Myocardial reperfusion injury. *N Engl J Med*. 2007; 357(11):1121-1135.
5. bsp; Bartel DP. Metazoan MicroRNAs. *Cell*. 2018;173(1):20-51.

6. Liu X, Dong Y, Chen S, et al. Circulating MicroRNA-146a and MicroRNA-21 Predict Left Ventricular Remodeling after ST-Elevation Myocardial Infarction. *Cardiology*. 2015;132(4): 233-241.
7. Hendgen-Cotta UB, Messiha D, Esfeld S, et al. Inorganic nitrite modulates miRNA signatures in acute myocardial in vivo ischemia/reperfusion. *Free Radic Res*. 2017;51(1): 91-102.
8. Bian B, Yu XF, Wang GQ, et al. Role of miRNA-1 in regulating connexin 43 in ischemia-reperfusion heart injury: a rat model. *Cardiovasc Pathol*. 2017;27:37-42.
9. Grabmaier U, Clauss S, Gross L, et al. Diagnostic and prognostic value of miR-1 and miR-29b on adverse ventricular remodeling after acute myocardial infarction- The SITAGRAMI-miR analysis. *Int J Cardiol*. 2017;244:30-36.
10. de Couto G, Gallet R, Cambier L, et al. Exosomal MicroRNA Transfer Into Macrophages Mediates Cellular Postconditioning. *Circulation*. 2017;136(2):200-214.
11. Chen Y, Zhao Y, Chen W, et al. MicroRNA-133 overexpression promotes the therapeutic efficacy of mesenchymal stem cells on acute myocardial infarction. *Stem Cell Res Ther*. 2017; 8(1):268.
12. Ge Z-W, Zhu X-L, Wang B-C, et al. MicroRNA-26b relieves inflammatory response and myocardial remodeling of mice with myocardial infarction by suppression of MAPK pathway through binding to PTGS2. *Int J Cardiol*. 2019;280:152-159.
13. Yang M, Kong DY, Chen JC. Inhibition of miR-148b ameliorates myocardial ischemia/reperfusion injury via regulation of Wnt/ β -catenin signaling pathway. *J Cell Physiol*. 2019;234(10):17757-17766.
14. Shi CC, Pan LY, Peng ZY, et al. MiR-126 regulated myocardial autophagy on myocardial infarction. *Eur Rev Med Pharmacol Sci*. 2020;24(12):6971-6979.
15. Horvath M, Horvathova V, Hajek P, et al. MicroRNA-331 and microRNA-151-3p as biomarkers in patients with ST-segment elevation myocardial infarction. *Sci Rep*. 2020;10(1):
16. Yang B, Zhao F, Yao L, et al. CircRNA circ_0006677 Inhibits the Progression and Glycolysis in Non-Small-Cell Lung Cancer by Sponging miR-578 and Regulating SOCS2 Expression. *Front Pharmacol*. 2021;12:657053.
17. Hong H, Yao S, Zhang Y, et al. In vivo miRNA knockout screening identifies miR-190b as a novel tumor suppressor. *PLoS Genet*. 2020;16(11):e1009168.
18. Chen Z, Yang L, Chen L, et al. miR-190b promotes tumor growth and metastasis via suppressing NLRC3 in bladder carcinoma. *FASEB J*. 2020;34(3):4072-4084.
19. Su Q, Liu Y, Lv X-W, et al. Inhibition of lncRNA TUG1 upregulates miR-142-3p to ameliorate myocardial injury during ischemia and reperfusion via targeting HMGB1- and Rac1-induced autophagy. *J Mol Cell Cardiol*. 2019;133:12-25.
20. Ali Sheikh MS. Overexpression of miR-375 Protects Cardiomyocyte Injury following Hypoxic-Reoxygenation Injury. *Oxid Med Cell Longev*. 2020;2020:7164069.
21. Uddin MN, Li M, Wang X. Identification of Transcriptional Markers and microRNA-mRNA Regulatory Networks in Colon Cancer by Integrative Analysis of mRNA and microRNA Expression Profiles in Colon Tumor Stroma. *Cells*. 2019;8(9):1054.

22. Mullany LE, Herrick JS, Wolff RK, et al. MicroRNA-transcription factor interactions and their combined effect on target gene expression in colon cancer cases. *Genes Chromosomes Cancer*. 2018;57(4):192-202.
23. Vizcaíno C, Mansilla S, Portugal J. Sp1 transcription factor: A long-standing target in cancer chemotherapy. *Pharmacol Ther*. 2015;152:111-124.
24. Safe S, Imanirad P, Sreevalsan S, et al. Transcription factor Sp1, also known as specificity protein 1 as a therapeutic target. *Expert Opin Ther Targets*. 2014;18(7):759-769.
25. Hu Y, Zhang C, Zhu H, et al. Luteolin modulates SERCA2a via Sp1 upregulation to attenuate myocardial ischemia/reperfusion injury in mice. *Sci Rep*. 2020;10(1):15407.
26. Xu Y, Wang B, Liu X, et al. Sp1 Targeted PARP1 Inhibition Protects Cardiomyocytes From Myocardial Ischemia-Reperfusion Injury via Downregulation of Autophagy. *Front Cell Dev Biol*. 2021;9:621906.
27. Sukhatme VP. The Egr transcription factor family: from signal transduction to kidney differentiation. *Kidney Int*. 1992;41(3):550-553.
28. Wang XT, Wu XD, Lu YX, et al. Egr-1 is involved in coronary microembolization-induced myocardial injury via Bim/Beclin-1 pathway-mediated autophagy inhibition and apoptosis activation. *Aging (Albany NY)*. 2018;10(11):3136-3147.
29. Pan J, Alimujiang M, Chen Q, et al. Exosomes derived from miR-146a-modified adipose-derived stem cells attenuate acute myocardial infarction-induced myocardial damage via downregulation of early growth response factor 1. *J Cell Biochem*. 2019;120(3):4433-4443.
30. Billah M, Ridiandries A, Rayner BS, et al. Egr-1 functions as a master switch regulator of remote ischemic preconditioning-induced cardioprotection. *Basic Res Cardiol*. 2019;115(1):3.
31. Chen E, Chen C, Niu Z, et al. Poly(I:C) preconditioning protects the heart against myocardial ischemia/reperfusion injury through TLR3/PI3K/Akt-dependent pathway. *Signal Transduct Target Ther*. 2020;5(1):216.
32. Tian Y, Song H, Jin D, et al. MST1-Hippo pathway regulates inflammation response following myocardial infarction through inhibiting HO-1 signaling pathway. *J Recept Signal Transduct Res*. 2020;40(3):231-236.
33. Zhu L, Li Q, Li Q, et al. MicroRNA-2861 and microRNA-5115 regulates myocardial ischemia-reperfusion injury through the GPR30/mTOR signaling pathway by binding to GPR30. *J Cell Physiol*. 2020;235(11):7791-7802.
34. Foglio E, Puddighinu G, Germani A, et al. HMGB1 Inhibits Apoptosis Following MI and Induces Autophagy via mTORC1 Inhibition. *J Cell Physiol*. 2017;232(5):1135-1143.
35. Yu J, Zhang X, Zhang Y. Astragaloside attenuates myocardial injury in a rat model of acute myocardial infarction by upregulating hypoxia inducible factor1alpha and Notch1/Jagged1 signaling. *Mol Med Rep*. 2017;15(6):4015-4020.
36. Haghighi A, Ricke-Hoch M, Stapel B, et al. STAT3, a key regulator of cell-to-cell communication in the heart. *Cardiovasc Res*. 2014;102(2):281-289.

37. Zhao GL, Yu LM, Gao WL, et al. Berberine protects rat heart from ischemia/reperfusion injury via activating JAK2/STAT3 signaling and attenuating endoplasmic reticulum stress. *Acta Pharmacol Sin.* 2016;37(3):354-367.
38. Chen PJ, Shang AQ, Yang JP, et al. microRNA-874 inhibition targeting STAT3 protects the heart from ischemia-reperfusion injury by attenuating cardiomyocyte apoptosis in a mouse model. *J Cell Physiol.* 2019;234(5):6182-6193.
39. Dang M, Zeng X, Chen B, et al. Soluble receptor for advance glycation end-products inhibits ischemia/reperfusion-induced myocardial autophagy via the STAT3 pathway. *Free Radic Biol Med.* 2019;130:107-119.
40. Yang Y, Hu W, Di S, et al. Tackling myocardial ischemic injury: the signal transducer and activator of transcription 3 (STAT3) at a good site. *Expert Opin Ther Targets.* 2017;21(2):215-228.
41. Yang X, Qin Y, Shao S, et al. MicroRNA-214 Inhibits Left Ventricular Remodeling in an Acute Myocardial Infarction Rat Model by Suppressing Cellular Apoptosis via the Phosphatase and Tensin Homolog (PTEN). *Int Heart J.* 2016;57(2):247-250.
42. Xue R, Lei S, Xia ZY, et al. Selective inhibition of PTEN preserves ischaemic post-conditioning cardioprotection in STZ-induced Type 1 diabetic rats: role of the PI3K/Akt and JAK2/STAT3 pathways. *Clin Sci (Lond).* 2016;130(5):377-392.
43. Li S, Ren J, Sun Q. The expression of microRNA-23a regulates acute myocardial infarction in patients and in vitro through targeting PTEN. *Mol Med Rep.* 2018;17(5):6866-6872.
44. Hu H, Wu J, Li D, et al. Knockdown of lncRNA MALAT1 attenuates acute myocardial infarction through miR-320-Pten axis. *Biomed Pharmacother.* 2018;106:738-746.
45. Mahmoud AH, Taha NM, Zakhary M, et al. PTEN gene & TNF-alpha in acute myocardial infarction. *Int J Cardiol Heart Vasc.* 2019;23:100366.
46. Ling H, Guo Z, Shi Y, et al. Serum Exosomal MicroRNA-21, MicroRNA-126, and PTEN Are Novel Biomarkers for Diagnosis of Acute Coronary Syndrome. *Front Physiol.* 2020;11: 654.

Figures

Figure 1

Identification of the candidate DEMs

Notes: (A) GSE31568 dataset; (B) GSE61741 dataset; (C) GSE148153 dataset. (D) Venn diagram indicated overlapping DEMs in the three datasets GSE148153, GSE61741, and GSE31568; (E) LogFC heatmap of the candidate DEMs. The $|\text{LogFC}| > 1.5$ and an adjusted P value < 0.05 were set as the

threshold to screen DEMs. Red represents upregulated DEMs, Blue represents downregulated DEMs, and Yellow represents no significant changes. The numbers in the box represent the logFC values.

Figure 2

Potential transcription factors of DEMs predicted by FunRich.

Figure 3

Potential target genes of DEMs predicted by miRNet.

Figure 4

GO annotation analysis for the target genes of DEMs in the biological process, cellular component, and molecular function.

Notes: (A) Barplot; (B) Dotplot. An adjusted $P < 0.05$ was identified as significantly changed GOs. The x-axis shows the adjusted P value (A) and gene ratio (B) of each term, and y-axis shows the GO annotation terms.

Figure 5

KEGG pathway analysis for the target genes of DEMs.

Notes: (A) Barplot; (B) Dotplot. An adjusted $P < 0.05$ was identified as significantly changed pathways. The x-axis shows the adjusted P value (A) and gene ratio (B) of each term, and y-axis shows the KEGG pathway terms.

Figure 6

PPI network of the top 30 hub genes for downregulated DEMs.

Figure 7

The miRNA-hub gene regulatory network.

Figure 8

The mRNA expression of the top 10 hub genes was determined from the GSE66360 dataset.

Notes: (A) STAT3; (B) PTEN; (C) TP53; (D) MYC; (E) ESR1; (F) MAPK3; (G) IGF1R; (H) HSP90AA1; (I) CTNNB1; (J) CASP3.

Figure 9

Flow chart of constructing miRNA-mRNA regulatory network in AMI plasma

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplementaryTableS1GSE31568.xlsx](#)
- [SupplementaryTableS2GSE61741.xlsx](#)
- [SupplementaryTableS3GSE148153.xlsx](#)
- [SupplementaryTableS4.xlsx](#)