FULL LENGTH ARTICLE

The transcriptome of circulating cells indicates potential biomarkers and therapeutic targets in the course of hypertension-related myocardial infarction

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Abstract Hypertension (HT) is the most common public-health challenge and shows a high incidence around the world. Cardiovascular diseases are the leading cause of mortality and morbidity among the elderly (age > 65 years) in the United States. Now, there is widespread acceptance of the causal link between HT and acute myocardial infarction (MI). This is the first data-mining study to identify co-expressed differentially expressed genes (co-DEGs) between HT and MI (relative to normal control) and to uncover potential biomarkers and therapeutic targets of HT-related MI. In this manuscript, HT-specific DEGs and MI-specific DEGs and differentially expressed miRNAs (DE-miRNAs) were identified in Gene Expression Omnibus (GEO) datasets GSE24752, GSE60993, GSE62646, and GSE24548 after data consolidation and batch correction. Subsequently, enrichment in Gene Ontology (GO) terms and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways as well as protein–protein interaction networks were identified, and single-gene gene set enrichment analysis was performed to determine the affected biological categories and networks. Cross-matching of the results on co-DE-miRNAs and predicted miRNAs targeting the co-DEGs was conducted and discussed as well. We found that MYC and HIST1H2BO may...
be associated with HT, whereas FCGR1A, FYN, KLRD1, KLRB1, and FOLR3 may be implicated in MI. Moreover, co-DEGs FOLR3 and NFE2 with predicted miRNAs and DE-miRNAs, especially miR-7 and miR-548, may be significantly associated and show huge potential as a new set of novel biomarkers and important molecular targets in the course of HT-related MI.

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Introduction

Hypertension (HT) is by far the most common public-health problem with a high incidence around the world, regardless of ethnicity, sex, and geographic disparities. It is the leading cause of premature death in advanced nations. The residual lifetime risk for the middle-aged and elderly is 90%, indicating a huge public-health burden. According to the anticipated changes in size and age composition, 29.2% of the world’s population are predicted to have HT by 2025. The rise is mainly due to drawbacks in preventive medicine and due to therapeutic inertia when blood pressure is uncontrolled. Thus, the burden of HT is now being transferred to low- and middle-income nations with backward disease surveillance and resources. Recent studies linked HT pathogenesis with concomitant risks of cardiovascular, cerebrovascular, and kidney complications, particularly in the elderly. In the United States, cardiovascular diseases (CVDs) are a serious complication in elderly individuals (age > 65 years) and an important cause of mortality and morbidity. Although mechanical complications of acute myocardial infarction (AMI) have been significantly reduced (to less than 1% of the cases) with new developments in pharmacotherapy, timely revascularization, and improved cardiac rehabilitation, the patients still face a poor prognosis, considering their worsened cardiac function and accelerated progression of heart failure after the operation. An immune reaction and inflammatory response play critical roles in ischemic cardiac injury, in the postinfarction repair process, and in ischemia/reperfusion injury after temporarily enhanced blood flow following percutaneous coronary intervention, which is usually too late for a risk assessment.

Valiđated cardiac biomarkers such as BNP and CRP provide only limited information about what is already known or being measured. In contrast, primary prevention of CVD depends mostly on the capacity to recognize at-risk individuals long before AMI. Novel molecular markers for early detection and continuous monitoring might help researchers to reveal initial dysregulation and the underlying mechanisms and will guide healthcare professionals to ensure formulation of the correct therapeutic regimen.

HT has grown in prevalence, and today, HT is widely accepted to be causally linked with AMI in the general population. Although CVD-related mortality does not increase along with any increases within the normal range of blood pressure, each additional 20 mmHg of systolic blood pressure or 10 mmHg of diastolic blood pressure leads to more than a 2-fold increase in the rate of death from ischemic heart disease in the age group “40–69 years.” With age, the annual absolute risk of HT-related CVDs, especially ischemic heart disease and AMI, increases exponentially. HT is usually a predictor of serious physical illnesses and even death in the early post-AMI period. Lowering of high blood pressure correlates with a lessened CVD burden, but the actual risk of major adverse cardiovascular events or all-cause deaths during a follow-up period as long as 330 days is not associated with a history of HT probably because timely recanalization and medication compliance might alleviate the adverse influences of prior HT. There is an inconsistent relation between blood pressure elevation recorded after stabilization of hemodynamics following acute ischemic events and repeated coronary events during long-term follow-up, whereas lower systolic blood pressure during chest pain episodes is always linked with lower mortality within 1 year from stenosis or occlusion of large vessels. According to the above-mentioned complex relation, the absolute value of blood pressure might not perfectly reflect the onset and development of myocardial infarction (MI); therefore, researchers thinking outside the box and exploring the integrated molecular mechanisms might broaden the understanding of HT-related MI from a different perspective.

As an emerging technology for characterizing the landscape of differentially expressed genes (DEGs), differentially expressed proteins, and differentially produced metabolites in the course of CVD, microarray gene expression data provide a comprehensive perspective on pathological mechanisms by integrating the data on dysregulated pathways and possible interactions. To compensate the shortage of tissue-based microarray profiles, particularly those of the human heart and cardiac vessels, Liew et al have compared the complexity of the blood transcriptome with gene expression levels in 9 organs of the human body. The results showed that expression levels of over 80% genes were consistent between any given tissue and blood, suggesting that peripheral blood is an ideal surrogate tissue to discover genes and pathways that play important roles in the pathogenesis and progression of such diseases. We herein conducted a peripheral-blood whole-genome microarray transcriptional analysis based on public datasets in a prospective cohort of patients with AMI by means of cross-platform integration to improve reproducibility and robustness of gene signature biomarkers. This is the first data mining study to identify coexpressed differentially expressed genes (co-DEGs) of HT and MI and to elucidate the molecular mechanisms and pathophysiology by means of HT-specific DEGs (HT-DEGs) and MI-specific DEGs (MI-DEGs). Gene set enrichment analysis (GSEA) was performed on the co-DEGs for pathway analysis. At the end of this paper, we demonstrate potential microRNAs (miRNAs; by studying overlaps of gene sets) specific for HT patients susceptible to MI.
Results

Identification of HT-DEGs and MI-DEGs

A total of 14,973 probes were identified, which were consistent with 46,674 genes in GSE-MI (GSE60993 and GSE62646) after the batch correction. Almost 10,001 probe sets were selected for the 22,283 genes of GSE-MI and GSE-HT (GSE24752), and MI-DEGs and HT-DEGs were confirmed. Patients with HT-complications, like diabetes, smoking, renal failure, coronary artery disease (CAD), stroke, peripheral artery disease (PAD), were not included. We found 70 DEGs in blood samples from patients that underwent emergency percutaneous coronary intervention as compared to control patients within 24 h of hospitalization, and we designated 93 DEGs as the HT-DEGs in hypertension patients. The volcano plot of DEG inclusion criteria is given in Fig. 1. Heatmaps of MI-DEGs in terms of an immune response, cellular signaling, and an inflammatory response were constructed for analysis of gene expression, and these data are displayed in Fig. 2A–C. Fig. 2D–F shows the gene expression values corresponding to an immune response, cell signaling, and T-cell activation among the above HT-DEGs.

Construction of interaction networks and functional enrichment evaluation

We identified 58 and 44 nodes in the PPI networks of MI-DEGs and HT-DEGs, respectively, and these data are presented in Fig. 3. Here, hub nodes (proteins) called high-affinity immunoglobulin γ Fc receptor I (FCGR1A; the degree of intranode connectivity (degree) = 14), a tyrosine protein kinase (FYN; degree = 13), transcription factor PU.1 (SPI1; degree = 12), natural killer cell antigen CD94 (KLRD1; degree = 10), HLA class I histocompatibility antigen α chain E (HLA-E; degree = 9), cytidine deaminase (CDA; degree = 8), killer cell lectinlike receptor subfamily B member 1 (KLRB1; degree = 7), actin-related protein 2 (ACTR2; degree = 6), and folate receptor γ (FOLR3; degree = 5) were detected among MI-DEGs because of the relatively higher degree. However, the genes including regulatory subunits of the 26S proteasome non-ATPase (PSMA, PSMB, PSMC, and PSMD; degree = 20–22) and Myc proto-oncogene (MYC; degree = 9) were regarded as hub genes in relation to HT maintenance.

In the DAVID database, the top 5 GO terms (biological processes) related to HT-DEGs were found to be primarily associated with amino acid transport (p value: 1.37E–06), positive regulation of interferon-gamma production (p value: 0.01), T cell receptor signaling pathway (p value: 0.02), interferon-gamma-mediated signaling pathway (p value: 0.03), and T cell costimulation (p value: 0.03). Among cellular components, there was a significant correlation with the MHC class II protein complex (p value: 0.07) and the integral component of lumenal side of endoplasmic reticulum membrane (p value: 0.09). In addition, the enriched GO terms from the category “molecular functions” were MHC class II receptor activity (p value: 0.04) and protein heterodimerization activity (p value: 0.05). With respect to MI-DEGs, the following biological processes (GO terms) were significantly enriched: defense response (p
value: 4.00E-06), immune response (p value: 1.19E-04), cell surface receptor signaling pathway (p value: 4.27E-04), natural killer cell mediated immunity (p value: 7.13E-03), inflammatory response (p value: 0.05), and positive regulation of angiogenesis (p value: 0.06). Among cellular components, there was enrichment of extracellular region (p value: 3.04E-04), integral component of membrane (p value: 0.02), and integral component of plasma membrane (p value: 0.02). The following molecular functions (GO terms) were found to be enriched: carbohydrate binding (p value: 4.37E-07), transmembrane signaling receptor activity (p value: 7.04E-03), receptor activity (p value: 7.38E-03), MHC class I protein complex binding (p value: 0.01), serine-type endopeptidase activity (p value: 0.01), and protein antigen binding (p value: 0.02; Fig. 4A and B).

KEGG pathway analysis results are illustrated in Fig. 4C, D. The results suggested that the set of HT-DEGs was mainly enriched in multiple pathways of inflammatory diseases, including systemic lupus erythematosus (p value: 2.20E-03), complement and coagulation cascades (p value: 4.30E-03), TGF-β signaling pathway (p value: 7.00E-03), allograft rejection (p value: 0.01), graft-versus-host disease (p value: 0.01), type 1 diabetes mellitus (p value: 0.01), autoimmune thyroid disease (p value: 0.02), inflammatory bowel disease (p value: 0.03), antigen processing and presentation (p value: 0.04), and Th1 and Th2 cell differentiation (p value: 0.05). In the set of MI-DEGs, inflammatory and immune-system-related KEGG terms were found to be enriched, including antigen processing and presentation (p value: 3.97E-04), natural killer cell
mediated cytotoxicity (p value: 2.89E-03), tuberculosis (p value: 8.71E-03), TNF signaling pathway (p value: 0.01), acute myeloid leukemia (p value: 0.04), adipocytokine signaling pathway (p value: 0.04), and PPAR signaling pathway (p value: 0.04; Fig. 4 C and D).

Analysis of enrichment with GO terms using the REACTOME database uncovered additional associations as confirmation and supplementary information. The HT-DEG set turned out to be enriched in such terms as complement cascade (p value: 1.07E-03), transcription of E2F targets under negative control by DREAM complex (p value: 1.97E-03), translocation of ZAP-70 to immunological synapse (p value: 1.97E-03), phosphorylation of CD3 and TCR zeta chains (p value: 2.64E-03), PD-1 signaling (p value: 2.89E-03), interferon gamma signaling (p value: 4.01E-03), "RUNX1 regulates genes involved in megakaryocyte differentiation and platelet function" (p value: 4.66E-03), downstream TCR signaling (p value: 4.79E-03), signaling by TGF-beta family members (p value: 5.36E-03), and TCF dependent signaling in response to WNT (p value: 8.61E-
Similar results were obtained for MI-DEGs, including neutrophil degranulation (p value: 5.53E-11), immunoregulatory interactions between a lymphoid and a non-lymphoid cell (p value: 6.96E-10), metabolism of angiotensinogen to angiotensins (p value: 7.29E-05), endogenous sterols (p value: 7.71E-03), peptide hormone metabolism (p value: 9.50E-03), ROS, RNS production in phagocytes (p value: 0.01), metabolic disorders of biological oxidation enzymes (p value: 0.01), interferon alpha/beta signaling (p value: 0.05), "RUNX1 and FOXP3 control the development of regulatory T lymphocytes" (p value: 0.05), and interleukin-6 signaling (Fig. 4E and F).
Biological-category enrichment and disease-related prediction scores for the co-DEGs

The Rat Genome Database (RGD, http://rgd.mcw.edu) is a valuable resource with a large amount of rat and human genetic data and resources, such as information on genes, quantitative trait loci, microsatellite markers, and rat strains. After recent system updates, the RGD can serve as a high-quality human-disease-centric resource based on emerging *H. sapiens* genomic sequence and annotation pipelines.\(^\text{15}\) By digging into disease-related connections between HT and MI, we found a high degree of overlap in the biological functions between the HT-specific and MI-specific gene sets. Proteins encoded by the related gene sets are located mostly on the plasma membrane. The progression of diseases was found to significantly affect some biological processes, such as multicellular organism development, signal transduction, and response to stress. The changes in cellular function mentioned above are mainly caused by changes in protein binding, catalytic activity, and receptor activity at the molecular level (Table 1).

Although detailed molecular targets and pathways during disease onset and development were not specifically identified, these data are remarkably similar to our results on biological-category enrichment in datasets GSE24752, GSE60993, and GSE62646 (as depicted in Fig. 2 and 4).

To generate gene–disease annotations in detail and to gain a deeper understanding of the genetic changes that are identical between HT patients and MI patients, the Venn map was constructed, and the co-DEGs between HT-DEGs and MI-DEGs were identified (Fig. 3C and Table 2). Of note, only 2 co-DEGs were found. The expression of FOLR3 and NFE2 was low in patients with HT (log2FC: −1.03, \(P\) value: 9.76E-03, and log2FC: −0.75, \(P\) value: 0.04, respectively), and just the opposite happened soon after AMI (log2FC: 0.66, \(p\) value: 0.02, and log2FC: −0.64, \(P\) value: 4.58E-07, respectively). In a comparison between HT and MI, the PPI network showed similar proteins interacting with FOLR3, and the same was true for NFE2, thus indicating possible similarity of upstream and downstream interactions and biological functions between the 2 diseases (Fig. 3D–G).

Table 1 Cardiovascular disease portal and gene ontology annotation overview of human species and human synteny.

| Disease      | Category | Term          | genes |
|--------------|----------|---------------|-------|
| Hypertension (755 total genes) | GO (Cellular Component) | Cell | 977  |
|              |          | Plasma membrane | 693  |
|              |          | Cytoplasm      | 452  |
|              |          | Nucleus        | 348  |
|              |          | Extracellular region | 265  |
|              | GO (Biological Process) | Multicellular organism development | 2k   |
|              |          | Signal transduction | 1k   |
|              |          | Response to stress | 762  |
|              |          | Anatomical structure morphogenesis | 593  |
|              |          | Cell differentiation | 530  |
|              |          | Cell death      | 505  |
| MI (339 total genes) | GO (Cellular Component) | Cell | 408  |
|              |          | Plasma membrane | 368  |
|              |          | Cytoplasm      | 270  |
|              |          | Extracellular space | 187  |
|              |          | Nucleus        | 187  |
|              | GO (Biological Process) | Multicellular organism development | 836  |
|              |          | Response to stress | 536  |
|              |          | Signal transduction | 470  |
|              |          | Anatomical structure morphogenesis | 370  |
|              |          | Cell death      | 363  |
|              |          | Cell differentiation | 295  |
|              | GO (Molecular Function) | Protein binding | 842  |
|              |          | Catalytic activity | 237  |
|              |          | Receptor activity | 201  |
|              |          | Signaling receptor binding | 200  |
|              |          | Peptidase activity | 145  |
|              |          | Nucleotide binding | 93   |

https://rgd.mcw.edu.
showed that the co-DEGs were associated with several CVDs and metabolic diseases, and these data are presented in Fig. 5. Given that the functional enrichment mentioned earlier was observed in a group of genes with significantly altered expression, we used the GSEA of the GEO datasets to explore the potential functions of FOLR3 and NFE2. Because the metric that we selected for ranking gene parameters (Signal2Noise or tTest) requires at least 3 samples for each phenotype, GSE24752 (the HT dataset) failed GSEA owing to too few samples \((n \leq 1\) for HT and \(n \leq 2\) for control) for both phenotypes. For MI analysis, we divided the expression profiling data into datasets “FOLR3 (or NFE2) lower expression” and “FOLR3 (or NFE2) higher expression” according to the median FOLR3 (or NFE2) expression level in the batch-corrected MI dataset (combined datasets GSE60993 and GSE62646). Comparison of transcript levels between the FOLR3 (or NFE2) higher expression and lower expression groups of blood samples from patients with MI revealed a number of DEGs. Our results showed that both FOLR3 and NFE2 expression significantly upregulated autophagy pathways and downregulated pathways related to energy metabolism, including alanine, aspartate, and glutamate metabolism; butanoate metabolism; the citrate cycle; the tricarboxylic acid (TCA) cycle; glycolysis; gluconeogenesis; and pyruvate metabolism (Fig. 6 and Table 3). Collectively, GO term and KEGG pathway enrichment analyses and GSEA of datasets GSE60993 and GSE62646 indicated that the low expression of FOLR3 and NFE2 affects the activity of cellular metabolism and influences autophagy and cell

| Terms | Total | Elements |
|-------|-------|----------|
| MI-DEGs | 65 | STAB1, FLOT2, KCTD12, DOK3, CYP27A1, NCF4, S100A12, ASGR2, TRIB1, ZFP36, LILRA2, GZMH, CTSD, TGFB3, CEBPB, KLRC3, AQP9, RNASE2, LOC441081, FAM20A, ACSL1, SOCS3, SH2D1A, HIST1H4C, TCN2, CDA, CSF3R, SAMD3, ZNF467, FAM101B, LRG1, SLCL1A1, ADM, TARP, SDAD1, SIGLEC9, LILRA5, FCGR1A, MCOLN2, CLC, LILRA3, TBC1D2, KLKD1, ANPEP, SPI1, GZMA, PADI4, CYP1B1, EGR1, P2RX1, DYSF, MCEMP1, GZMK, C1orf21, BCL3, MLF2, CR1, KLKB1, KLRC2, MS4A3, KLRG1, WAS, KLRF1, HP, HSPA1A |
| HT-DEGs | 90 | ZDHHC14, NKAPP1, ACIN1, PVRIG, LINCO0473, FAM1868, LOC102724275, C4BPA, SLCA41, LOC101928893, ZNF891, COQ3, PSMB6, TMEM43, PLA1A, C3AR1, LINCO06899, HLA-DPB1, MLH3, HOTAIR1M, EZF5, IL36A, LOC100506922, RP11-39S16.3, RP11-466A19.8, ORZL13, HIP1, DTHD1, LMTK3, RP11-554J4.1, KCN1V, ACPI1, HLA-DPA1, XK, LINCO0403, HIST1H2B0, MYC, ANK54B, C1orf1126, RNOH, VPREB3, LHBP, SPEF1, WDFC6, NOG, GGT6, RBM53-A53, ND6, OTUB2, HHEX, LOC285556, LOC643085, ERICH4, CSN1S2AP, PLEKHF2, PRDX6, FAM32A, ATF1, HERC2, SLCA6A14, POU6F2-A5, FAM170A, RP11-184E9.2, 2, TMEM55A, RP6-994M1.2, AKR1D1, FUC1D1, TIMM1, CD8, RP11-184B15.2, C1orf91, TRAC, LINCO0551, LOC100507537, CTC-510F12.4, PCTP, AX747191, IRF8, WWA5A, CTBP1-A5, LOC100506563, TCL1A, RP11-568N6.1, ZNF385B, CHRM3-A52, HIST1H2AE, MAGEB6, SKAP2 |
| Overlapped DEGs | 2 | NFE2, FOLR3 |

Figure 5  GSEA of expression of co-DEGs in patients with MI. (A) Expression profiling data were divided into datasets “NFE2 lower expression” and “NFE2 higher expression” according to the median NFE2 expression level. (B) Expression profiling data were divided into datasets “FOLR3 lower expression” and “FOLR3 higher expression” according to the median FOLR3 expression level in the batch-corrected MI dataset (combined datasets GSE60993 and GSE62646). Visualization was implemented in GSEA 3.0 software. The final map was generated by means of R packages “plyr,” “ggplot2,” “grid,” and “gridExtra” for removal of any general or noninformative smaller networks to simplify the final diagram.
death, thereby contributing to changes in the overall expression level of genes after aggressive AMI.

Prediction of miRNAs targeting co-DEGs and analysis of the actual expression levels

Table 4 shows the top 5 carefully predicted miRNAs targeting each co-DEG involved in HT-related MI as a result of combined application of mirDIP, miRWalk, and TargetScan tools. These data gave us an ingenious way to understand how predicted miRNAs correlate with the progression of HT-related MI. GSE24548 contains a miRNA expression profile of platelet samples from 4 patients with their very first AMI and from 3 controls. By modulating the predicted miRNAs and differentially expressed miRNAs (DE-miRNAs) after MI, we hope to determine a more realistic miRNA–mRNA network around key miRNAs (hsa-miR-7-5p, log2FC: 0.82, P value: 2.80E-03, and hsa-miR-548-5p, log2FC: 1.05, P value: 1.14E-04) during MI (Fig. 7).

Discussion

Detecting HT is necessary to prevent AMI. However, inadequate blood pressure monitoring, medication noncompliance, an unhealthy lifestyle, and poor sensitivity to blood pressure fluctuations in the elderly limit effective target control and even cause HT emergencies.4,5,16 Identification of markers and associations between HT and MI is therefore worthy of attention and may reveal a target for primary prevention and community therapy. In this study, biological functions such as the inflammatory and immune response,

Table 3  GSEA enrichment results for FOLR3 and NFE2C genes with original data obtained from GSE60993 和 GSE62646.

| Gene     | Regulation | Term                                         | ES     | NES     | P value   |
|----------|------------|----------------------------------------------|--------|---------|-----------|
| FOLR3    | Up         | KEGG_REGULATION_OF_AUTOPHAGY                 | 0.519914| 1.668857| 0.008180  |
|          | Up         | KEGG_PYRUVATE_METABOLISM                     | −0.58483| −1.67831| 0.010395  |
|          | Up         | KEGG_BUTANOATE_METABOLISM                    | −0.57011| −1.67309| 0.010309  |
|          | Up         | KEGG_ALANINE ASPARATE_AND_GLUTAMATE_METABOLISM| −0.50376| −1.66442| 0.020666  |
|          | Up         | KEGG_GLYCOLYSIS_GLUconeogenesis              | −0.4773 | −1.65101| 0.017647  |
|          | Up         | KEGG_PRIMARY_IMMUNODEFICIENCY                | −0.73625| −1.59229| 0.012605  |
|          | Up         | KEGG_THYROID_CANCER                          | −0.49717| −1.5756 | 0.047325  |
|          | Up         | KEGG_CITRATE_CYCLE_TCA_CYCLE                | −0.5862 | −1.54846| 0.069106  |
|          | Up         | KEGG_REGULATION_OF_AUTOPHAGY                 | 0.519914| 1.695634| 0.012000  |
|          | Up         | KEGG_PYRUVATE_METABOLISM                     | −0.58483| −1.75248| 0.012121  |
|          | Up         | KEGG_BUTANOATE_METABOLISM                    | −0.57011| −1.72362| 0.010204  |
|          | Up         | KEGG_ALANINE ASPARATE_AND_GLUTAMATE_METABOLISM| −0.50376| −1.70064| 0.009921  |
|          | Up         | KEGG_GLYCOLYSIS_GLUconeogenesis              | −0.4773 | −1.64576| 0.005769  |
|          | Up         | KEGG PRIMARY_IMMUNODEFICIENCY                | −0.73625| −1.61349| 0.009843  |
|          | Up         | KEGG_CITRATE_CYCLE_TCA_CYCLE                | −0.5862 | −1.58633| 0.062124  |
|          | Up         | KEGG_THYROID_CANCER                          | −0.49717| −1.56898| 0.039293  |
|          | Up         | KEGG_WNT SIGNALING_PATHWAY                   |        |         | 0.062500  |
cellular signaling as well as T-cell activation and development were found to be closely related to the maintenance and onset of HT and to AMI through combined mining of public databases. A few hub genes—directly or indirectly coregulating the endocrine and cardiovascular systems—were found among the HT-DEGs through Comparative Toxicogenomics Database-mediated prediction of the PPI network and diseases. For example, a distinctive feature of HT in the heart is hypertrophy, meaning limited capacity for adaptation. This age-related mechanical load in the myocardium is mainly due to diminished induction of proto-oncogene MYC on the molecular level. Increased MYC expression is observed in hypertrophied hearts, whereas a heart-specific knockdown of MYC expression postpones and reverses the progression of cold-induced cardiac hypertrophy with decreased cardiac output, left ventricle wall thickness, and decreased heart weight. HIST1H2BO is a core histone among the proteins wrapped around DNA and forms the nucleosome structure of the chromosomal fiber. Changes in HIST1H2BO expression are seen in differentiated macrophages and systemic lupus erythematosus, an autoimmune disease induced by prolonged exposure of apoptotic cells to the immune system. Dysregulation of HIST1H2BO expression is accompanied by abnormal inflammatory infiltration, and the causes of this dysregulation in HT are yet to be studied. Cardiac troponins, CK-MB, total CK, and myoglobin are already widely used as key diagnostic and prognostic markers in clinical practice. We also found several candidate marker genes whose expression changed significantly in blood samples from patients with MI as early as within 4 h of admission. Short-type peptidoglycan recognition protein 1 gene (PGLYRP1) encodes an innate-immunity protein that directly breaks down the structure of microbial cell wall peptidoglycan (PGNs) and plays an important part both in antibacterial defenses and several inflammatory diseases. FYN-dependent phosphorylation of a nonreceptor protein tyrosine kinase activates immunoreceptor signaling. FYN deficiency is protective, whereas FYN activation is detected in patients with lupus nephritis, a severe complication of systemic lupus erythematosus. The SPI1 transcription factor has been proven to be a key modulator of hematopoiesis processes and inhibits the self-renewal function of hematopoietic stem cells, whereas human leukocyte antigen E (HLA-E), a nonclassical major histocompatibility complex class I molecule, has been identified as a marker of hematological cancers at terminal stages.

Additionally, research attention should be focused on FOLR3 and NFE2: the 2 co-DEGs of HT and MI. A loss of function or variants of folate receptor FOLR3 may affect folate availability, and folic acid has been reported to reduce serum homocysteine levels and (in some cases) blood pressure. Hyperhomocysteinemia above the normal range (5–15 μmol/L) has been identified as an independent predictor of CVDs and HT, in addition to reducing the integrity of vascular endothelial cells and smooth muscle cells that maintain the integrity of blood vessels as well as renal function. Elevated homocysteine levels induce a hypercoagulation state, makes a clot more resistant to fibrinolysis, and contributes to inflammation and oxidative stress: the 2 key mechanisms underlying the pathological changes during CVD. Earlier studies suggest that NFE2 acts as a critical regulator of globin gene expression. NFE2 knockout mice feature a major defect in megakaryocyte biogenesis and severely impaired platelet production, whereas an abnormal increase in NFE2 expression is observed in hematological disorders such as polycythemias. Recent studies shed light on various functional roles of NFE2 in nonhematopoietic tissues. Hepatic NFE2 overexpression has been found to promote gluconeogenesis.

### Table 4

| Genes | Predicted miRNAs | Category | Term | genes | miRNAs | P value |
|-------|-----------------|----------|------|-------|--------|---------|
| FOLR3 | hsa-miR-7-5p | KEGG | Fatty acid biosynthesis | 4 | 2 | 2.42e-19 |
|       | hsa-miR-548-5p | Hippo signaling pathway | 46 | 5 | 6.31e-06 |
|       | hsa-miR-6077 | Adherens junction | 29 | 5 | 1.92e-04 |
|       | hsa-miR-876-5p | Estrogen signaling pathway | 21 | 5 | 2.66e-03 |
|       | hsa-miR-1290 | Protein processing in endoplasmic reticulum | 18 | 5 | 1.77e-03 |
| NFE2  | hsa-miR-146b-5p | KEGG | Cocaine addiction | 14 | 4 | 5.22e-04 |
|       | hsa-miR-2115-3p | Thyroid hormone synthesis | 16 | 3 | 1.40e-03 |
|       | hsa-miR-518F-5p | ErbB signaling pathway | 25 | 4 | 1.86e-03 |
|       | hsa-miR-7515 | Circadian rhythm | 11 | 3 | 0.029 |
|       | hsa-miR-329-3p | Vasopressin-regulated water reabsorption | 15 | 4 | 0.043 |
|       | hsa-miR-329-3p | Vasopressin-regulated water reabsorption | 15 | 4 | 0.043 |
|       | hsa-miR-329-3p | Vasopressin-regulated water reabsorption | 15 | 4 | 0.043 |

**Table 4** The Gene Ontology (GO) terms and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways enrichment among predicted miRNAs and Co-DEGs.
and lipid deposition and to cause hyperglycemia in normal mice through the FAM3A-ATP-Akt pathway. The NFE2 transcription factor was identified more than 25 years ago, yet its roles in HT and myocardial tissues remain unclear. In this study, our dataset analysis revealed that this positive relation warrants further research on a direct link between FOLR3 or NFE2 and HT or MI. Notably, in patients with MI, GSEA showed higher levels of FOLR3 and NFE2 accompanied by an increasing expression of autophagy-related genes and a decreasing level of energy metabolism-related genes such as genes of the TCA cycle. This mechanism is similar to the compensatory increase in BNP levels in response to the relief of heart failure symptoms. Additional observational research should be conducted.

MiRNAs are well-known powerful regulators of gene expression. Their potential to serve as circulating biomarkers and for gene therapy with delivery by an adenovirus, plasmid, or lentiviral vector is widely accepted in terms of HT and MI. By identifying the genes in the overlap between the set of predicted miRNAs targeting the co-DEGs and the set of DE-miRNAs specific to patients with MI, we found that miR-7 and miR-548 may be potential miRNA markers of HT-related MI. Previous research has confirmed that miR-7 and miR-26b are upregulated in the serum of HT patients with left ventricular hypertrophy, whereas miR-375, miR-7, and miR-29b expression is significantly altered in patients with primary aldosteronism, a common cause of human HT. In vitro overexpression of miR-7b inhibits ischemia/reperfusion-induced apoptosis of H9C2 neonatal rat cardiac cells by targeting the HIF1a/p-P38 pathway. On the other hand, IFN-λ1 (also known as IL-29), predicted and validated as the direct target of miR-548, is a major cytokine recently proven to suppress the migratory capacity of neutrophils and the formation neutrophil extracellular traps (NETs), which are a major mediator of thromboinflammation. Polyphosphate released from activated platelets results in NET formation in patients with ST-segment elevation MI via mTOR inhibition and autophagy induction; these 2 pathways can be counteracted by IL-29 treatment, inhibiting NET formation. At this point, our biological-category enrichment analysis gave rise to reasonable speculation about the cause of changes after MI with a complete closed loop. Decreased expression of miR-548 may upregulate 2 cardioprotective target genes (FOLR3 and IL-29 mRNAs), thereby reducing blood pressure and thrombus burden. This phenomenon may explain the enrichment results that we obtained regarding inflammation, immune-system-related biological processes, the TCA cycle, folic acid metabolism-associated molecular function, and abnormalities in such pathways as autophagy and the mTOR cascade.

Here, we suggest a new set of novel biomarkers and important molecular targets during the progression of HT and MI, including 2 co-DEGs (FOLR3 and NFE2 mRNAs) and 2 miRNAs (miR-7 and miR-548). This is the first data-mining study to identify co-expressed differentially expressed genes (co-DEGs) between HT and MI. Our biological-category enrichment analysis gave rise to reasonable speculation about the cause of changes after MI with a complete closed loop. Decreased expression of miR-548 may upregulate 2 cardioprotective target genes (FOLR3 and IL-29 mRNAs), thereby reducing blood pressure and thrombus burden.

PCR and ELISA may validate these markers for assessing the risk of HT-related MI. Experiments with in vitro and in vivo models as well as prospective clinical studies will be indispensable for validation of these markers for diagnosis and theragnostic.

Materials and methods

Microarray data and preprocessing

mRNA expression profiles (datasets GSE24752, GSE60993, and GSE62646) and an miRNA expression profile (the GSE24548 dataset) were retrieved from the Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/) and were based on platform GPL570 (Affymetrix Human Genome U133 Plus 2.0 Array, Affymetrix Inc., Santa Clara, CA, USA), GPL6884 (illumina HumanWG-6 v3).

Figure 7 Hierarchical clustering analysis of DE-miRNAs. (A) Results of hierarchical clustering analysis of expression of MI-specific DE-miRNAs. (B-C) Microarray expression levels of miR-7 and miR-538 in GSE24548 in detailed representation. Orange: higher expression, blue: lower expression.
0 Expression Beadchip, Illumina Inc., San Diego, CA, USA), GPL6244 (Affymetrix Human Gene 1.0 ST Array, Affymetrix Inc.), and GPL8227 (Agilent-019,118 Human miRNA Microarray 2.0 G4470B, Agilent Technologies, Palo Alto, CA, USA). Additionally, GSE24752 contains data on peripheral-blood samples from 3 patients with HT and 3 normotensive controls. The expression profiles of 17 blood samples (from patients with acute coronary syndrome visiting an emergency department within 4 h) and of 7 blood samples (from normal controls included in the GSE60993 dataset) were used to identify AMI-related gene expression differences and to determine enrichment with biological categories, together with 28 peripheral blood mononuclear cell samples from patients with ST-segment elevation MI on admission and 14 samples from patients with stable coronary artery disease without a history of MI as a control group included in the GSE62646 dataset, as complementary data processing. On the basis of the results, mRNA expression profiling data on platelets from 4 patients with very first acute MI and from 3 controls included in GSE24548 were selected for subsequent analysis of mRNA–miRNA regulatory networks.

**Screening of DEGs**

To assess and transform raw datasets, Perl software (Version 5.28.2) and R packages "affy," "limma," and "sva" were applied to conduct log₂ transformation and background correlation.⁴⁰ Datasets GSE60993 and GSE62646 were combined by batch correction to eliminate the variance due to the interexperiment differences caused by subjective and objective factors, for more accurate and reliable results. Original P values were adjusted by the Benjamini–Hochberg method. However, the lowest P value (0.000123) was greater than the inverse of the total gene number (0.0001205) in GSE24752, meaning that the adjusted P values were unreliable. Because alterations of RNA levels are usually lower in peripheral-blood samples and peripheral blood mononuclear cells than in other tissues,⁴¹ we used the criteria |log₂ FC| > 1 (where FC is fold change) and original P < 0.05 to normalize the results and identify the DEGs in all the mRNA and miRNA datasets. Co-DEGs for HT-DEGs and MI-DEGs were identified in the overlap between these 2 gene sets in a Venn diagram.

**Construction of interaction networks and functional enrichment**

The Database for Annotation Visualization and Integrated Discovery (DAVID, https://david.ncifcrf.gov/) online database was utilized to annotate and visualize Gene Ontology (GO) enrichment using R package “GOplot.” Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway and REACTOME enrichment analyses were conducted and visualized using R packages “clusterProfiler” and “ReactomePA.”⁴²,⁴³ A P value cutoff <0.05 was assumed to indicate significant enrichment of co-DEG sets. To probe the potential pathways with respect to the cardiovascular effect of co-DEGs at the single-gene level, GSEA 3.0 (http://software.broadinstitute.org/gsea/index.jsp) software was used to analyze the combined dataset of GSE60993 and GSE62646, which is the transcription profile adjusted via array analysis of patients with MI according to a standard protocol.⁴⁴,⁴⁵ DEG-encoded proteins were identified, and protein–protein interaction (PPI) network data were obtained using the Search Tool for the Retrieval of Interacting Genes database (STRING, http://string-db.org). The Cytoscape software (Version 3.6.1) was employed for the visualization of intranode connectivity degrees and interactions (or edges) among the candidate DEGs, with the following inclusion criterion: confidence score >0.4.

**Identification and verification of miRNAs targeting DEGs**

Online target prediction software microRNA Data Integration Portal (mirDIP, http://ophid.utoronto.ca/mirDIP), TargetScan (version 7.1, http://www.targetscan.org/vert_71/), and miRWalk (http://mirwalk.umm.uni-heidelberg.de/) were used in combination to predict miRNAs targeting the co-DEGs. We selected top 5 candidate miRNAs of each co-DEG according to a higher prediction score from 2 or more prediction tools. The most representative and valuable predicted miRNAs for the co-DEGs as well as the DE-miRNAs from GSE24548 were studied via a Venn diagram to determine the overlap between these two gene sets.

**Identification of genes associated with CVDs or metabolic diseases**

The Rat Genome Database (RGD) Disease database (http://rgd.mcw.edu/wg/portals/) was utilized to determine the integrated gene landscape and enrichment with biological categories for Homo sapiens HT or MI. The Comparative Toxicogenomics Database (http://ctdbase.org/) was employed to predict novel associations between co-DEGs and CVDs or metabolic diseases via a computational prediction score.

**Availability of data and material**

The microarray datasets GSE60993, GSE62646, GSE24752, and GSE24548 can be obtained from the NCBI-GEO online database (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi).

**Authors contribution**

Wei Z.L. designed the study and performed the bioinformatics analysis. Wei Z.L., Li Q.L., and Yin Y. wrote this manuscript. Wei Z.H. and Xu B. were in charge of language editing. All the coauthors read and approved the final manuscript.

**Conflict of Interests**

The authors declare no conflict of interests.
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List of abbreviations

HT hypertension
AMI acute myocardial infarction
ACS acute coronary syndrome
CVD cardiovascular diseases
GEO Gene Expression Omnibus
KEGG Kyoto Encyclopedia of Genes and Genomes
DAVID The Database for Annotation Visualization and Integrated Discovery
STRING Search Tool for the Retrieval of Interacting Genes database
DEG differentially expressed gene
CO-DEG co-expressed differentially expressed genes
GO Gene Ontology
RGD The Rat Genome Database
I/R ischemia/reperfusion
PCI percutaneous coronary intervention
SBP systolic blood pressure
DBP diastolic blood pressure
MACE major adverse cardiovascular events
GSEA Gene Set Enrichment Analysis
mirDIP microRNA Data Integration Portal
CICH cold-induced cardiac hypertrophy
FOLR3 folate receptor 3
NFE2 nuclear factor, erythroid-derived 2

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