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Research article

Integrated network analysis to explore the key mRNAs and lncRNAs in acute myocardial infarction

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Abstract: Acute myocardial infarction (AMI) is the most severe cardiovascular event in the world. However, the molecular mechanisms underlying AMI remained largely unclear. Recently, long non-coding RNAs (lncRNAs) were reported to play important roles in human diseases. In the present work, we analyzed a public dataset GSE48060 to confirm key lncRNAs and mRNAs in AMI. We observed 4835 mRNAs and 442 lncRNAs were significantly differently expressed in AMI. Then, we for the first time constructed PPI networks and lncRNA co-expression networks in AMI. The protein-protein interaction (PPI) networks revealed several mRNAs such as RHOA, GNB1, GNG, RAC1, FBXO32, DET1, MEX3C and HECTD1 functioned as key regulators in AMI. LncRNA co-expression network analysis showed 8 lncRNAs (CA5BP1, LOC101927608, BZRAP1-AS1, EBLN3, FGD5-AS1, HNRNPU-AS1, LINC00342, and LOC101927204) played key roles in AMI. Gene ontology (GO) analysis demonstrated these differently expressed lncRNAs were associated with more signaling pathways, such as regulating transcription, protein amino acid phosphorylation, signal transduction, development. Taken together, our research unveiled a series of key lncRNAs and mRNAs in AMI. Several lncRNAs, including CA5BP1, LOC101927608, BZRAP1-AS1, EBLN3, FGD5-AS1, HNRNPU-AS1, LINC00342, and LOC101927204 were identified as key lncRNAs. PPI networks were constructed to reveal key mRNAs in AMI. These results provided useful information for exploring novel molecular target therapy for AMI.

Keywords: differentially expressed gene; long non-coding RNA; acute myocardial infarction;

protein-protein interaction analysis

1. Introduction

Acute myocardial infarction (AMI) is one of the most severe cardiovascular events and causes more than a third of deaths in developed nations annually [1]. Of note, the global burden of acute myocardial infarction has shifted to low- and middle-income countries, including China [2]. AMI was divided into two kinds of histological categories, ST-elevation myocardial infarction (STEMI) and non-STEMI (NSTEMI) [3]. Cardiac troponin (cTn) isoforms I and T had emerged as the "gold standard" for the early diagnosis of AMI, which were detectable within 2−3 h and highly sensitive and specific for myocardial injury [4–6]. Of note, the mechanism underlying AMI progression remained unclear. Classic cardiovascular risk factors, including hypertension, diabetes, and smoking, had been showed to play a crucial role in AMI progression [7,8]. Recently, a few studies reported some mRNAs such as BNP and CRP were dysregulated in AMI progression [9,10]. Therefore, there was still an active demand to find new regulators to unveil the molecular mechanisms of AMI and provide new biomarkers for AMI.

More than 80%- of the human genome encodes non-coding transcripts [11]. In AMI, increasing studies reported showed non-coding RNAs, including miRNAs and lncRNAs, played important roles in AMI [12,13]. For example, inhibitions of microRNA-16 protect against AMI by reversing beta2-adrenergic receptor downregulation [14]. Lately, lncRNAs, a novel kind of ncRNAs containing longer than 200 nts, had gained increased attention [15]. Devaux et al. and Yang et al. reported a cluster of lncRNAs are dysregulated in peripheral blood cells of AMI patients [13,16]. The circulating lncRNA urothelial carcinoma-associated 1 (UCA1) was down-regulated within 3 days after the onset of AMI [17]. These reports suggested the potential prognostic value of lncRNAs in AMI. However, the knowledge of the role of lncRNAs in AMI is still limited.

In this work, we confirmed differentially expressed lncRNAs and mRNAs in the AMI by analyzing GSE48060. PPI network and lncRNA co-expression networks were built to determine hub mRNAs and lncRNAs. After that, GO and KEGG pathway analysis was utilized to study the potential functions of DEGs. This work would give important messages to determine the potential candidate biomarkers for diagnosis, prognosis, and drug targets in AMI.

2. Materials and method

2.1. Microarray data

According to Suresh et al.'s reports [18], the dataset of GSE48060 has downloaded the Gene Expression Omnibus database (GEO) (Dataset link: https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE48060). In this dataset, it contains 31 whole blood samples acquired from AMI patients and 21 controls samples acquired from normal echocardiogram AMI patients.

2.2. LncRNA classification pipeline

In this work, a pipeline was utilized to identify the lncRNA expression pattern in AMI, which was described by Zhang et al [19]. Briefly, the Affymetrix HG-U133 Plus 2.0 probe set ID was used to map to the NetAffx Annotation Files (HG-U133 Plus 2.0 Annotations, CSV format, release 31, 08/23/10). Second, the probe sets that were assigned with a RefSeq transcript ID in the NetAffx annotations were extracted. For the probe sets with RefSeq IDs, we only retained those labeled as "NR_" (NR indicates non-coding RNA in the RefSeq database). Third, we filtered the probe sets obtained in step 2 by filtering out pseudogenes, rRNAs, microRNAs and other short RNAs including tRNAs, snRNAs and snoRNAs. Finally, 2448 annotated lncRNA transcripts with corresponding Affymetrix probe IDs were generated.

2.3. Differentially expressed genes

The raw datasets of AMI were collected from GEO. The raw data was analyzed by utilizing the R software limma package [20]. The differential expression genes were satisfied with fold changes ≥ 2 and P values <0.05.

2.4. GO & KEGG pathway analysis

The Database for Annotation Visualization and Integrated Discovery (DAVID; http://david.ncifcrf.gov) (version 6.8) integrating the information of biological functions of genes and analysis tools was used to unravel the GO and KEGG pathway of targets. [21].

2.5. Co-expression networks and protein-protein interaction networks construction and analysis

In this study, the Pearson correlation coefficient between lncRNA and mRNA was calculated according to their expression value. The co-expressed DEG-lncRNA pairs with the absolute value of the Pearson correlation coefficient ≥ 0.75 were selected and the co-expression network was established using Cytoscape software. We applied the analysis of the interactions among mRNAs by utilizing STRING online software was utilized to analyze [22] and the combined score > 0.4 was utilized as the cut-off criterion [23]. The PPI network was built by utilizing Cytoscape software [24].

2.6. Statistical analysis

Because the distribution of expression data is normal，statistical comparisons between groups of normalized data were performed using the T-test according to the test condition. $p < 0.05$ was selected statistical significance with a 95% confidence level.

3. Results

3.1. Identification of DEGs and lncRNAs in AMI

In our work, a public AMI mRNA expression profiling (GSE48060), which included 31 AMI patients and 21 controls blood samples, was analyzed to identify differentially expressed genes (DEGs) in AMI. DEGs analysis showed totally 4835 DEGs were determined through AMI patients

samples, containing 2497 up-regulated genes and 2348 down-regulated genes. The heat map of the DEGs in AMI and control samples is shown in Figure 1A.

We further compared the lncRNA expression patterns between FL and DLBCL. We found 300 lncRNAs were up-regulated and 142 lncRNAs were down-regulated in AMI paralleled with normal samples (Figure 1B).

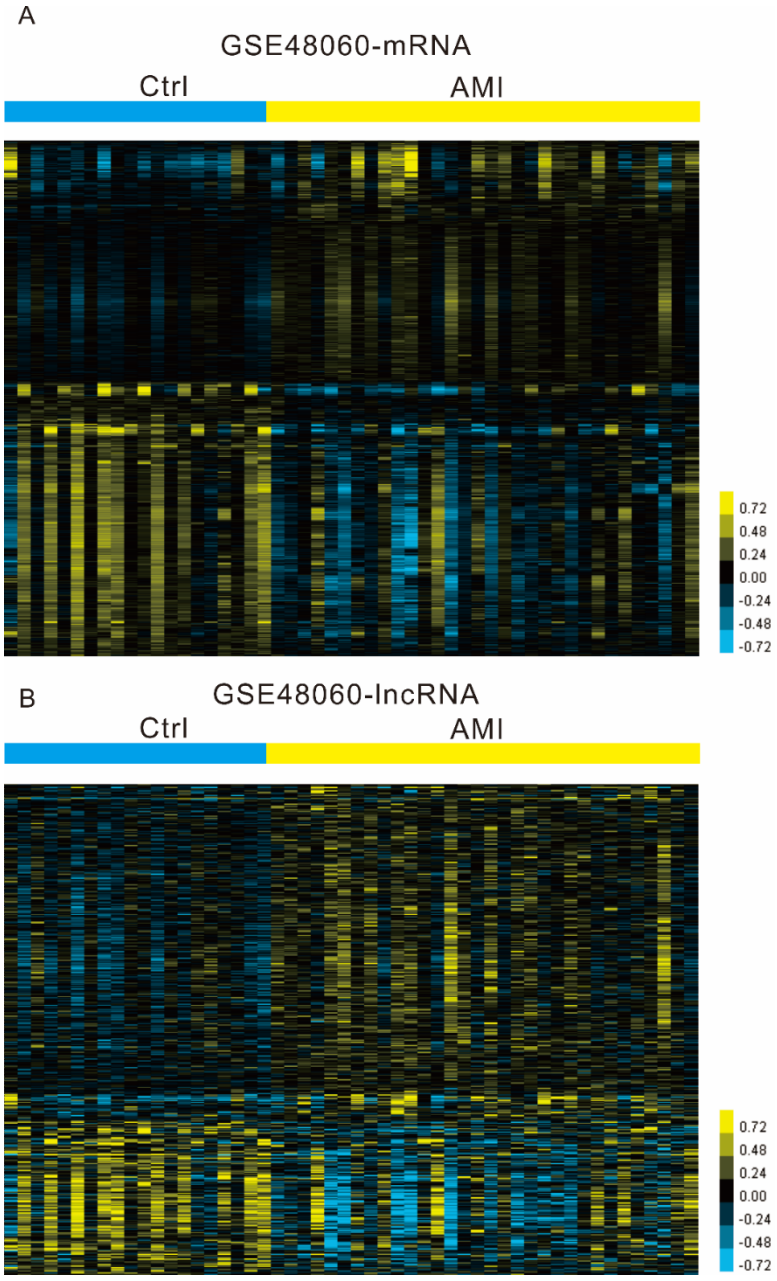

Figure 1. Identification of DEGs and lncRNAs in AMI (Acute Myocardial Infarction). (A) Hierarchical clustering analysis shows differential genes expression in the AMI by using GSE48060. DEGs analysis shows 2497 up-regulated genes and 2348 down-regulated genes in AMI. (B) Hierarchical clustering analysis shows differential lncRNAs expression in the AMI by using GSE48060. Differential lncRNAs analysis shows 300 lncRNAs up-regulated and 142 lncRNAs down-regulated in AMI.

3.2. PPI network analysis of DEGs in AMI

Based on the STRING database [25], we constructed PPI networks for up- and down-regulated mRNAs in AMI. The cut-off criterion of this analysis was that the score > 0.9. As shown in Figure 2, the up-regulated PPI network included 598 nodes and 1735 edges. 8 significant modules (score >10) were identified in the up-regulated PPI network using the MCODE plug-in of Cytoscape. RHOA (degree = 43), GNB1 (degree = 41), GNG7 (degree = 41), and RAC1 (degree = 26) were identified as hub nodes in PPI network (Figure 2).

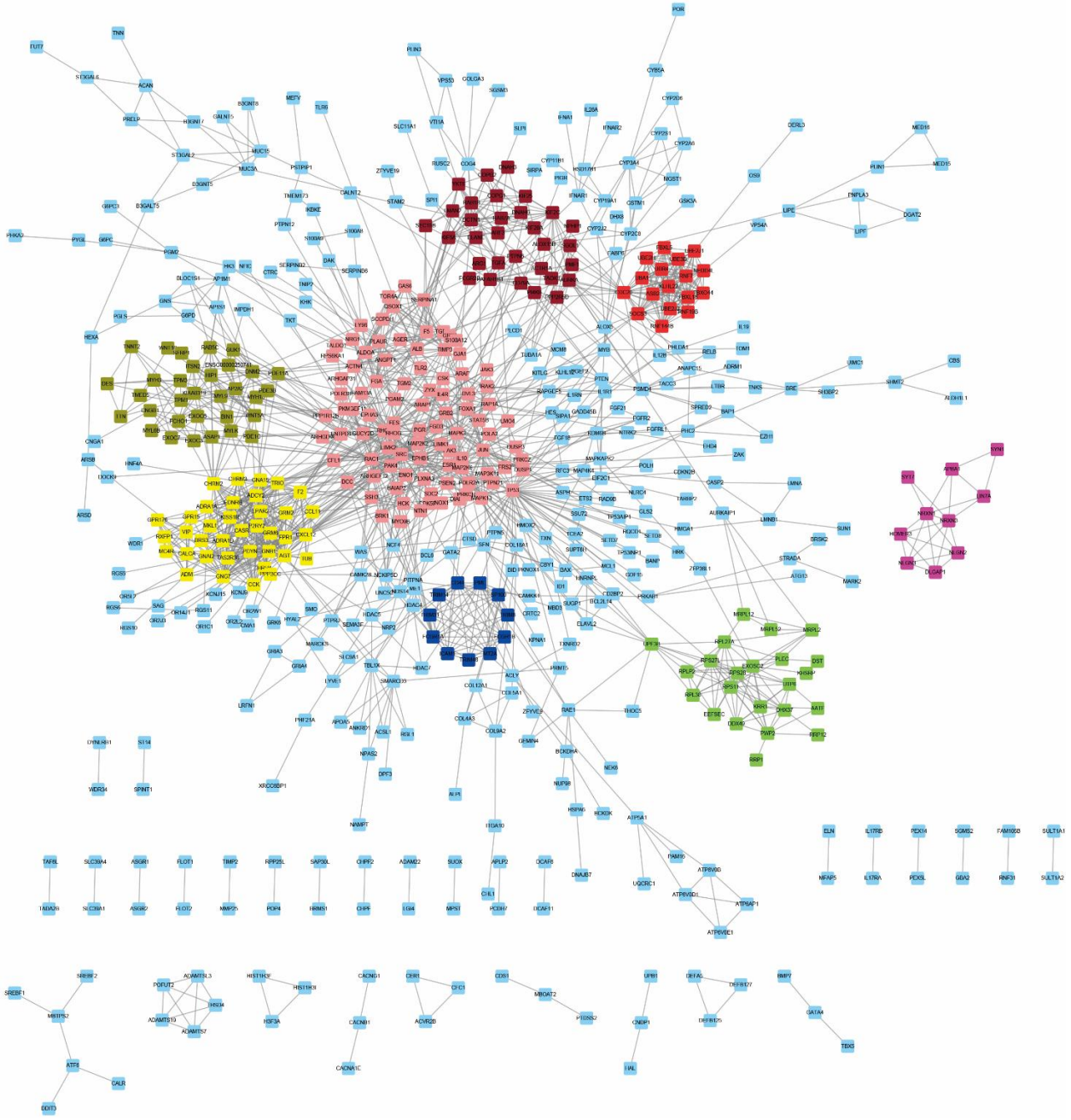

Figure 2. The up-regulated PPI network analysis of DEGs in AMI. Construction of PPI networks for up-regulated genes in AMI compared to control samples.

We found down-regulated PPI network included 662 nodes and 3114 edges. Meanwhile, nine significant modules with score >10 were identified in down-regulated PPI (Figure 3). The nodes that had higher degrees including CDC23 (degree = 40), FBXO32 (degree = 37), DET1 (degree = 36), MEX3C (degree = 35), HECTD1 (degree = 34), HERC4 (degree = 33), POLR2K (degree = 33), CUL5 (degree = 31), UNKL (degree = 30) (Figure 3).

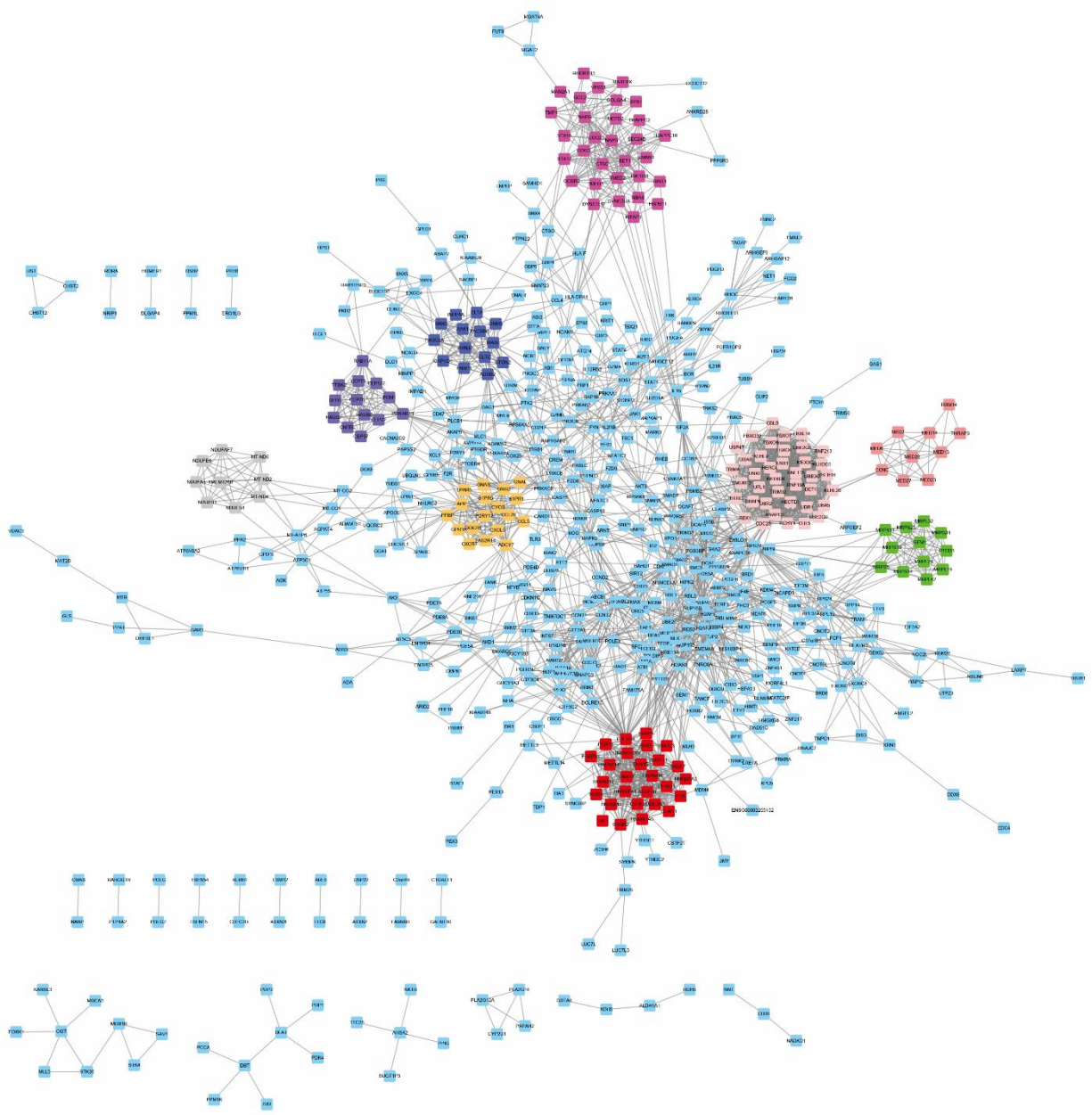

Figure 3. The down-regulated PPI network analysis of DEGs in AMI. Construction of PPI networks for down-regulated genes in AMI compared to control samples.

3.3. Co-expression network analysis of differently expressed lncRNA in AMI

It is worth noting that the roles of dys-regulated lncRNAs in AMI are still dimness. Therefore,

the co-expression network of lncRNAs was built to unveil their potential functions in AMI. In this research, only the co-expressed DEG-lncRNA pairs (Pearson correlation coefficient ≥ 0.75) were selected to construct the co-expression network. The up-regulated lncRNA associated network contained 49 lncRNAs and 502 differentially expressed genes (Figure 4). Meanwhile, 71 lncRNAs and 1156 DEGs were included in the down-regulated lncRNA linked network totally (Figure 5).

A part of lncRNAs were well-known and reported to play important roles in many types of human diseases, such as GAS5, TUG1 and MALAT1. However, most of these lncRNAs were never reported in human diseases. Several lncRNAs were identified as key regulators in AMI by regulating more than 50 DEGs, such as up-regulated CA5BP1 and LOC101927608, and down-regulated BZRAP1-AS1, EBLN3, FGD5-AS1, HNRNPU-AS1, LINC00342, and LOC101927204.

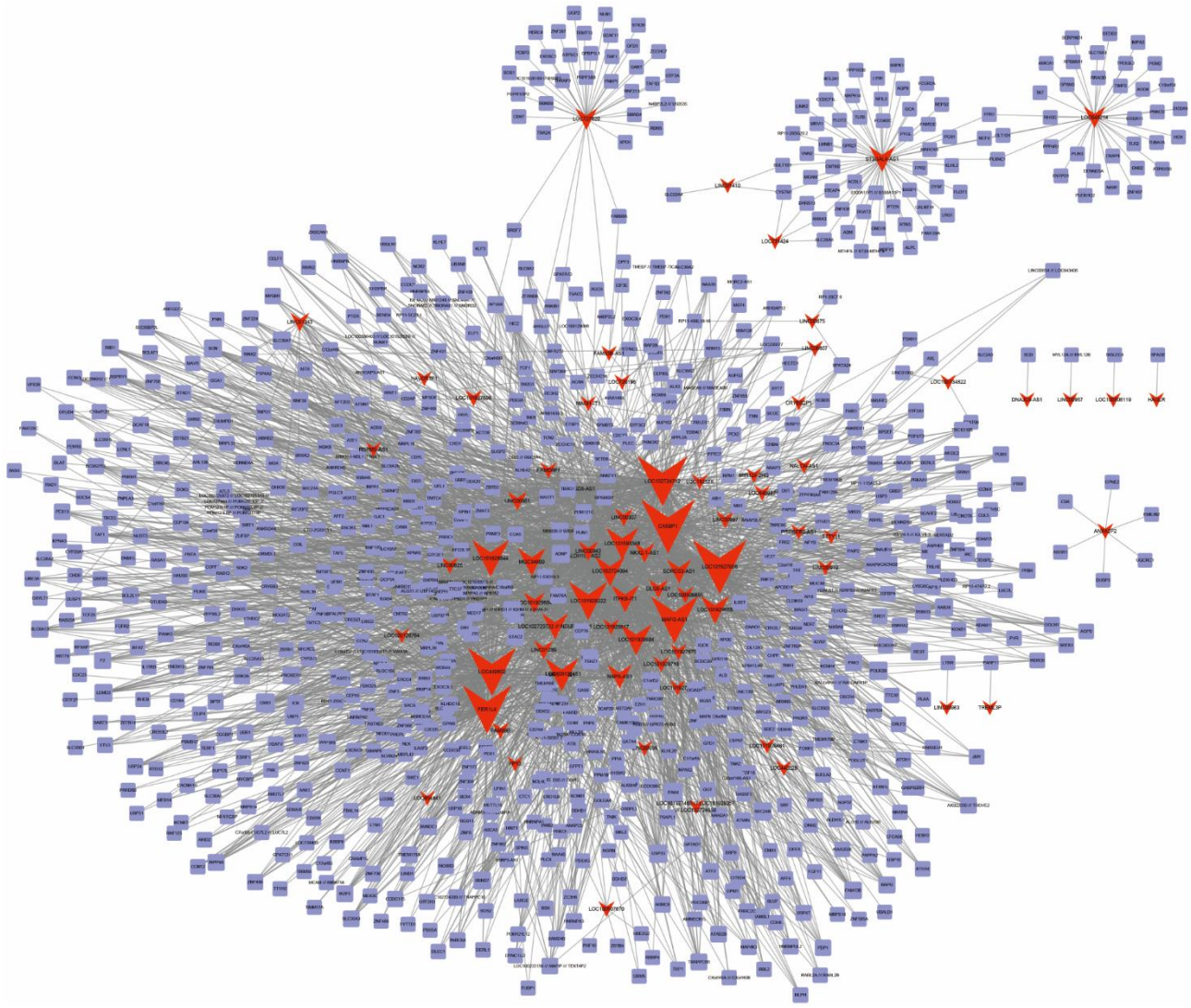

Figure 4. The up-regulated lncRNA associated network. Construction of up-regulated lncRNAs mediated the co-expression network in AMI compared to control samples. Red nodes, up-regulated lncRNAs; purple nodes, differentially expressed genes.

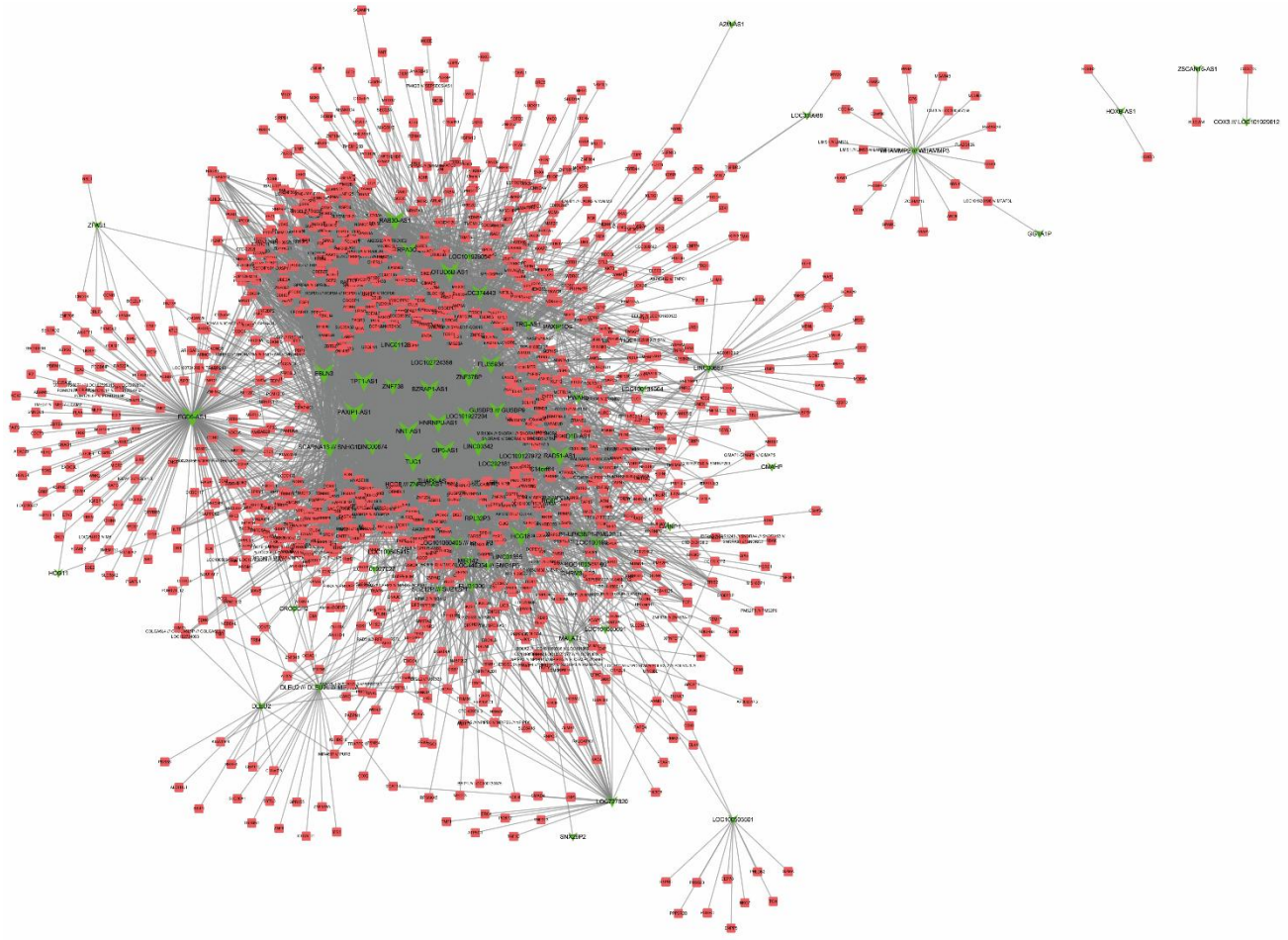

Figure 5. The down-regulated lncRNA associated network. Construction of down-regulated lncRNAs mediated the co-expression network in AMI compared to control samples. Green nodes, down-regulated lncRNAs; pink nodes, differentially expressed genes.

3.4. GO and KEGG Analysis of differentially expressed lncRNAs

Furthermore, for obtaining differentially expressed mRNAs, we utilized GO and KEGG analysis. GO analysis demonstrated that the dys-regulated lncRNAs were associated with regulating transcription, protein amino acid phosphorylation, development, interspecies interaction between organisms, cell cycle, signal transduction, cell adhesion, intracellular signaling cascade, protein transport, apoptosis, ubiquitin-dependent protein catabolism, negative regulation of cell proliferation, modification-dependent protein catabolism, RNA splicing, cell division, nuclear mRNA splicing, DNA repair, response to DNA damage stimulus (Figure 6A-6B).

The results of the KEGG pathway indicated that upregulated genes were associated with pathways associated with Ubiquitin-mediated proteolysis, Insulin signaling pathway, Tight junction, Axon guidance, Basal transcription factors, Regulation of actin cytoskeleton, MAPK signaling pathway, and ErbB signaling pathway. Downregulated genes were mainly associated with T cell

receptor signaling pathway, MAPK signaling pathway, Chronic myeloid leukemia, Cell cycle, ErbB signaling pathway, Axon guidance, Ubiquitin mediated proteolysis, Insulin signaling pathway, p53 signaling pathway (Figure 6C-6D).

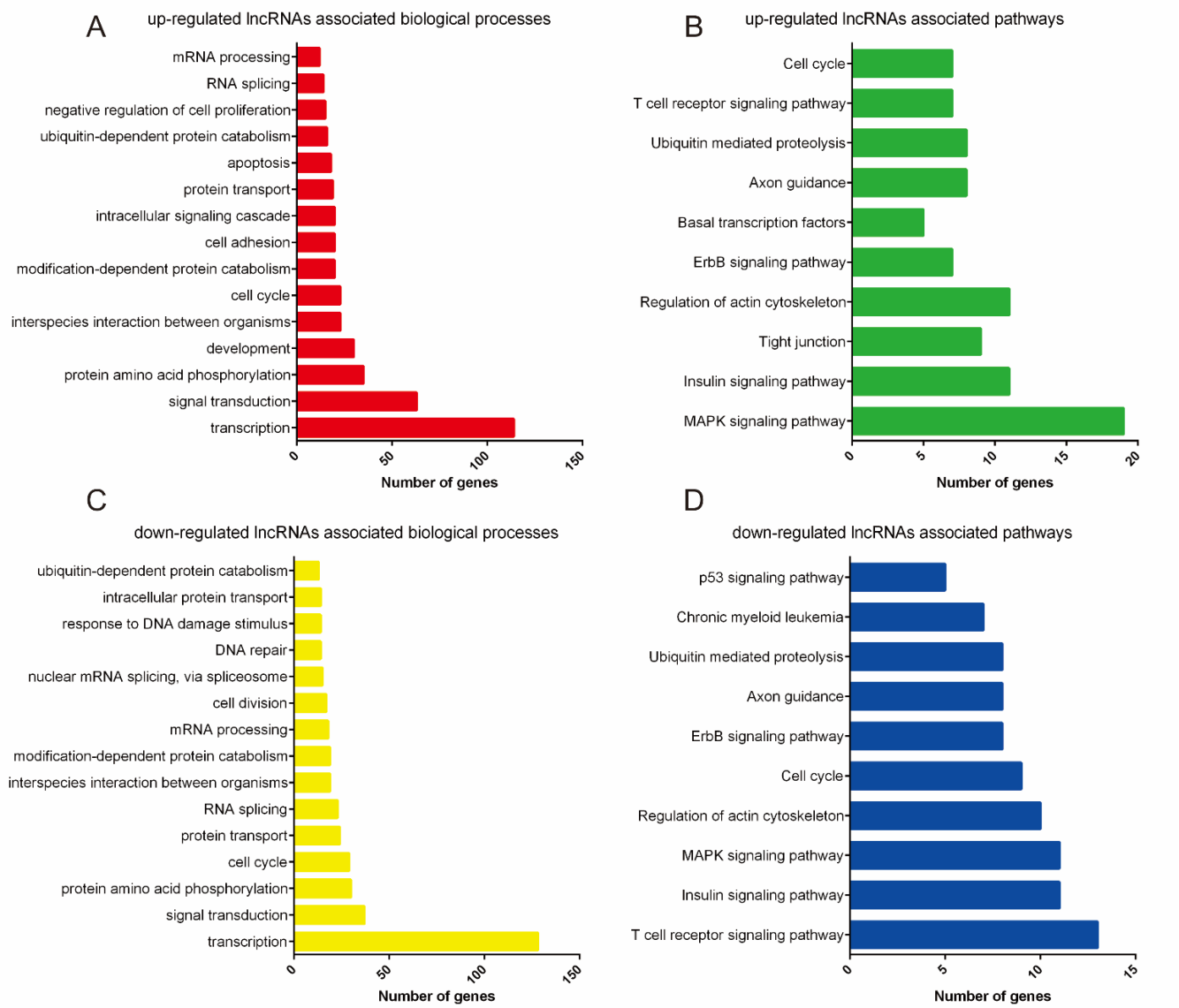

Figure 6. GO and KEGG Analysis of differentially expressed lncRNAs. (A) GO analysis shows up-regulated lncRNA associated biological processes. (B) GO analysis shows down-regulated lncRNA associated biological processes. (C) KEGG pathway analysis shows up-regulated lncRNA associated pathways. (D) KEGG pathway analysis shows down-regulated lncRNA associated pathways.

4. Discussion

Bioinformatics analysis, such as PPI network analysis, co-expression analysis, and KEGG analysis, had been suggested as useful tools to reveal disease-specific mechanisms and widely applied to identify novel diagnostic and prognostic biomarkers for human diseases [26]. Most

importantly, there was still an active demand to determine new regulators to find the molecular mechanisms and provide new biomarkers for AMI. In our work, we reanalyzed a public AMI mRNA expression profiling GSE48060. A total of 2497 up-regulated mRNAs and 2348 down-regulated mRNAs were identified in AMI patients. PPI networks were constructed and the network analysis revealed several key regulators in AMI, such as RHOA, GNB1, GNG7, RAC1, CDC23, FBXO32, DET1, and MEX3C.

Recently, the important roles of lncRNAs were revealed in human disease progression [27]. LncRNAs could regulate expression and activity of protein through transcriptional, post-transcriptional, posttranslational and/or epigenetic regulation [28,29]. In AMI, a few groups had showed dysregulated lncRNAs could serve as biomarkers [30]. MIAT was the first reported lncRNA which confers risk of myocardial infarction [31]. Zhang et al. also found that Zinc finger antisense 1 (ZFAS1) and Cdr1 antisense (CDR1AS) showed significant differential expression between AMI patients and control subjects [32]. The Comprehensive whole-genome screen of the transcriptome was also performed to reveal differently expressed genes and lncRNAs in AMI. For example, Devaux et al. and Yang et al. reported a cluster of lncRNAs are dysregulated in peripheral blood cells of AMI patients [13,16]. Hence, the functions of most lncRNAs in AMI was unknown.

In this research, we found 300 lncRNAs were up-regulated and 142 lncRNAs were down-regulated in AMI compared to normal samples. Among them, some lncRNAs had been reported in human diseases, including GAS5, TUG1 and MALAT1. Most dysregulated lncRNAs remained unclear. Therefore, the co-expression network of lncRNAs was built to show their potential roles in AMI. We found 49 lncRNAs and 502 differentially expressed genes were included in the up-regulated lncRNA associated network. Meanwhile, 71 lncRNAs and 1156 DEGs were included in the down-regulated lncRNA associated network. Several lncRNAs were identified as key regulators in AMI by regulating more than 50 DEGs, such as up-regulated CA5BP1 and LOC101927608, and down-regulated BZRAP1-AS1, EBLN3, FGD5-AS1, HNRNPU-AS1, LINC00342, and LOC101927204. KEGG and GO analysis were also performed. GO analysis demonstrated that the dysregulated lncRNAs were mainly involved in regulating transcription, signal transduction, development, interspecies interaction between organisms, cell cycle, cell adhesion, intracellular signaling cascade, protein transport, apoptosis, ubiquitin-dependent protein catabolism, RNA splicing. KEGG pathways showed the dysregulated lncRNAs were associated with Regulation of the actin cytoskeleton, T cell receptor signaling pathway, MAPK signaling pathway, Cell cycle, ErbB signaling pathway, Ubiquitin mediated proteolysis, Chronic myeloid leukemia, p53 signaling pathway, Insulin signaling pathway, Tight junction, Basal transcription factors.

Several limitations in present study should be noted. First, the present study analyzed a public dataset GSE48060 to identify AMI related mRNAs and lncRNAs. A much larger number of public datasets are necessary to support in silico analysis. Moreover, these results were mainly based on bioinformatics analysis. Despite this study provide novel and useful information for us to understand the potential functions of lncRNAs in AMI, further validation of our analysis using clinical samples is still needed.

5. Conclusion

In conclusion, a public dataset GSE48060 was utilized to confirm differentially expressed lncRNAs and mRNAs in the AMI. PPI networks were built to unveil key mRNAs in AMI. We also

conducted lncRNA co-expression networks to shoe the functions of these novel lncRNAs. Several lncRNAs, including CA5BP1, LOC101927608, BZRAP1-AS1, EBLN3, FGD5-AS1, HNRNPU-AS1, LINC00342, and LOC101927204 were identified as key lncRNAs. KEGG and GO analysis were revealed these dysregulated lncRNAs widely participated in regulating a series of biological processes, such as transcription, signal transduction and MAPK pathway. We thought this work would provide information to study new molecular therapy for AMI.

Conflict of interest

The authors declare no financial conflicts of interest.

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