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Identification of lncRNA-Mediated ceRNA Network Reveals Potential Biomarkers for Early Stage Acute Myocardial Infarction

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ABSTRACT Acute myocardial infarction (AMI) is a leading cause of. Hence, in this study, the researchers aimed to identify long non-coding RNA (lncRNA) biomarkers for early stage AMI based on lncRNA-mediated competitive endogenous RNA (ceRNA) network (LMCN). Seed lncRNAs were selected from the LMCN through network topological centrality analysis. Subsequently, a sub-LMCN was extracted from LMCN based on Biclique algorithm. Ultimately, Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses were conducted on nodes in the sub-LMCN. Researchers found 45 lncRNAs, 1,756 mRNAs and 2,323 ceRNA interactions in the LMCN, of which 9 seed lncRNAs were obtained. A sub-LMCN with 22 lncRNAs, 341 mRNAs, and 811 ceRNA interactions were extracted. A total of 12 GO terms and 6 pathways were denoted as functional genes of early stage AMI. The seed lncRNAs and their functional genes might be potential biomarkers for early stage AMI treatment.

INTRODUCTION

Acute myocardial infarction (AMI) is a global leading cause of morbidity and mortality in cardiovascular diseases, despite the rate having significantly declined over the past decade (Bank et al. 2016). The most common symptom is chest pain or discomfort which may travel into the shoulder, arm, back, neck, or jaw. Its progress is a result of several major trends, including improvements in risk stratification, more widespread use of an invasive strategy, implementation of care delivery systems prioritising immediate revascularisation through percutaneous coronary intervention, advances in antiplatelet agents and anticoagulants, and greater application of secondary prevention strategies (Reed et al. 2013). Importantly, an early and correct diagnosis might warrant immediate initiation of reperfusion therapy to potentially reduce the mortality rate (Stengaard et al. 2016). Biomarkers, used to establish a diagnosis in patients with AMI, have emerged largely from targeted analyses of known myocardial proteins and become more and more important for diagnosis of AMI (Li et al. 2017b). For instance, creatine kinase MB isoenzymes, cardiac myoglobin, and troponins have been widely applied in clinical diagnosis (Langhans et al. 2014). However, investigating new biomarkers with high sensitivity and specificity in early diagnosis of AMI never stop.

Recently, long non-coding RNAs (lncRNAs), which are non-proteincoding and greater than 200 nucleotides in length, have received considerable attention (Hao et al. 2017). Owing to development of high-throughput technology, lncRNAs have been uncovered in numerous of biological processes, especially chromatin modification, the regulation of cell apoptosis and invasion, reprogramming of induced pluripotent stem cells and genomic imprinting (Qiu et al. 2017; Xie et al. 2017; Pan et al. 2016; Liu et al. 2017). In addition, it had been demonstrated that lncRNAs took active participated in competing endogenous RNAs (ceRNAs) regulations for purpose of corresponding to other RNA transcripts simultaneously (Li et al. 2017a; Wang et al. 2017). Nonetheless, the functions of lncRNAs in AMI are not well characterized, and the identification of lncRNA biomarkers is challenging.

Therefore, in the present study, the researchers aimed to explore lncRNA biomarkers for early stage AMI based on an lncRNA-mediated

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ceRNA network (LMCN). To achieve this goal, firstly, the LMCN was constructed by Pearson correlation coefficient (PCC) algorithm dependent on lncRNA and mRNA expression data, and miRNA-target interactions. Secondly, seed lncRNAs of the LMCN were identified through network topological centrality analysis. Thirdly, a sub-LMCN was extracted from the LMCN by seed lncRNA expansion based on the Biclique algorithm. Finally, Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses were conducted on nodes in the sub-LMCN to explore significant functional gene sets of early stage AMI. These biomarkers might be potential markers for target treatment of early stage AMI, and give a hand for revealing pathological mechanism underlying this disease.

METHODOLOGY

Preparing lncRNA, miRNA and mRNA Expression Data

In this paper, lncRNA-miRNA and mRNA-miRNA interactions were collected from online confirmed small non-coding RNAs (sRNAs) target Base (starBase) v2.0 (http://starbase.sysu.edu.cn/). Herein, starBase manages a comprehensive exploration for miRNA-target interactions that curated from those published studies manually (Yang et al. 2011), and identifies RNA-RNA and protein-RNA interaction networks from 108 CLIP-Seq (PAR-CLIP, HITS-CLIP, iCLIP, CLASH) datasets systematically (Li et al. 2013).

Meanwhile, the researchers prepared a dataset (E-GEOD-29532) for lncRNAs and mRNAs of early stage AMI from ArrayExpress database (http://www.ebi.ac.uk/arrayexpress/). Specifically, E-GEOD-29532 was comprised of 6 normal controls and 49 early stage AMI samples, and presented on Affymetrix GeneChip Human Exon 1.0 ST Array version 1 [HuEx-1_0-st-v1] Platform. Prior to analysis, the researchers performed pre-treatments on the dataset and converted the pre-processed data on probe level into gene symbols. Consequently, a total of 14,451 genes were identified in the dataset.

In order to build a close relationship for lncRNA-miRNA and mRNA-miRNA interactions with AMI, genes in the dataset were taken intersections with the two specific kinds of interactions. A new expression dataset with 8,471 mRNAs and 51 lncRNAs were obtained. Subsequently, the researchers captured interactions containing any gene of the new dataset from the lncRNA-miRNA and mRNA-miRNA interactions mentioned above. Ultimately, 265,782 miRNA-mRNA or miRNA-target interactions, and 598 lncRNA-miRNA interactions were determined for further study.

Identifying ceRNA Interactions

Hypergeometric test takes advantage of a hypergeometric distribution to compute the statistical significance of having drawn a specific successes from aforementioned population (Skala 2013). Thus, the researchers executed the hypergeometric test to enable assessment of the significance for the common miRNAs between each lncRNA and mRNA (Sumazin et al. 2011), and to capture ceRNA interactions based on the 265,782 miRNA-target interactions and 598 lncRNA-mRNA interactions. Supposing that N was the total number of miRNAs, of which K and M were the numbers of miRNAs associated with the current lncRNA and mRNA, the P value was calculated in order to evaluate the enrichment significance for that function as following:

$$P = 1 - \sum_{t=0}^{c} \frac{\binom{K}{t}\binom{N-K}{M-t}}{\binom{N}{M}}$$

Of which c was the common miRNA number shared by the lncRNA and mRNA. Of note, multiple miRNAs belonging to the same family were combined into one, and the hypergeometric test counted every miRNA family only once. Importantly, all P values were adjusted by false discovery rate (FDR) implemented in Benjamini and Hochberg method (Benjamini et al. 2001). Only lncRNA-mRNA interactions which met to the threshold of $P < 0.01$ were considered to be ceRNA interactions for early stage AMI.

Constructing LMCN

Although ceRNA interactions were identified as described above, the interacted strength between lncRNA and mRNA in a ceRNA interaction was still unknown. Therefore, PCC algorithm was employed to measure the co-expression probability for the competing lncRNA-mRNA pairs (Nahler 2009). Assuming i and j stood for two variables of a ceRNA interaction under a

specific condition, the PCC was computed according to the formula:

$$PCC(i,j) = \frac{cov(i,j)}{\sigma_i \sigma_j}$$

Where cov(i, j) represented the covariance of i and j; σ_i and σ_j stood for the standard deviations for i and j, respectively. We should note that the PCC for a ceRNA interaction across normal and AMI condition was different. The difference of PCC value across normal and AMI condition was defined as a weight for this ceRNA interaction. If the weight for a ceRNA interaction was more than 0.90, it would be reserved for constructing a LMCN for early stage AMI. Besides, the LMCN was visualized by Cytoscape v3.1.0 (http://www.cytoscape.org/) (Morris et al. 2014). Cytoscape is an open source for integrating biomolecular interaction networks with high-throughput expression data and other molecular states into a unified conceptual framework (Shannon et al. 2003).

Selecting Seed lncRNAs

To the best of the researchers' knowledge, topological centrality is shown to be effective for identifying essential molecules in well-characterized interaction networks (Prifti et al. 2010). Thus the researchers conducted the topological centrality analysis to understand the functionality of complex systems of lncRNAs and mRNAs in the LMCN. Degree quantifies the local topology of each node, by summing up the number of its adjacent nodes (Haythornthwaite 1996). It gives a simple count of the number of interactions of a given node. The nodes at the top of degree distribution (>99.5% quantile) in the significantly perturbed networks were defined as seed nodes. What's more, the researchers investigated the relationship between the number of nodes and node degree distribution using the Network Analyzer 2.7 plugin (Assenov et al. 2008) in Cytoscape v3.1.0, and the fitting coefficient R^2 was produced. Since networks in general are modular and scale-free, a good R^2 indicates that the network has a power-law (or scale-free) degree distribution (Ravasz et al. 2002; Rifai and Ridker 2001).

Extracting Sub-LMCN

To further investigate significant mRNAs or target genes regulated by the seed lcnRNAs in details, a synergistic, competing lncRNA module (sub-LMCN) was extracted from the LMCN using the Biclique algorithm (Binkele-Raible et al. 2010). The sub-LMCN was a complete bipartite graph in which an edge was realized from every vertex of a set to every vertex of a target gene set. In particular, vertices of such graph could be partitioned into two subsets X and Y such that no edge had both endpoints in the same subset, and every possible edge that connected vertices in different subsets was part of the graph (Diestel 2005). Briefly, it was a bipartite graph (x_1, y_1, S) such that for every two vertices $x_1 \varepsilon X$ and $y_1 \varepsilon Y$, $x_1 y_1$ is an edge in S. Every two graphs with the same notation were isomorphic.

Functional Enrichment Analysis

For purpose of exploring significant gene sets enriched by seed lncRNAs and their target genes in the sub-LMCN, functional enrichment analyses were carried out on them, including the Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis.

GO Enrichment Analysis

GO (http://www.geneontology.org) provides structured, controlled vocabularies and classifications that cover several domains of molecular and cellular biology and are freely available for community use in the annotation of genes, gene products and sequences (Consortium 2004). In this work, GO functional enrichment analysis was conducted using the Database for Annotation, Visualization and Integrated Discovery (DAVID) based on the guilt by association strategy. DAVID (https://david.ncifcrf.gov/) is a web tool providing a comprehensive set of functional annotation for researchers to understand the biological meaning behind a large number of genes (Huang da et al. 2009). Furthermore, the Fisher's exact test (Routledge 2005) was employed to evaluate the significances of GO terms between normal controls and AMI patients of early stage. The P values were corrected relying on FDR through Benjamini and Hochberg approach (Benjamini et al. 2001). Finally, GO terms with $P < 0.01$ were regarded as statistically significant between normal controls and AMI patients of early stage.

Pathway Enrichment Analysis

As described above, the researchers conducted pathway enrichment analysis for target genes of lncRNAs of sub-LMCN according to the KEGG implemented in DAVID. Here, the KEGG database (http://www.genome.jp/kegg/) is a collection of manually drawn pathway maps for metabolism, genetic information processing, environmental information processing such as signal transduction, various other cellular processes and human diseases (Kanehisa 2000). Besides, the Fisher's exact test (Routledge 2005) was employed to identify the significant pathways between AMI patients and normal controls. The threshold of significance was defined as $P < 0.01$ which were adjusted by FDR based on Benjamini and Hochberg method (Benjamini et al. 2001).

RESULTS

LMCN

In this section, the researchers used the hypergeometric test to identify ceRNA interactions from 598 lncRNA-miRNA interactions and 265,782 miRNA-target interactions. A P value was implemented to compute the significance of the shared miRNAs between each lncRNA-mRNA pair. The result showed 34,586 ceRNA interactions involved in 51 lncRNAs and 8,125 mRNAs were obtained when setting the thresholding as $P < 0.01$. With the goal of assessing the strength between lncRNA and mRNA in a ceRNA interaction, a weight was calculated for each ceRNA pair identified above dependent on the PCC method. Consequently, significantly co-expressed ceRNA interactions with weight > 0.90 were reserved to construct a LMCN for early stage AMI, and then were graphically visualized by Cytoscape v3.1.0 (Fig. 1). As a result, the LMCN was consisted of 45 lncRNAs, 1,756 mRNAs and 2,323 ceRNA interactions. In detail, weight distribution for ceRNA interactions in the LMCN was displayed in Figure 2A. We found that the weight ranged from 0.90 to 1.65, especially the section of 1.00 ~ 1.20. From a global view, the distribution between the number of interactions and their weights accorded with normal distribution approximately.

Seed lncRNAs

With an attempt to investigate the biological importance for nodes in the LMCN, topological degree centrality analysis was performed. As described in Figure 2B, the node degree distribution revealed a good power law distribution ($R^2 = 0.99999$), which indicated the LMCN for early stage AMI was a scale-free network. Particularly, the lncRNA nodes were typically in the central region of the network, while the mRNAs nodes were typically in the outside layer. In consequence, nodes with high degree belonged to lncRNAs, whereas the degrees for mRNAs were smaller than 5. In brief, there was a statistical significance with $P < 0.01$ for node degree distribution of lncRNAs when comparing with that of mRNAs (Fig. 2C).

On the basis of ceRNA regulatory behavior, the lncRNAs in the LMCN were more likely to have central regulatory roles than mRNAs, especially those with high degree. Therefore, the lncRNAs at the top of degree distribution (> 99.5% quantile) were defined as seed lncRNAs. Specifically, a total of 9 seed lncRNAs were detected (Fig. 2B), containing LINC00839 (Degree = 265), EPB41L4A-AS1 (Degree = 241), TAPT1-AS1 (Degree = 207), TTTY15 (Degree = 164), NPSR1-AS1 (Degree = 148), SNHG12 (Degree = 129), MCM3 AP-AS1 (Degree = 92), LINC00242 (Degree = 87) and MALAT1 (Degree = 79).

Sub-LMCN

Since seed lncRNAs might be more significant than the others in the LMCN for early stage AMI and a network with too large scale might be too generic, thus the researchers extracted a sub-LMCN which exhibited more details of how the lncRNAs synergized with competing mRNAs from the LMCN dependent on the Biclique algorithm. The sub-LMCN was illustrated in Figure 3, of which 22 lncRNAs, 341 mRNAs, and 811 ceRNA interactions were comprised. Furthermore, the degree distribution for nodes in this sub-LMCN was clarified (Fig. 4). Degree distribution of all nodes reflected a power-law distribution ($R^2 = 0.99935$), which suggested that the sub-network was a scale free network. Apart from the 9 seed lncRNAs, the other 13 lncRNAs also were mapped to the sub-LMCN. The total

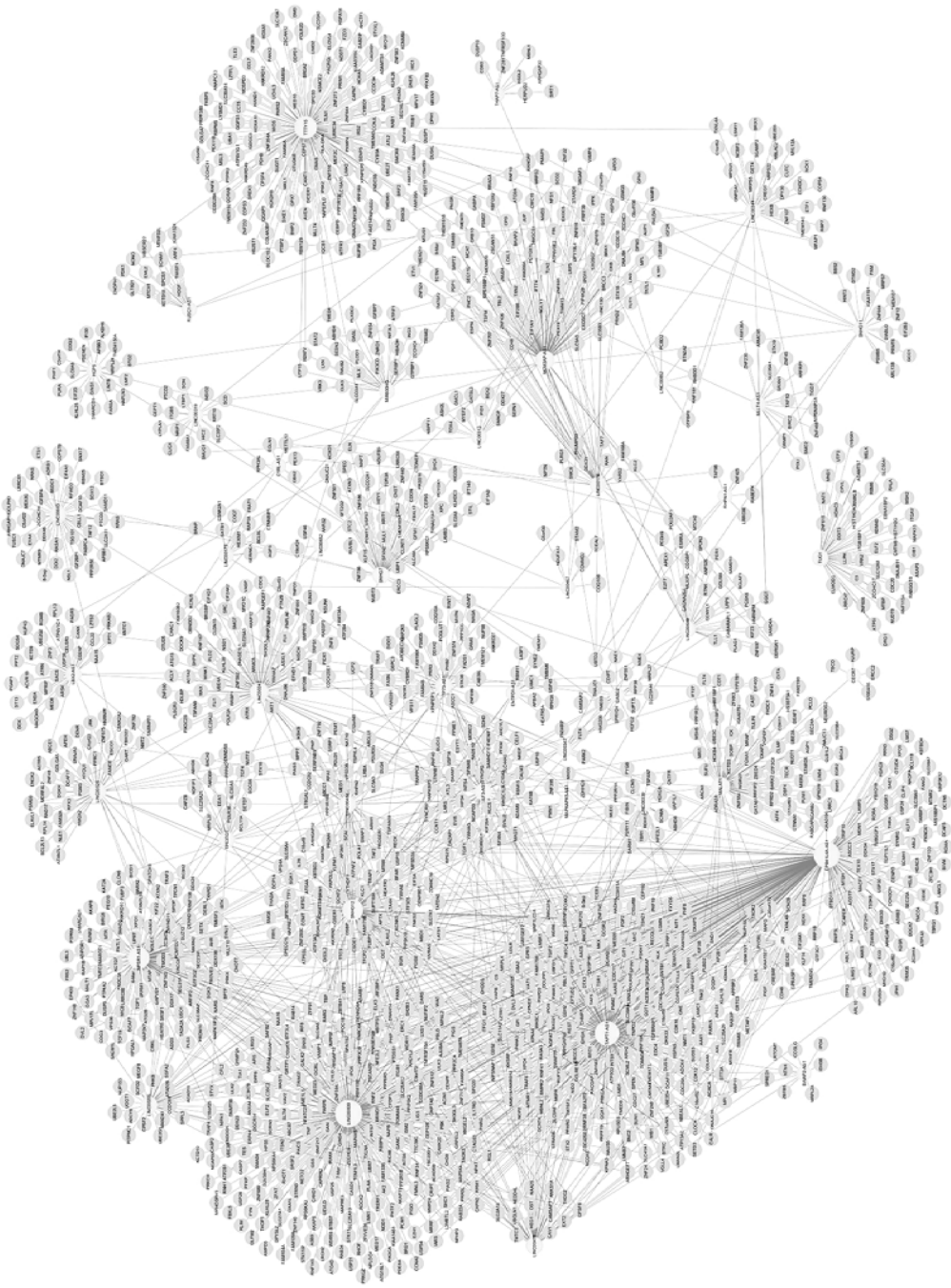

Fig. 1. The lncRNA-mediated ceRNA network (LMCN) of early stage acute myocardial infarction (AMI). There were 45 lncRNAs, 1,756 mRNAs and 2,323 ceRNA interactions. Besides, ceRNA interactions indicated the interactions between lncRNA and mRNA with weight > 0.9
Source: Author

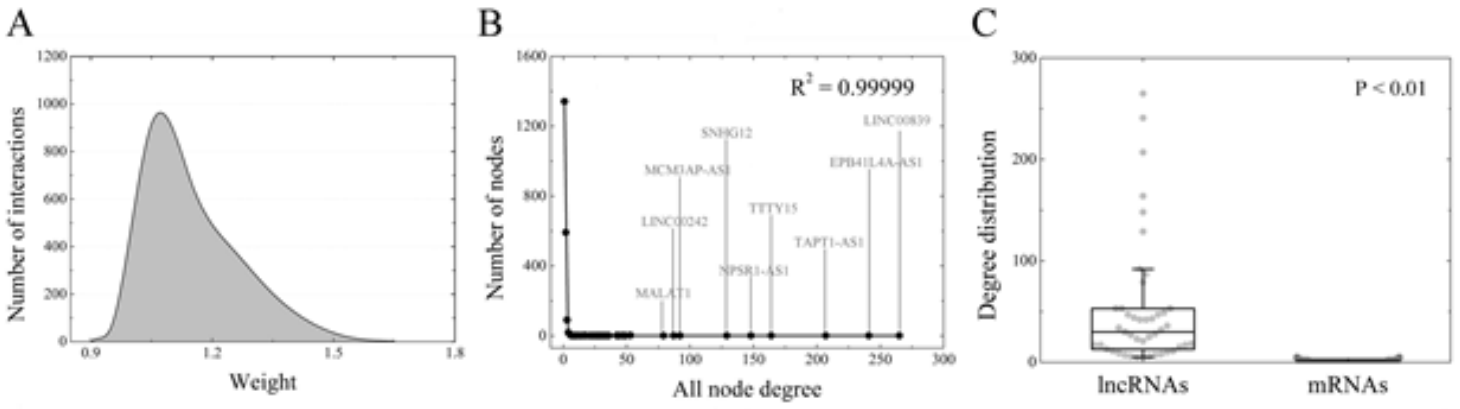

Fig. 2. Properties for the lncRNA-mediated ceRNA network (LMCN). A: Weight distribution of ceRNA interactions in the LMCN accorded with normal distribution approximately. B: Degree distribution of all nodes reflected a power-law distribution (R^2 = 0.99999). C: Comparison across degree distribution of lncRNAs and mRNAs nodes in the LMCN (P < 0.01)
Source: Author

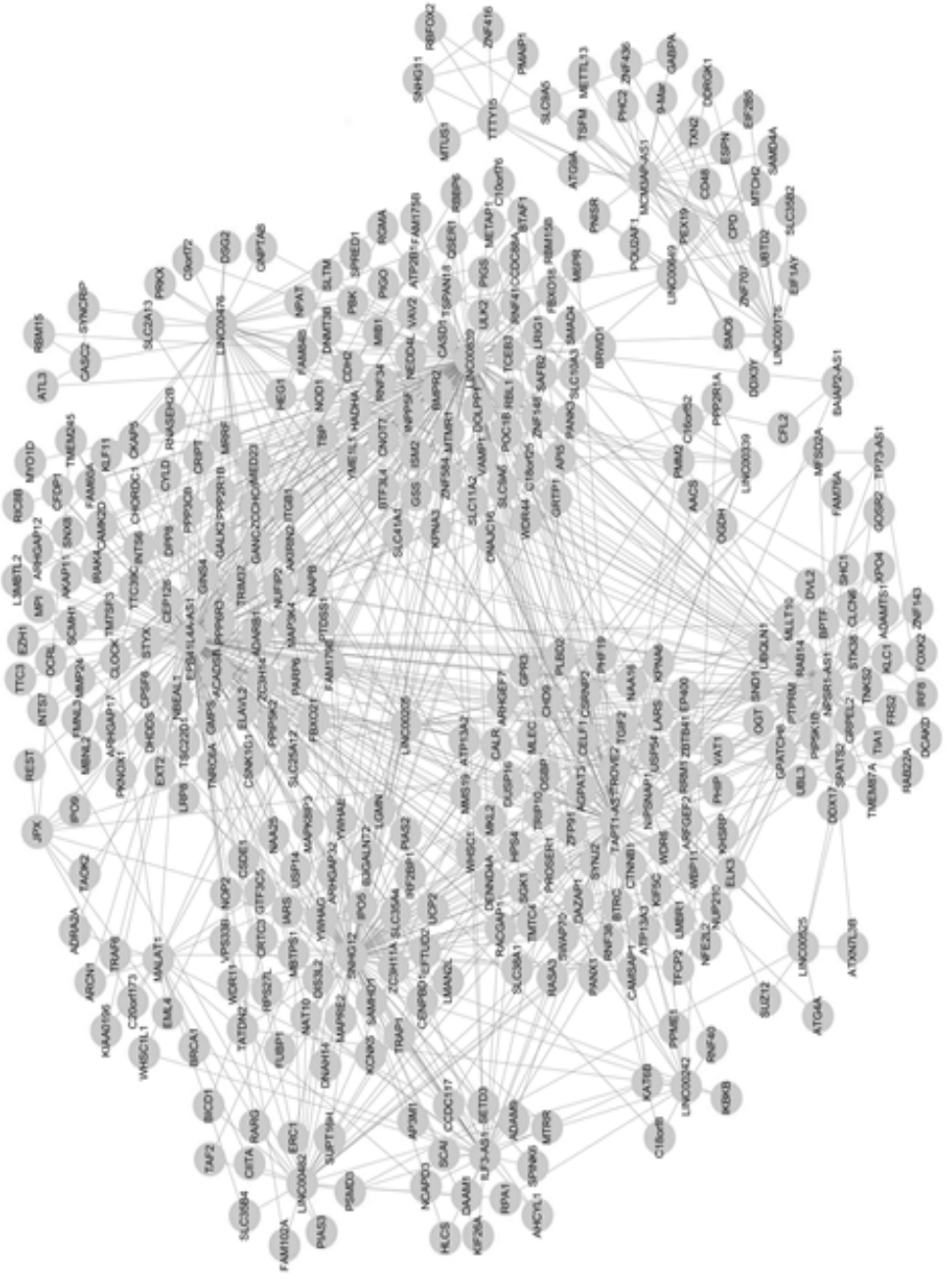

Fig. 3. Sub-lncRNA-mediated ceRNA network (LMCN) extracted from the LMCN. It was comprised of 22 lncRNAs, 341 mRNAs, and 811 ceRNA interactions
Source: Author

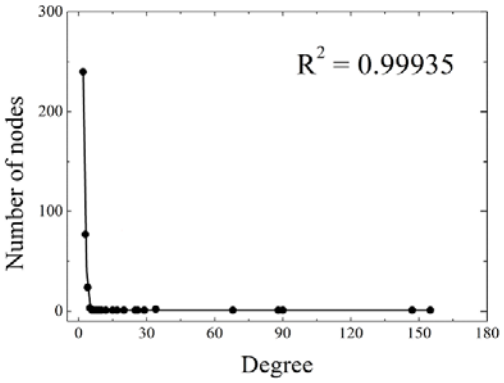

Fig. 4. Topological property for the sub-LMCN. Degree distribution of all nodes reflected a power-law distribution (R^2 = 0.99935)
Source: Author

22 lcnRNAs together regulated 341 mRNAs, and these interactions might play key roles in various biological processes of early stage AMI progression. Hence the researchers identified their functional gene sets in the next part.

Functional Gene Sets

As described above, GO and pathway enrichment analyses were carried out on nodes in the sub-LMCN, respectively. The results showed that a total of 12 GO terms were obtained under the threshold of P < 0.01 (Table 1). The most significant five terms were response to endoplasmic reticulum stress (P = 3.39E-07, Count = 52), peptidyl-lysine modification (P = 1.20E-05, Count = 69), histone modification (P = 1.35E-05, Count = 73), mRNA processing (P = 2.22E-05, Count = 77), and posttranscriptional regulation of gene expression (P = 2.38E-05, Count = 76).

The result of KEGG pathway enrichment analysis displayed that 6 significant pathways with P < 0.01 were obtained (Table 2). In details, Protein processing in endoplasmic reticulum (P = 3.65E-12, Count = 48), SNARE interactions in vesicular transport (P = 7.84E-10, Count = 54), Amino sugar and nucleotide sugar metabolism (P = 3.30E-07, Count = 29), AMPK signaling pathway (P = 2.78E-05, Count = 35), and Neuroactive ligand-receptor interaction (P = 3.53E-04, Count = 40) were the top 5 ones in descending order of P values. Specifically, all of seed lncRNAs except for SNHG12 were enriched in Protein processing in endoplasmic reticulum, while SNARE interactions in vesicular transport had 7 of 9 seed lncRNAs apart from LINC00242 and MALAT1.

DISCUSSION

MiRNAs, a endogenous small RNAs 21-25 nucleotides in length, can pair with the 32 untranslated region sites in mRNAs of protein-coding genes to downregulate their expression (Tsiachris et al. 2018), and they play important roles in various physiological and pathologic processes (Fan et al. 2017; Yan et al. 2017). LncRNAs interact with miRNAs and indirectly regulate miRNA targets. Particularly, by sharing common miRNA-binding sites with mRNAs, lncRNAs compete with miRNA target genes for miRNA molecules, thereby relieving miRNA-mediated target repression (Wang et al. 2015; Zhang et al. 2017). However, molecular mechanisms underlying these interactions are still largely unknown. Thus, in this study, ceRNA interactions between competing lncRNAs and mRNAs by mediation of miRNAs were studied. A LMCN was constructed for early stage AMI, which comprising 45 lncRNAs, 1,756 mRNAs and 2,323 ceRNA

Table 1: GO terms with P < 0.01

ID	*GO term*	*P value*	*Count*
GO:0034976	Response to endoplasmic reticulum stress	3.39E-07	52
GO:0018205	Peptidyl-lysine modification	1.20E-05	69
GO:0016570	Histone modification	1.35E-05	73
GO:0006397	mRNA processing	2.22E-05	77
GO:0010608	Posttranscriptional regulation of gene expression	2.38E-05	76
GO:0006986	Response to unfolded protein	3.09E-05	36
GO:0035966	Response to topologically incorrect protein	3.79E-05	38
GO:0034620	Cellular response to unfolded protein	3.88E-04	30
GO:0035967	Cellular response to topologically incorrect protein	3.91E-04	32
GO:0071900	Regulation of protein serine/threonine kinase activity	1.59E-03	73
GO:1903311	Regulation of mRNA metabolic process	1.70E-03	28
GO:0030968	Endoplasmic reticulum unfolded protein response	1.99E-03	28

Table 2: KEGG pathways with P < 0.01

ID	*Pathway*	*P value*	*Count*	*Seed lncRNA*
hsa04141	Protein processing in endoplasmic reticulum	3.65E-12	48	LINC00839, EPB41L4A-AS1, TAPT1-AS1, TTTY15, NPSR1-AS1, MCM3AP-AS1, LINC00242, MALAT1
hsa04130	SNARE interactions in vesicular transport	7.84E-10	54	LINC00839, EPB41L4A-AS1, TAPT1-AS1, TTTY15, NPSR1-AS1, SNHG12, MCM3AP-AS1
hsa00520	Amino sugar and nucleotide sugar metabolism	3.30E-07	29	LINC00839, EPB41L4A-AS1, TAPT1-AS1, TTTY15
hsa04152	AMPK signaling pathway	2.78E-05	35	LINC00839, EPB41L4A-AS1, TAPT1-AS1, NPSR1-AS1, SNHG12, LINC00242
hsa04080	Neuroactive ligand-receptor interaction	3.53E-04	13	EPB41L4A-AS1, MALAT1
hsa04722	Neurotrophin signaling pathway	9.28E-04	40	LINC00839, EPB41L4A-AS1, TAPT1-AS1, NPSR1-AS1, SNHG12, LINC00242
hsa04740	Olfactory transduction	4.09E-03	9	LINC00839, EPB41L4A-AS1, TAPT1-AS1, NPSR1-AS1

interactions. In addition, the degree distribution of entire nodes revealed power law distribution, which indicated that the AMI-associated LMCN was a scale-free network. These results suggested that the LMCN was similar to many biological networks and was well organized by a core set of lncRNA-mRNA competing principles into structured rather than random networks (Cao et al. 2016; Zhu et al. 2017).

One of the fundamental problems in network analysis is to determine the importance of a particular vertex in a network (Xiao et al. 2017). In the current work, the researchers accessed topological degree analysis to select important nodes with informative signatures, and 9 seed lncRNAs were obtained, including LINC00839, EPB41L4A-AS1, TAPT1-AS1, TTTY15, NPSR1-AS1, SNHG12, MCM3AP-AS1, LINC00242 and MALAT1. In brief, expression profiling of LINC00839 (long intergenic non-protein coding RNA 839) was related to nasopharyngeal nonkeratinizing carcinoma (Zhang et al. 2016). While SNHG1 (small nucleolar RNA host gene 1) was identified as a novel predictor for event-free survival in neuroblastoma, but the functional characterization and clinical implication of LINC00839 in this tumor were still unknown (Sahu et al. 2014). In addition, EPB41L4A-AS1 (Erythrocyte membrane protein band 4.1 like 4A) might play certain roles in human cancers, such as aneurysm of the thoracoabdominal aorta (Zhao et al. 2014). Meanwhile, Marta et al revealed that TAPT1-AS1 (transmembrane anterior posterior transformation 1 antisense RNA 1) was differentially expressed in a large fraction of multiple myeloma samples (Marta et al. 2015). Above all, the researchers may infer that lncRNAs often regulated the expressions of tumor genes, but there is rare study which focused on their functions in other diseases. According with the researchers' results, it is the first time to uncover the correlations between seed lncRNAs and early stage AMI.

Although the data of large-scale protein interaction is keeping accumulated with the development of high throughput technology, a certain number of significant nodes and interactions are not tested (Rice et al. 2017). Besides, a network with too large-scale might be too generic and not representative. This type of difficulty might be resolved to some extent by utilizing sub-networks of the complex network (Wu et al. 2014). What's more, lncRNAs and mRNAs connected to seed lncRNAs might be more significant than the others. Hence the researchers extracted a sub-LMCN from the LMCN by seed lncRNA expansions utilizing the Biclique algorithm. Furthermore, GO and KEGG pathway enrichment analyses were conducted on 22 lncRNAs and 341 mRNAs in the sub-LMCN, and the results showed that a total of 12 GO terms and 6 pathways were denoted as functional gene sets of early stage AMI.

CONCLUSION

The researchers have identified seed lncRNAs and their functional gene sets for AMI relying on network-based GBA method. The findings might provide potential biomarkers for ear-

ly stage AMI treatment, and reveal potential mechanism underlying this disease.

RECOMMENDATIONS

However, how these seed lncRNAs affect each other is still unknown, and thus future study should focus on this aspect.

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