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Construction ceRNA network of regulated ferroptosis in doxorubicin induced myocardial injury

Hongwei Ye [1, 2], **Yuping Li** [1, 2], **Lu Li** [1, 2], **Yuhui Huang** [1, 2], **Jiahui Wang** [2, 3], **Qin Gao** [Corresp. 1, 2]

[1] Department of Physiology, Bengbu Medical College, Bengbu, Anhui, China

[2] Key Laboratory of Basic and Clinical Cardiovascular Diseases, Bengbu Medical College, Bengbu, Anhui, China

[3] Department of Anatomy, Bengbu Medical College, Bengbu, Anhui, China

Corresponding Author: Qin Gao
Email address: bbmcgq@126.com

Background. Ferroptosis and long-noncoding RNAs (lncRNAs) play crucial roles in doxorubicin (DOX) induced myocardial injury (DIMI). Nevertheless, there is no research to construct competing endogenous RNAs (ceRNAs) network between lncRNAs and ferroptosis related key gene. So our research was designed to screen ferroptosis related genes from differentially expressed mRNAs in DIMI and construct lncRNAs regulated ferroptosis related key gene ceRNAs network.

Methods. The male mice were injected with DOX intraperitoneally to induce myocardial injury, myocardial injury was evaluated by Hematoxylin and Eosin (HE) staining, and ferroptosis related protein -- glutathione peroxidase 4 (GPx4) protein expression was detected. The differentially expressed lncRNAs and mRNAs were detected by microarray, and the ferroptosis related genes were screened to construct protein-protein interaction network (PPI), the highest score gene were identified by Cytoscape software, miRNAs bound to key genes and lncRNAs bound to miRNAs were predicted, then the obtained lncRNAs were intersected with differentially expressed lncRNAs detected by microarray. Finally, the lncRNA/miRNA/mRNA ceRNA network of the highest score gene regulating ferroptosis in DIMI was constructed. The expressions of the key components in ceRNA network were detected by qRT-PCR.

Results. Compared with the control group, in the DOX group, myocardial enzymes and HE staining showed that myocardium structure was changed, GPx4 protein expression was decreased. The differentially expressed 10492 lncRNAs and 6727 mRNAs in the DOX group were detected via microarray. Among them, 115 ferroptosis related genes were obtained to construct PPI networks, and Becn1 was identified as the key gene. Finally, the ceRNA network including Becn1, 3 miRNAs and 4 lncRNAs was constructed by predicting data of Starbase database. The relative expressions of these components in ceRNA net were up-regulated and consistent with Microarray results.

Conclusions. Based on the microarray detection results and bioinformatics analysis, we screened ferroptosis related gene Becn1 and constructed the lncRNA/miRNA/mRNA ceRNA network of regulated ferroptosis in DIMI.

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[1] Department of Physiology, Bengbu Medical College, Bengbu, Anhui, China

[2] Key Laboratory of Basic and Clinical Cardiovascular Diseases, Bengbu Medical College, Bengbu, Anhui, China

[3] Department of Anatomy, Bengbu Medical College, Bengbu, Anhui, China

Corresponding Author:

Qin Gao

2600 Donghai Avenue, Bengbu Medical College, Bengbu, Anhui, 233030, P. R. China

E-mail address: bbmcgq@126.com

Abstract

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Introduction

Doxorubicin (DOX) is a classical first-line anti-tumor drug, and is widely used in clinical to treat acute leukemia, lung cancer, breast cancer, bladder cancer, gastric cancer, liver cancer and other tumors due to its high efficiency and wide spectrum(Zhu & Lin, 2021). However, the dose-dependent cardiotoxicity limits the clinical application of DOX. In view of the universality and importance of DOX in clinical antitumor therapy, it is of great significance to explore the potential mechanisms of DOX induced myocardial injury (DIMI) and seek for the effective measures to prevent the happening of cardiotoxicity.

Ferroptosis as a novel form of cell death has attracted widespread attention in recent years. It is characterized by excessive accumulation of intracellular lipid reactive oxygen species (ROS) and lipid peroxidation induced by glutathione peroxidase4 (GPx4) inactivation. Studies have shown that ferroptosis is closely related to the occurrence and development of Alzheimer's disease, tumor and stroke(Liu et al., 2020). Ferroptosis plays an important role in cardiovascular diseases, such as resveratrol protects against myocardial I/R injury via reducing oxidative stress and attenuating ferroptosis(Li et al., 2022), ferritinophagy-mediated ferroptosis is involved in the development of sepsis-induced cardiac injury(Li et al., 2020). Ferroptosis also plays a crucial role in DIMI. It has

been reported that mitochondrial dependent ferroptosis plays a key role in the progression of DIMI (Tadokoro et al., 2020a). Epigallocatechin gallate pretreatment alleviates DOX induced ferroptosis and cardiotoxicity by upregulating AMPKα2 and activating adaptive autophagy(He et al., 2021), knockout of TRIM21 can reduce DOX cardiotoxicity by inhibiting ferroptosis(Hou et al., 2021). However, the signaling pathways and the pathogenesis of DOX-mediated ferroptosis and cardiac failure remain largely unknown.

Long-noncoding RNAs (lncRNAs) are a subset of non-coding RNAs, the more and more emerging evidences have suggested that lncRNAs could serve as sponges for miRNAs through miRNA response elements, resulting in alterations in miRNAs-regulated mRNA levels, and the lncRNA/miRNA/mRNA competing endogenous RNAs (ceRNA) network is reported to be one of the important mechanisms in the development and progression of cardiovascular diseases. The ceRNA regulatory mechanism is also involved in DIMI. Xia et al found that lncRNA-MALAT1/miR-92A-3p/ATG4a partially mediated the protective effect of exosomes secreted by hypoxic pretreated mesenchymal stem cells on DOX-induced cardiac injury(Xia et al., 2020a). Therefore, the enrichment and discovery of lncRNA/miRNA/mRNA ceRNA networks may help to reveal the potential function of lncRNAs involved in DIMI.

So, in this study, firstly, we detect the differentially expressed lncRNAs and mRNAs in DIMI by Microarray analysis, and then screen ferroptosis related genes through bioinformatics analysis, we aim to construct the ceRNA network to explore the potential mechanism, and want to provide the new clues for studies on the role of lncRNAs in regulating ferroptosis in DIMI.

Materials and methods

Animals

Male C57BL/6J mice (body weight of 18-22 g) were purchased from Henan Skbex Biotechnology Co., LTD.. All animals were maintained in the SPF animal laboratory, and housed using standard cages in the environment of the standard humidity/temperature and a 12h-12h light-dark cycle and fed free access to sterile rodent food and water. After acclimatization to the environment for one week, the mice were used for the experiment. All animal experiments were approved by the Animal Management and Ethics Committee of Bengbu Medical College (Permit number: [2022] 024), and the care and treatment of the animals were carried out in strict accordance with the Regulations on the Management of Experimental Animals.

The twelve mice were randomized into two groups: control group (CON) and DOX group (DOX), each consisted of six mice. The mice were given DOX (15mg/k[illegible]u et al., 2019), purchased from Dalian Meilun Biotechnology Co., LTD.) in the DOX group, and given the same dose saline in the CON group through single intraperitoneal injection. After 3 days of intraperitoneal injection of DOX or saline, blood was collected through eye vessels by removing eyeballs when the mice were anesthetized with 1.5% isoflurane via a mask. Then, the animals were sacrificed by cervical dislocation, and heart tissues were excised for further detection.

Serum lactic dehydrogenase (LDH) and myocardial-bound creatine kinase (CK-MB) levels detection

The mice were anesthetized with isoflurane to collect blood through eye vessels by removing eyeballs. Serum LDH and CK-MB levels of the two groups were determined according to the kit instructions (purchased from Nanjing Jiancheng Bioengineering Institute).

Histopathology observation through hematoxylin and eosin (HE) staining method

The heart was removed immediately after anesthesia and cleaned with PBS at 4℃, the left

ventricular myocardium was selected and fixed with 4% paraformaldehyde for 48 h, dehydrated by gradient ethanol, embedded in paraffin, sliced (0.5mm) and stained with hematoxylin and eosin. The histopathologic damages of myocardial tissue were observed under light microscope (Nikon Eclipse E100). All sections were assessed for the presence of myocardial injury in a blinded fashion.

Western blot analysis

Mouse myocardial tissue (40mg) was homogenized in ice-cold RIPA lysate (500μl) containing PMSF, and centrifuged at 12000*g for 5 mins to collect supernatant. The concentration of total protein was determined by BCA kit, the obtained total protein was added into western blot loading buffer, and boiled for 5 min at 95℃, the total protein in each groups were separated by SDS-PAGE with 10% polyacrylamide gel for 2 h, then electro-transferred to PVDF membranes, and blocked by 5% skim milk, and incubated with primary antibody of anti-GPx4 (Abcam, 1:3000) and anti-GAPDH (Absin, 1:5000) at 4℃ overnight, then the membranes were incubated with second antibody (Absin, 1:8000) at room temperature for 1 hour, and the membranes were washed by TBST for 4 times, finally, the bounds were stained with ECL reagent, and was visualized using the BIO-RAD ChemiDoc Touch Imaging System (BIO-RAD, USA). The relative protein expression level was calculated by the ratio of GPx4/GAPDH.

Detection of differentially expressed lncRNAs and mRNAs by microarray

Total RNA of myocardial tissue in each groups were isolated using TRIzol reagent (Invitrogen, Grand Island, NY, USA), RNA isolation methods had been described previously by our laboratory(Hu et al., 2017). The differentially expressed lncRNAs and mRNAs were analyzed by Kangchen Biotech Co., Ltd. (Shanghai, China) using Mouse lncRNA Microarray V4.0. Briefly, each RNA sample was transcribed into fluorescent cRNA, and was labeled, hybridized to the

lncRNA expression microarray, microarray images were analyzed using Agilent Feature Extraction software. The differentially expressed mRNAs and lncRNAs in DOX group were defined according to the thresholds of an absolute fold change. The threshold values we used to define up-regulation or down-regulation were fold change >1.5 and P< 0.05.

Screening and biological function analysis of ferroptosis related genes

Ferroptosis related genes were obtained from FerrDb database (http://www.zhounan.org/ferrdb/), which were intersected with the differentially expressed mRNAs in DOX group to screen the ferroptosis related genes. These genes that were screened out and analyzed using the DAVID (https://david.ncifcrf.gov/summary.jsp) database to obtain its biological function in Gene Ontology (GO) functional annotations and Encyclopedia of Genes and Genomes (KEGG) signal pathway. P<0.05 indicated that the GO analysis or KEGG pathway analysis were significantly enriched.

Construction of protein-protein interaction network (PPI) and screening the highest score gene

The PPI was constructed using the String database (https://cn.string-db.org/) for genes involved in regulating ferroptosis in DIMI[illegible]e data of PPI were imported into Cytoscape software (version 3.9.1) to visualize PPI network, then the high score ferroptosis related genes in PPI network were scored using the CytoHubba plugin, and the highest score gene was screened out.

Construction of ceRNA network

The miRNAs binding with the highest score ferroptosis related gene were predicted using the Starbase database (https://starbase.sysu.edu.cn/index.php), and the lncRNAs binding with the predicted miRNA were predicted, and the predicted lncRNA was intersected with the differentially expressed lncRNAs, finally LncRNA/miRNA/mRNA ceRNA network was obtained, and

visualized using Cytoscape software (version 3.9.1).

Detection the expression of the components in ceRNA network by qRT-PCR

The expression of the components in ceRNA network were detected by qRT-PCR using a SYBR Green QPCR Supermix (Bio-Rad). The result of each sample was normalized by GAPDH and the data were calculated by 2−ΔΔCt method. The primers of the key components in ceRNA network and GAPDH were designed and synthesized by GenechemBio (Shanghai, China), and listed in Table 1.

Statistical analysis

All research data were shown as mean±SD. Independent Student's t test was used to analyze the difference between CON and DOX groups. The statistical analysis was carried out with GraphPad Prism software 8.0 (GraphPad Software Inc.). $P<0.05$ was considered statistically significant.

Results

Changes of serum LDH and CK-MB levels

In comparison with the CON group, the levels of serum LDH and CK-MB were significantly increased in the DOX group (Fig. 1), these results indicated that DOX induced myocardial injury.

Changes of myocardial histological observation by HE staining

The HE staining results showed in the CON group, the myocardial tissue was uniformly stained with red staining in cytoplasm, blue staining in the nucleus, complete cell membrane, compact arrangement, and no interstitial edema. Compared with the CON group, in the DOX group, the myocardial tissue was not uniformly stained, with weakly staining cytoplasm and nucleus staining in damaged areas, and the cells were fragmented with incomplete cell membrane, and myocardial fibers were loosely arranged, interstitial edema was observed. These results suggested that DOX caused myocardial structural changes.

Changes of GPx4 protein expression in heart tissue

Compared with the CON group, the expression of GPx4 protein, a marker of ferroptosis, was decreased in the DOX group, the result suggested that DOX induced the happening of ferroptosis.

Detection of differentially lncRNAs and mRNAs

Seven myocardial samples (3 in CON group and 4 in DOX group) were collected for microarray analysis. The differentially expressed lncRNAs and mRNAs were shown using cluster heatmaps and volcano plots (Fig. 4) in DOX and CON groups. Compared with the CON group, the differential expression of lncRNAs including 6111 up-regulated and 4381 down-regulated lncRNAs, and the differentially expressed 2191 up-regulated and 4536 down-regulated mRNAs with more than 1.5 fold change were identified in the DOX group.

Ferroptosis related genes and biological function

There were 388 ferroptosis related genes obtained by FerrDb database, which were intersected with 6727 differentially expressed mRNAs obtained by Microarray analysis, and 115 ferroptosis related genes in the DOX group were obtained (Fig. 5A). Gene Ontology (GO) analysis, including biological processes, molecular functions and cellular components, and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways of these 115 genes were performed to reveal the potential biological function through DAVID database. GO analysis results showed that 150 biological process (BP) items, 33 cellular Component (CC) items and 36 molecular function (MF) items were enriched. The top 10 GO enrichment items were shown in a bar graph in Fig. 5B, where the lipid metabolism process (GO:0006629) in BP item, mitochondria (GO:0005739), peroxisome (GO:0005777), membrane (GO:0016020) in CC item, oxidoreductase activity (GO:0016491), 2 iron, 2 sulfur cluster binding (GO:0051537), iron-sulfur cluster binding (GO:0051536) and iron ion transmembrane transporter activity (GO:0005381) in MF item were closely associated with

ferroptosis. In KEGG pathway analysis results, 64 related signaling pathways were enriched, the top 10 pathways were shown in the form of bubble diagram in Fig. 5C, among which, ferroptosis was one of the important pathways. The above bioinformatics analysis results suggested that these 115 genes were involved in ferroptosis.

PPI network and high score genes

The PPI network of 115 ferroptosis related genes in the DOX group was constructed through String database, and the relationship between them were obtained (Fig. 6A). PPI network data was imported into Cytoscape software, and the genes closely related to ferroptosis of PPI network were scored by maximal clique centrality (MCC) algorithm through CytoHubba plugin. Ten genes were displayed in Fig. 6B, among them, the score of Becn1 was the highest, and the Microarray analysis results showed that Benc1 was the most significantly up-regulated gene among the 115 ferroptosis related genes. Therefore, we speculated that Becn1 might play an important role in mediating ferroptosis involved in DIMI.

Construction of ceRNA network of the highest score gene Becn1

The miRNAs binding to Becn1 gene in DIMI were predicted using the Starbase database, and the prediction conditions were set as follows: predicted programs were miRanda and miRmap. The result showed that there were 6 miRNAs that could bind with Becn1. Then, the lncRNAs binding to each of the 6 miRNAs obtained above were predicted, the prediction conditions were set as follows: CLIP Data is medium stringency (≧2), and there were 19 lncRNAs binding to miRNA were obtained. Finally, these predicted lncRNAs were intersected with differentially expressed lncRNAs to obtain lncRNA/miRNA/mRNA ceRNA network. The results showed that the ceRNA network was composed of Becn1, 3 miRNAs and 4 lncRNAs, there are Rian/mmu-miR-145a-5p/Becn1, Tug1/mmu-miR-145a-5p/Becn1, Malat1/mmu-miR-30e-5p/Becn1 and H19/mmu-

miR-299a-3p/Becn1 axes respectively. The data of ceRNA network were imported into Cytoscape software for visual display (Fig. 7).

The expressions of the major components in ceRNA network

The qRT-PCR results showed that the expressions of Becn1 at mRNA level and 4 lncRNAs in the ceRNA network were significantly up-regulated in the DOX group compared with the CON group (Fig. 8A), the same trend with Microarray results (Fig. 8B). Hence, the expression levels of Becn1 mRNA and 4 lncRNAs met with the conditions to construct ceRNA network.

Discussion

Doxorubicin belonged to the first-line and broad-spectrum clinical antitumor drug. Because to its cardiotoxic side-effects, the incidence of heart failure in cancer patients is increased. Therefore, to explore the mechanisms of DOX induced myocardial injury and find the effective preventive measures can reduce the incidence of myocardial injury and improve the survival rate of cancer patients. Although numerous studies have elucidated the mechanisms underlying DIMI, the exact mechanism remains to do further research. Recent studies have found that ferroptosis is involved in DIMI(Fang et al., 2019; Tadokoro et al., 2020a), and alleviating ferroptosis may be a possible therapeutic strategy to prevent DIMI(Kitakata et al., 2022). Non-coding RNAs play an important role in DIMI(Zhao et al., 2018; Hu et al., 2019; Lu et al., 2020; Zhan, Hu & Wang, 2020), and the ceRNA regulation mechanism is one of the crucial ways (Xia et al., 2020b), but few reports investigate the ceRNA network on regulating ferroptosis in DIMI. Therefore, this study aims to construct the lncRNA/miRNA/mRNA ceRNA network through bioinformatics analysis technology in DIMI, it will provide the potential mechanisms and biomarkers for finding the effective therapeutic targets.

In this study, we used DOX to induce myocardial injury in mice model. The results showed that in comparison with the CON group, in the DOX group, the levels of serum LDH and CK-MB levels were significantly increased 3 days after DOX intervention. HE staining showed that the cardiomyocyte membranes and nucleus were abnormally stained with the destroyed structure. The changes of myocardial enzymes and histology suggested that DOX could cause myocardial injury. Meanwhile, we observed GPx4 expression in the DOX group was significantly lower than in the CON group, it suggested that ferroptosis was involved in DIMI, which was consistent with previous reports(Tadokoro et al., 2020b; Li et al., 2021). Based on the above animal model, further research was carried out to identify the potential ceRNA mechanisms in DIMI.

In recent years, lncRNAs causes more and more attention in disease development, recent investigations demonstrate that lncRNAs plays a key role in cardiovascular disease, which may be biomarkers or even the therapeutic targets for cardiovascular diseases(Wang et al., 2021). In this study, firstly, the differentially expressed lncRNAs and mRNAs were detected by Microarray analysis in DIMI. The results showed that there were 10492 differentially expressed lncRNAs (6111 up-regulated and 4381 down-regulated) and 6727 differentially expressed mRNAs (2191 up-regulated and 4536 down-regulated) in the DOX group compared with the CON group. The Microarray analysis results suggest that these differentially expressed lncRNAs and mRNAs may be involved in DIMI, and the possible mechanism requires further study.

Ferroptosis as a new programmed mode of cell death has been reported involved in DIMI(Li et al., 2021; Kitakata et al., 2022; Chen et al., 2022). Whether ferroptosis related genes involved in the differentially expressed mRNAs also participate in DIMI? caused our interesting. The 388 ferroptosis related genes were obtained from the FerrDb database and intersected with the differentially expressed mRNAs, as a result, the 115 ferroptosis related genes were screened. In

order to further explore the potential biological functions of the 115 ferroptosis related genes, GO analysis and KEGG pathway analysis were performed using the DAVID database. GO analysis results shows that the lipid metabolism process (GO:0006629) in BP item, mitochondria (GO:0005739), peroxisome (GO:0005777), membrane (GO:0016020) in CC item, oxidoreductase activity (GO:0016491), 2 iron, 2 sulfur cluster binding (GO:0051537), iron-sulfur cluster binding (GO:0051536) and iron ion transmembrane transporter activity (GO:0005381) in MF item were closely associated with ferroptosis. KEGG pathway analysis showed that ferroptosis was one of the important signaling pathways involved in DIMI. The above biological function analysis results suggested that ferroptosis was involved in DIMI, which was closely related to these 115 genes. Then the PPI network was obtained using the String database to analyze the interaction relationships between these 115 genes, and the ferroptosis related genes were scored by CytoHubba plugin in Cytoscape software. The result showed that Becn1 was the highest score gene. Combined with the Microarray analysis results, it showed that Becn1 mRNA was highly expressed in the DOX group, and it was the most up-regulated gene among the 115 ferroptosis related genes. Therefore, we speculated that Becn1 could play an important role in regulating ferroptosis in DIMI. Since previous studies reported that Becn1 was an important autophagy related gene, but the recent finding of Kang et al. revealed that Becn1 was meanwhile a new driver of ferroptosis, which promoted ferroptosis through formatting Becn1-SLC7A11 complex to inhibit the cysteine and glutamate antiporter system X_c^- activityin cancer cells(Kang et al., 2018). It also reported that Becn1 promoted hepatic stellate cells ferroptosis by suppressing xCT-driven Gpx4 expression(Tan et al., 2022), and Becn1 haploinsufficiency protecte against low ambient temperature-induced myocardial remodeling and contractile dysfunction through inhibiting ferroptosis(Yin et al., 2020). Nonetheless, whether Becn1 participates in DIMI by promoting

ferroptosis has not been reported. So we want to seek for the likely connection of Becn1 and ferroptosis in DIMI.

Prior study has proposed a ceRNA hypothesis that mRNA, miRNA and lncRNA could crosstalk with each other to form a regulatory network(Salmena et al., 2011). In our study, 10,492 differentially expressed lncRNAs were detected by Microarray analysis in the DOX group, accumulating evidence has suggested that lncRNAs could sponge miRNAs through ceRNA mechanism resulting in alterations in the miRNAs-regulated mRNA levels, so we further investigate the ceRNA mechanisms between these differentially expressed lncRNAs and Becn1 in regulating ferroptosis in DIMI. To systemically explore the potential ceRNA mechanisms between these differentially expressed lncRNAs and Becn1, miRNAs bound with Becn1 and lncRNAs bound with miRNAs were predicted respectively by Starbase database, then the predicted lncRNAs were intersected with the differentially up-regulated lncRNAs to obtain lncRNA/miRNA/mRNA ceRNA network, the results showed there were Rian/mmu-miR-145a-5p/Becn1, Tug1/mmu-miR-145a-5p/Becn1, Malat1/mmu-miR-30e-5p/Becn1 and H19/mmu-miR-299a-3p/Becn1 axes respectively. There have been reported that lncRNA Rian, lncRNA Tug1, lncRNA Malat1 and lncRNA H19 all serve as ceRNA involved in multiple biological process, such as apoptosis(Wu et al., 2020; Yao et al., 2020), ferroptosis(Liang et al., 2022; Zhang et al., 2022) and pyroptosis(Sun, Mao & Ji, 2021; Kang et al., 2022). Furthermore, we also measured the expressions of Becn1 and the 4 lncRNAs (lncRNA Rian, Tug1, Malat1 and H19) by qRT-PCR, the results of Becn1 at mRNA expression and the 4 lncRNAs met the conditions to construct ceRNA network. All above further hinted that the ceRNA network we established has the theoretical credibility.

Conclusions

In summary, we identified the differentially expressed lncRNAs and mRNAs in DIMI by Microarray analysis, screened the highest score gene Becn1 that regulated ferroptosis by bioinformatics analysis methods, and constructed the potential lncRNA/miRNA/mRNA ceRNA regulatory network. Our findings may be as a potential mechanism and candidate biomarker for therapeutic target of DIMI, it can provide the objective for further research on the mechanism of DIMI. For future, we will continue to validate and investigate how the Becn1 regulating ferroptosis related ceRNA regulatory network in DOX induced myocardial injury.

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Competing Interests

The authors declare that they have no competing interests.

Data Availability

The following information was supplied regarding data availability:

Raw data are available in the NCBI Gene Expression Omnibus: GSE207737. Additional data are available as a Supplemental File.

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Table 1(on next page)

Primer sequences of components in ceRNA network and GAPDH

Abbreviations: F: Forward; R: Reverse.

TABLE 1 Primer sequences of components in ceRNA network and GAPDH

Gene name	Category	**Prime sequence**	**Product length**
Becn1	mRNA	F:5' GGTCCTGGGCGGAAGTCTT3' R:5' CTTAGACCCCTCCATGCCTCA3'	166
Rian	lncRNA	F:5' GTCCCACAGAGCATCACTATCA3' R:5' TGTCTGTATCGTCCCTCCTTCT3'	241
Tug1	lncRNA	F:5'AGTGAACTACGGTACTTGCCAT3' R:5'CCAGGTGAAGAATCACAGAAGT3'	105
Malat	lncRNA	F:5'GATTGTAAAGGGAGGTTTTGTGA3' R:5'TCTCCAAATACTAGCCTAACCTCA3'	159
H19	lncRNA	F:5'CCCACCTCATTTGTCTTTATTC3' R:5'TGAGTCTGCTCTTTCAAAATGTT3'	80
GAPDH		F:5'CACTGAGCAAGAGAGGCCCTAT3' R:5'GCAGCGAACTTTATTGATGGTATT3'	144

Abbreviations: F: Forward; R: Reverse.

Figure 1

Serum LDH (A) and CK-MB (B) levels in each group (n=6, mean±SD).

**P<0.01 vs. the CON group.

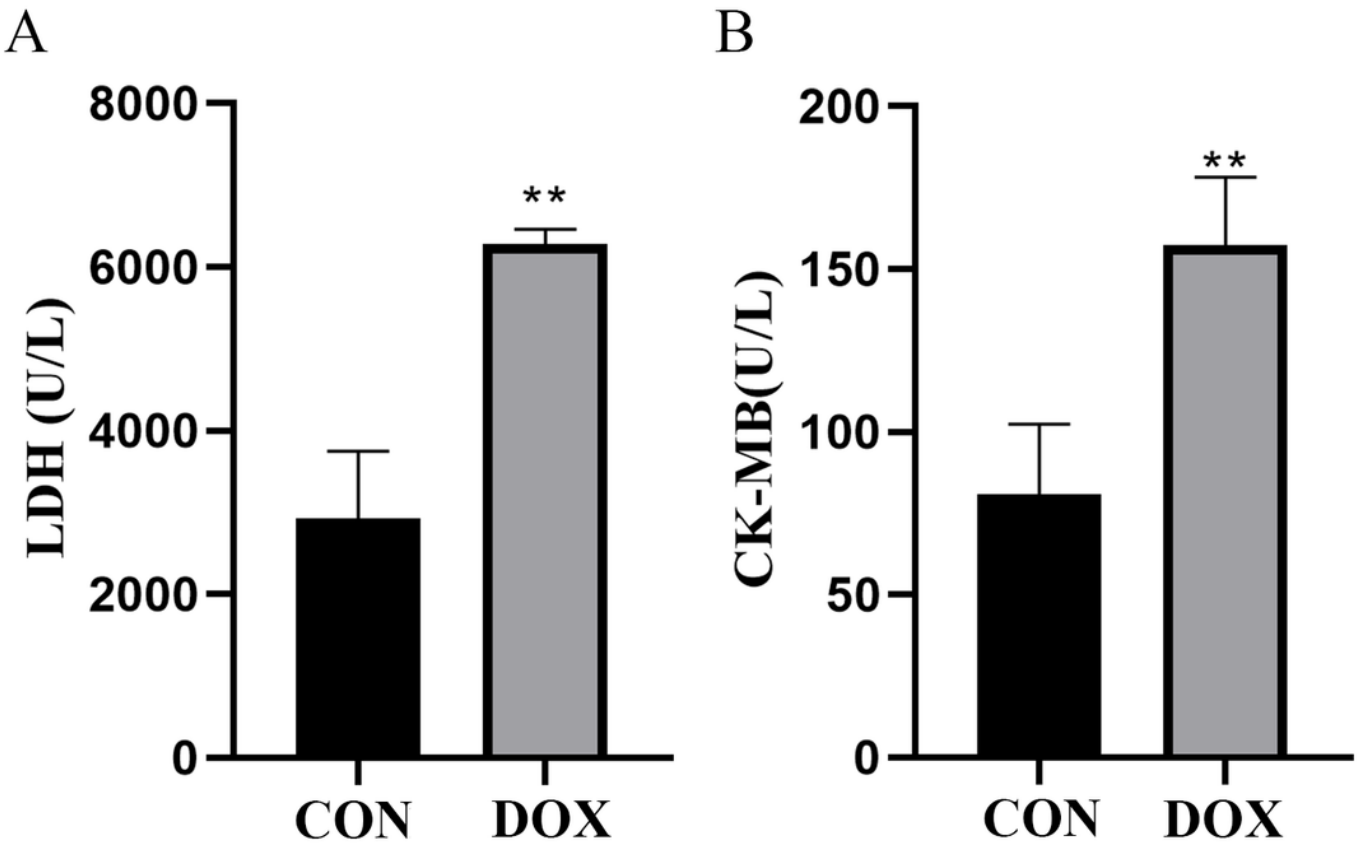

Figure 2

Typical HE staining pictures of mice myocardial tissue in each group (×200).

CON: Con group; DOX: DOX group.

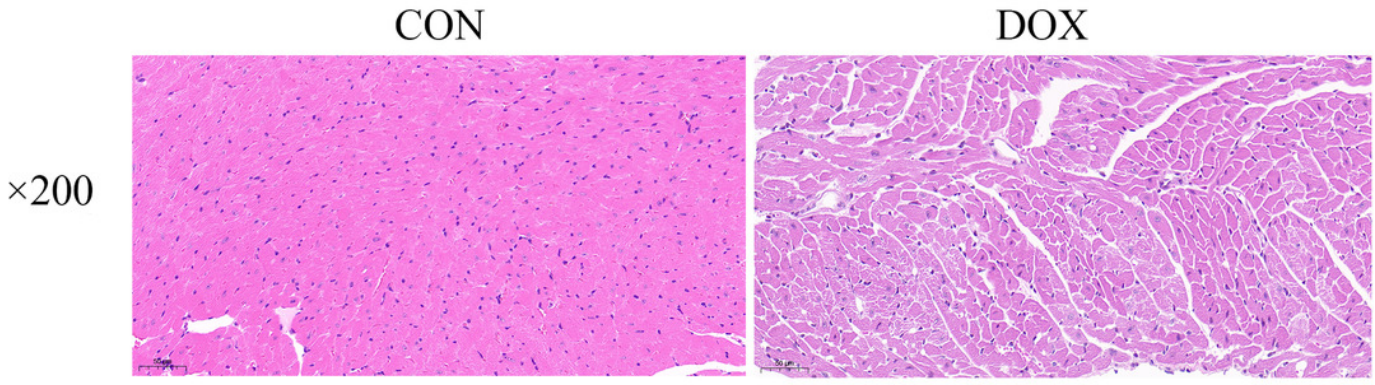

Figure 3

The GPx4 protein expression in mouse myocardium (n=3, mean±SD).

A: Representative western blot band; B: The relative protein expression levels of GPx4. *P<0.05 vs. the CON group.

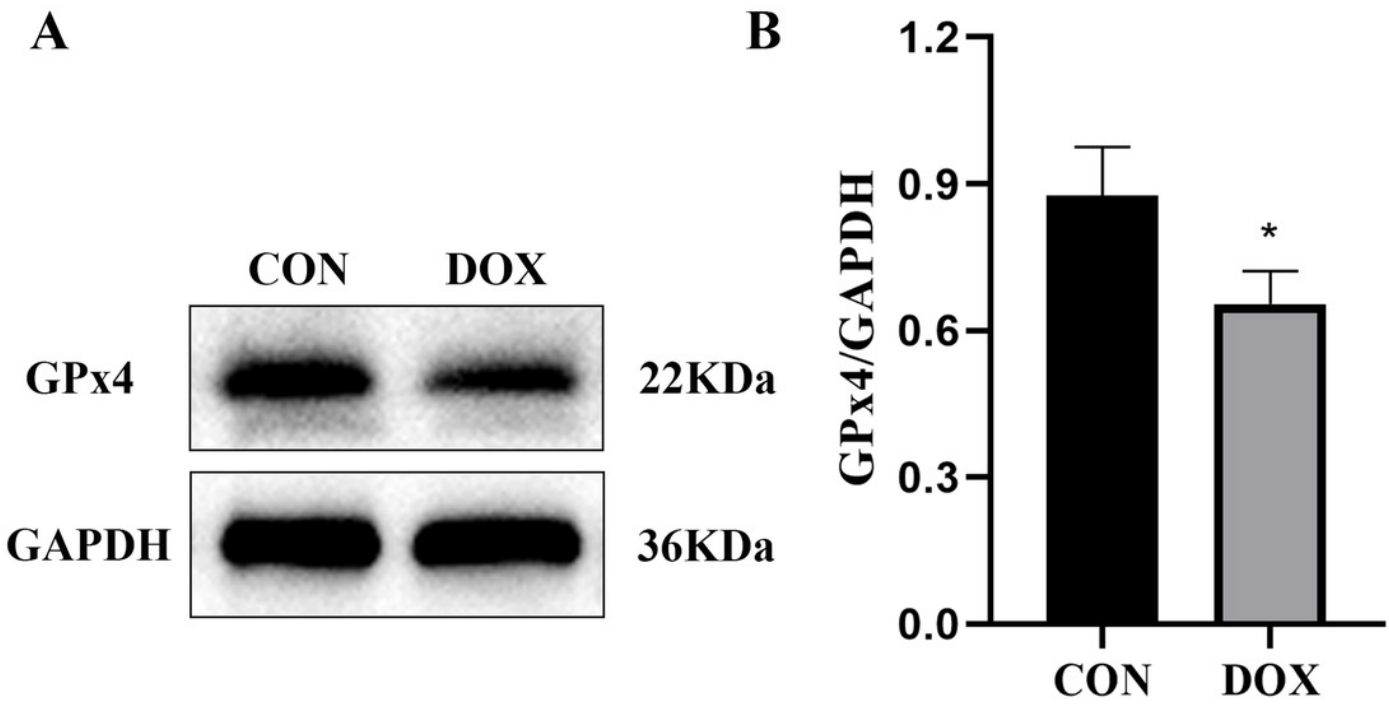

Figure 4

The expression profiles of lncRNAs and mRNAs in mouse myocardium between CON and DOX group.

A: Hierarchical clustering of the differentially expressed lncRNAs; B: Hierarchical clustering of the differentially expressed mRNAs; C: Volcano plots of differentially expressed lncRNAs; D: Volcano plots of differentially expressed mRNA. The red and green shades represent a high and low relative expression.

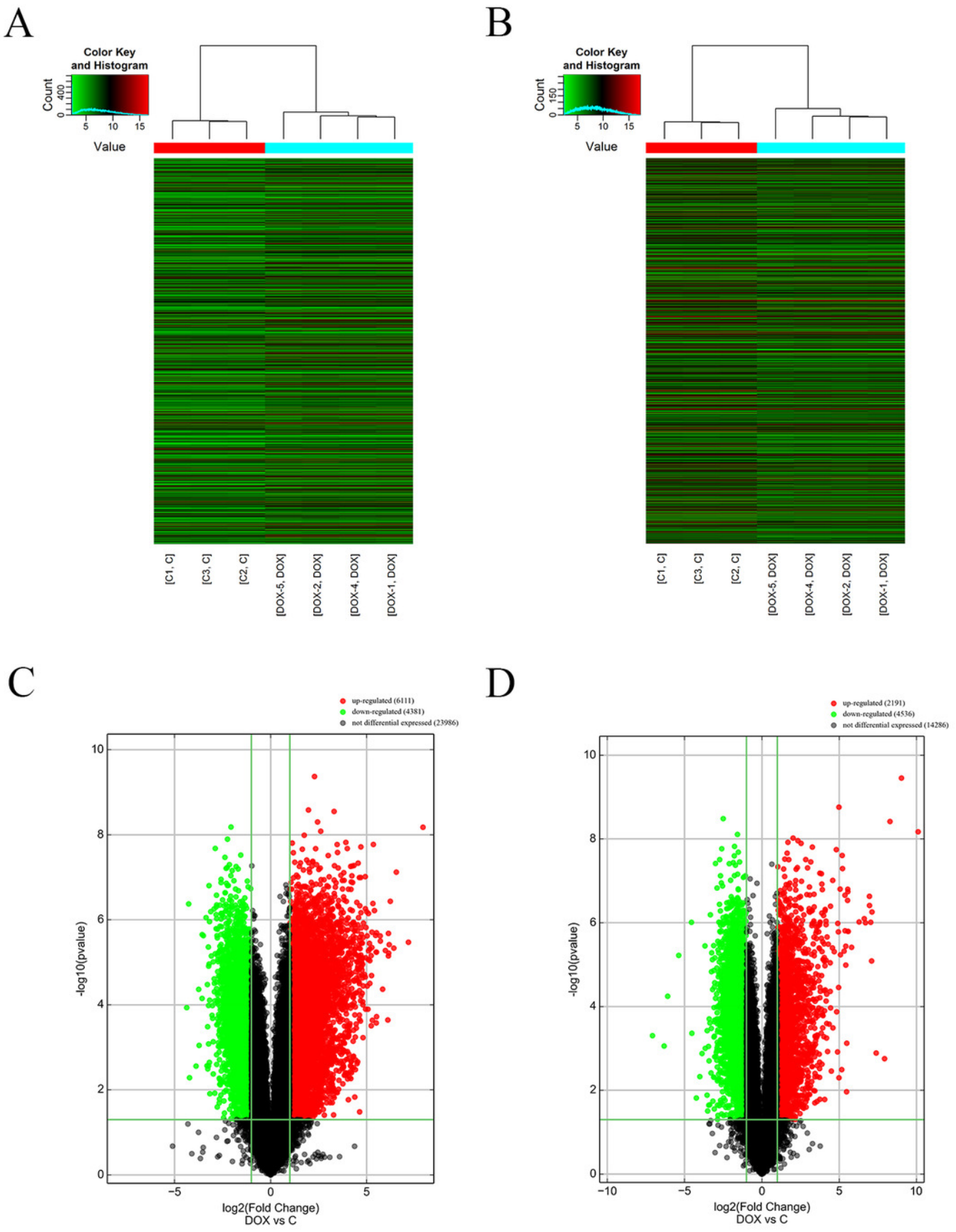
A
Color Key and Histogram
Count
Value
[C1, C]
[C3, C]
[C2, C]
[DOX-5, DOX]
[DOX-2, DOX]
[DOX-4, DOX]
[DOX-1, DOX]
B
Color Key and Histogram
Count
Value
[C1, C]
[C3, C]
[C2, C]
[DOX-5, DOX]
[DOX-4, DOX]
[DOX-2, DOX]
[DOX-1, DOX]
C
up-regulated (6111)
down-regulated (4381)
not differential expressed (23986)
-log10(pvalue)
log2(Fold Change)
DOX vs C
D
up-regulated (2191)
down-regulated (4536)
not differential expressed (14286)
-log10(pvalue)
log2(Fold Change)
DOX vs C

Figure 5

Screening of ferroptosis-related genes (A) and biological function Analysis (B, C).

A: Venn diagram, the blue areas represent ferroptosis-related genes in the FerrDb database, the orange areas represent differentially expressed mRNAs; B: GO analysis of ferroptosis-related genes in DIMI, the top 10 GO terms were listed; C: KEGG pathway analysis of ferroptosis-related genes in DIMI, the top 10 pathways were listed.

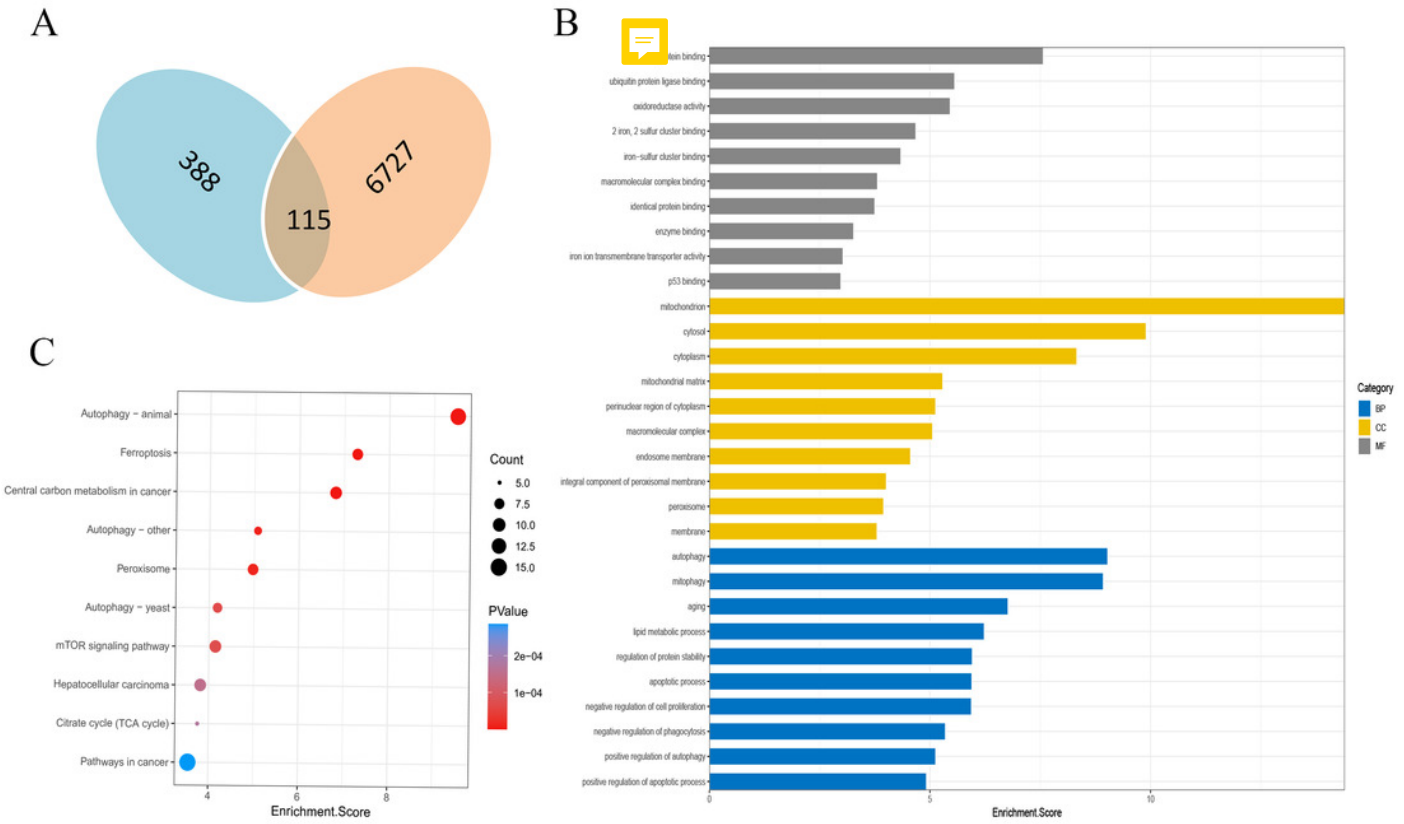

Figure 6

PPI network of ferroptosis-related genes (A) and key genes (B) in DIMI.

A: PPI network of ferroptosis-related genes; B: The top 10 key genes.

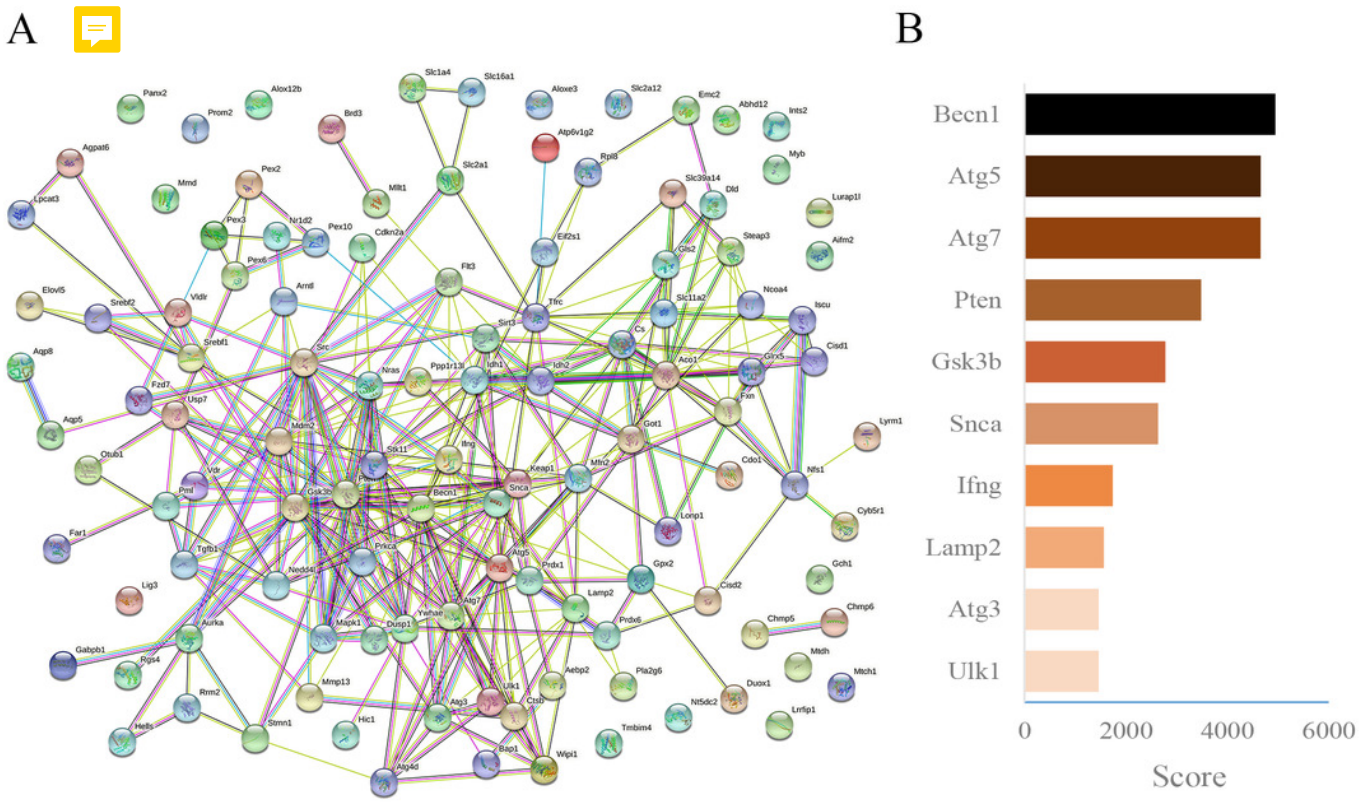

Figure 7

The ceRNA network of the highest score gene Benc1.

Red circle represents Becn1, green diamond represents miRNAs, blue arrow represents lncRNAs.

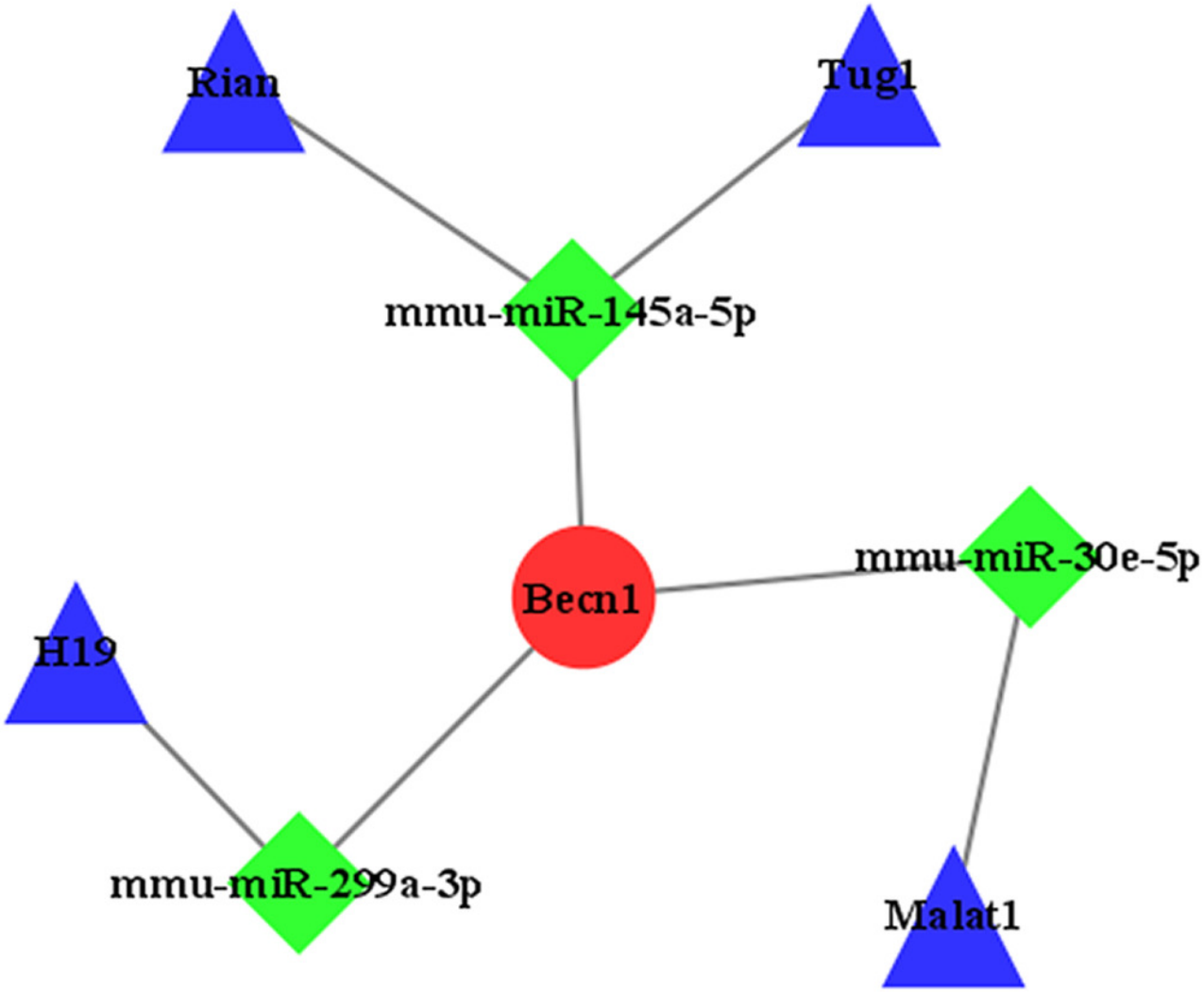

Figure 8

The expression of components in ceRNA network.

(n=3 in the CON group and n=4 in the DOX group, **mean**±SD). A: Relative expression of mRNA and lncRNAs; B: The expression of mRNA and lncRNAs compared to microarray results. *P<0.05, **P<0.01 vs. the CON group.