European Review for Medical and Pharmacological Sciences 2024; 28: 1641-1650

Identifying critical genes and pathways of doxorubicin-induced cardiomyopathy *via* bioinformatics analysis

G. FAN[1,2], X. SHI[1], C. HUO[1], H. ZUO[1]

[1]Cardiology Department, Xianyang Central Hospital, Xianyang, Shaanxi Province, China
[2]Clinical Research Center, Xianyang Central Hospital, Xianyang, Shaanxi Province, China

Abstract. – OBJECTIVE: The pathogenesis of doxorubicin (DOX) induced cardiomyopathy (DCM) is still uncertain. We aimed to identify the critical genes and pathways involved in DCM based on bioinformatics analysis.

MATERIALS AND METHODS: The GSE59672 and GSE23598 mice heart tissue microarray data were obtained from Gene Expression Omnibus (GEO) database. The "limma" package of R software was used to screen the differently expressed genes (DEGs). GO (Gene Ontology) and KEGG (Kyoto Encyclopedia of Genes and Genomes) analyses were performed on DEGs by using "clusterProfiler" package in R software. The PPI (Protein - Protein Interaction) network of DEGs constructed by STRING online database and thereby the top 15 hub genes selected by cytoHubba in Cytoscape software. The hub genes interaction was performed by GeneMANIA online database. The "Corrplot" R package was employed to assess hub genes correlation.

RESULTS: Finally, a total of 492 and 501 DEGs were screened in GSE59672 and GSE23598 datasets, respectively. GO analyses revealed that DEGs were mainly involved in the regulation of extracellular matrix organization, metabolic process, regulation of collagen-containing extracellular matrix.

KEGG pathway analyses indicated that DEGs were mainly involved in protein digestion and absorption, ECM-receptor interaction, phagosome, and *p53* signaling pathway. Finally, the 8 hub genes were identified, including *Col1a1, Col3a1, Col1a2, Col6a1, Ptprc, Tyrobp, Itgb2,* and *Ctss*.

CONCLUSIONS: The present study identified a series of key genes, including *Col1a1, Col3a1, Col1a2, Col6a1, Ptprc, Tyrobp, Itgb2*, and *Ctss*. In addition, important pathways were also discovered. The results of this study may provide a novel molecular mechanism and potential therapeutic targets for DCM.

Key Words:
Doxorubicin, Cardiomyopathy, Critical genes, Pathways.

Introduction

The malignant tumor remains the most common reason of death globally. Cardiovascular diseases and carcinoma are the principal causes of morbidity and mortality in developed regions[1]. Doxorubicin (DOX), an anthracycline antibiotic drug, is the most widely used clinical chemotherapeutic agent for the treatment of several cancers[2]. As with the advancement of medical science and the increasing aging population, the number of people suffering from cancer will continue to rise worldwide. However, the clinical benefit of DOX in patients with cancer is limited by the potential risk of DOX-induced cardiomyopathy (DCM). Cancer and heart disease will cause substantial public health and economic burden. Therefore, it is crucial to better understand the pathogenesis and molecular mechanism of DCM.

DCM can be divided into acute and chronic types: acute DCM usually appears within 2-3 days from the first DOX treatment, and its characteristics often include premature beats, tachycardia, and acute left ventricular failure; chronic DCM emerges from 30 days to more than 10 years since the start of DOX administration[3]. Acute DCM is generally reversible but can also manifest as myocardial damage or progress to chronic DCM. Whereas chronic DCM usually leads to heart failure and impaired heart function[4]. When the cumulative dosage of DOX reaches or exceeds 500 mg/m^2 of body surface area, it leads to increased ROS production, which in turn disrupts cardiomyocyte homeostasis[5]. It is certified[6] that DOX medication results in alterations in the structure of sub-cellular organelles, such as swelling of mitochondria, myofibrillar loss, and cytoplasmic vacuolization. The impaired function of the organelles will negatively affect cardiomyocyte metabolism and thus promote the progression of DCM[6]. DOX treatment usually induces an immune

Corresponding Authors: Gang Fan, MD, Ph.D; e-mail: nfgcardiology@126.com;
Hong Zuo, MD; e-mail: 1316033998@qq.com

response with the release of a series of cytokines. Previous studies[7,8] indicated that DOX elevates Toll-like receptor 2, which then induces the nuclear factor kappa B (NF-kB) and ultimately increases apoptosis. Currently, although relatively extensive studies in the literature have been conducted on DCM, its exact pathogenesis remains uncertain, and there is a lack of specific and effective therapeutic agents for the treatment of DCM.

As with the development and maturation of gene chips, genomics, transcriptomics, proteomics, metabonomics, and bioinformatics have generated biological big data. Analyzing biological big data may enable the understanding of diseases from traditional pathology to the genetic level and promote the birth and development of precision medicine. However, bioinformatics data mining for DCM has not been reported. Therefore, the present study aimed to perform bioinformatics-based identification of candidate hub genes that participate in DCM based on the GEO database.

In our study, we applied a gene microarray of mouse DOX-induced cardiomyopathy from the GEO database to screen out DEGs and then annotate differential gene functions and evaluate gene-enriching signaling pathways. Our study may provide a novel molecular mechanism and potential therapeutic targets for DCM.

Materials and Methods

Data Sourcing

The GSE59672 and GSE23598 mice heart tissue datasets were downloaded from the Gene Expression Omnibus (GEO) database of the National Center for Biotechnology Information (NCBI)[9]. GSE59672 included three DOX-treated mice and three control mice, and the platform was GPL1261 Affymetrix Mouse Genome 430 Version 2.0 Array (available at: https://bioconductor.org/packages/release/data/experiment/html/Affymoe4302Expr.html). GSE23598 included 2 DOX-treated mice and 2 control mice, and the platform was the same.

Identification of DEGs

The DEGs of GSE59672 and GSE23598 were screened out using the "limma" package of R software (available at: https://bioconductor.org/packages/release/bioc/html/limma.html). DEGs were considered as *p*-values lower than 0.05 and |log2foldChange(logFC)|>1. The "ggplot2" package (available at: https://cran.r-project.org/web/packages/ggplot2/index.html) was utilized to generate volcano and PCA (Principal Component Analysis) plots. The "pheatmap" package (available at: https://cran.r-project.org/web/packages/pheatmap/index.html) was employed to make a heatmap to visualize the DEGs.

Functional and Pathway Enrichment Analysis of DEGs

GO functional annotation and KEGG pathway enrichment analyses of DEGs were conducted for DEGs by using "clusterProfiler" package in R software (available at: https://bioconductor.org/packages/release/bioc/html/clusterProfiler.html). Significant enrichment was considered as a gene count of 2 or more and a *p*-value lower than 0.05.

Construction of the PPI Network

The PPI network of DEGs was constructed by the STRING online database (STRING: functional protein association network, available at: https://string-db.org), and thereby, the hub genes ranked by the score identified by CytoHubba plug-in Cytoscape software (Version 3.9.1, available at: https://apps.cytoscape.org/apps/cytohubba). We also used multiple topological analysis algorithms, including MCC, MNC, Degree, and EPC, to predict and explore the top 15 hub genes. The intersection of screened genes was achieved by the Venny online tool (available at: https://csbg.cnb.csic.es/BioinfoGP/venny.html).

Gene Function Interaction Analyses

The hub genes interaction was performed by GeneMANIA (available at: https://genemania.org) to predict correlations among co-localization, shared protein domains, co-expression, prediction, and pathways.

Analysis of Hub Gene Correlation with R Software

To verify the similarity between hub genes, we used the "Hmisc" package of R software (available at: https://cran.r-project.org/web/packages/Hmisc/index.html) to calculate the correlation matrix and its significance level and the "Corrplot" R package (available at: https://cran.r-project.org/web/packages/corrplot/index.html) was applied to create a correlation heat map.

Statistical Analysis

The data are presented as the mean ± SD and were analyzed by using SPSS software, version 17.0 (SPSS Inc., Chicago, IL, USA). A *p*-value lower than 0.05 was defined as significant.

Results

Identification of DEGs

The PCA analysis results showed that the PC1 and PC2 values were able to distinguish between the difference between DOX and control groups (Figure 1A-B). Finally, a total of 492 DEGs, including 276 upregulated and 216 downregulated DEGs, were identified in GSE59672. 230 upregulated and 271 downregulated DEGs were screened in GSE23598 (Figure 1C-D). 201 co-DEGs, including 111 upregulated and 90 downregulated DEGs, were identified by taking the intersection of DEGs from GSE59672 and GSE23598 datasets (Figure 1E-F). The top 30 DEGs of the GSE59672 and GSE23598 datasets were shown with a heat map (Figure 2A-B).

GO Annotation of DEGs

The co-DEGs significantly enriched in 54 GO terms, including 20 BP terms (extracellular structure organization, extracellular matrix organization, external encapsulating structure

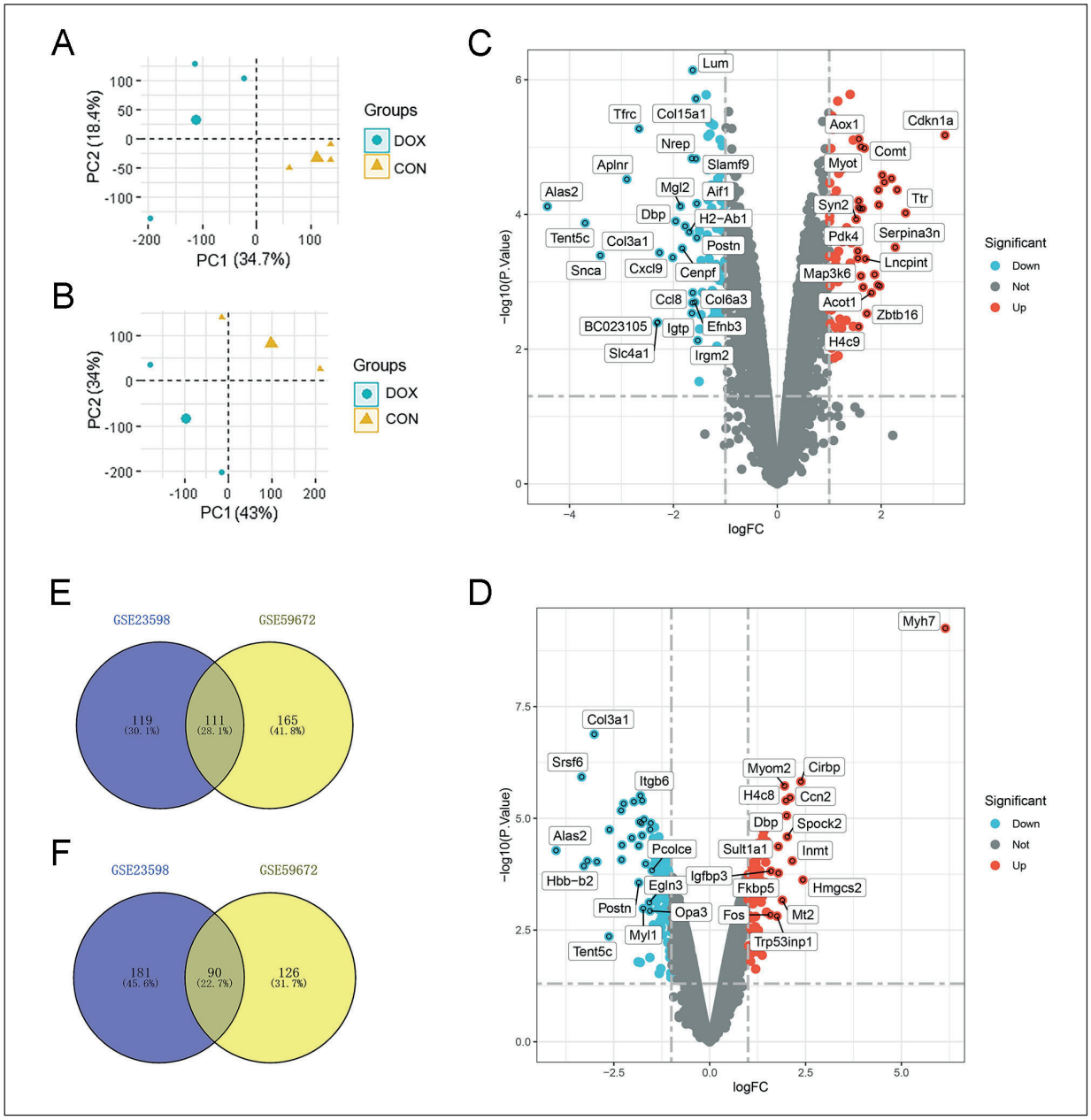

Figure 1. **A**, PCA plot of GSE59672; (**B**) PCA plot of GSE23598; (**C**) Volcano plot of DEGs in GSE59672; (**D**) Volcano plot of DEGs in GSE23598. Venn plot upregulated (**E**) and downregulated (**F**) genes in both datasets, respectively.

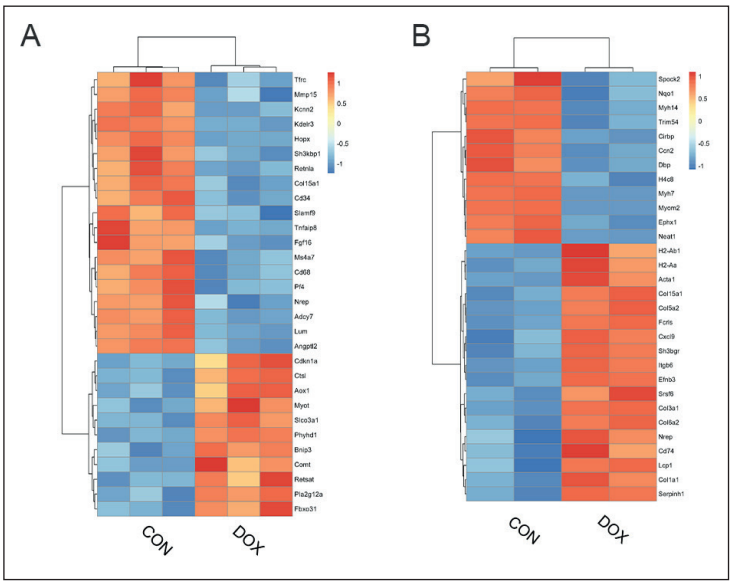

Figure 2. Heat map plots. **A**, Heat map plot of GSE59672. **B**, Heat map plot of GSE23598. DOX: Doxorubicin; CON: Normal or Control.

organization, response to extracellular stimuli, and cell-substrate adhesion) (Figure 3A), 13 CC terms (collagen-containing extracellular matrix, collagen trimer, an anchored component of membrane) (Figure 3B), and 21 MF terms (glycosaminoglycan binding, extracellular matrix structure constituent, heparin-binding, sulfur compound binding, and growth factor binding) (Figure 3C).

KEGG Pathway Enrichment Analysis of DEGs

The co-DEGs significantly enriched in 5 KEGG pathways, including protein digestion and absorption, phagosome, ECM-receptor interaction, Toxoplasmosis, and *p53* signaling pathway (Figure 3D).

PPI Network and Hub Genes

We uploaded the DEGs to the STRING online database, and thereby, the PPI network analysis was conducted. The PPI network of the DEGs was screened by STRING, including 195 nodes and 652 edges, with a PPI enrichment *p*-value<1.0e-16 (Figure 4A). MCODE, a plug-in with Cytoscape software (available at: https://apps.cytoscape.org/apps/mcode), was used to conduct module analysis to detect critical clustering modules. Two modules were retrieved from the PPI network. Module 1 included 33 nodes and 483 edges with a cluster score (density times the number of members) of 15 (Figure 4B). Module 2, with 8 nodes and 56 edges, had a score of 8 (Figure 4B). The PPI network results were further analyzed by cytoHubba plug-in Cytoscape software. We also used multiple topological analysis algorithms, including MCC (Figure 4C), EPC (Figure 4D), Degree (Figure 4E), and MNC (Figure 4F), to predict and explore the top 15 hub genes. The intersection of these 15 genes from the four algorithms by venny online tool revealed 8 candidate hub genes: *Col1a1, Col3a1, Col1a2, Col6a1, Ptprc, Tyrobp, Itgb2,* and *Ctss* (Figure 4G).

Hub Genes Interaction Analyses by GeneMANIA

PPI analysis of the eight hub genes and their 20 interacting genes was performed by GeneMANIA to predict correlations among co-localization, shared protein domains, interaction, prediction, and other pathways (Figure 5). The predicted genes are located in the outer circle, and the hub genes are in the inner circle. The results indicate that these genes are enriched in collagen-containing extracellular matrix, extracellular matrix organization, complex of collagen trimers, extracellular structure organization, cellular response to acid chemical, response to amino acid, and growth factor binding.

Hub Genes Correlation Analyses by R

Hub genes are selected both by Degree and MCC topological analysis algorithms. Thbs2 was negatively correlated with the remaining hub gene clusters (Figure 6). However, other hub genes were positively correlated. The correlation coefficients between most of the genes in the turquoise module were greater than 0.60, indicating that the correlation between the pivotal genes is high. The above

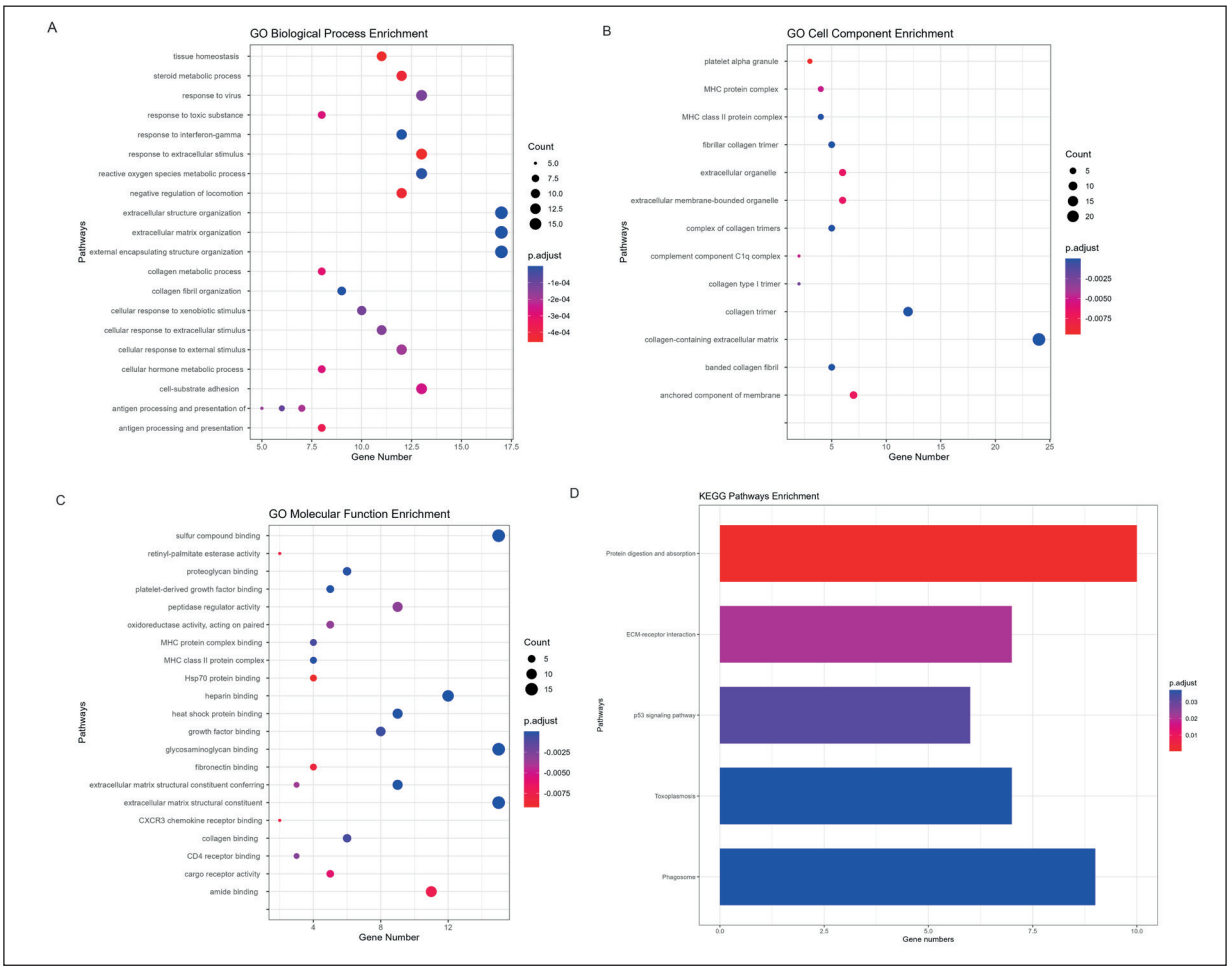

Figure 3. Gene Ontology (GO) analysis of DEGs. **A**, Biological process (BP) enrichment analysis; (**B**) Cell component (CC) analysis; (**C**) Molecular function (MF) enrichment analysis; (**D**) KEGG analysis of DEGS.

results indicate that the key genes interact closely with each other, and the upstream and downstream regulatory effects may be closely linked.

Discussion

Our study identified a number of key genes, including *Col1a1, Col3a1, Col1a2, Col6a1, Ptprc, Tyrobp, Itgb2,* and *Ctss*. We also uncovered important pathways that were most closely associated with DCM in mice, including protein digestion and absorption, phagosome, ECM-receptor interaction, and the *p53* signal pathway.

The prevalence and incidence of DOX-induced cardiomyopathy (DCM) is still increasing and thus leads to severe clinical consequences all over the world. Therefore, further exploring and better understanding the genetic and molecular basis of DCM is urgent and of great importance. The progress of sequencing technology and the development of microarray science provided an opportunity to investigate the potential biomarkers and therapeutics of DCM by bioinformatics analysis.

To the best of our knowledge, this is the first bioinformatics study mining two GEO datasets to identify candidate hub genes and pathways that participate in DCM. In the present study, a total of 492 DEGs, including 276 upregulated and 216 downregulated DEGs, were identified in the GSE59672 dataset. 501 DEGs, including 230 upregulated and 271 downregulated DEGs, were screened in the GSE23598 dataset. Finally, 201 co-DEGs of GSE59672 and GSE23598 datasets, including 111 upregulated and 90 downregulated DEGs, were identified. To study the biological function of DEGs, we conducted further analyses of GO, KEGG pathway enrichment, PPI network, and hub gene identification. We screened potential key genes for DCM, including

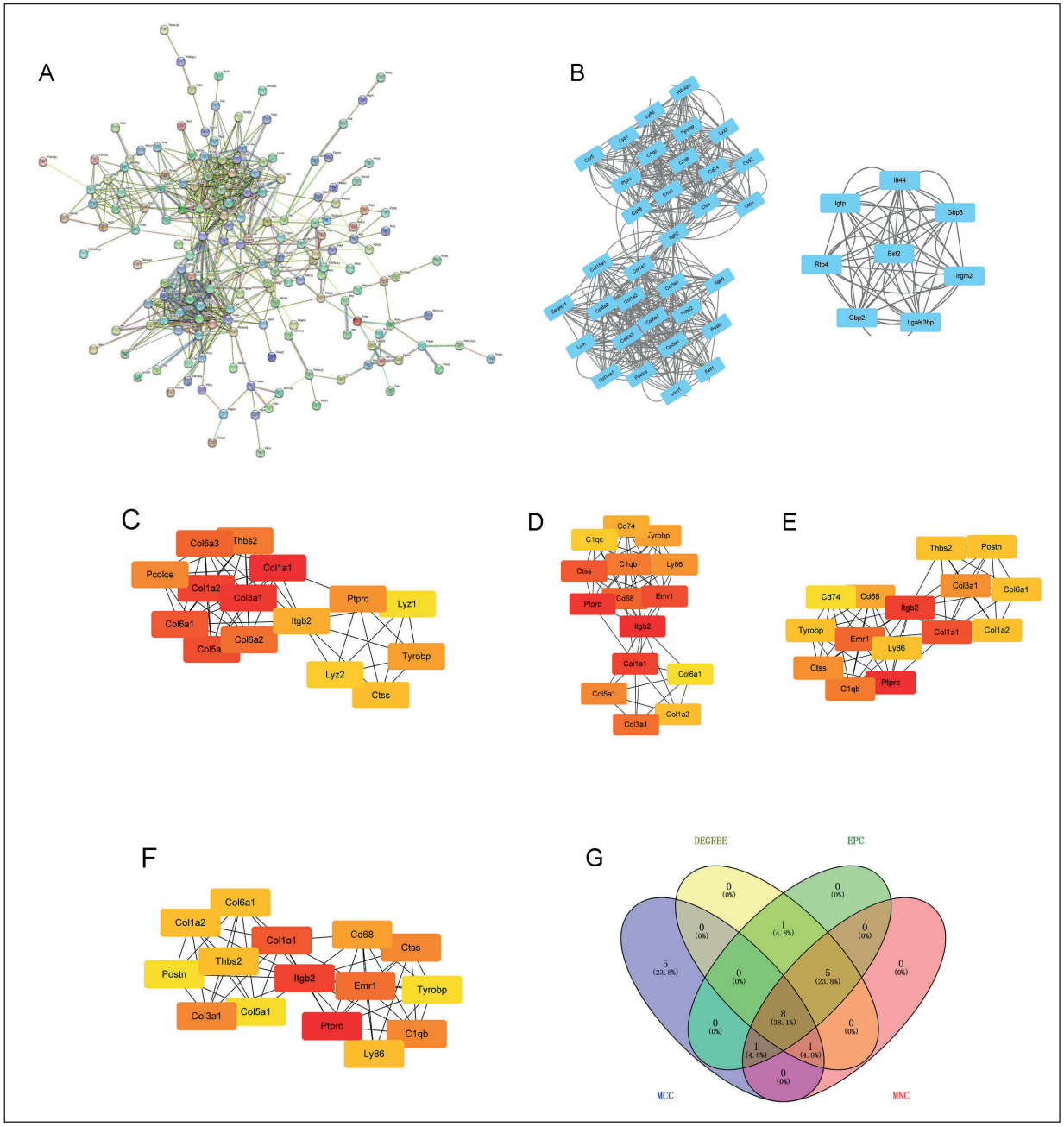

Figure 4. **A**, PPI network of the screened DEGs constructed by STRING; (**B**) Significant gene module and enrichment analysis of the modular genes; (**C**) Hub genes screened by MCC; (**D**) Hub genes screened by EPC; (**E**) Hub genes screened by Degree; (**F**) Hub genes screened by MNC; (**G**) Identification of 8 candidates for hub genes by Venn intersection.

Col1a1, Col3a1, Col1a2, Col6a1, Ptprc, Tyrobp, Itgb2, and *Ctss*. *Col1a1* (Collagen type I alpha 1), a member of the collagen family, modulates cell proliferation, metastasis, apoptosis, and cisplatin resistance cellular process. *Col1a1* is also related to cancer progression and prognosis[9,10]. Knockdown *Col3a1* (collagen type III alpha 1) inhibits cell proliferation, migration, invasion, and immune escape[11]. It is concluded that *Col1a2* is involved in cancer and heart failure development[12]. *Col6a1, Col6a2*, and *Col6a3* are all Collagen VI genes; it is suggested that Collagen VI genes are associated with myopathies[13,14]. *Ptprc* (Protein tyrosine phosphatase receptor type C), also known as *CD45*, is a transmembrane glycoprotein. *Ptprc* is an important regulator of T and B cell antigen receptor-mediated activation[15]. A study from Spain[16] suggested that *Ptprc* was

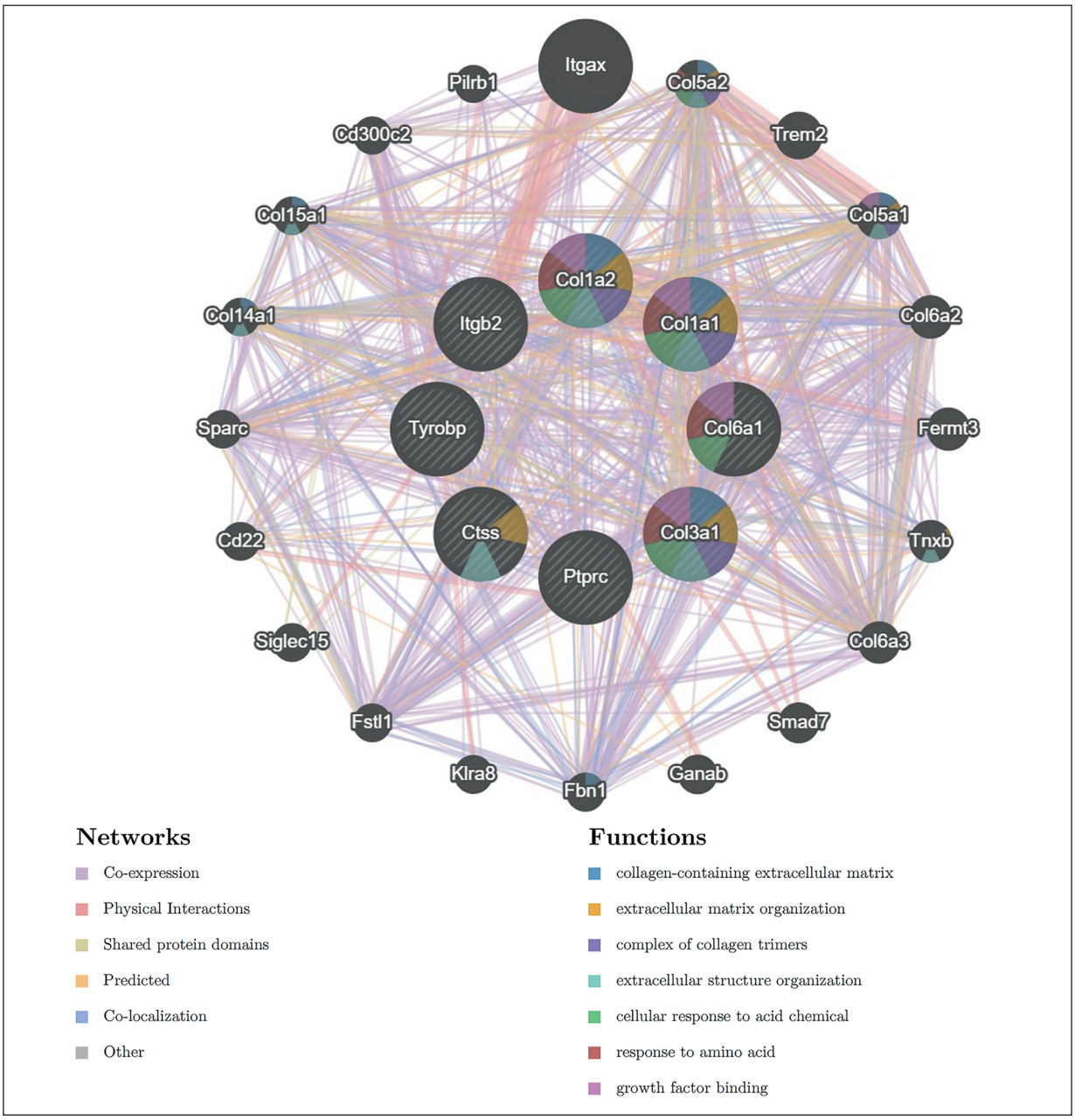

Figure 5. The gene-gene interaction network for DEGs was analyzed using the GeneMANIA database. The 20 most frequently changed neighboring genes are shown. The predicted genes are located in the outer circle, and hub genes are in the inner circle.

upregulated in arrhythmogenic cardiomyopathy patients. *Tyrobp* and related genes are mainly engaged in immunoregulatory mechanisms, such as NK cell-mediated cytotoxicity and macrophage fusion. This suggested that immune response may be involved in the pathogenesis of *DCM17*. *Itgb2* (Integrin beta 2), one of the integrin subunits, was previously demonstrated to be exclusively expressed in leukocytes. It promotes leukocyte adhesion to the endothelium and the ensuing extravasation[18,19]. Zhang et al[20] reported that *Itgb2* participates in cell communication and is involved in cardiac development. *Ctss* (cathepsin S) is a member of the cysteine protease cathepsin family, which is synthesized as an inactive proenzyme in the endoplasmic reticulum and is activated by the removal of the endosomal and lysosomal compartments. It has been reported[21] that *Ctss* regulates the pathogenesis of cardiovascular disease through mediating extracellular matrix protein degradation and cell-to-cell communication. The hub genes interaction analyses indicate that these genes are

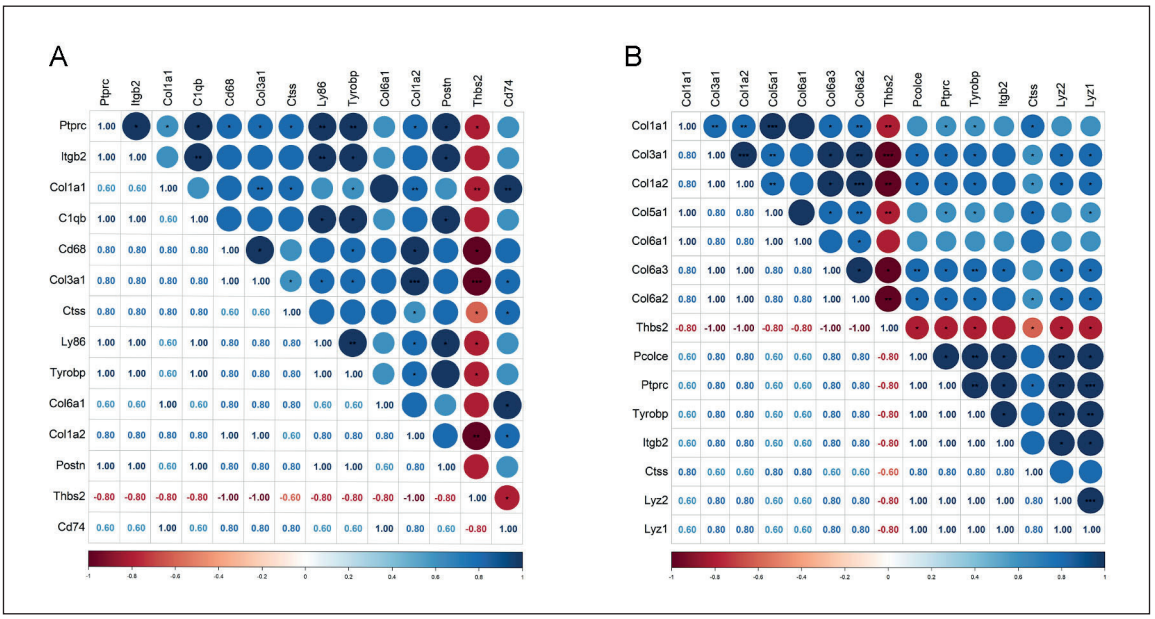

Figure 6. **A**, Heatmap of co-expression correlation between the hub genes which selected both by "Degree"; (**B**) Heatmap of co-expression correlation between the hub genes which selected both by "MCC".

enriched in collagen-containing extracellular matrix, extracellular matrix organization, complex of collagen trimers, extracellular structure organization, cellular response to acid chemical, response to amino acid, and growth factor binding.

GO enrichment results of DEGs revealed that extracellular structure organization, collagen-containing extracellular matrix, and glycosaminoglycan binding are crucial for DCM. Perestrelo et al[22] reported that pathological extracellular matrix prevents cell homing and promotes heart failure. KEGG enrichment results of DEGs illustrated that protein digestion and absorption, phagosome, ECM-receptor interaction, Toxoplasmosis, and *p53* signaling pathway are involved in the pathogenesis of DCM. Kirschner et al[23] found that protein digestion and absorption significantly decreased in congestive heart failure patients. Abdullah et al[24] affirmed that DOX-induced mice cardiomyopathy promoted the accumulation of autophagosomes and autolysosomes, inhibited autophagy flux, and resulted in mitochondria dysfunction. Another bioinformatics study[25] revealed that ECM-receptor interaction is involved in the development of cardiac hypertrophy. The transcription factor *p53* functions as a gatekeeper, regulating a myriad of genes to maintain normal cell functions. Basal *p53* is also necessary to maintain normal heart architecture and physiological function[26]. Knockdown *p53* level can prevent dilated cardiomyopathy[27]. Lee et al demonstrated that mesenchymal stem cell-derived small extracellular vesicles protect cardiomyocytes from doxorubicin-induced cardiomyopathy by inhibiting *p53* signaling[28]. The hub genes we screened have been largely unreported in heart disease and are expected to be novel targets for DCM. Moreover, those genes are mainly involved in regulating immune response, protein degradation, and matrix remodeling, which provide new mechanisms for DCM.

Limitations

As the study is an analytical study based on the GEO database, the chip data were obtained from mice rather than humans. Thus, the key genes and pathways determined in this study will have to be compared and validated with human data in the future. Furthermore, due to funding and time constraints, this study was not validated *in vitro* and *in vivo*. Future research may confirm and promote the application of our study's conclusions.

Conclusions

The present study identified a series of key genes, including *Col1a1, Col3a1, Col1a2, Col6a1, Ptprc, Tyrobp*, *Itgb2*, and *Ctss*. In addition, we also discovered important pathways, including protein digestion and absorption, phagosome, ECM-receptor interaction, and *p53* signaling pathway that were

also most closely correlated with mouse doxorubicin-induced cardiomyopathy (DCM). Our results may provide a more detailed molecular mechanism and potential therapeutic targets for DCM.

Authors' Contributions

Conceptualization, G. Fan, and H. Zuo; formal analysis, G. Fan, X. Shi and C. Huo; data curation, X. SHI and C. Huo; writing–original draft preparation, G. Fan; writing–review and editing, G. Fan, X. Shi, and C. Huo; visualization, G. Fan and H. Zuo; supervision, G. Fan, and H. Zuo. All authors have read and agreed to the published version of the manuscript.

Funding

This research received no external funding.

Informed Consent

Not applicable.

Ethics Approval

Not applicable.

Data Availability

The data used in this study are from the GSE59672 and GSE23598 datasets of the GEO database.

Conflicts of Interest

The authors declare no conflict of interest.

ORCID ID

Gang Fan: 0000-0002-6616-3916.

References

1) Curigliano G, Cardinale D, Dent S, Criscitiello C, Aseyev O, Lenihan D, Cipolla CM. Cardiotoxicity of anticancer treatments: Epidemiology, detection, and management. CA Cancer J Clin 2016; 66: 309-325.
2) Sohail M, Sun Z, Li Y, Gu X, Xu H. Research progress in strategies to improve the efficacy and safety of doxorubicin for cancer chemotherapy. Expert Rev Anticancer Ther 2021; 21: 1385-1398.
3) Chatterjee K, Zhang J, Honbo N, Karliner JS. Doxorubicin cardiomyopathy. Cardiology 2010; 115: 155-162.
4) Zhang M, Yang H, Xu C, Jin F, Zheng A. Risk Factors for Anthracycline-Induced Cardiotoxicity in Breast Cancer Treatment: A Meta-Analysis. Front Oncol 2022; 12: 899782.
5) Pereira GC, Silva AM, Diogo CV, Carvalho FS, Monteiro P, Oliveira PJ. Drug-induced cardiac mitochondrial toxicity and protection: from doxorubicin to carvedilol. Curr Pharm Des 2011; 17: 2113-2129.
6) Singal PK, Li T, Kumar D, Danelisen I, Iliskovic N. Adriamycin-induced heart failure: mechanism and modulation. Mol Cell Biochem 2000; 207: 77-86.
7) Frantz S, Kelly RA, Bourcier T. Role of TLR-2 in the activation of nuclear factor kappaB by oxidative stress in cardiac myocytes. J Biol Chem 2001; 276: 5197-5203.
8) Wang S, Kotamraju S, Konorev E, Kalivendi S, Joseph J, Kalyanaraman B. Activation of nuclear factor-kappaB during doxorubicin-induced apoptosis in endothelial cells and myocytes is pro-apoptotic: the role of hydrogen peroxide. Biochem J 2002; 367: 729-740.
9) Barrett T, Troup DB, Wilhite SE, Ledoux P, Rudnev D, Evangelista C, Kim IF, Soboleva A, Tomashevsky M, Edgar R. NCBI GEO: mining tens of millions of expression profiles--database and tools update. Nucleic Acids Res 2007; 35: D760-765.
10) Li X, Sun X, Kan C, Chen B, Qu N, Hou N, Liu Y, Han F. COL1A1: A novel oncogenic gene and therapeutic target in malignancies. Pathol Res Pract 2022; 236: 154013.
11) Yang F, Lin L, Li X, Wen R, Zhang X. Silencing of COL3A1 represses proliferation, migration, invasion, and immune escape of triple negative breast cancer cells via down-regulating PD-L1 expression. Cell Biol Int 2022; 46: 1959-1969.
12) Huang K, Zhang X, Duan J, Wang R, Wu Z, Yang C, Yang L. STAT4 and COL1A2 are potential diagnostic biomarkers and therapeutic targets for heart failure comorbided with depression. Brain Res Bull 2022; 184: 68-75.
13) Fan Y, Liu A, Wei C, Yang H, Chang X, Wang S, Yuan Y, Bonnemann C, Wu Q, Wu X, Xiong H. Genetic and clinical findings in a Chinese cohort of patients with collagen VI-related myopathies. Clin Genet 2018; 93: 1159-1171.
14) Caria F, Cescon M, Gualandi F, Pichiecchio A, Rossi R, Rimessi P, Cotti Piccinelli S, Gallo Cassarino S, Gregorio I, Galvagni A, Ferlini A, Padovani A, Bonaldo P, Filosto M. Autosomal recessive Bethlem myopathy: A clinical, genetic and functional study. Neuromuscul Disord 2019; 29: 657-663.
15) Al Barashdi MA, Ali A, McMullin MF, Mills K. Protein tyrosine phosphatase receptor type C (PTPRC or CD45). J Clin Pathol 2021; 74: 548-552.
16) Hall CL, Gurha P, Sabater-Molina M, Asimaki A, Futema M, Lovering RC, Suarez MP, Aguilera B, Molina P, Zorio E, Coarfa C, Robertson MJ, Cheedipudi SM, Ng KE, Delaney P, Hernandez JP, Pastor F, Gimeno JR, McKenna WJ, Marian AJ, Syrris P. RNA sequencing-based transcriptome profiling of cardiac tissue implicates novel putative disease mechanisms in FLNC-associated arrhythmogenic cardiomyopathy. Int J Cardiol 2020; 302: 124-130.
17) Lu J, Peng Y, Huang R, Feng Z, Fan Y, Wang H, Zeng Z, Ji Y, Wang Y, Wang Z. Elevated TYROBP

expression predicts poor prognosis and high tumor immune infiltration in patients with low-grade glioma. BMC Cancer 2021; 21: 723.

18) Lee SH, Corry DB. Homing alone? CD18 in infectious and allergic disease. Trends Mol Med 2004; 10: 258-262.

19) Tan SM. The leucocyte beta2 (CD18) integrins: the structure, functional regulation and signalling properties. Biosci Rep 2012; 32: 241-269.

20) Zhang L, Shi X, Gu C, Chen B, Wang M, Yu Y, Sun K, Zhang R. Identification of cell-to-cell interactions by ligand-receptor pairs in human fetal heart. Biochim Biophys Acta Mol Basis Dis 2020; 1866: 165917.

21) Wu H, Du Q, Dai Q, Ge J, Cheng X. Cysteine Protease Cathepsins in Atherosclerotic Cardiovascular Diseases. J Atheroscler Thromb 2018; 25: 111-123.

22) Perestrelo AR, Silva AC, Oliver-De La Cruz J, Martino F, Horvath V, Caluori G, Polansky O, Vinarsky V, Azzato G, de Marco G, Zampachova V, Skladal P, Pagliari S, Rainer A, Pinto-do OP, Caravella A, Koci K, Nascimento DS, Forte G. Multiscale Analysis of Extracellular Matrix Remodeling in the Failing Heart. Circ Res 2021; 128: 24-38.

23) Kirschner SK, Deutz NEP, Rijnaarts I, Smit TJ, Larsen DJ, Engelen M. Impaired intestinal function is associated with lower muscle and cognitive health and well-being in patients with congestive heart failure. JPEN J Parenter Enteral Nutr 2022; 46: 660-670.

24) Abdullah CS, Alam S, Aishwarya R, Miriyala S, Bhuiyan MAN, Panchatcharam M, Pattillo CB, Orr AW, Sadoshima J, Hill JA, Bhuiyan MS. Doxorubicin-induced cardiomyopathy associated with inhibition of autophagic degradation process and defects in mitochondrial respiration. Sci Rep 2019; 9: 2002.

25) Meng X, Cui J, He G. Bcl-2 Is Involved in Cardiac Hypertrophy through PI3K-Akt Pathway. Biomed Res Int 2021; 2021: 6615502.

26) Men H, Cai H, Cheng Q, Zhou W, Wang X, Huang S, Zheng Y, Cai L. The regulatory roles of p53 in cardiovascular health and disease. Cell Mol Life Sci 2021; 78: 2001-2018.

27) Chen SN, Lombardi R, Karmouch J, Tsai JY, Czernuszewicz G, Taylor MRG, Mestroni L, Coarfa C, Gurha P, Marian AJ. DNA Damage Response/TP53 Pathway Is Activated and Contributes to the Pathogenesis of Dilated Cardiomyopathy Associated With LMNA (Lamin A/C) Mutations. Circ Res 2019; 124: 856-873.

28) Lee JY, Chung J, Byun Y, Kim KH, An SH, Kwon K. Mesenchymal Stem Cell-Derived Small Extracellular Vesicles Protect Cardiomyocytes from Doxorubicin-Induced Cardiomyopathy by Upregulating Survivin Expression via the miR-199a-3p-Akt-Sp1/p53 Signaling Pathway. Int J Mol Sci 2021; 22.