

Differentially Expressed Autophagy-Related Genes are Potential Biomarkers and Therapeutic Targets in Atrial Fibrillation

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Research

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Abstract

Background: Autophagy is an evolutionary conserved important process for the turnover of intracellular substances in eukaryotes, and is closely related to the development of cardiovascular disease. However, the impact of autophagy-related genes (ARGs) on the occurrence and development of atrial fibrillation (AF) is still unclear.

Methods: We downloaded two data sets from the Gene Expression Omnibus (GEO) database, GSE14975 and GSE31821. After merging the data of the two microarrays, adjusting the batch effect, and integrating the differentially expressed genes (DEGs) with ARGs to obtain differentially expressed autophagy-related genes (DEARGs). Functional and pathway enrichment analyses were carried out based on Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG). Use the STRING database to construct a protein-protein interaction (PPI) network.

Results: Compared with the control group, we finally identified 11 DEARGs (CDKN1A, CXCR4, DIRAS3, HSP90AB1, ITGA3, PRKCD, TP53INP2, DAPK2, IFNG, PTK6, and TNFSF10) in AF using $[\log_2 \text{ (fold change)}] > 0.5$ and $P < 0.05$. In the pathway enrichment analysis, the most significantly enriched pathway was the autophagy pathway.

Conclusions: Our study demonstrates that these 11 potential crucial ARGs, especially PRKCD, TP53INP2 and DAPK2, may be potential biomarkers and therapeutic targets in AF, which will help the personalized treatment of AF patients.

Introduction

Atrial fibrillation (AF) is the most common clinically sustained cardiac rhythm disorder. There is a high prevalence of AF all over the world, the highest in North America, Europe, China and Southeast Asia, with approximately 270–360 cases per 100,000 people [1]. At present, treatments of AF mainly include pharmacologic treatment, direct current (DC) cardioversion, endovascular catheter and surgical ablation therapy [2]. Although the wide array of treatment options, each has its limitations. Current therapies available only attenuate symptoms but are not able to cure the disease, imposing a serious burden on AF patients' quality of life. Therefore, there is an urgent need to improve our understanding of AF pathogenesis and to find new therapeutic targets to cure AF.

Autophagy is the main intracellular degradation system. In the process of autophagy, the cytoplasm is encapsulated in a double-membrane structure of autophagosomes, and autophagosomes fuse with lysosomes to form autophagolysosomes, which are degraded in autophagosomes [3]. A lot of evidence shows that autophagy response is closely related to the occurrence and development of cardiovascular disease [4, 7]. For example, ischemia injury activates autophagy of cardiomyocytes, enabling cardiomyocytes to cope with nutritional stress and improving cell survival rate during ischemia-reperfusion injury [8]. For coronary heart disease, enhanced autophagy can not only protect the myocardium against ischemia but also prevent the remodeling of the heart after ischemia [9]. Therefore, the relationship between autophagy and AF has also attracted the attention of researchers.

Some studies on autophagy and AF have been carried out in recent years [10, 12]. Observation of 170 patients in sinus rhythm who had undergone elective coronary artery bypass grafting, Garcia et al. [13] found that impaired autophagy plays an important role in the occurrence of postoperative AF. Yuan et al. [14] indicated that there is AMPK-dependent autophagy in the occurrence of AF. Their subsequent research found that autophagy can induce atrial electrical remodeling through ubiquitin-dependent selective degradation of Cav1.2 [15]. However, the understanding of the role of autophagy in the occurrence and development of AF is far from enough. Not only that, at present, but there is also a lack of research on the use of large-scale autophagy-related genes (ARGs) expression profile to screen and identify AF biomarkers and therapeutic targets.

The purpose of this study is to deeply understand the potential clinical application value of ARGs in AF through the Gene Expression Omnibus (GEO) database using bioinformatic methods. We combined the differentially expressed genes (DEGs) in samples from patients with AF and the control group with the expression profile of ARGs and systematically analyzed the expression status of ARGs and their impact on the occurrence and development of AF.

Results

Data preprocessing and differential expression analysis

A detailed flow chart for the specific process of analysis was shown in Fig. 1. A total of 54 675 probes were obtained from GSE14975 and GSE31821. After preprocessing, 21 644 genes were identified. Considering the criteria for $[\log_2 \text{ (fold change)}] > 0.5$ and $P < 0.05$, we finally obtained a total of 611 significantly DEGs, of which 309 were up-regulated and 302 were down-regulated. The clustering heatmap is shown in Supplementary Fig. 1.

PPI network and functional GO terms and pathway enrichment analyses of DEGs

Altogether, 202 nodes and 389 interaction pairs were identified in the PPI network (Supplementary Fig. 2). According to the view that highly connected genes were noted to possibly play important roles in diseases, we calculated the connectivity between the nodes through R software and the results are displayed in Fig. 2. Here, the first 6 nodes are all members of the collagen family, including collagen type I alpha 1 chain (COL1A1, degree = 21), collagen type III alpha 1 chain (COL3A1, degree = 17), collagen type IV alpha 1 chain (COL4A1, degree = 16), collagen type IV alpha 2 chain (COL4A2, degree = 16), collagen type V alpha 1 chain (COL5A1, degree = 15) and collagen type IX alpha 3 chain (COL9A3, degree = 15) are considered as hub genes in related to AF maintaining.

To investigate the biological effects of DEGs, we performed GO and KEGG functional enrichment analyses, the top 3 GO terms related biological processes were collagen catabolic process (enrich factor: 6.62; P-value: 3.148e-07), collagen fibril organization (enrich factor: 7.92; P-value: 1.713e-05) and negative regulation of cell-cell adhesion (enrich factor: 7.00; P-value: 3.780e-07), the results are shown in the Supplementary Fig. 3A.

KEGG pathway analysis data appear in Supplementary Fig. 3B and 3C. The results suggesting that DEGs were significantly enriched in pathways of protein digestion and absorption (P-value: 4.24e-08), amoebiasis (P-value: 4.72e-06) and IL-17 signaling pathway (P-value: 0.00037).

Identification Of Deargs

Data were further analyzed by R software, take the intersection of DEGs expression profile and 232 ARGs to identify DEARGs.

Functional Enrichment Of The Deargs

To further explore the biological functions of the DEARGs, functional enrichment and pathway analyses were performed and the results are presented in Fig. 4A, 4B and 4C.

GO enrichment showed that changes in the biologic process (BP) of DEARGs were mainly enriched in the protein phosphorylation, protein kinase activation and apoptotic signaling pathway. (Fig. 4A, Table 2)

The results of KEGG enrichment analysis showed that pathways of DEARGs mainly involve pathways in autophagy, necroptosis and bladder cancer (Fig. 4B and 4C). It is worth to mentioning that it is directly enriched into autophagy, which proves the correctness of the analysis results.

Table 1

The DEARGs of merged data of 611 DEGs and 232 ARGs with the use of criteria of \log_2 (fold change) > 0.5 and $P < 0.05$

Gene names	log2 FC	AveExpr	t	P-Value	adj. P. Val	B
CDKN1A	0.6755	8.0689	2.7433	0.0136	0.7170	-2.8357
CXCR4	0.8774	6.3564	2.8709	0.0104	0.6877	-2.6383
DAPK2	-0.6586	7.1420	-4.1998	0.0006	0.4001	-0.5576
DIRAS3	0.8147	6.0380	2.3157	0.0330	0.7937	-3.4772
HSP90AB1	0.6056	9.8253	2.3393	0.0314	0.7870	-3.4427
IFNG	-0.9761	3.2009	-2.4571	0.0247	0.7607	-3.2693
ITGA3	0.8263	5.0535	2.3481	0.0309	0.7870	-3.4300
PRKCD	0.9270	5.1578	2.2153	0.0403	0.8028	-3.6217
PTK6	-0.8182	3.8179	-3.7636	0.0015	0.5276	-1.2334
TNFSF10	-0.7106	8.0994	-2.5080	0.0223	0.7607	-3.1933
TP53INP2	0.5637	9.7921	3.7557	0.0015	0.5276	-1.2457

DEARGs, differentially expressed autophagy-related genes; DEGs, differentially expressed genes; ARGs, autophagy-related genes; log2 FC, log2 (fold change); AveExpr, average expression; adj. P. Val, adjust P-value

Table 2
Significant enriched GO terms and pathways of DEARGs

Term	count	Genes	P-Value	adj. P. Val	Q value
GO terms					
GO:0001932 (BP)	regulation of protein phosphorylation	7	HSP90AB1/DIRAS3/PTK6/CDKN1A/IFNG/CXCR4/PRKCD	0.000001317	0.0001825
GO:0045859 (BP)	regulation of protein kinase activity	6	HSP90AB1/DIRAS3/PTK6/CDKN1A/CXCR4/PRKCD	0.000002055	0.0001708
GO:0043549 (BP)	regulation of kinase activity	6	HSP90AB1/DIRAS3/PTK6/CDKN1A/CXCR4/PRKCD	0.000002858	0.0001485
GO:0097190 (BP)	apoptotic signaling pathway	5	DAPK2/TNFSF10/CDKN1A/IFNG/PRKCD	0.000005167	0.0001464
KEGG Pathway					
hsa04140	Autophagy - animal	3	DAPK2/ PRKCD/ TP53INP2	0.000376076	0.029412614
hsa04217	Necroptosis	3	HSP90AB1/ IFNG/ TNFSF10	0.000582428	0.029412614
hsa05219	Bladder cancer	2	CDKN1A/ DAPK2	0.000891239	0.030005034
GO, Gene Ontology; DEARGs, differentially expressed autophagy-related genes; BP, biological process; adj. P. Val, adjust P-value					

Discussion

Although some studies have shown that autophagy is involved in the occurrence and development of AF, ARGs have not been comprehensively analyzed to explore its clinical significance. In the present study, we used bioinformatics tools to analyze the integrated data of gene expression profiles from two GEO datasets to identify key ARGs related to the therapeutic targets of AF patients. We found that 11 ARGs (CDKN1A, CXCR4, DIRAS3, HSP90AB1, ITGA3, PRKCD, TP53INP2, DAPK2, IFNG, PTK6, TNFSF10) under the criteria of $[\log_2(\text{fold change})] > 0.5$ and $P < 0.05$ were differentially expressed in AF patient myocardial tissue samples. Furthermore, we also found several important pathways, suggesting that these pathways may play an important role in the mechanism of AF.

CXCR4 is found on chromosome 2q22.1 and encodes a CXC chemokine receptor specific for stromal cell-derived factor-1. Upadhye et al. [20] indicated that CXCR4 is over-expressed in humans with low coronary artery plaque burden. The interaction between CXCL12 and CXCR4 can inhibit ABCA1-dependent cholesterol efflux from macrophages, aggravating atherosclerosis [21]. The role of CXCR4 in AF is unclear. In this study, we found that CXCR4 is highly expressed in the myocardial tissue of AF patients.

Our study revealed that CDKN1A, DIRAS3, HSP90AB1, ITGA3 and PRKCD expression levels were all up-regulated in AF samples compared to control samples. CDKN1A is located on chromosome 6p21 and encodes a potent cyclin-dependent kinase inhibitor. This gene mainly plays an important role in cellular metabolic pathways [22]. DIRAS3, located on chromosome 1p31, encodes a member of the ras superfamily. The protein may play an autophagy effect in some cancer cells by regulating the autophagosome initiation complex [23]. HSP90AB1 encodes a member of the heat shock proteins 90 family; these proteins are involved in protein folding and degradation and signal transduction. The 90 kDa heat-shock protein encoded by this gene is thought to play an important role in inflammation. ITGA3 encodes a member of the integrin alpha chain family of proteins. PRKCD is located on chromosome 3p21 and encodes a member of the protein kinase C family of serine- and threonine-specific protein kinases. The protein can positively or negatively regulate apoptosis [24]. However, the relationship between these genes and cardiovascular disease has not been studied yet. Functional enrichment analyses in the present study indicated that CDKN1A, CXCR4, DIRAS3, HSP90AB1 and PRKCD were all enriched in GO terms (biological processes) of regulation of protein phosphorylation, protein kinase activity and kinase activity.

TP53INP2 is located on chromosome 20q11.22. The protein encoded by this gene is crucial in the formation and processing of autophagosomes [25]. DAPK2 is located on chromosome 15q22.31 and encodes a protein that belongs to the serine/threonine protein kinase family. Overexpression of this gene was shown to induce cell apoptosis [26]. KEGG pathway enrichment analysis showed that PRKCD, TP53INP2 and DAPK2 were enriched in the autophagy pathway. Therefore, it is extremely necessary to have a more thorough understanding of the role of these three genes in the autophagy pathway in the occurrence and development of AF.

IFNG is located on chromosome 12q15 and encodes a soluble cytokine that is a member of the type II interferon class. The encoded protein is secreted by cells of the innate immune system and the adaptive immune system. In the Framingham heart study, IFNG was significantly associated with a higher body mass index (BMI) [27]. A study published in 2015 by Barsova et al. showed that the genetic effect of IFNG and/or its biallelic combination on myocardial infarction was discovered and replicated in Russians [28]. In this study, we found that the IFNG expression level was down-regulated in the myocardial tissue of AF patients. These results revealed that IFNG was related with cardiovascular disease and might be a potential biomarker of AF.

Besides, we also screened out the other two genes PTK6 and TNFSF10. Similarly, no one has reported their relationship with cardiovascular disease before. PTK6 encodes a cytoplasmic non-receptor protein kinase that can be used as an intracellular signal converter in epithelial tissue. Akt plays an important role in the cardiovascular system, and the PTK6 metabolic pathway is an atypical pathway that affects the activity of Akt [29]. TNFSF10 is located on chromosome 3q26.31, the protein encoded by this gene is a cytokine that belongs to the tumor necrosis factor (TNF) ligand family. The binding of this protein to the receptor has been shown to trigger the activation of MAPK8/JNK, caspase 8 and caspase 3 [30]. Like the IFNG, the expressions of these two genes were down-regulated in AF patients.

In the current study, we discussed 11 potential crucial DEARGs involved in the occurrence and development of AF, suggesting that these genes may serve as potential biomarkers and therapeutic targets for AF. However, there are still some limitations in this study. Firstly, our study was mainly conducted using patient data available in public database. These findings need to be verified in clinical trials. In addition, the mechanisms of ARGs regulating the initiation and progression of AF need to be further validated by RT-qPCR in clinical samples. Finally, the working mechanism of these genes is not yet fully understood, so more evidence is needed to discover its biological basis.

Conclusion

In summary, our study shows that these 11 ARGs have great potential as biomarkers and therapeutic targets in AF. The crucial genes (PRKCD, TP53INP2 and DAPK2) identified in the autophagy pathway provide new possibilities for further identifying the susceptibility of AF and finding useful therapeutic targets. However, the results of this study need to be further verified to aid individualized clinical treatment.

Materials And Methods

Data collection and preprocessing

GSE14975 and GSE31821 (10 samples in GSE14975 and 6 samples in GSE31821) were downloaded from GEO (<http://www.ncbi.nlm.nih.gov/geo>) [16] and the sample platforms used were GPL570 (HG-U133_Plus_2) Affymetrix Human Genome U133 Plus 2.0 Array.

The data were preprocessed as follows: to annotate the data, the probe names were converted into gene names with ActivePerl 5.28.1 software (<https://www.activestate.com/products/perl/downloads/>). Then we generated a synthetic dataset from GSE14975 and GSE31821 datasets. For the new dataset, eliminate probes without related gene symbols, and calculate the average expression value of those gene symbols with multiple probes. The "sva" package of R software was used to remove batch-effects for new dataset batch correction.

Screening of DEGs

The DEGs were screened with R software limma package version 3.44.3 (<http://bioconductor.org/packages/release/bioc/html/limma.html>) under the criteria of $|\log_2(\text{fold change})| > 0.5$ and $P < 0.05$.

Protein-protein interaction (PPI) network and functional analysis of DEGs

PPI networks of DEGs were analyzed using the STRING online tool (STRING database, version 11.0; https://string-db.org/cgi/input.pl?sessionId=1fJdSiXTVpOd&input_page_show_search=on) [17] to further predict protein functional associations and protein-protein interactions. It might provide insights into the underlying molecular mechanism of the beginning or development of diseases. An interaction with a confidence score > 0.70 was considered statistically significant. The specific process of DEGs function and pathway enrichment analysis is consistent with that described by differentially expressed ARGs (DEARGs).

Autophagy-related genes

We obtained a total of 232 ARGs from the Human Autophagy-dedicated Database (HADb, <http://autophagy.iu/clustering/index.html>), which provides a more detailed list of human genes involved in autophagy.

Identification of DEARGs

Data were further analyzed by R software, take the intersection of DEGs expression profile and 232 ARGs to identify DEARGs.

Functional and pathway enrichment analysis of DEARGs

Through Gene Ontology (GO) enrichment analysis, we can comprehensively understand the biological process, cellular component and molecular function of DEARGs enriched. Use the bohao online enrichment tool (<http://enrich.shbio.com/index/ga.asp>) to perform GO enrichment analysis on DEARGs. Terms of which $P < 0.05$ were statistically significant.

Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment was performed to search for relevant important pathway information of previously identified DEARGs. ClusterProfiler version 3.16.1 (<https://bioconductor.org/packages/release/bioc/html/clusterProfiler.html>) [18] was used to perform KEGG pathway enrichment analysis [19] in R software. Pathways of which $P < 0.05$ were statistically significant.

Declarations

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Author contribution

Jiao Zhou and Yunlong Dong conceived and designed the research; Xiang Cai and Yunlong Dong collected and analyzed the data; Hongbo Yang created all tables and figures; Jiao Zhou and Zhuxinyue Xie drafted the manuscript; Guo Tao made critical revision of the manuscript. All authors have read and approved the final manuscript.

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Data availability

The data analyzed in the present study can be accessed the GEO (<https://www.ncbi.nlm.nih.gov/geo/>) website.

Ethics approval and consent to participate

Not necessary.

Consent for publication

Not applicable.

Competing interests

The authors declare that there is no conflict of interest.

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Figures

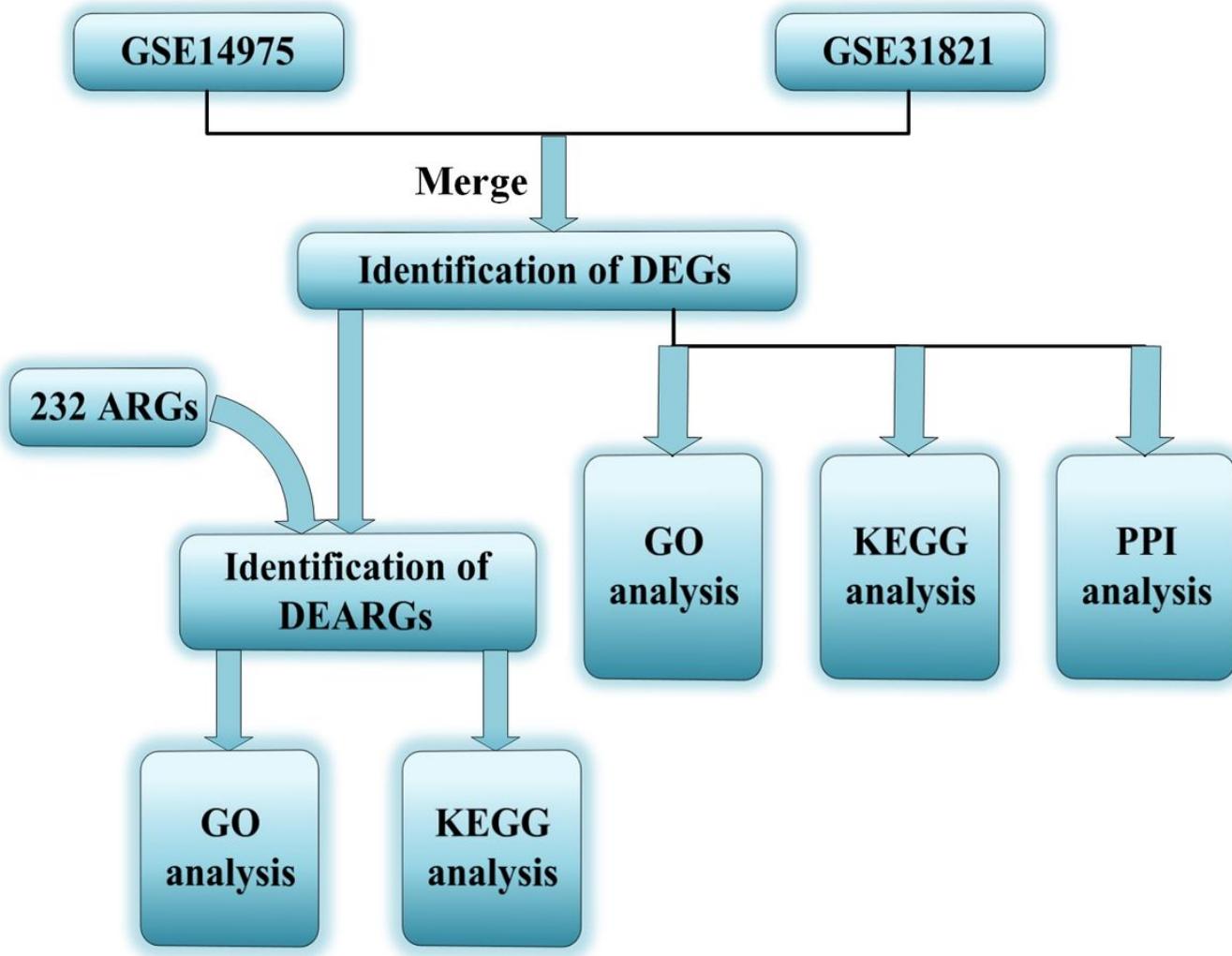


Figure 1

The flow chart shows the design of the present study. DEGs, differentially expressed genes; ARGs, autophagy-related genes; DEARGs, differentially expressed autophagy-related genes; PPI, protein-protein interaction; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes

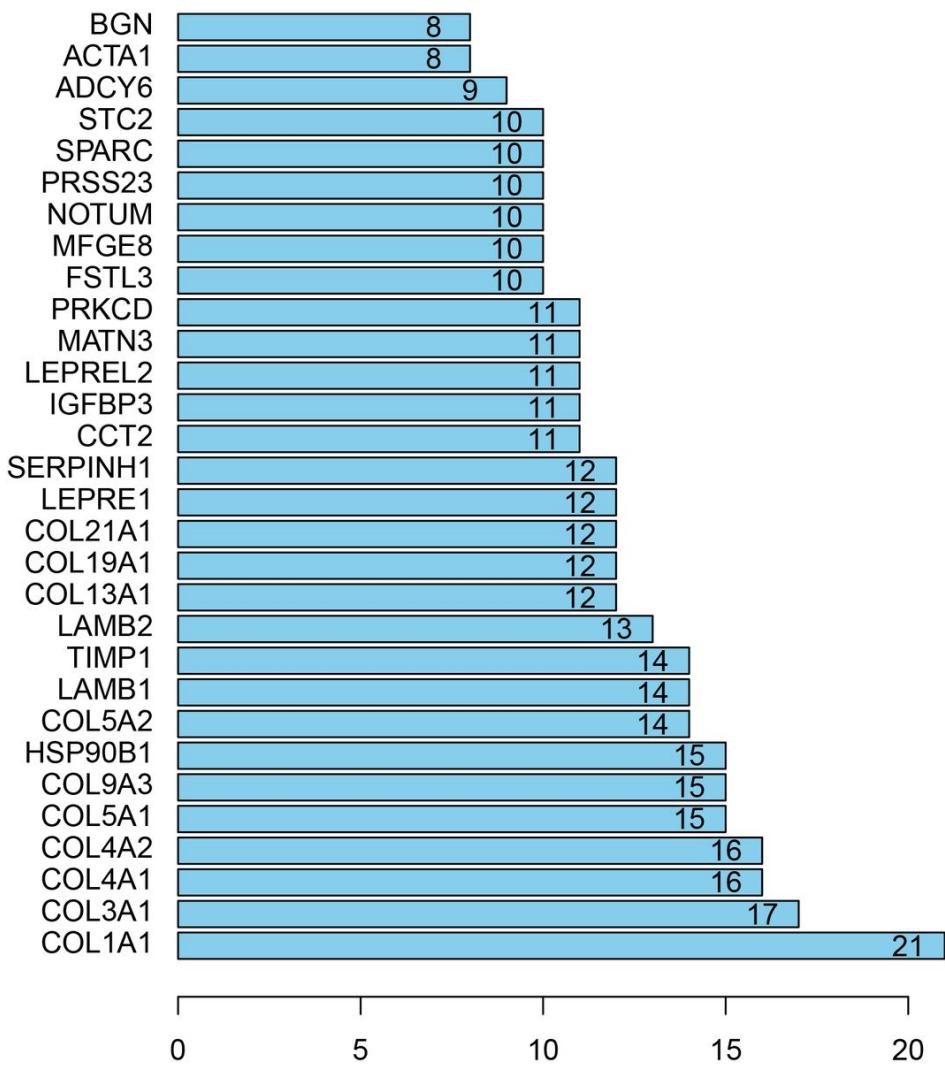


Figure 2

Top 30 nodes of PPI networks of DEGs between control samples and AF samples. PPI, protein-protein interaction; DEGs, differentially expressed genes; AF, atrial fibrillation

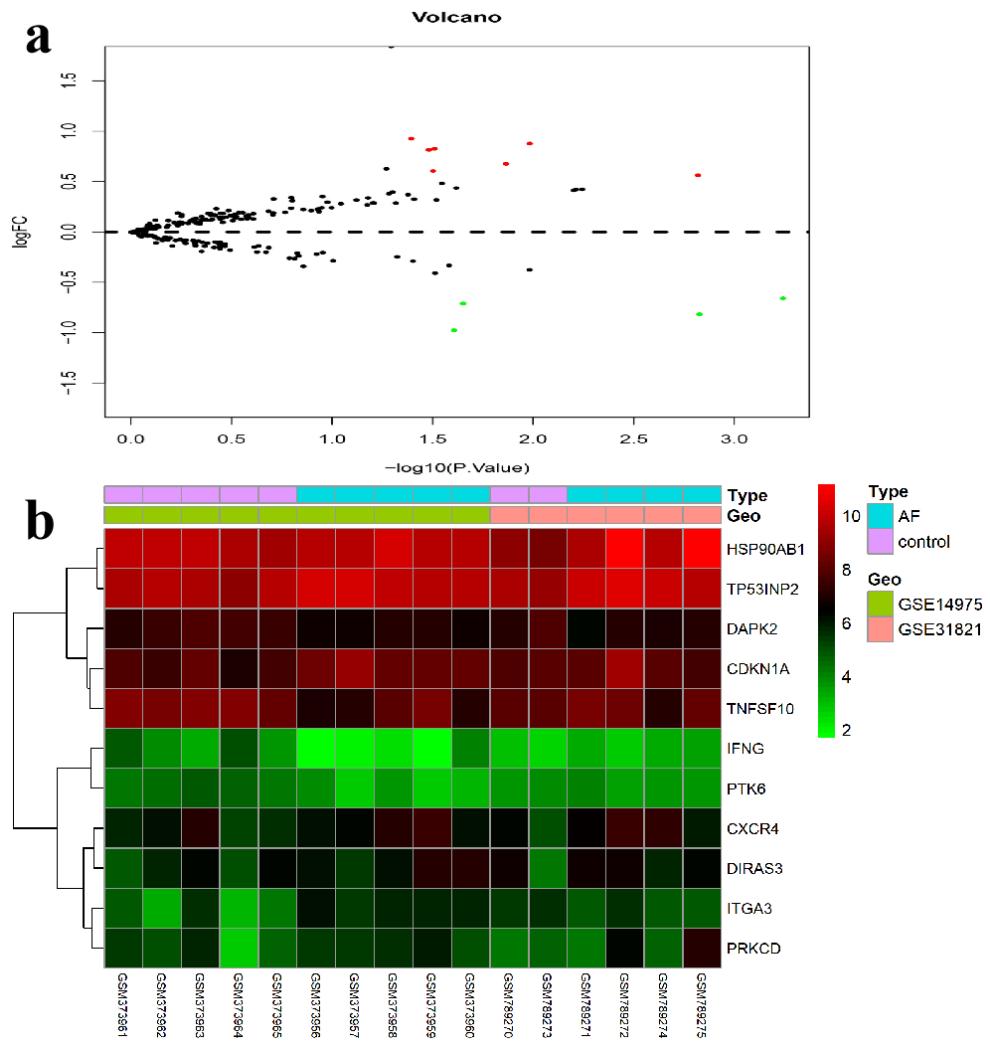


Figure 3

Volcano map (A) show DEARGs, with red dots representing up-regulated genes, green dots representing down-regulated genes, and the remaining black dots representing no differences gene. Heat map (B) of 11 DEARGs in AF samples and control samples. DEARGs, differentially expressed autophagy-related genes; AF, atrial fibrillation

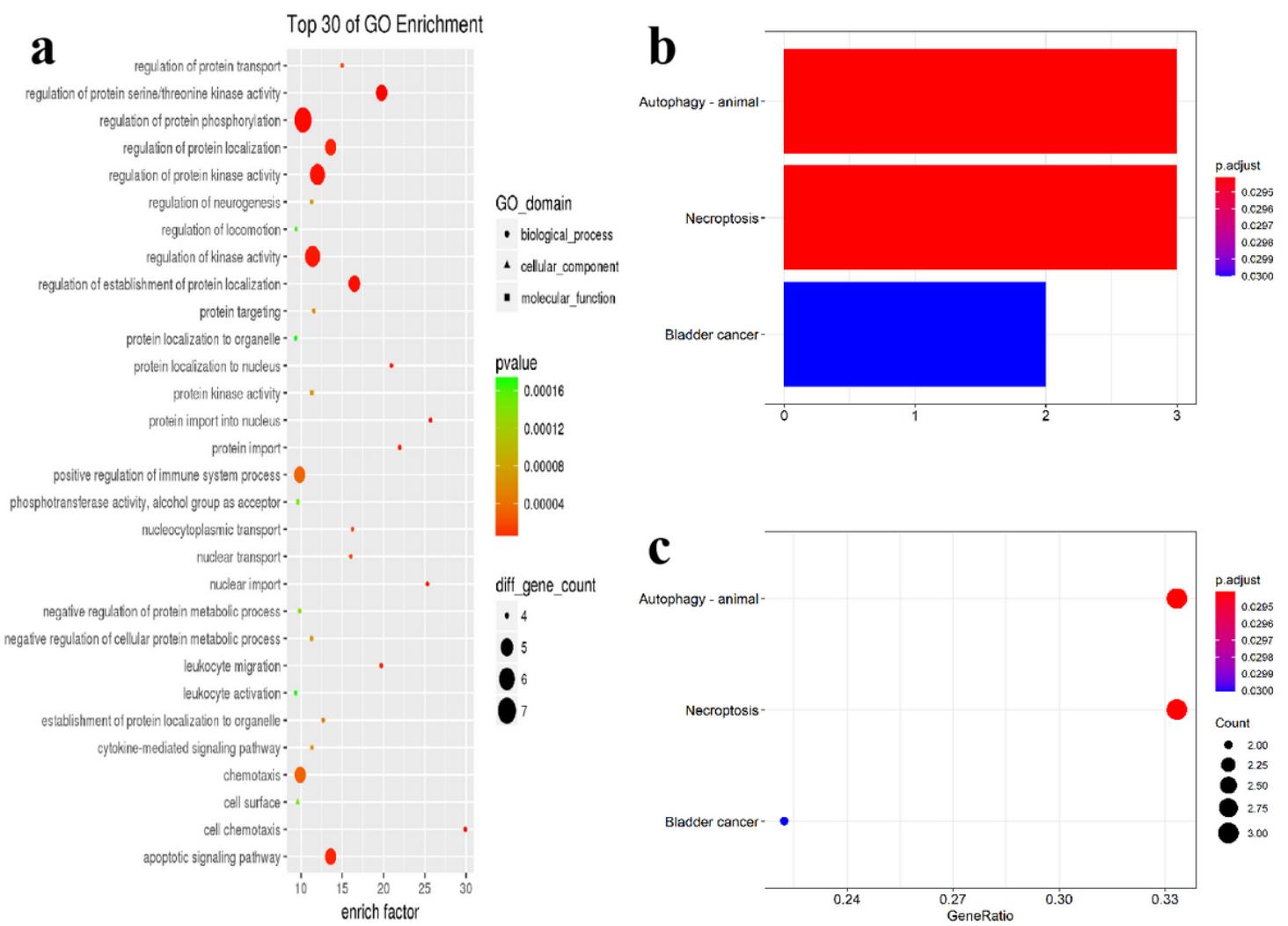


Figure 4

GO (A) analysis shows the biological processes, cellular components and molecular functions involved in DEARGs. Bar plot (B) and dot plot (C) show KEGG pathway enrichment of DEARGs. GO, Gene Ontology; DEARGs, differentially expressed autophagy-related genes; KEGG, Kyoto Encyclopedia of Genes and Genomes

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SUPPLEMENTARYFIGURE1.jpg](#)
- [SUPPLEMENTARYFIGURE2.jpg](#)
- [SUPPLEMENTARYFIGURE3.png](#)