Exploring the Mechanism of Quercetin for the Treatment of Atrial Fibrillation by GEO Gene Chip Combined with Network Pharmacology and Molecular Docking

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Abstract

Atrial fibrillation (AF) is a common atrial arrhythmia for which there is no specific therapeutic drug. Quercetin (Que) has been used to treat cardiovascular diseases such as arrhythmias. In this study, we explored the mechanism of action of Que in AF using network pharmacology and molecular docking. The chemical structure of Que was obtained from Pubchem. TCMSP, Swiss Target Prediction, Drugbank, STITCH, Binding DB, Pharmmapper, CTD, GeneCards, DISGENET and TTD were used to obtain drug component targets and AF-related genes, and extract AF and normal tissue by GEO database differentially expressed genes by GEO database. The top targets were IL6, VEGFA, JUN, MMP9 and EGFR, and Que for AF treatment might involve the lipid and atherosclerosis pathway, the role of AGE-RAGE signaling pathway in diabetic complications, MAPK signaling pathway and IL-17 signaling pathway. In addition, molecular docking showed that Que binds strongly to key targets and is differentially expressed in AF. This study systematically elucidated the key targets of Que treatment for AF and the specific mechanisms, providing a new direction for further basic experimental exploration and clinical treatment.

Introduction

Atrial fibrillation (AF) is by far the most common type of atrial arrhythmia and is a risk factor for increased cardiovascular mortality.¹ The prevalence of AF increases with age, doubling every 10 years after age 50 years and reaching 10% in patients ≥ 80 years. Furthermore, the number of patients with AF is expected to increase significantly in the coming decades.^{2,3} Oral anticoagulant drugs are currently one of the main treatment modalities for AF, such as rivaroxaban and dabigatran.^{4,5} In addition, relevant studies have reported that AF patients will increase significantly in the next few decades. Therefore, exploring new drugs to reduce the danger of bleeding in patients with AF, improve the cardiac function of patients and the complications of AF requires further research.

Quercetin (Que) is widely found in vegetables and fruits, and Que is also found in several herbal medicines. The therapeutic potential of Que has been widely studied because of its ability to inhibit low-density lipoprotein (LDL) oxidation, reduce adhesion molecules and other inflammatory markers, prevent neuronal oxidation and inflammatory damage, and platelet anti-aggregation.^{6,7} Que has been reported to show significant therapeutic effects in animal models of heart failure, arrhythmias and hypertension in various cardiovascular diseases.⁸⁻¹¹ Recent studies have found that in a rat model of arrhythmia after myocardial ischemia and reperfusion injury, the administration of Que significantly reduced the time to arrhythmia as well as lactate dehydrogenase (LDH) levels of myocardial injury.¹² Based on these comprehensive data, Que is one of the new therapeutic approaches for the prevention of cardiovascular diseases, but there is still lack of the related research in vitro and in vivo as well as clinical studies of Que for AF treatment, so we think it is necessary to do the systematic and in-depth studies on the molecular mechanisms of Que for AF treatment.

Network pharmacology is to further analyze the relationship between drugs, diseases and targets by collecting various database data, data analysis and simulation.¹³ Therefore, by using a network

pharmacological analysis method, we investigated the targets of action in Que for the treatment of AF and analyzed their relevant target biological pathways, which laid a good foundation for further in-depth study of the mechanism of action of Que for the treatment of AF. The detail flow chart of this study is shown in Figure 1.

Results

Information on potential targets of Que and AF

The chemical structure of quercetin is shown in Figure 2A. Que potential targets were obtained by TCMSP, PharmMapper, Swiss Target Prediction, Drugbank, Stitch and Binding database databases, and a total of 449 were obtained by screening de-duplication and integration. A total of 2201 disease targets were screened using CTD, GeneCards, Disgenet and TTD databases, and 3096 differential genes between normal and AF patients were obtained using the GEO database, of which 462 were upregulated and 2634 were downregulated. From the volcano plot (Figure 2B) and the difference plot (Figure 2C), it can be seen that there are significant genetic differences between normal human atrial tissues and atrial tissues from AF patients. Combining drug targets, disease targets and GEO targets, 65 core targets, including IL-6, EGFR, MMP9, ESR1 (etc) were obtained by online mapping of Venn diagram (Figure 2D).

PPI network and Key Targets of Que for AF

The Que and AF intersection targets were imported into String database, species limited to human, and the protein interactions network was obtained, which was further imported into Cytoscape for topological analysis, and the results suggested that there were 65 nodes and 575 edges in the network, and the key core targets were obtained using cytoHubba plugin (Figure 2E), ranking the top 10 key targets for treating AF targets IL-6, VEGFA, JUN, MMP9, EGFR, CCND1, CXCL8, PTGS2, ESR1 and MYC, and the detailed information of the top 10 targets ranked by the key target Degree value is shown in Table 1.

GO function analysis and classification

To further clarify the mechanism of action of quercetin in the treatment of AF, we classified 65 core targets into proteins using the PANTHER database (Figure 3A), found that they were mainly focused on protein modifying enzymes (20.4%), gene-specific transcriptional regulators (18.40%), and metabolite interconverting enzymes (18.40%). 65 core genes were introduced into the R package, and the GO functional results (Figure 3B), the biological process (BP) was mainly focused on: response to lipopolysaccharide, response to molecule of bacterial origin, regulation of inflammatory response, response to oxidative stress, and epithelial cell proliferation, cell composition (CC) is mainly focused on: vesicle lumen, membrane raft, membrane microdomain, membrane region, focal adhesion, molecular functions (MF) mainly focus on: DNA-binding transcription factor binding, RNA polymerase II-specific DNA-binding transcription factor binding, cytokine receptor binding, monocarboxylic acid binding, and growth factor binding. In addition, we used the MCODE tool to identify highly interconnected clusters in the PPI network, and the MCODE clusters were classified into three types, including regulation of smooth

muscle cell proliferation (Figure 3C), positive regulation of protein import into nucleus (Figure 3D), negative regulation of intracellular signal transduction (Figure 3E).

KEGG pathway analysis and core pathway network construction

The pathways of action were clarified by KEGG enrichment analysis of 65 intersecting genes, with 157 enrichment terms, of which the top 30 most significant pathways (Figure 4A), the core targets may affect the lipid and atherosclerosis, AGE-RAGE signaling pathway in diabetic complications, MAPK signaling pathway, and IL-17 signaling pathway, suggesting that Que may be effective in treating AF by modulating the above signaling pathways.

The top 10 relevant pathways of Que for AF were screened (Table 2) and imported into Cytoscape to construct the “Que-Major Pathways-AF” network (Figure 4B). After screening out the KEGG-enriched pathways that were not related to AF disease, it was found that the important pathway in Que for AF treatment was probably related to MAPK signaling pathway, and the detailed pathway map was obtained by R package analysis (Figure 4C), in which the red area was signal upregulation.

Molecular docking and differential analysis of core targets

The five core targets with high Degree values were selected for molecular docking to verify the binding activity of Que active ingredients to potential anti-AF targets. The average docking affinity of the results was -5.042 kcal/mol, as detailed in Table 3, where the larger the absolute value of the docking affinity, the more stable the binding proved to be, indicating that Que has strong binding to the core targets. The detailed diagram of docking with each key target site is shown in Figure 5.

Using the difference data of control group and AF group in GEO database, the differential expression of the first five core targets in Que treatment of AF diseases (Figure 6). VEGFA was significantly upregulated, IL-6, JUN and VEGFA were significantly downregulated compared to controls, while MMP9 targets were not statistically significant, which was considered to be related to the small sample size. The above results suggest that the core targets are closely related to the pathological changes of AF.

Discussion

One study found that the increased risk of cardiovascular disease in some populations was associated with low dietary intake of flavonoids, including Que.¹⁴ It has been found that Que played the anti-ischemic and anti-arrhythmic effects in clinical studies,^{9–11} it leads to great interest and concern of scientists, but the specific mechanisms of Que in the treatment of AF remains unclear. In this study, we investigated the mechanism of Que in the treatment of AF based on network pharmacology analysis. In this study, 449 Que potential targets and 2201 AF targets were obtained from each database respectively, 3096 AF differential genes were obtained from the GEO database. The intersection of the 3 was taken to obtain 65 core genes for Que treatment of AF. The above core genes were screened for the top 5 hub target genes of IL-6, VEGFA, JUN, MMP9 and EGFR by Degree value. GO analysis confirmed that Que had

the potential to play an important role in the pathogenesis of AF through multiple biological pathways, and KEGG analysis obtained key pathways of Que for AF, mainly involving lipid and atherosclerosis pathways, the AGE-RAGE signaling pathway in diabetic complications, MAPK signaling pathway and IL-17 signaling pathway, etc. Through further screening, we found that MAPK pathway plays a key role in the treatment of AF.

IL-6 plays a key role in immune, tissue regeneration and metabolism. It was shown that IL-6 in the left atrium was found to promote early atrial fibrosis through the pSTAT3/STAT3 signaling pathway by establishing postoperative AF mice, and similarly higher IL-6 concentrations were found in the pericardial drainage fluid of patients with postoperative AF in a prospective clinical study.¹⁵ Wu reported inhibiting IL-6 inflammatory factor release reduced early atrial fibrosis and its duration in SD rats with postoperative AF.¹⁶ Studies based on clinical follow-up investigations have found that measurement of IL-6 concentrations could provide an independent prognostic signal as well as a predictor of recurrence for mortality in AF patients.^{17,18} Therefore, IL-6 has the potential to be a therapeutic target for AF. The next key gene VEGFA is a key factor in the growth process of blood vessels and endothelial cells. Its main function is to induce endothelial cell proliferation and inhibit apoptosis.¹⁹ It is well known that AF can lead to endothelial dysfunction,²⁰ and Wang reported that the plasma levels of VEGFA were significantly elevated in AF patients,²¹ suggesting that endothelial dysfunction may be one of the etiologies of AF, VEGFA may be a potential therapeutic target for AF.

The next gene is Transcription factor JUN, It has been shown that the transcription factor complex AP-1 plays a major role in cardiac hypertrophy, and knockout of c-jun pure mice develops in a process leading to cardiac malformations,²² activation of c-jun impaired cell-cell communication between atrial myocytes and ultimately promotes the development of atrial arrhythmias.^{23,24} Therefore, JUN has the potential to be a therapeutic target for AF. The next gene MMP9 is a matrix metalloproteinase that plays an important role in local protein hydrolysis, cell migration, apoptosis and signaling in the extracellular matrix. During AF development, overproduction of reactive oxygen species activated MAPK signaling pathway further increasing MMP9 expression.²⁵ Moe GW reported inhibiting MMP9 expression in canine AF model improved cardiomyocyte hypertrophy and reduced myocardial fibrosis, as well as reduced AF duration.²⁶ Recent clinical studies have found that the plasma MMP9 level in AF patients were higher than that in controls, which suggested MMP9 could be as a target for the treatment of AF.^{17,27} The last of the first five core genes is EGFR, whose main function is to regulate cell growth, proliferation and apoptosis, and is a key regulator of cardiac organogenesis and adult heart.²⁸ In AF fibrotic atrial tissues, EGFR expression was increased, probably due to the cleavage by pro-fibrotic metalloproteinases during atrial remodeling.²⁹ The plasma level of EGFR in clinical AF patients was significantly higher than that in the control group,³⁰ These results suggest that EGFR plays an important role in AF. Overall, the top five core genes were found to be supported by previous studies based on network pharmacology studies.

The results of KEGG enrichment analysis suggested that the lipid and atherosclerosis pathway, AGE-RAGE signaling pathway in diabetic complications, MAPK signaling pathway and IL-17 signaling

pathway played the major roles in the treatment of AF with Que. One of the main causative factors of atherosclerosis and cardiovascular disease is dyslipidemia, which is considered one of the major risk factors for AF, and several clinical studies had demonstrated that LDL and total cholesterol were negatively correlated with the occurrence of AF.^{31–33} It is suggested that lipids and atherosclerosis may be involved in the pathogenesis of AF. The AGE-RAGE signaling pathway is an important link in the development of diabetic disease, and this signaling pathway directly mediates diabetic vascular calcification and vasoconstriction-diastolic dysfunction, and a meta-analysis showed that type 2 diabetes increased the risk of AF by 40%.³⁴ Recent studies had found that the AGE-RAGE signaling pathway was associated with the development of AF,^{35,36} and that when AGE bind to RAGE, it produced inflammatory cytokines and cell adhesion molecules that further induced structural and electrical remodeling of the atria. Raposeiras-Roubín found significantly higher AGE and RAGE plasma levels in AF patients than in normal subjects, and several prospective studies have also shown that RAGE predicts future cardiovascular events and death in diabetic patients.³⁷ This suggesting that this pathway may mediate the progression of AF.

MAPK signaling pathway plays an important role in cell growth, proliferation and fibroblast growth. Li found that atrial structural remodeling in an AF mouse model could be attenuated by decreasing MAPK expression.³⁸ It has been demonstrated that activation of phosphorylated MAPK significantly increases susceptibility to AF,³⁹ in detail MAPK pathway (Figure 4C), red upregulated targets get inflammation and apoptosis play an important role in the development of AF disease. Inflammation is one of the important pathogenic mechanisms of AF, and there is an increasing evidence supporting the involvement of inflammation and immune response in the development and progression of AF. Many studies have found that IL-17 signaling pathway stimulates the release of pro-inflammatory cytokines, which promote myocardial fibrosis and thus induce the development of AF.^{40,41} In addition, IL-17 promotes AF development in rats model with aseptic pericarditis by promoting cardiac inflammatory expression and atrial fibrosis.^{40,42} Therefore, the results of the present study suggest that these four signaling pathways may be involved in the mechanism of Que treatment of AF.

GO analysis revealed the biological processes mainly focused on responses to lipopolysaccharide, regulation of inflammatory responses, phosphatidylinositol-mediated signaling, responses to oxidative stress and responses to epithelial cell proliferation related to the aforementioned pathway-mediated biological processes, which are also consistent with the aforementioned pathway-mediated biological processes. Molecular docking also suggested strong binding of Que to the above key targets and also validated the therapeutic effect of Que on AF targets at the molecular level.

Conclusions

In summary, we have clarified the key target of Que treatment of AF through network pharmacology, obtained the core signaling pathways for the treatment of AF through GO and KEGG analysis and screening, and validated the binding of Que to the key targets using molecular docking technology.

However, the main limitation of this study is the lack of drug intervention in AF animal model experiments and verification of key pathways. Therefore, this study can provide a basis for further research in basic experiments, as well as new ideas and methods for drug development.

Methods

Acquisition of Que targets

Using the TCMSP (<http://tcmssp.com/>),⁴³ PharmMapper (<http://www.lilab-ecust.cn/pharmmapper/>),⁴⁴ SwissTargetPrediction (<http://www.swisstargetprediction.ch/>),⁴⁵ Drugbank (<https://go.drugbank.com/>),⁴⁶ Binding database (<http://www.bindingdb.org/bind/index.jsp>),⁴⁷ STITCH (<http://stitch.embl.de/cgi/input.pl?UserId=MxcokIPgQVMY&sessionId=032YjbzG5Gio>) and other databases to obtain Que targets, default HumanProtein Targets Only (Retrieved on:2021.7.10).

Acquisition of AF related targets

The CTD (<http://ctdbase.org/>),⁴⁸ GeneCards (<https://www.genecards.org/>),⁴⁹ DISGENET(<https://www.disgenet.org/>),⁵⁰ Therapeutic Target Database (<http://db.idrblab.net/ttd/>),⁵¹ were used to “Atrial fibrillation” was used as a keyword to search for the relevant genes. The collected targets were combined and eliminated by the median algorithm to obtain disease targets. GSE31821 data were imported into the online analysis tool GEO2R for processing to obtain differentially expressed genes. The gene screening criteria were $P < 0.05$ and $|\log FC| \geq 0.5$ to obtain differentially expressed genes between normal tissues and AF, and further volcano maps of differentially expressed genes were drawn.⁵²

Cross-tabulation of drugs, diseases and GEO

By using the online mapping tool (<http://www.ehbio.com/test/venn/#/>), intersections of drug targets, disease targets and differential genes can be obtained.

Construction of protein-protein interaction network (PPI)

The intersecting genes were imported into the String database⁵³ (<https://string-db.org/cgi/input.pl>) to construct the protein-protein interaction network (PPI), and the species selectors, exported in tsv format. The tsv was imported into Cytoscape 3.7.2 software (<http://www.cytoscape.org/>) for adjustment as well as to obtain key targets using the cytoHubba plugin. Simultaneous protein functional classification of the intersecting targets using the Panther database.

GO analysis and KEGG pathway and Que-primary pathway-AF network construction

To further understand the function and role in signaling pathways of the intersecting genes, the gene abbreviations (Symbol) were transformed into gene ID (EntrezID), and the results were analyzed visually by the R language package,⁶ as well as the gene ontology (GO) of the above intersecting genes, GO analysis can further explain the role of the intersecting genes in molecular function (MF), biological

process (BP) and cellular component (CC) of AF resistance. The Kyoto encyclopedia of genes and genomes (KEGG) enrichment analysis was used to investigate the major anti-AF signaling pathways involved in the crossover genes. The core target network of “Que-major pathway-AF” was also constructed.

Molecular Docking and differential analysis of core targets

The known key targets were molecularly docked to Que to obtain docking affinities reflecting their stability. The PDB database was used to download the core protein molecular structures and PUBCHEM to download the structures of Que. The protein and Que structures were imported into Auto Dock Tools software for processing and molecular docking, and intermolecular docking affinities were calculated and PyMol 2.4.0 was used for visualization.^{54,55} Changes in core targets were analyzed using differential expression between the normal and AF groups from the GEO database. GraphPad Prism software was used for graphical visualization. Values are expressed as mean $\pm$ SD.

Declarations

Conflicts of interest

The authors declare that there is no conflict of interest.

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Tables

Table 1 Key target information table of Que in the treatment of AF

Uniprot ID	Gene name	Protein name	Degree
P05231	IL-6	Interleukin-6	47
P15692	VEGFA	Vascular endothelial growth factor A	45
P05412	JUN	Transcription factor AP-1	41
P00533	EGFR	Epidermal growth factor receptor	38
P14780	MMP9	Mitogen-activated protein kinase 1	38
P24385	CCND1	G1/S-specific cyclin-D1	38
P10145	CXCL8	Interleukin-8	38
P35354	PTGS2	Prostaglandin G/H synthase 2	37
P03372	ESR1	Estrogen receptor	35
P01106	MYC	Myc proto-oncogene protein	35

Table 2 KEGG pathway enrichment analysis of Que against AF

Pathway	Enrichment	P value	Symbols	Count
AGE-RAGE signaling pathway in diabetic complications	29.23	7.01E-22	NOX4, VEGFA, CCND1, BAX, JUN, IL-6, PRKCA, STAT1, IL-1B, SELE, CSCL8, TGFB1, THBD, SERPINE, COLA1A, COL3A1, JAK2, MAPK14, CD42	19
Lipid and atherosclerosis	35.38	1.45E-20	PTK2, MMP9, CAMK2B, PPARG, FOS, BAX, JUN, IL6, NFKBIA, PRKCA, IL1B, SELE, CXCL8, NFE2L2, CXCL2, CASP1, JAK2, MAPK14, HSP90AA1, CCL5, RHOA, CDC42, HSPA42	23
Fluid shear stress and atherosclerosis	23.08	1.51E-13	PTK2, MMP9, VEGFA, FOS, JUN, IL1B, SELE, THBD, NFE2L2, NQ01, MAPK14, HSP90AA1, RHOA, GSTO1, ACTB	15
Proteoglycans in cancer	26.15	2.33E-13	PTK2, MMP9, CAMK2B, VEGFA, CND1, PRKCA, MYC, TGFB1, COL1A1, FGFR1, ESR1, PRKACA, MAPK14, RHOA, CDC42, ACTB	17
Human cytomegalovirus infection	24.61	1.41E-11	PTK2, PTGS2, VEGFA, CCND1, BAX, IL6, NFKBIA, PRKCA, MYC, IL1B, CXCL8, PRKACA, MAPK14, CCL5, RHOA	16
Kaposi sarcoma-associated herpesvirus infection	21.54	2.64E-10	VEGFA, CCND1, FOS, BAX, JUN, IL6, NFKBIA, STAT1, MYC, CXCL8, CXCL2, JAK2, MAPK14	14
Hepatitis B	20.00	3.34E-10	FOS, BAX, JUN, IL6, NFKBIA, PRKCA, STAT1, MYC, CXCL8, TGFB1, JAK2, MAPK14	13
MAPK signaling pathway	24.62	7.75E-10	MAPT, INSR, VEGFA, FOS, JUN, PRKCA, MYC, IL1B, HSPB1, TGFB1, FGFR1, PRKACA, MAPK14, CDC42, HSPA2	16
Salmonella infection	21.54	6.95E-09	BAX, JUN, IL6, NFKBIA, MYC, IL1B, CXCL8, CASP1, MAPK14, HSP90AA1, RHOA, CDC42, ACTB	14
Chemical carcinogenesis - receptor activation	20.00	9.16E-09	VEGFA, CCND1, FOS, JUN, PRKCA, MYC, PPARA, JAK2, ESR1, PRKACA, HSP90AA1, GSTO1	13

Table 3 Molecular docking results

Compound	Target	PDB	Energy (kcal/mol)
Quercetin	IL-6	4O9H	-4.7
Quercetin	VEGFA	4G1W	-6.47
Quercetin	JUN	1JUN	-3.64
Quercetin	MMP9	5TH6	-5.15
Quercetin	EGFR	3IKA	-5.25

Figures

Figure 1

Graphical abstract

Figure 2

Protein-protein interaction (PPI) networks construction for target proteins of Que against AF. (A) The chemical structure of the quercetin. (B) GEO Volcano Map. Red upregulates the target, green downregulates the target, (C) GEO Difference Chart Differential. Gene expression in normal subjects (green) vs AF patients (purple). (D) Venn diagram of Que AF and GEO. (E) PPI network of Que against AF. The larger the circle and the darker the color, the stronger the correlation with the therapeutic target.

Figure 3

Bioinformatics analysis of target proteins of Que against AF. **(A)** Panther classification categorized target proteins of Que against AF. The figures next to the pie chart indicate the percentage of protein in the given functional class. **(B)** GO, BP, CC and MF enrichment analysis of interselection targets. The intensity of the color represents the adjusted p value, and the bubble size corresponds to the number of genes. **(C, D, E)** The Molecular Complex Detection (MCODE) algorithm has been used to identify densely connected network components.

Figure 4

KEGG pathway enrichment analysis(A) KEGG enrichment analysis of interselection targets. The intensity of the color represents the adjusted p value. (B) Que target-major pathway-AF. The middle purple circle is the relevant pathway and the outer circle is the relevant target in the pathway. (C) MAPK detailed pathway map. The red area is the MAPK upregulation target of the key pathway of AF for Que treatment.

Figure 5

Docking pattern of Que with key target molecules. (A) IL-6 and Que molecular docking, (B) VEGFA and Que molecular docking, (C) JUN and Que molecular docking, (D) MMP9 and Que molecular docking, (E) EGFR and Que molecular docking.

Figure 6

The core action targets of Que on AF were differentially expressed in the GEO data set. Left atrial healthy controls n = 5, AF group n = 5. Values are expressed as mean $\pm$ SD. Compared with Control, *P < 0.05 and **P < 0.01. (A) IL-6 differential expression, (B) VEGFA differential expression, (C) JUN differential expression, (D) EGFR differential expression, (E) MMP9 differential expression.