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Original Article

Danqi soft capsules alleviate myocardial ischemia/reperfusion injury through inhibiting apoptosis-related signaling pathways

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Abstract: Objective: This study aimed to explore the mechanisms of Danqi Soft Capsules (DQ) in reducing myocardial ischemia/reperfusion injury (MI/RI) through network pharmacology, molecular docking, and experimental validation. Methods: The TCMSP database was used to screen for active ingredients of DQ and their potential targets, and compare them to MI/RI-related targets to construct a "drug-active ingredient-target" network. The protein-protein interaction (PPI) network was constructed using the STRING database; and Gene Ontology (GO) functional enrichment analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis were performed. Molecular docking experiments verified the binding affinity between DQ's active ingredients and apoptosis-related target proteins, and cellular experiments validated DQ's anti-apoptotic effects in the H9c2 cardiomyocyte hypoxia/reoxygenation model. Results: Network pharmacology analysis identified 66 active ingredients and 240 potential targets, of which 105 were related to MI/RI. PPI network analysis screened out 10 core targets. GO and KEGG analyses indicated that these targets were related to the pathways of cell apoptosis. The molecular docking experiment confirmed that the active ingredient had a strong binding affinity with the core target, with the binding affinity between tumor necrosis factor (TNF) and tanshinone IIA being -9.2 kcal/mol, and that between tumor protein (TP) 53 and quercetin being -8.6 kcal/mol. Cellular experimental results showed that the cell apoptosis rate in the DQ-treated group was lower than in the model group, with the protective effect in the high-dose group being slightly better than the low-dose group. Conclusion: This study revealed that DQ alleviates MI/RI by inhibiting cell apoptosis, providing a scientific basis for the clinical application of DQ and offering new directions for drug development.

Keyword: Myocardial ischemia-reperfusion injury, danqi soft capsules, network pharmacology, molecular docking, apoptosis

Introduction

Cardiovascular diseases (CVD) are one of the leading causes of death in the 21st century, and acute myocardial infarction (AMI), caused by acute or prolonged coronary ischemia and hypoxia leading to myocardial tissue necrosis, is a serious threat to patients' lives [1]. Although timely reperfusion is currently the most effective treatment to reduce the area of myocardial infarction, this therapeutic approach can also lead to myocardial ischemia/reperfusion injury (MI/RI). This means that after the blood supply is restored, patients may suffer more severe structural damage to the heart and disturbances in the energy metabolism of myocardial cells, and may even experience irreversible damage such as an enlarged infarct area and life-threatening arrhythmias [2]. The physiologic mechanisms of MI/RI encompass multiple biological processes, including apoptosis, oxidative stress, and autophagy [3]. Among these,

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cardiomyocyte apoptosis serves as a pivotal factor in the pathogenesis of MI/RI, with approximately 50% of MI/RI cases demonstrating a direct correlation with cardiomyocyte apoptosis [4]. Myocardial ischemia can induce apoptosis of cardiomyocytes, while reperfusion can further exacerbate this process and induce the expression of various apoptosis-related factors and inflammatory factors, thereby promoting abnormal apoptosis of cardiomyocytes [5]. Currently, the main drugs for treating myocardial cell apoptosis caused by MI/RI are antioxidants, anti-inflammatory drugs, and anti-apoptotic drugs. The antioxidant N-acetylcysteine can scavenge reactive oxygen species and alleviate oxidative stress, but its therapeutic effect is limited [6]. Anti-inflammatory drugs, such as nonsteroidal anti-inflammatory drugs and glucocorticoids, can inhibit inflammatory factors and reduce cardiomyocyte apoptosis, but they may also cause gastrointestinal bleeding and liver dysfunction in patients [7]. Although anti-apoptotic drugs such as belinostat can regulate the expression of apoptosis-related proteins, they lack specificity and may interfere with other normal apoptotic processes *in vivo*, thus causing adverse reactions [8]. Therefore, new therapeutic strategies and drugs need to be explored to more effectively inhibit cardiomyocyte apoptosis and reduce the incidence and severity of MI/RI.

Danshen soft capsule (DQ) is a traditional Chinese medicine compound consisting of Salvia miltiorrhiza (Danshen) and Notoginseng (Sanqi). Tanshinone, salvianolic acid, volatile oil, polysaccharide, and other active ingredients have been found in Danshen, according to studies. Active components found in Sanqi include sugars, amino acids, flavonoids, and saponins [9, 10]. Whereas Sanqi is frequently used to dissipate blood stasis and hemostasis, reduce swelling and discomfort, and clear heat, Danshen's primary effectiveness is in promoting blood circulation, regulating menstruation, and removing blood stasis and pain. The combination of these two herbs can enhance each other's effect. DQ are commonly used in traditional Chinese medicine to improve blood circulation and eliminate blood stasis, thereby enhancing the blood supply to the myocardium [11]. DQ has been employed in contemporary medical studies to treat cardiovascular conditions such myocardial ischemia and coronary heart disease. By controlling autophagy, it has been demonstrated in studies to enhance cardiac function and shield cardiomyocytes from harm [12]. Therefore, in-depth exploration of the mechanism of DQ may provide a theoretical foundation for the discovery of new cardiovascular medicines with stronger targeting and better therapeutic efficacy.

A new area of pharmacology and bioinformatics called "network pharmacology" uses a comprehensive network approach to examine the intricate relationships between substances, proteins, genes, and illnesses. It effectively explores drugs and discovers a potential "compound-protein/gene-disease" pathway. This work aims to establish a scientific foundation for clinical intervention by revealing the fundamental molecular mechanism of DQ against MI/RI. The interaction network between the active components of DQ and their targets was systematically analyzed in this study using a multidisciplinary integration strategy. By targeting apoptosis-related signaling pathways, DQ was discovered to have a dose-dependent protective effect and to effectively prevent MI/RI. This study provides a theoretical foundation for the creation of novel therapeutic medications for MI/RI.

Materials and methods

Collection of potential DQ-targeted genes

The chemical composition information was obtained by Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP, https://old.tcmsp-e.com/tcmsp.php) using the keywords "Danshen" and "Sanqi". Candidate compounds were filtered to meet the criteria of oral bioavailability (OB) ≥ 30% and drug-likeness (DL) ≥ 0.18. The action targets were predicted using TCMSP. The names of the related proteins were all mapped to the official nomenclature of the UniProt database (https://www.uniprot.org/). Using Cytoscape, a "drug active component-target" network was constructed.

Collection of MI/RI-related genes

A search was done for MI/RI-related targets in the GeneCards (https://www.genecards.org/), OMIM (https://www.omim.org/), and DisGeNET (https://disgenet.com/) databases using the keyword "Myocardial ischemia reperfusion injury". Candidate genes were collected based on

the following criteria: DisGeNET score gad ≥ 0.1, and GeneCards relevance score ≥ 6.99 (median).

Construction of DQ-related genes protein-protein interaction (PPI) network

The target proteins of active ingredients from Danshen and Sanqi were compared with the MI/RI target database using the Venny online tool (https://bioinfogp.cnb.csic.es/tools/venny/) to screen out shared targets. For further analysis of the PPI network, these identified shared targets were entered into the STRING (https://cn.string-db.org/), with the species specified as "Homo sapiens" and the threshold set to "highest confidence" > 0.9. After that, the PPI network was built and visualized with Cytoscape. During the in-depth analysis and evaluation of the PPI network, the CytoNCA plugin was applied, adopting "Degree" as the scoring standard.

Enrichment analysis of DQ-related genes

The identified common targets were uploaded to the DAVID database (https://david.ncifcrf.gov), with "Homo sapiens" selected as the species for conducting Gene Ontology (GO) functional enrichment analysis, focusing on the main biological processes, cellular components, and molecular functions. The KOBAS platform (http://bioinfo.org/kobas) was used to perform Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis to reveal the distribution and function of the related genes in biological pathways. Subsequently, the ggplot package in R language was employed to visualize these enrichment analysis results.

Molecular docking

To confirm how strongly DQ's active components bind to apoptosis-related target proteins, molecular docking analyses were conducted on 5 key active ingredients and their corresponding apoptosis-associated target proteins. The 2D and 3D molecular structures of these active ingredients were retrieved from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) and stored in SDF format. Next, ChemBio3D software was used to perform energy minimization on the 3D small molecule structures, which were then saved in mol2 format. After undergoing processes, the optimized small molecules were converted to pdbqt format using AutoDockTools. For the target proteins, their 3D structures were obtained from the PDB database. Using PyMOL, water molecules and original ligands were removed from the crystal structures, and the processed proteins were imported into AutoDockTools. The actual docking simulations were carried out with AutoDockVina software, while PyMOL was used to examine and analyze the interaction modes revealed in the docking outcomes.

Cell culture and modeling

Four groups were used in the experiment: control group, model group, model group + low-dose DQ, and model group + high-dose DQ. To prepare the hypoxia/reoxygenation model of rat H9c2 (Meisen Chinese Tissue Culture Collections, China) cardiomyocytes, the cells were first taken out from the incubator at 37°C and 5% CO_2 to observe their growth until the cell confluence reached 70%-80%. Then, the high-glucose DMEM culture medium in the 100 mm culture dish was aspirated and the cells were rinsed three times with PBS. Subsequently, an appropriate amount of glucose-free DMEM culture medium was added, mixed well, and the cells were placed into an anaerobic sealed box with the lid tightly closed. Nitrogen gas (containing 95% N_2 + 5% CO_2) was introduced into the sealed box from a nitrogen cylinder for 15 minutes to displace the air. After that, the connection port was closed, and the sealed box was placed in an incubator at 37°C and 5% CO_2 for 4 hours of sealed culture. After the hypoxia phase, the cells were taken out from the sealed box, and a pre-prepared glucose solution was added to adjust the sugar concentration in the 100 mm culture dish to the same as that of the high-glucose DMEM culture medium. The mixture was shaken well again and then placed back into the CO_2 incubator for further cultivation for 2 hours. After 2 hours, the cells were taken out from the CO_2 incubator, completing the preparation of the hypoxia/reoxygenation model. Before the preparation of the hypoxia/reoxygenation model, the model + low-dose DQ (DQ-L) group was treated with Danshen solution diluted with Earl's balanced salt solution (300 μg/mL), and the model + high-dose DQ (DQ-H) group was treated with 600 μg/mL, both for 2.5 hours. The control group of rat H9c2 cardiomyocytes did not require hypoxia/

reoxygenation treatment. They were simply taken out from the CO_2 incubator, and when the cell confluence reached 70%-80%, RNA extraction was performed.

Apoptosis detection

Following trypsin digestion, cells were rinsed twice with PBS at 4°C. Subsequently, the cells were resuspended in DMEM containing 10% FBS and stained with Annexin V and dead cell reagent (Muse™ Cell Analyzer, Merck Millipore, Darmstadt, Germany) Flow cytometry was then performed to measure the apoptosis rate.

Quantitative real-time polymerase chain reaction

Using the SteadyPure Kit (Accurate Biotechnology, Hunan) we extracted the total RNA. One microgram of RNA was reverse transcribed using the Evo M-MLV RT Premix (Accurate Biotechnology, Hunan). Primers were synthesized by Sangon Biotech Co., Ltd. (Shanghai, China) (Table S1). RT-qPCR was performed using SYBR Green Premix Pro Taq HS (Accurate): 95°C for 30 s; 40× (95°C for 5 s, 60°C for 30 s). The internal reference was β-actin. The data were calculated by $2^{-\Delta\Delta Ct}$.

Statistical analysis

Experimental data were visualized and analyzed by GraphPad Prism 9.5 and SPSS 26.0 statistical software. Data were displayed as the mean ± standard deviation (SD) of three independent replicates. The Bonferroni post hoc test in conjunction with one-way analysis of variance (ANOVA) was used for comparisons between various groups. Statistical significance was defined as $P<0.05$ in all analyses.

Results

Target prediction and analysis

In the TCMSP database, a total of 65 major active components of DanShen and 8 major active components of SanQi were screened out. After removing duplicates and applying these filtering criteria, it was found that 6 active components lacked corresponding targets. Therefore, 66 candidate active components of DanShen and SanQi were finally selected. A total of 932 targets for the candidate components of DanShen and 253 targets for the candidate components of SanQi were collected. After removing duplicates, 240 targets were obtained. By introducing these 66 candidate active components and 240 targets into Cytoscape software, the DanShen-SanQi active drug-active component-target network was constructed (**Figure 1A**).

Queries were conducted in the DisGeNET, GeneCards, and TTD databases targeting the targets associated with MI/RI. After removing redundancy, 766 MI/RI related targets were obtained. 105 common targets were found by comparing the targets of the 66 drug active ingredients with the 766 MI/RI-related targets (**Figure 1B**).

Protein-protein network analysis

The PPI network was built using the 105 verified DQ treatment targets. There are 123 edges and 25 nodes in the PPI network (**Figure 2A**). **Figure 2B** presents the top 10 therapeutic targets ranked by their degree of weighting.

GO and KEGG analyses

KEGG and GO analyses showed that DQ's key targets for MI/RI treatment mainly clustered in apoptosis-related pathways. This indicated that DQ might treat MI/RI through multiple pathways, with apoptosis-related pathways being crucial. The GO analysis refined the molecular functions of DQ targets. These functions were not only closely related to the apoptotic process but also extensively involved in crucial aspects such as cellular signal transduction, gene expression regulation, and the maintenance of protein stability. In this context, the molecular functions closely associated with apoptosis are particularly significant, as they may be crucial for the diverse therapeutic effects of DQ on MI/RI (**Figure 3A**; **Table 1**).

KEGG analysis indicated that the DQ target was associated with the apoptotic signaling pathway, including the TNF, NOD-like receptor, HIF-1 and the PI3K-Akt signaling pathway (**Figure 3B**). Additionally, the DQ target was also related to the inflammatory response pathways, such as the IL-17 and the NF-κB signaling pathway, which are the core mechanisms of inflammation regulation after MI/RI (**Figure 3B**).

Subsequently, an active component-target-pathway network was constructed based on the 10 key targets, as well as the related 11

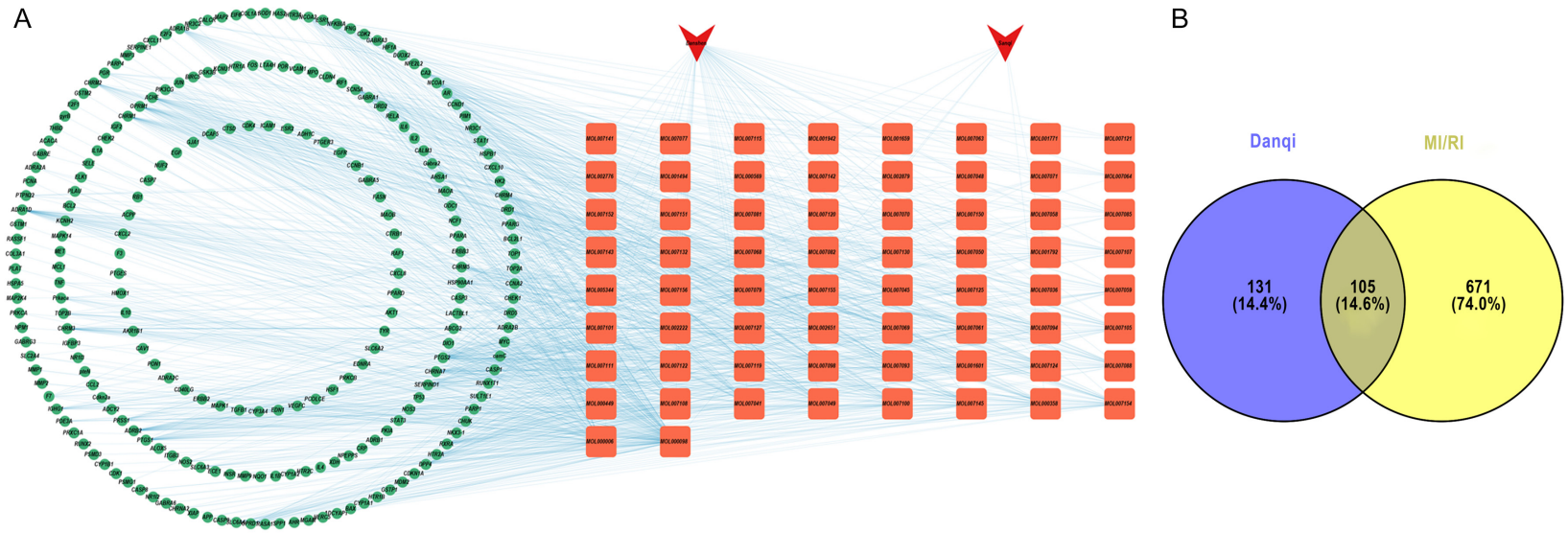

Figure 1. Prediction and Analysis of Targets for Danqi Soft Capsules (DQ) and myocardial ischemia/reperfusion injury (MI/RI) Targets. A. DQ-active components-target genes network. The red V-shape represents the main drug, the orange rectangle represents the bioactive components, and the green circle represents the target genes. B. Venn diagram of the intersection of bioactive component targets and MI/RI targets.

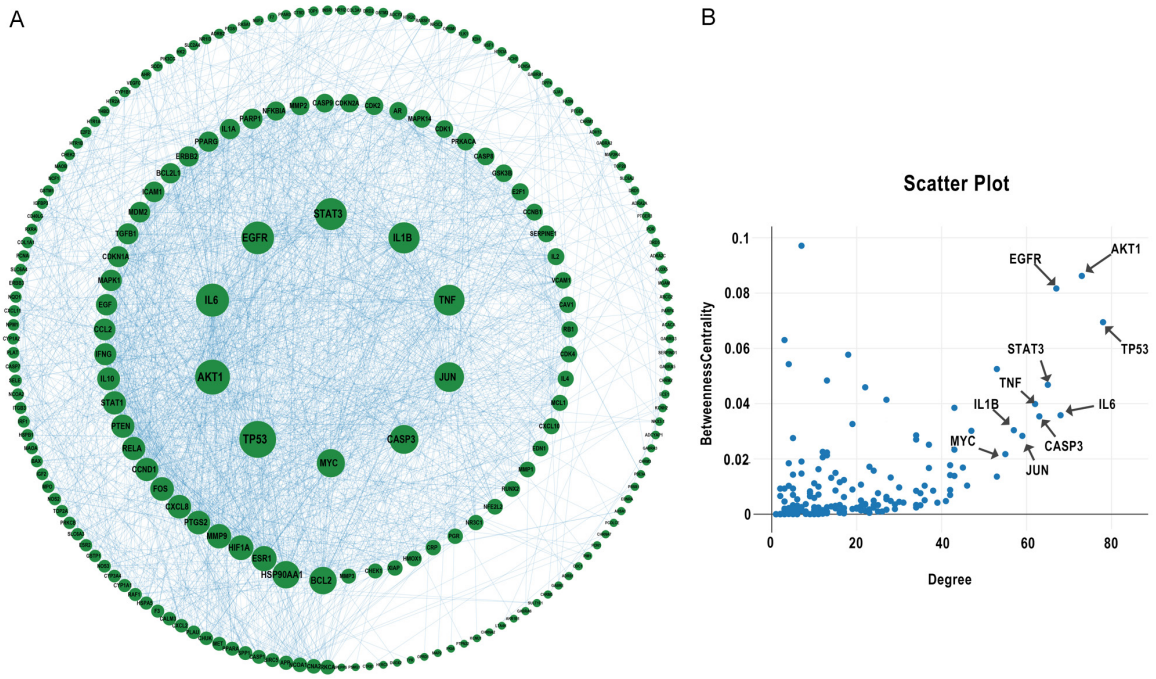

Figure 2. Protein-protein interaction (PPI) network analysis. A. The PPI network of the 105 key targets. Green circles represent key targets, with the size of the circle increasing as the degree increases; blue lines represent the connections between targets. B. Degree-Betweenness centrality scatter plot.

pathways and 6 active components (**Figure 3C**). Among the active components, quercetin (MOL000098), luteolin (MOL000006), and tanshinone IIA (MOL007154) had the highest degrees, which were 9, 7, and 4, respectively.

Results of molecular docking analysis

In the PPI network, the top 7 core targets have been identified as TP53, AKT1, IL6, EGFR, STAT3, CASP3, and TNF. These targets were then docked with the top 3 active components from the active component-target-pathway network, which are quercetin, luteolin, and tanshinone IIA, to assess the binding affinity of these complexes (**Table 2**). All of the complexes had a binding affinity greater than 5 kcal/mol, with 9 showing a particularly strong binding affinity greater than 8 kcal/mol. Specifically, the binding affinity between TNF and tanshinone IIA was approximately -9.2 kcal/mol, TP53 bound with quercetin at about -8.6 kcal/mol, and CASP3 bound with tanshinone IIA at around -8.5 kcal/mol. Additionally, TP53 had a similar binding affinity of -8.5 kcal/mol with luteolin, while EGFR bound with both luteolin and quercetin at -8.3 kcal/mol. Furthermore, STAT3 bound with tanshinone IIA at approximately -8.0 kcal/mol, and AKT1 bound with luteolin at the same affinity of -8.0 kcal/mol (**Figure 4**).

DQ's role in reducing apoptosis in hypoxia/reoxygenation-stressed H9c2 cells

Flow cytometry analysis revealed that the apoptosis rate in the model group was higher than in the control group. However, the apoptosis rates in the Model + DQ-L group and Model + DQ-H group were reduced (**Figure 5**).

Effect of DQ on key targets

The RT-PCR results (**Figure 6**) showed that TP53, AKT1, IL6, EGFR, STAT3, CASP3, TNF, JUN, and IL1B in the Model group were upregulated compared to the Control group. Treatment with DQ reversed the abnormal expression of TP53, AKT1, IL6, EGFR, STAT3, CASP3, TNF, JUN, and IL1B in the Model group. Moreover, in TP53, EGFR, STAT3, IL1B, TNF, and CASP3, the Model + DQ-H group had a better effect than the Model + DQ-L group.

Discussion

MI/RI is a prevalent occurrence during the treatment of myocardial infarction. Ischemic

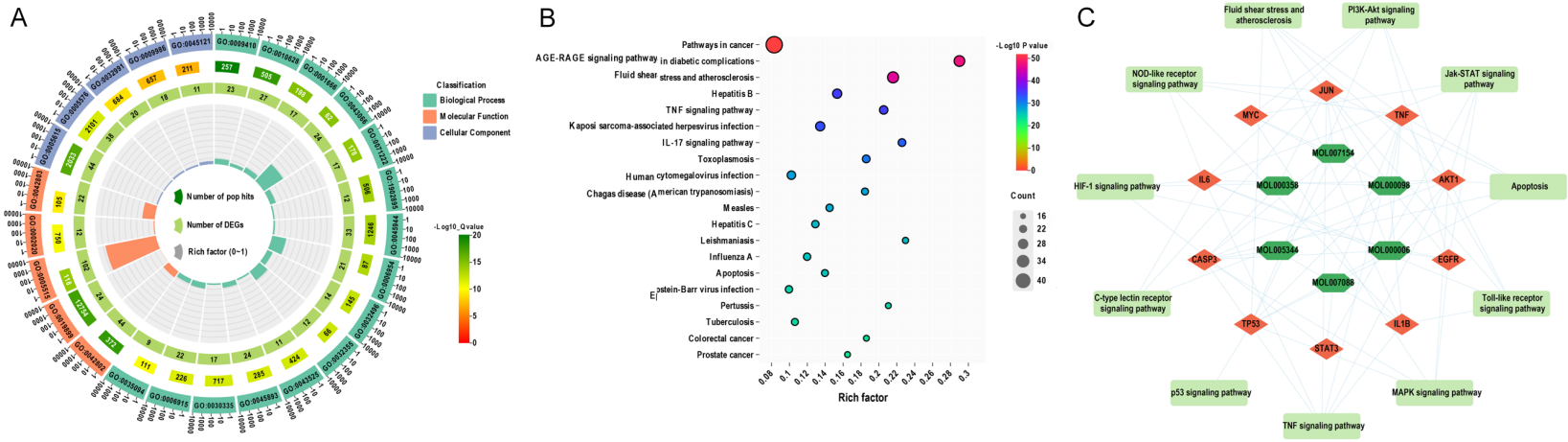

Figure 3. Gene Ontology (GO) analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis of the key targets. A. GO analysis circle plot. B. KEGG analysis bubble plot. C. Active component-target-pathway network. Green hexagons represent active components, orange diamonds represent targets, and cyan rectangles represent pathways.

Table 1. Gene Ontology (GO) Identifier (ID) associated with the GO term

ID	Term	ID	Term
GO:0009410	response to xenobiotic stimulus	GO:0006915	apoptotic process
GO:0010628	positive regulation of gene expression	GO:0035094	response to nicotine
GO:0001666	response to hypoxia	GO:0042802	identical protein binding
GO:0043066	negative regulation of apoptotic process	GO:0019899	enzyme binding
GO:0071222	cellular response to lipopolysaccharide	GO:0005515	protein binding
GO:1902895	positive regulation of miRNA transcription	GO:0002020	protease binding
GO:0045944	positive regulation of transcription by RNA polymerase II	GO:0042803	protein homodimerization activity
GO:0006954	inflammatory response	GO:0005615	extracellular space
GO:0032496	response to lipopolysaccharide	GO:0005576	extracellular region
GO:0032355	response to estradiol	GO:0032991	protein-containing complex
GO:0043525	positive regulation of neuron apoptotic process	GO:0009986	cell surface
GO:0045893	positive regulation of DNA-templated transcription	GO:0045121	membrane raft
GO:0030335	positive regulation of cell migration		

Table 2. Docking results of targets with corresponding components

	Quercetin	Luteolin	Tanshinone IIA
TP53 (6GGA)	-8.6 kcal/mol	-8.5 kcal/mol	-8.1 kcal/mol
AKT1 (1UNQ)	-6.7 kcal/mol	-8.0 kcal/mol	-7.4 kcal/mol
EGFR (3W2S)	-8.3 kcal/mol	-8.3 kcal/mol	-7.9 kcal/mol
IL6 (1ALU)	-6.8 kcal/mol	-6.9 kcal/mol	-7.8 kcal/mol
CASP3 (2XYG)	-7.9 kcal/mol	-7.9 kcal/mol	-8.5 kcal/mol
STAT3 (421A)	-7.5 kcal/mol	-7.5 kcal/mol	-8 kcal/mol
TNF (2E7A)	-7.6 kcal/mol	-7.9 kcal/mol	-9.2 kcal/mol

Note: TP53, tumor protein p53; AKT1, AKT serine/threonine kinase 1; EGFR, epidermal growth factor receptor; IL6, interleukin 6; CASP3, caspase 3; STAT3, signal transducer and activator of transcription 3; TNF, tumor necrosis factor.

myocardial reperfusion can lead to further damage to the heart structure and the function of myocardial cells [2]. When treating MI/RI, traditional Chinese medicine is beneficial pharmacologically [13, 14]. DQ is often used alone or in combination with compound preparations for the treatment of MI/RI, which can improve symptoms and reduce the incidence of severe complications [12, 15]. Using a network pharmacology approach, molecular docking, and experimental validation, this study demonstrated that DQ has broad regulatory effects across a variety of targets and pathways. Particularly noteworthy is that the key active components of DQ are related to targets such as cell apoptosis and inflammation, and exhibit strong binding affinity. This discovery provides a new perspective for understanding the therapeutic characteristics of DQ in the treatment of MI/RI.

The main components of DQ include Danshen and Sanqi. Danshen has a rich chemical composition, including phenolic compounds, triterpenoids, and polysaccharides [16]. Sanqi contains various bioactive components such as saponins, polysaccharides, and peptides [17]. Seven active components of Sanqi and 65 major active components of Danshen were effectively discovered through this investigation using the TCMSP. After removing duplicates, these filtering criteria were applied again, and it was found that 6 active constituents lacked corresponding targets. Ultimately, 66 candidate active constituents of Danshen and Sanqi were selected, many of which have been proven to have cardioprotective effects. For instance, quercetin, which has the highest degree in the active component-target-pathway network, has been shown to exert significant antioxidant effects by directly scavenging free radicals and enhancing the activity of antioxidant enzymes, effectively alleviating oxidative stress-induced damage to the heart [18]. Its cardioprotective mechanisms include the inhibition of inflammatory factor formation and the regulation of pathways such as NF-κB, with the result that cardiomyocyte inflammation is reduced. In addition, it protects mitochondrial function and inhibits apoptosis through PPARγ/NF-κB regulation during myocardial ischemia-reperfusion injury [19]. Moreover, it has been demonstrated to inhibit cardiac hypertrophy, lower blood pres-

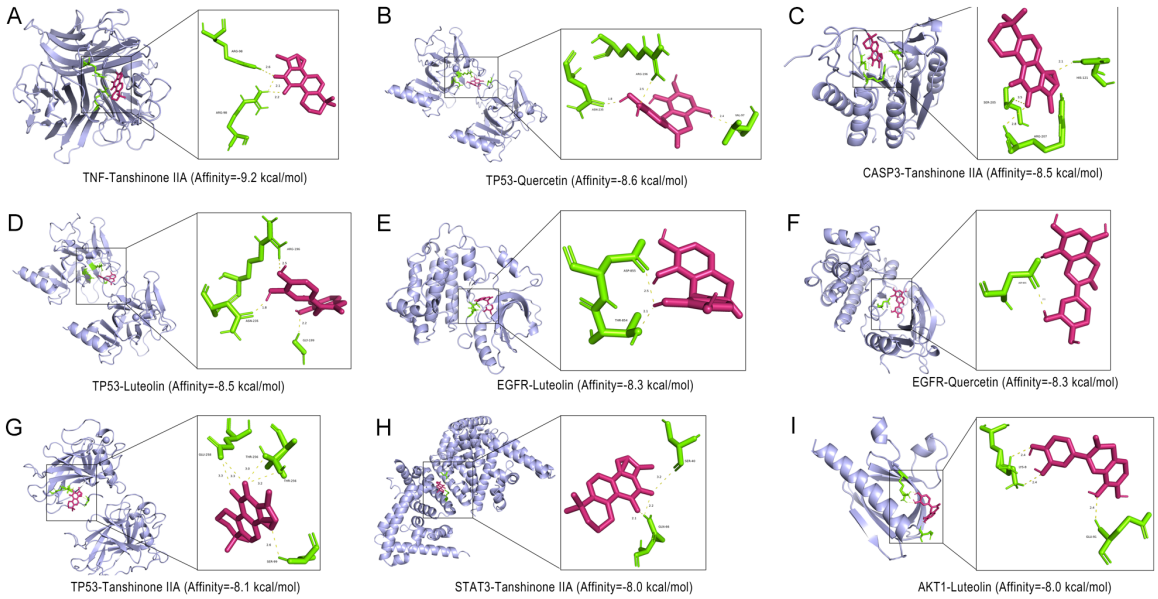

Figure 4. Molecular docking verification. A. Tumor necrosis factor (TNF)-tanshinone IIA binding site. B. Tumor protein p53 (TP53)-Quercetin binding site. C. Caspase 3 (CASP3)-Tanshinone IIA binding site. D. Tumor protein p53 (TP53)-Luteolin binding site. E. Epidermal growth factor receptor (EGFR)-Luteolin binding site. F. EGFR-Quercetin binding site. G. TP53-Tanshinone IIA binding site. H. Signal transducer and activator of transcription 3 (STAT3)-Tanshinone IIA binding site. I. AKT1-Luteolin binding site.

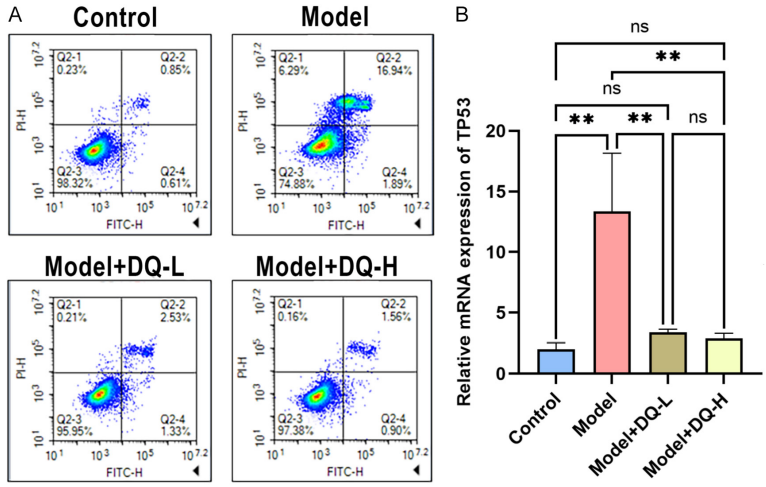

Figure 5. Flow cytometry analysis of cell apoptosis results. A. Cell distribution diagram. B. Quantification diagram of apoptotic cells. Different letters indicated significant differences within groups, while the same letters indicated no significant differences within groups. $^{**}P<0.01$.

sure, dilate coronary arteries, and improve cardiac function, while alleviating drug-induced cardiac toxicity through the regulation of the SIRT3/PARP-1 pathway [20]. In a similar manner, luteolin has been demonstrated to exert a multitude of cardioprotective effects. It activates the PI3K/Akt signaling pathway, inhibits GSK3β/Fyn, initiates Nrf-2 activation and nuclear translocation, upregulates antioxidant genes like HO-1, and reduces oxidative stress damage [21]. It also protects cardiomyocytes from doxorubicin or starvation-induced damage via Akt/mTOR and ERK pathways. In MI/RI, luteolin markedly diminishes the myocardial infarction area, enhances hemodynamics, reduces cardiomyocyte apoptosis, and lowers levels of the oxidative stress marker MDA and the inflammatory factor TNF-α [22]. Similarly, tanshinone IIA has been shown to mitigate oxidative stress by interacting with reactive oxygen species and via the SIRT1-AMPK pathway [20]. It also decreases intracellular Ca^{2+} levels, inhibits L-type Ca^{2+} channels, and safeguards the myocardium through the PI3K/Akt pathway, thereby improving mitochondrial function [23]. Additionally, it exerts anti-inflammatory effects by suppressing inflammatory factors and cytokine expression, reducing myocardial necrosis and left ventricular remodeling, and slowing cardiac

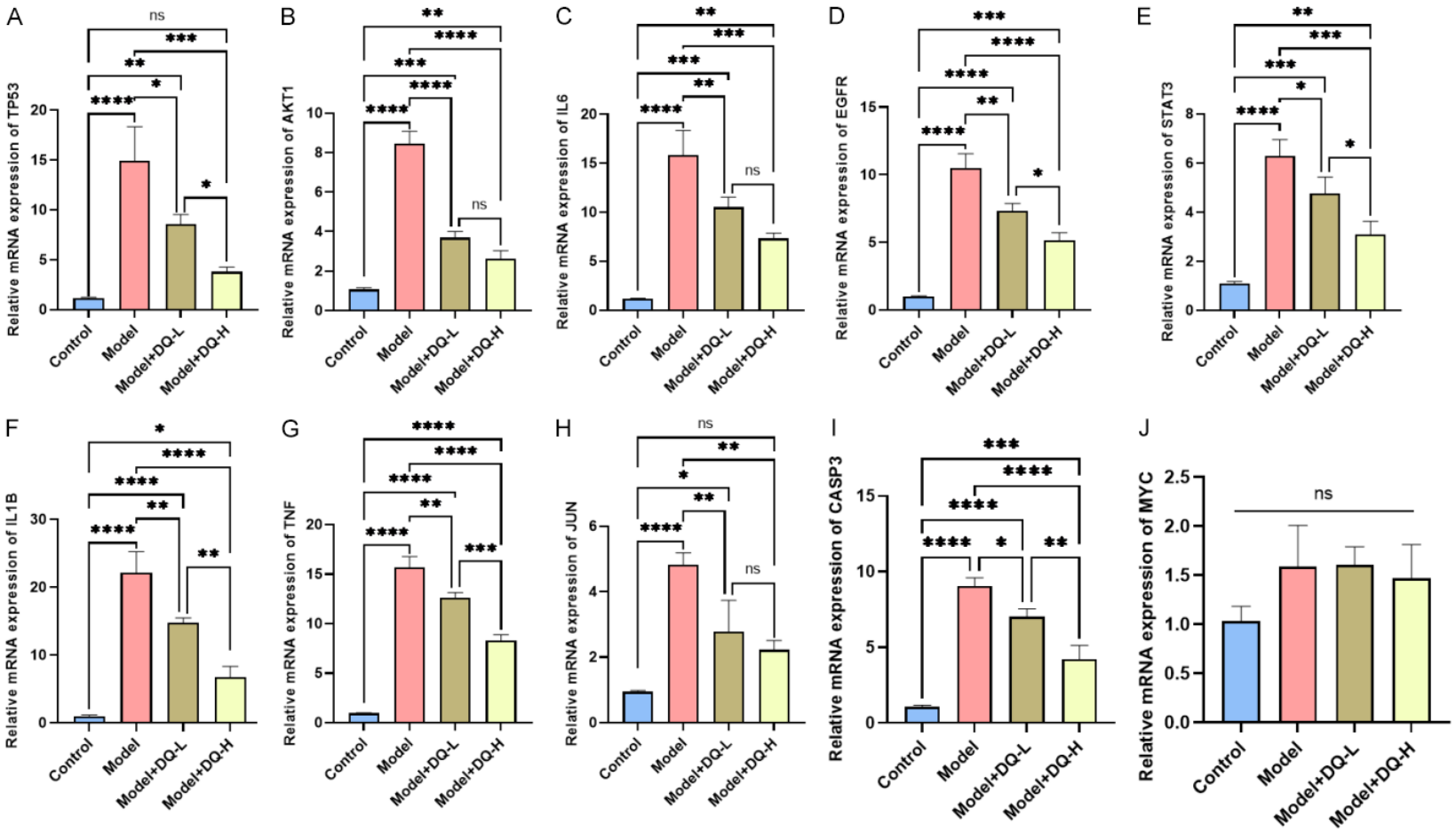

Figure 6. Effect of DQ on key targets mRNAs. Relative mRNA expression of (A) tumor protein p53 (TP53), (B) AKT serine/threonine kinase 1 (AKT1), (C) interleukin 6 (IL6), (D) epidermal growth factor receptor (EGFR), (E) signal transducer and activator of transcription 3 (STAT3), (F) interleukin 1 beta (IL1B), (G) tumor necrosis factor (TNF), (H) c-jun proto-oncogene (JUN), (I) caspase 3 (CASP3), (J) v-myc avian myelocytomatosis viral oncogene homolog (MYC). Different letters indicated significant differences within groups, while the same letters indicated no significant differences within groups. **P*<0.05, ***P*<0.01, ****P*<0.001, *****P*<0.0001.

fibrosi [24]. Collectively, the active constituents of DQ contribute to cardiac protection through multiple mechanisms. Subsequently, the study further explored the mechanisms of these components and their potential therapeutic targets. To summarize, the active constituents of DQ have been demonstrated to be crucial in protecting the heart through a range of mechanisms.

Following this, a comparison was conducted between the target genes of DQ's active components and the MI/RI target gene library, identifying 105 overlapping targets. Subsequent enrichment analyses on these 105 shared targets indicated that the key targets of DQ in treating MI/RI are concentrated in several critical signaling pathways and inflammatory response pathways. The fact that these targets are concentrated in multiple pathways, with a significant number of them being directly involved in apoptosis-related processes, indicates that while DQ may act through multiple pathways, those related to apoptosis are particularly prominent and likely play a central role in its therapeutic effects. In the TNF signaling pathway, TNF-α activates the caspase-8 and p38MAPK signaling pathways through its type 1 receptor TNFR1, forming the pro-apoptotic complex IIa, and induces necroptosis when caspase-8 activity is inhibited, thereby exerting pro-apoptotic effects in MI/RI [25]. Meanwhile, by encouraging the release of inflammatory factors, the NLRP3 inflammasome indirectly activates NF-κB and other pathways within the NOD-like receptor signaling pathway, intensifying the inflammatory response and triggering cardiomyocyte apoptosis [26]. The PI3K/Akt pathway, when activated, can reduce mitochondria-mediated cell apoptosis and maintain mitochondrial integrity. The expression of p-PI3K and p-AKT is downregulated by hypoxia-reoxygenation, while MI/RI increases reactive oxygen species production, which raises oxidative stress, modifies mitochondrial membrane potential, releases mitochondrial cytochrome c into the cytoplasm, and activates caspase-3 in the apoptotic pathway [27]. The inhibition of HIF-1α expression during ischemia-reperfusion is also largely caused by mitochondrial malfunction and oxidative stress. In the HIF-1 signaling pathway, HIF-1α reduces cardiomyocyte apoptosis by improving mitochondrial function and reducing cellular oxidative stress. During MI/RI, the expression level of HIF-1α in myocardial cells decreases, and the activation of HIF-1α and PDK1 can alleviate myocardial injury [28]. The inflammatory signaling pathway stimulates various cells to produce inflammatory factors, activates immune cells such as macrophages and neutrophils, and enhances their infiltration in the ischemia-reperfusion area, thereby aggravating myocardial injury [29]. The correlation between inflammation and apoptosis indicates that DQ may affect the apoptotic process by regulating the inflammatory response.

This study constructed a protein interaction network to conduct in-depth analysis of 105 common targets, and finally selected the core therapeutic targets (TP53, AKT1, IL6, EGFR, STAT3, CASP3, TNF, JUN, IL1B, MYC). Biological processes like signal transmission, inflammatory response, and cell death are significantly influenced by these basic targets. In drug design, the higher the binding affinity of small molecule drugs to the target, the more stable the binding, and the more significant the biological activity [30]. The molecular docking experiments in this study revealed that the binding affinity of flavonoid IIA to TNF reached -9.2 kcal/mol, indicating that it may affect the cell apoptosis and inflammation process in myocardial injury through regulating the TNF pathway [31]. In addition, the binding of quercetin to AKT1 may regulate cell apoptosis by influencing the PI3K-Akt signaling pathway [32]. As a key protein in the regulation process, the binding of luteolin to CASP3 may affect myocardial cell apoptosis [33]. These findings reveal the potential of DQ active ingredients in regulating the key signaling pathways of MI/RI, providing a mechanism basis for its treatment.

The DQ-L and DQ-H groups had lower apoptosis rates, in line with the outcomes anticipated by the molecular docking analysis, the high-dose group's protective effect was marginally superior to that of the low-dose group. DQ protects cardiomyocytes and preserves the structure as well as function of the coronary artery by lowering the rate of apoptosis. The better protective effect of the high-dose group may be related to the higher concentration of the active components in the cells. In this study, molecular docking predicted the strong binding affinity, and the cell experiments confirmed the regulatory effect of DQ on cardiomyocyte apoptosis in vitro. These results indicate that DQ alleviates

MI/RI by targeting the apoptosis pathway, proving its dose-dependent efficacy, and providing strong evidence for its clinical application.

A potential mechanism of DQ in MI/RI was uncovered by this investigation. There were 240 possible targets and 66 active components in all. Among them, 10 core targets were related to the apoptotic-related pathways. Molecular docking and cell experiments confirmed the anti-apoptotic effect of DQ, providing a scientific basis for clinical application and new drug development. The research does have some limitations. First, no animal trials were used in this study to perform *in vivo* verification. Second, exploration of other related mechanisms was relatively limited.

Conclusion

This study, through network pharmacology, identified 66 active components and 240 potential targets of DQ. Among them, 105 were related to myocardial MI/RI. PPI identified 10 core targets. These targets were linked to apoptotic signaling pathways, according to GO and KEGG studies. Furthermore, the significant binding affinity of the active components with the key targets was validated by molecular docking. Additional cell studies confirmed DQ's anti-apoptotic action on cardiomyocytes. These discoveries open up new avenues for the creation of novel drugs and give the clinical use of DQ a scientific foundation.

Disclosure of conflict of interest

None.

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Table S1. Primer sequences

Primer		Sequences 5'-3'
TP53	Forward:	GGAAATTTGCGTGTGGAGTATTT
	Reverse:	GTTGTAGTGGATGGTGGTACAG
AKT1	Forward:	CTTCTATGGCGCTGAGATTGT
	Reverse:	GCCCGAAGTCTGTGATCTTAAT
IL6	Forward:	CCAGGAGAAGATTCCAAAGATGTA
	Reverse:	CGTCGAGGATGTACCGAATTT
EGFR	Forward:	GCCTCCAGAGGATGTTCAATAA
	Reverse:	TGAGGGCAATGAGGACATAAC
STAT3	Forward:	GAGAAGGACATCAGCGGTAAG
	Reverse:	CAGTGGAGACACCAGGATATTG
IL1B	Forward:	ATGGACAAGCTGAGGAAGATG
	Reverse:	CCCATGTGTCGAAGAAGATAGG
TNF	Forward:	CCAGGGACCTCTCTCTAATCA
	Reverse:	TCAGCTTGAGGGTTTGCTAC
JUN	Forward:	TTCTATGACGATGCCCTCAAC
	Reverse:	TCAGGGTCATGCTCTGTTTC
CASP3	Forward:	AGATGGCTTGCCAGAAGATAC
	Reverse:	CTGCAAAGGGACTGGATGAA
MYC	Forward:	CATACATCCTGTCCGTCCAAG
	Reverse:	GAGTTCCGTAGCTGTTCAAGT

Note: TP53, tumor protein p53; AKT1, AKT serine/threonine kinase 1; IL6, interleukin 6; EGFR, epidermal growth factor receptor; STAT3, signal transducer and activator of transcription 3; IL1B, interleukin 1 beta; TNF, tumor necrosis factor; JUN, c-jun proto-oncogene; CASP3, caspase 3; MYC, v-myc avian myelocytomatosis viral oncogene homolog.