

Combining bioinformatics and network pharmacology to explore diagnostic biomarkers and mechanisms associated with mitochondria in diabetic nephropathy using *Rhodiola Rosea*

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Abstract

This study aimed to explore diagnostic biomarkers associated with mitochondria in the treatment of diabetic nephropathy (DN) using *Rhodiola Rosea* (RR) and to elucidate the underlying regulatory mechanisms. The target genes corresponding to the active ingredients of RR, mitochondria-related genes, and DN-related genes were intersected with differentially expressed genes from GSE96804 to obtain mitochondria-related pharmacopoeia genes (MRPGs). Based on these genes, a pharmacological network was constructed. Additionally, the biological pathways enriched by the diagnostic biomarkers, their correlation with the DN immune infiltration profile, and their binding ability to RR were analyzed. A total of 24 MRPGs against DN were identified using Venn analysis. Subsequently, six diagnostic biomarkers were screened by machine learning, namely CAT, CASP3, CPT1A, OGDH, CYP27B1, and ALDH9A1. Immune infiltration analysis showed eight differential immune cells between the DN and control groups, with macrophages M2 and neutrophils correlating with all diagnostic biomarkers. Based on the pharmacological network and molecular docking, two targeting relationship pairs were identified: CAT-RUTIN and ALDH9A1-RUTIN-2, both demonstrating good binding capacity. This study identified six diagnostic biomarkers for DN based on the effects of RR and mitochondrial function. The findings revealed the active components and potential molecular therapeutic mechanisms of RR in treating DN.

Introduction

Diabetic nephropathy (DN), also known as diabetic kidney disease (DKD), is a common microvascular complication of diabetes, a major cause of end-stage renal disease (ESRD), and one of the leading causes of death in diabetic patients [1, 2]. DN is characterized by a persistent elevation of albuminuria, progressive elevation of blood pressure, and progressive decline in renal function [3, 4]. DN accounts for 30–50% of all chronic kidney disease cases and affects 285 million people worldwide [5]. Epidemiological surveys predict that DN will become the seventh leading cause of death globally by 2030 [6].

DN is a multifactorial pathological process with a complex pathogenesis that includes renal hemodynamic abnormalities, oxidative stress, inflammatory response, hypoxia, metabolic disorders, hormone synthesis imbalance, and renin-angiotensin-aldosterone system (RAAS) [7]. Current therapeutic strategies for DN focus on controlling blood glucose, lipid, and blood pressure levels, inhibiting the RAAS system, and reducing proteinuria to slow the progression of DN [8, 9]. However, due to the extremely complex and multifactorial pathogenesis of diabetic puncture, not all patients can be effectively treated [8, 10]. Therefore, there is an urgent need to find new diagnostic and therapeutic targets for DN.

Mitochondria are the main energy center in the cells and are an important storage for the production of adenosine triphosphate (ATP). Mitochondria are also involved in regulating cellular metabolism, maintaining redox balance, and controlling cell death [11]. As a high-energy metabolism-demanding organ, the kidney has the second-highest mitochondrial abundance after the heart, and mitochondrial homeostasis plays a critical role in maintaining its structure and function [12]. Mitochondrial dysfunction

leads to defective mitochondrial fusion, reduced ATP synthesis, causing cell injury and apoptosis, and excessive production of reactive oxygen species (ROS). Mitochondrial dysfunction also disrupts the integrity of the glomerular filtration membrane, which in turn affects the onset and progression of DN [13]. Additionally, mitochondria can be selectively removed by autophagy, which plays a protective role in the kidney, and its dysfunction can lead to renal cell dysfunction and induce the progression of DN [14].

Han et al. found that activation of the mitochondrial ROS-TXNIP/NLRP3/IL-1 β biological axis led to renal tubular injury in DN [15]. Jiang et al. showed that activation of hypoxia-inducible factor-1 α (HIF-1 α) in renal tubular cells played an important protective role against renal injury in DN [16]. This protective effect may be mediated through the upregulation of heme oxygenase-1 (HO-1), which regulates mitochondrial dynamics. Previous studies have found that mitochondrial carnitine palmitoyltransferase 2 (CPT2) is involved in N ϵ -(carboxymethyl)-lysine (CML)-mediated DN [17]. Mitochondrial mitofusin 2 (MFN2) can exert a protective effect against DN by inhibiting histone acetylation at the DN collagen IV promoter [18]. However, the mitochondria-related genes (MRGs) and their related regulatory mechanisms in DN are largely unknown.

In recent years, the role of Traditional Chinese Medicine (TCM) in regulating glucose and lipid metabolism, reducing renal injury, delaying nephropathy, and preventing glomerular sclerosis and fibrosis has been gradually revealed. TCMs work through multi-target and multi-pathway intervention strategies, exerting overall regulation and synergistic effects, and have certain advantages in the prevention and individualized treatment of DN [19]. *Rhodiola Rosea* (RR) is a functional medicinal plant with properties such as inhibition of oxidative stress, inflammation, and prevention of altitude sickness. It is expected to be used as a therapeutic agent for renal diseases [20]. Recent studies have found that RR may play a beneficial role in DN mice through Sirt1/PGC-1 α mediated mitochondrial biogenesis [21]. However, there are limited studies linking the therapeutic effects of RR in DN to mitochondrial function.

Therefore, this study utilized bioinformatics and network pharmacology to explore the diagnostic biomarkers associated with mitochondria and their regulatory mechanisms in the treatment of DN by RR. The findings may provide insights into a better understanding of the pathogenesis of DN.

Results

Acquisition of RR active ingredients and target genes

In this study, 35 active ingredients of RR were obtained from the BATMAN-TCM database (Supplementary Table 1). Each ingredient was then screened for target genes with Confidence Scores ≥ 0.60 , Likelihood Ratio = 13.26, and Druggable Scores ≤ 0.1 . Subsequently, 31 active ingredients were obtained with a record of target genes, with a total of 622 target genes (Supplementary Table 2).

Identification of mitochondria-related pharmacopoeia genes (MRPGs) and exploration of potential biological

functions

Through the analysis of differential expression, 5097 differentially expressed genes (DEGs), including 2085 upregulated genes and 3012 downregulated genes, were identified between DN and control groups in GSE96804 (Fig. 1A). Following this, 24 MRPGs were obtained by intersecting the 5097 DEGs, 1136 MRGs, 622 target genes, and 20647 DN-related genes (DNRGs) (Fig. 1B). To elucidate the underlying biological processes of MRPGs, Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomics (KEGG) enrichment analyses were performed. As shown, the GO enrichment analysis revealed a total of 206 functional entries enriched for MRPGs, including processes such as amino-acid betaine metabolic process, mitochondrial matrix, electron transfer activity, among others (Fig. 1C). Meanwhile, in KEGG enrichment analysis showed that MRPGs were enriched in 35 signal pathways, such as tryptophan metabolism, fatty acid degradation, among others (Fig. 1D).

Construction of protein-protein interaction (PPI) and RR-active ingredient-MRPGs networks based on MRPGs

The interactions of MRPGs at the protein level were explored by constructing a PPI network, as shown in Fig. 2A. A total of 21 nodes and 48 edges were identified in the PPI network, including interactions such as ALDH2-SDHB and ALDH9A1-GATM. Subsequently, to investigate the relationship between MRPGs, RR, and active ingredients, an RR-active ingredient-MRPGs network was constructed. This network comprised 42 nodes and 65 edges, with 1 node representing RR, 17 nodes representing active ingredients, and 24 nodes representing MRPGs (Fig. 2B).

Selection of 6 diagnostic biomarkers by machine learning

Potential diagnostic biomarkers were identified from MRPGs using Least Absolute Shrinkage and Selection Operator (LASSO) logistic regression, Support Vector Machine-Recursive Feature Elimination (SVM-RFE), and Random Forest (RF) algorithms. In the LASSO model constructed with 10-fold cross-validation, the minimum error value corresponded to 10 genes, including CAT, CASP3, CPT1A, OGDH, CYP27B1, ALDH9A1, MAOA, SDHB, VDAC3, and NDUFV3 (Fig. 3A-B). Similarly, the SVM-RFE algorithm, validated by 10-fold cross-validation, identified eight genes with the most accuracy and smallest estimation error, including CAT, CASP3, CPT1A, OGDH, CYP27B1, ALDH9A1, VDAC3, and CHDH (Fig. 3C-D). Additionally, the RF algorithm selected the top 20 genes with the highest weights based on scalar importance assessment (Fig. 3E). Subsequently, the intersection of genes predicted by these three machine-learning algorithms was performed, resulting in six diagnostic biomarkers, namely CAT, CASP3, CPT1A, OGDH, CYP27B1, and ALDH9A1 (Fig. 3F).

The results of immune cell infiltration

To analyze immune patterns in the DN and control groups, the proportion of 22 immune cells in each sample calculated by the gene expression matrix using CIBERSORT algorithm. Box plots depicting

differences in immune cell infiltration revealed significant disparities between the DN and control groups. Specifically, T cells CD8, Macrophages M1, Macrophages M2, Dendritic cells resting, B cells memory, and Mast cells resting were notably elevated in the DN group compared to the control group, while Mast cells activated and Neutrophils exhibited the opposite trend (Fig. 4A).

Upon analyzing the correlation between immune cells and diagnostic biomarkers, we found two types of immune cells that correlated with all six diagnostic biomarkers: Neutrophils and Macrophages M2. Remarkably, Neutrophils displayed a significant positive correlation with CAT, CPT1A, OGDH, CYP27B1, and ALDH9A1, while demonstrating a negative correlation with CASP3. Conversely, Macrophages M2 showed the exact opposite pattern (Fig. 4B).

Functional enrichment analysis and pharmacological network construction based on diagnostic biomarkers

To explore the signal pathways enriched by diagnostic biomarkers, KEGG pathway enrichment analysis was performed for the six diagnostic biomarkers, as illustrated in Fig. 5A. Four pathways were identified: alcoholic liver disease, tryptophan metabolism, fatty acid degradation, and carbon metabolism.

Subsequently, to further examine the relationship between diagnostic biomarkers, active ingredients, and related pathways, a network of pharmacology pathways was constructed based on RR, active ingredients, diagnostic biomarkers, and their enriched pathways. This network comprised 23 nodes and 41 edges, including RR, six diagnostic biomarkers, 12 active ingredients, and four pathways (Fig. 5B).

Prediction of RR-diagnostic biomarkers binding capacity by molecular docking

To investigate the binding ability of RR's core active ingredients to diagnostic biomarkers, genes with the highest node degree in the pharmacological network (CAT and ALDH9A1) and the core active ingredient RUTIN were selected for molecular docking. Figure 6 illustrated that RUTIN exhibited the strongest and most stable binding affinity with CAT, with a free energy of binding of -13.1 kcal/mol, followed by RUTIN with ALDH9A1, with a free energy of binding of -9.1 kcal/mol. These findings suggest that RR possessed a strong binding ability to these targets.

Discussion

DN is a serious complication of diabetes mellitus and a major cause of ESRD worldwide. Exploring the pathogenesis and treatment of DN has been a hot research topic. RR and its extracts exhibit a variety of biological functions such as antidiabetic, anticancer, antiaging, antioxidant, anti-inflammatory, and immunomodulatory properties, and are also known as "Golden Root" or "Rosebud Root" [22].

Mitochondria play a crucial role in energy storage and provision to cells and have numerous essential physiological functions. Insufficient ATP supply and excessive production of ROS due to mitochondrial dysfunction are closely linked to the development of DN [23]. Therefore, identifying diagnostic

biomarkers associated with mitochondria and their regulatory mechanisms involved in the treatment of DN by RR may improve DN outcomes.

In this study, 24 MRPGs were obtained. These genes were involved in pathways such as amino-acid betaine metabolism and the mitochondrial matrix, which are associated with impaired amino-acid metabolism and mitochondrial damage in DN [24, 25]. Subsequently, through three machine learning algorithms, diagnostic biomarkers associated with RR treatment processes involving mitochondria were further identified, namely CAT, CASP3, CPT1A, OGDH, CYP27B1, and ALDH9A1. They were significantly enriched in signaling pathways such as fatty acid degradation. Immune infiltration analysis revealed that immune cells, such as Neutrophils and Macrophages M2, correlated significantly with the six diagnostic biomarkers. The constructed pharmacological network and molecular docking showed that RUTIN exhibited a good binding ability with CAT and ALDH9A1.

Among the six diagnostic biomarkers, CAT is catalase, a member of the antioxidant enzyme family in the body, which breaks down hydrogen peroxide into non-toxic substances and plays an important role in maintaining the balance of the redox state in the body. Hou et al. observed elevated levels of CAT activation in db/db diabetic mice treated with the mitochondria-targeted antioxidant peptide SS31 [26]. A proteomics study showed that the antioxidant protein disulfide-bond A oxidoreductase-like protein (DsbA-L) could ameliorate oxidative damage in DN renal tubules by modulating CAT activity and enhancing peroxisome abundance and function [27]. These findings, along with our results, suggest that CAT is closely related to DN pathogenesis. Salidroside, the main active ingredient of RR, has a protective effect against vascular disease and promotes CAT expression levels [28]. RUTIN, a flavonoid with antidiabetic properties, increases CAT levels in patients with type 2 diabetes mellitus, improving their blood pressure antioxidant enzyme levels, and quality of life [29]. Our current study also found an association between RUTIN and CAT, and the molecular docking results showed that the free energy of binding between the two was -13.1 kcal/mol, suggesting that they have a better binding capacity and RUTIN could directly target CAT. Therefore, it is hypothesized that RR may target CAT through its core active ingredient, RUTIN, to maintain redox homeostasis in DN patients, thereby reducing oxidative damage.

CPT1A is a pivotal rate-limiting enzyme for fatty acid oxidation, and its overexpression has a protective effect against DN, as evidenced by its ability to ameliorate albuminuria and glomerulosclerosis, and to attenuate glomerular lipid deposition and podocyte injury. CPT1A promotes lipid depletion through the fatty acid pathway to reduce lipotoxicity; it also anchors Bcl2 to the mitochondrial membrane, thus preventing cytochrome C release and inhibiting the mitochondrial apoptosis process [30]. Another study has indicated that the FOXA1-CPT1A axis can be targeted by the antihyperglycemic drug Canagliflozin to ameliorate fatty acid oxidation and iron death in renal tubular epithelial cells in DN [31]. Interestingly, these findings were similar to the "Fatty acid degradation" pathway enriched by the diagnostic biomarkers in the present study, further confirming the role of diagnostic biomarkers in the regulation of fatty acid metabolism in DN. Therefore, we conclude that improving fatty acid metabolism is important for improving renal function in DN.

CASP3 encodes cysteine-3 protease, which often plays a key role in the apoptotic phase. The expression level of CASP3 is significantly reduced by reagent drugs such as salidroside and saxagliptin, which attenuate the degree of renal apoptosis and pathological outcomes in patients with DN, thus preventing the progression of DN [20, 32]. OGDH belongs to a group of mitochondrial proteins located in the mitochondrial matrix and has been linked to diabetes, obesity, impaired insulin sensitivity, cardiovascular disease risk, and Charcot-Marie-Tooth neuropathy [33, 34]. CYP27B1 is the only enzyme capable of activating vitamin D in the kidney, and vitamin D reduces the hyperactivity of the renin-angiotensin system and improves β -cell function. Additionally, vitamin D affects insulin secretion, which is regulated by the opening and closing of calcium channels, inactivates inflammatory cytokines associated with insulin resistance, and promotes the expression of calmodulin, thereby preventing apoptosis [35, 36]. These findings invariably indicate the closeness of the association between CASP3, OGDH, CYP27B1, and DN, and we believe that abnormalities in these diagnostic biomarkers predispose to the onset and progression of DN.

ALDH9A1 is a common tumor suppressor substrate and a potential risk factor for kidney cancer [37, 38]. No studies related to DN have been found, whereas our study presents the first association between ALDH9A1 and DN and reveals the potential effect of ALDH9A1 in predicting the treatment of DN by RR through molecular docking analysis.

An important driver of DN progression is inflammation, and activation of the immune response promotes an inflammatory environment, which in turn leads to renal fibrosis and progressive loss of renal function [39, 40]. Macrophages, effector cells involved in the innate immune response, infiltrate the renal tissues of DN patients, mediating histological damage and decreasing glomerular filtration rate [41]. Macrophages are divided into two main subpopulations, M1 and M2. Macrophages M1 respond to inflammatory stimuli in the early stages of DN, exacerbating the inflammatory response through the production of inflammatory mediators. Macrophages M2, transformed from Macrophages M1 in the later stages of the disease, exhibit anti-inflammatory and tissue repair-promoting properties. However, in the diabetic setting, Macrophages M2 may become compromised, leading to a dysfunctional balance between macrophage subpopulations, thereby promoting renal degeneration and fibrosis [42, 43]. Neutrophils are a common leukocyte type, and their accumulation may cause patients with DN to experience recurrent episodes of acute kidney injury, resulting in progressive loss of renal function [39, 44]. In a study by Chen et al. investigating the effects of the Chinese herb Tangshen Formula (TSF) on interstitial cells of Cajal in the colon of diabetic rats, diabetic rats exhibited elevated expression of caspase-3 and a decreased total ratio of Macrophages M2 compared to control rats [45]. This phenomenon was also observed in an autoimmune function study of mouse pancreatic islets by Nikolic et al [46]. This paper also analyzed the relationship between DN and immune cells, identifying eight significantly different immune cells, including Macrophages M1, Macrophages M2, and Neutrophils. Our immune infiltration analysis showed a strong correlation between Macrophages M2 and CASP3. Therefore, understanding immune mechanisms in DN may help generate new therapeutic targets to inhibit renal injury in DN.

However, this study has some limitations. Specifically, the research relied on data from public databases and lacked detailed clinical information. Additionally, the diagnostic biomarkers identified in this study were only validated through computer simulation, lacking in vitro and in vivo experimental validation. Therefore, obtaining clinical samples for further investigation is necessary to confirm the therapeutic mechanisms and effects of RR on DN.

In conclusion, this study identified six diagnostic biomarkers associated with mitochondria in the treatment of DN by RR, namely CAT, CASP3, CPT1A, OGDH, CYP27B1, and ALDH9A1. RR may mitigate the progressive decline in renal function in DN by targeting CAT and ALDH9A1 through its core active ingredient RUTIN, suggesting its potential as a therapeutic agent for DN. Additionally, it has been observed that fatty acid metabolism and multiple immune cells, such as macrophages, may play crucial roles in the onset and progression of DN. These findings provide valuable insights into the broad application of RR in the clinical management of DN.

Materials and methods

Data source

The DN dataset GSE96804 (GPL17586 platform), which contains 20 glomeruli from the unaffected portions of tumor nephrectomies (controls) and 41 glomeruli from kidneys with DN (DN group), was downloaded from the Gene Expression Omnibus (GEO) database. To obtain MRGs, 1136 MRGs were retrieved from the MitoCarta3.0 database [47]. A total of 20,647 DN-related genes (DNRGs) were then obtained from the Comparative Toxicogenomics Database (CTD) [48] by searching with the keyword "Diabetes nephropathy".

Acquisition for target proteins of active ingredients in RR

The active ingredients of RR were obtained from the BATMAN-TCM database [49], and the target proteins of each active ingredient were predicted using this database. The screening criteria were set with a Confidence Score ≥ 0.60 , Likelihood Ratio = 13.26, and Druggable Score ≥ 0.1 .

Identification and functional enrichment analysis of MRPGs

DEGs between the DN and control groups in GSE96804 were explored using the "limma" R package (version 3.34.7) with $|\log_2FC| > 0.263$ and adjusted $p < 0.05$ as the filtering conditions. Subsequently, DEGs, MRGs, DNRGs, and target proteins of RR obtained from the above analyses were intersected using the "VennDetail" R package (version 3.4.4). The genes in the intersection were recorded as MRPGs. To explore the biological processes associated with the MRPGs, GO and KEGG pathway enrichment analyses were performed using the "clusterProfiler" R package (version 3.14.3). The significant enrichment results were screened with a Benjamini & Hochberg-adjusted p -value of < 0.05 .

Construction of PPI and RR-active ingredients-MRPGs networks

A PPI network was constructed using the STRING database [50] (version 11.5) and visualized by CytoScape software [51] (version 3.6.1) to observe MRPG interactions at the protein level, with the threshold set at a combined score > 0.4. Based on the RR-active ingredient and active ingredient-MRG relationship pairs, a network of RR-active ingredient-MRPGs was constructed using CytoScape software to explore the relationships between MRPGs, active components, and RR.

Screening of diagnostic biomarker through machine learning

Machine learning algorithms can generate more detailed models for biomarker screening. In this study, three machine learning algorithms were used to filter the MRPGs: LASSO logistic regression, SVM-RFE, and RF. First, LASSO regression analyses was performed using the "glmnet" R package (version 4.1-7) with 10 folds, and family = "binomial" was set for fitting. Next, SVM-RFE feature selection was performed using the "e1071" R package (version 1.6-8) with 10 folds. Then, the algorithm provided by the "randomForest" R package (version 4.6–14) was used to set 500 trees for RF. Finally, the overlapped genes from the three machine-learning algorithms were used as diagnostic biomarkers and included in the next step of the study.

Immune cell infiltration analysis

The proportions of 22 immune infiltrating cells in all samples from GSE96804 were assessed by the CIBERSORT algorithm, and their differences between DN and control groups were compared using a T-test. Next, a Spearman correlation analysis was performed using the "corrplot" R package (version 0.92) to explore the relationship between diagnostic biomarkers and immune cells, and heatmaps were drawn to illustrate this. In addition, the "clusterProfiler" R package (version 3.14.3) was used to analyze the KEGG pathways enriched by the screened diagnostic biomarkers.

Establishment of the pharmacological networks

Based on the RR-active ingredient and active ingredient-diagnostic biomarkers, the RR-active ingredient-target protein network was obtained. The pathway-target protein relationship was established by enriching KEGG results for the diagnostic biomarkers. Subsequently, these two networks were integrated to obtain the RR-active ingredient-target protein-pathway network. This pharmacological network was visualized using CytoScape software to demonstrate the pathway regulation mechanism of the relevant target proteins of the important components of TCM.

Molecular docking analysis

To explore the potential of RR for the treatment of DN, molecular docking of core active components of RR with diagnostic biomarkers was performed using Autodock software [52] (version 4.2.6). Firstly, the 3D crystal structure of the protein encoded by the diagnostic biomarkers was retrieved through the Protein Data Bank (PDB) database [53], and its water molecules and primitive ligands were removed using PyMOL software (version 2.0) to obtain the target protein. Next, the active ingredient targeting the diagnostic biomarkers in the pharmacological network constructed above was used as the core active

ingredient and imported into the PubChem database [54] to obtain its 3D structure. Finally, molecular docking of the target protein and the core active ingredient was performed via Autodock software, and the results were presented using LigPlot + software [55] (version .2.2).

Statistical analysis

Bioinformatics analyses were performed with R software and $p < 0.05$ indicated significant differences.

Declarations

Data availability

The data of the study are available from BATMAN-TCM database, PubChem database, Protein Data Bank (PDB) database, STRING database, Comparative Toxicogenomics Database, MitoCarta3.0 database and Gene Expression Omnibus (GEO) database.

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Author contributions

Ruihua Wang designed the study and is responsible for the main frame of the manuscript. Xiujie Yang, Ruman Yang, Xiaoyu Wang and Yuanyuan Guo collected the data and undertook the analysis. Ziyuan Zhang and Yaling Hu took charge of the polish of the manuscript. Jinai Fang provided the overall guidance .

Competing interests

The authors declare no competing interests.

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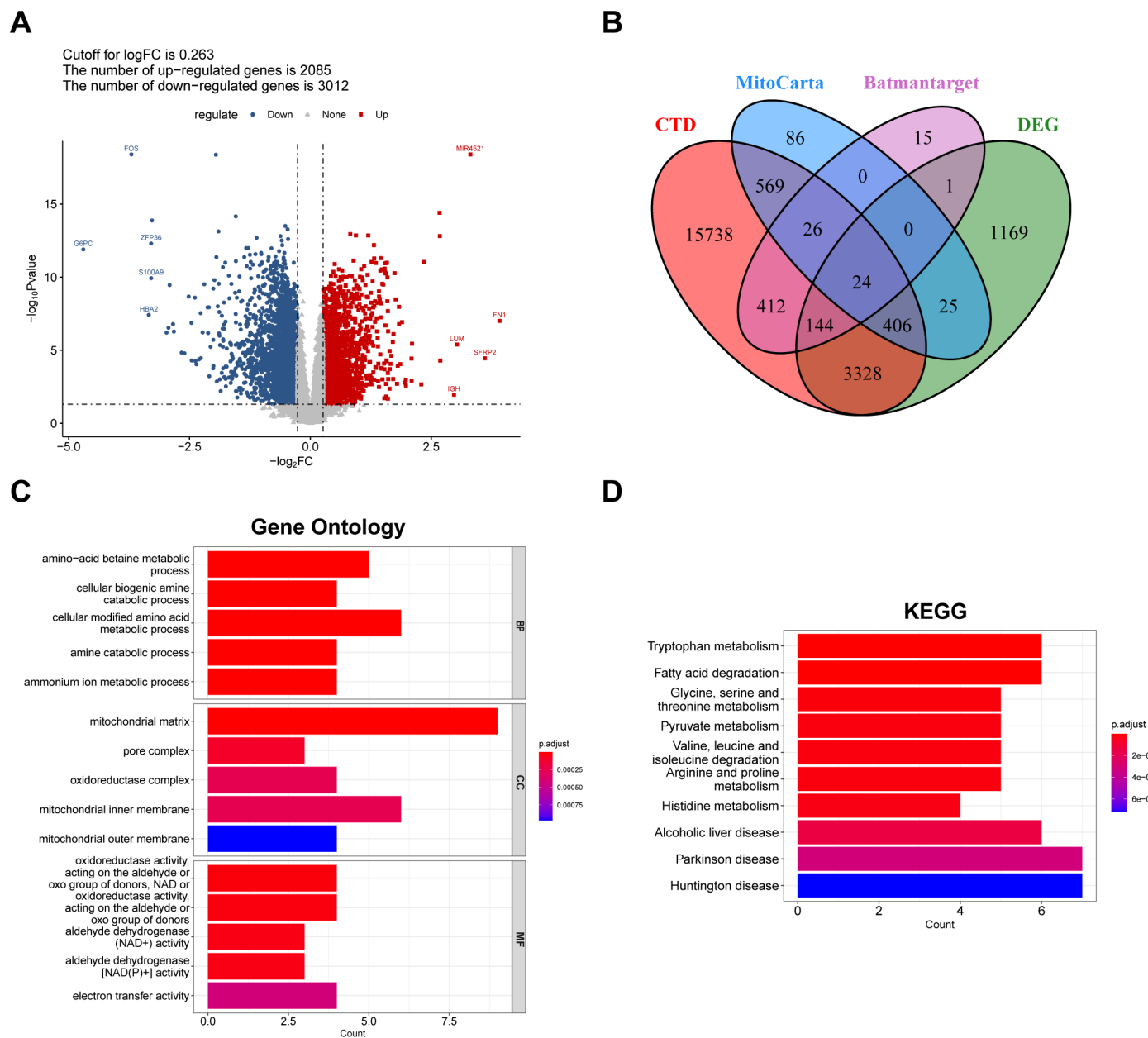
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Figures



D KEGG pathway analysis of MRPGs in the Venn diagram.

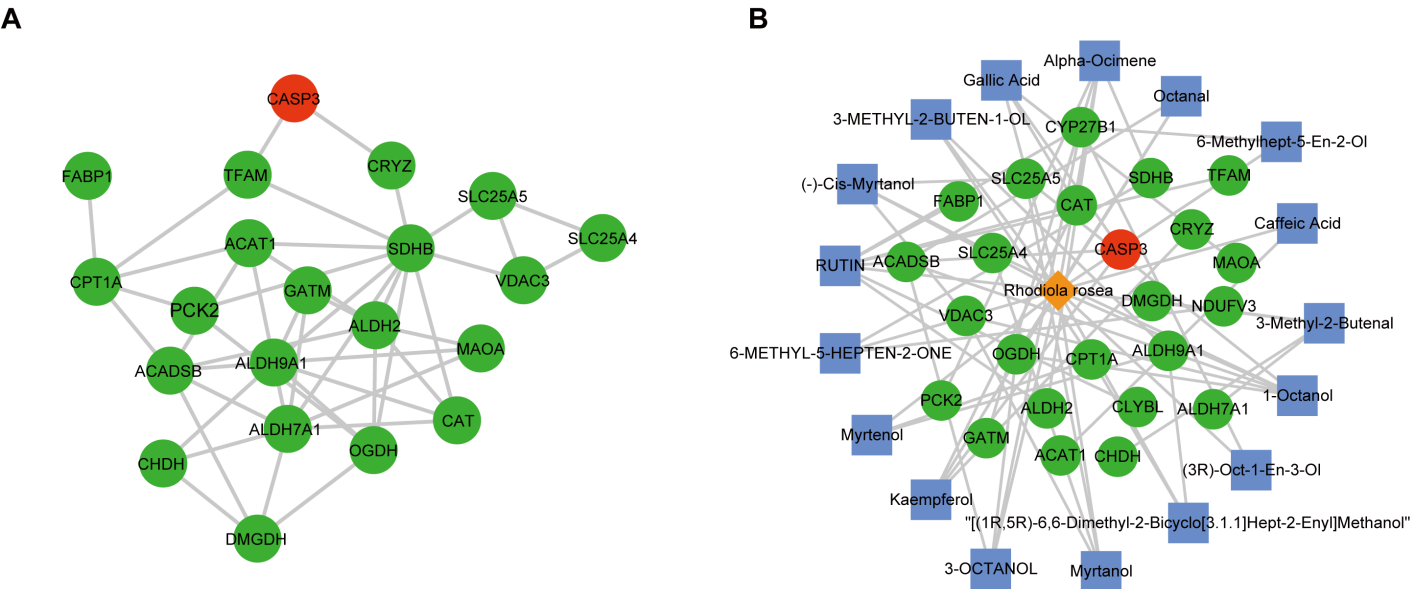


Figure 2

Network construction of MRPGs targeting DN.

A PPI network of MRPGs, red circles indicate upregulated genes and green circles indicate downregulated genes.

B RR active ingredient-MRPG network, yellow diamonds are RR, blue squares are active ingredients, red circles indicate upregulated genes, and green circles indicate downregulated genes.

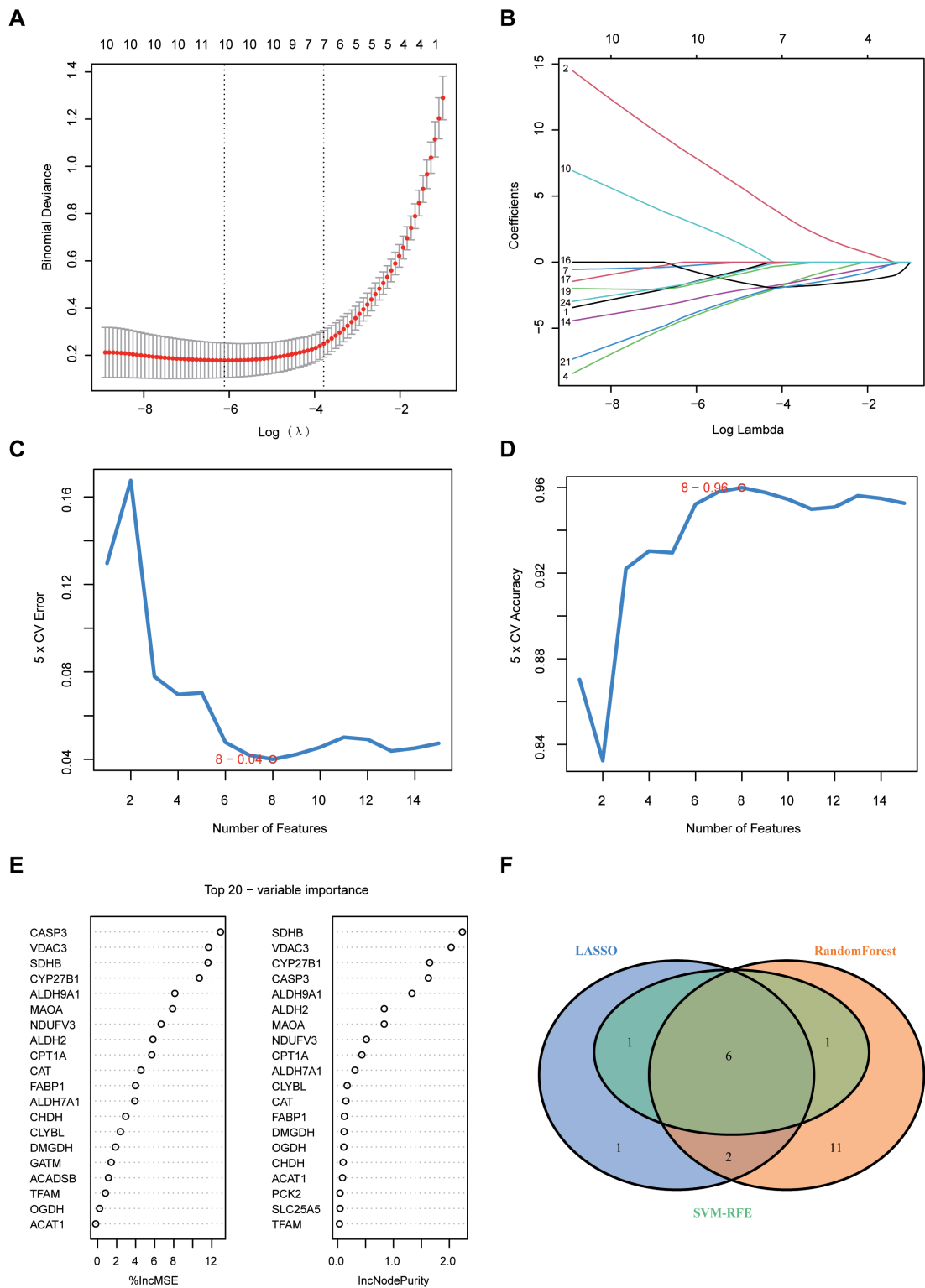


Figure 3

Diagnostic biomarkers of DN identified from MRPGs by machine learning algorithm.

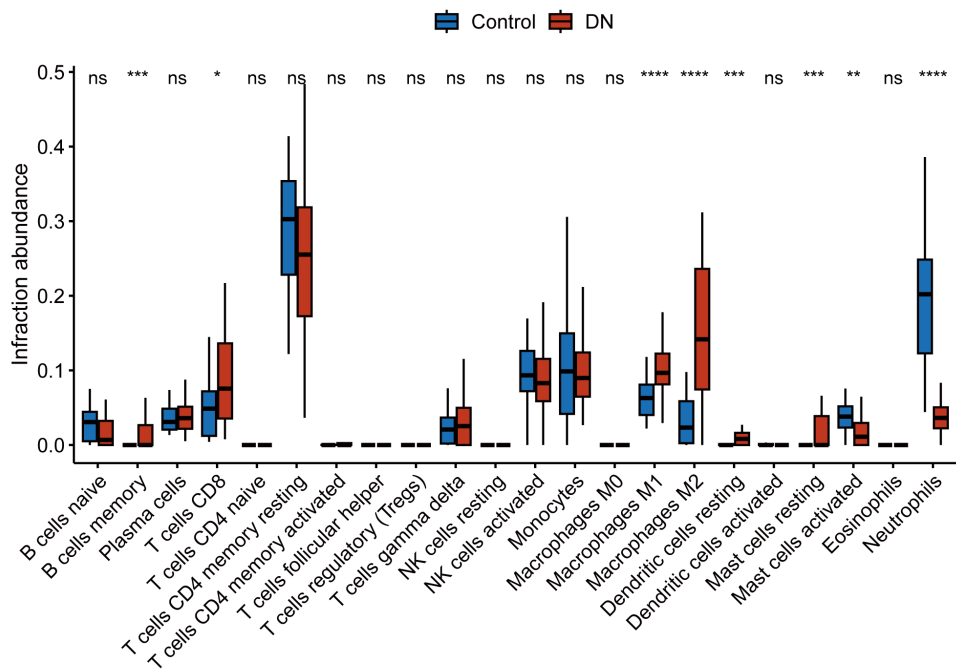
A-B Ten genes predicted by LASSO regression analysis.

C-D The eight genes screened by SVM-RFE algorithm.

E Importance ranking of the top 20 genes screened by the RF algorithm.

F Venn diagram showing diagnostic biomarkers between LASSO, SVM-RFE, and RF.

A



B

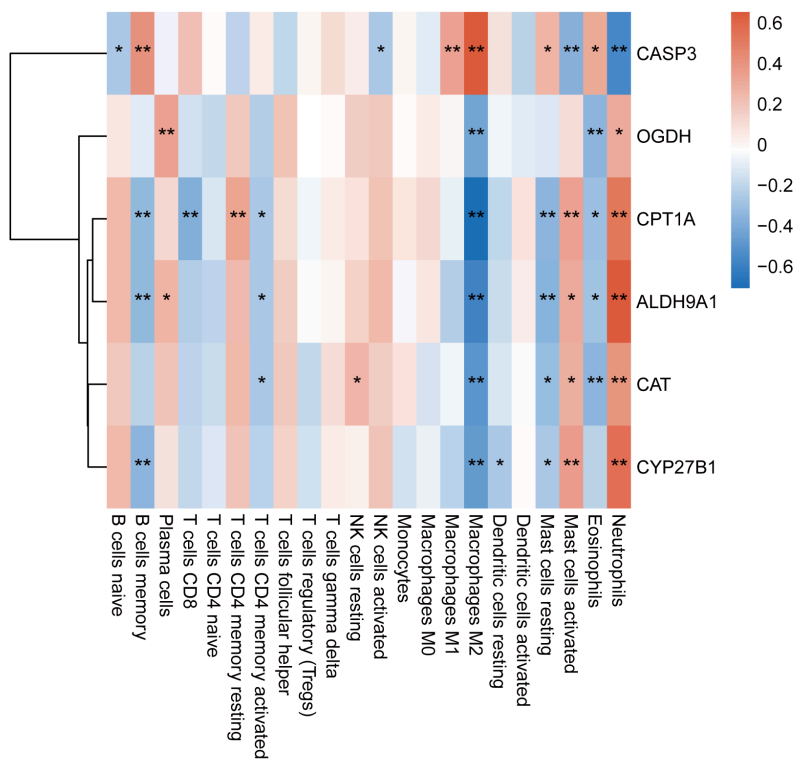


Figure 4

Immune infiltration analysis of DN.

A Expression abundance of immune cells in control and DN groups. (* p < 0.05, ** p < 0.01, *** p < 0.001 and **** p < 0.0001).

B Correlation between the diagnostic biomarkers and immune cells.

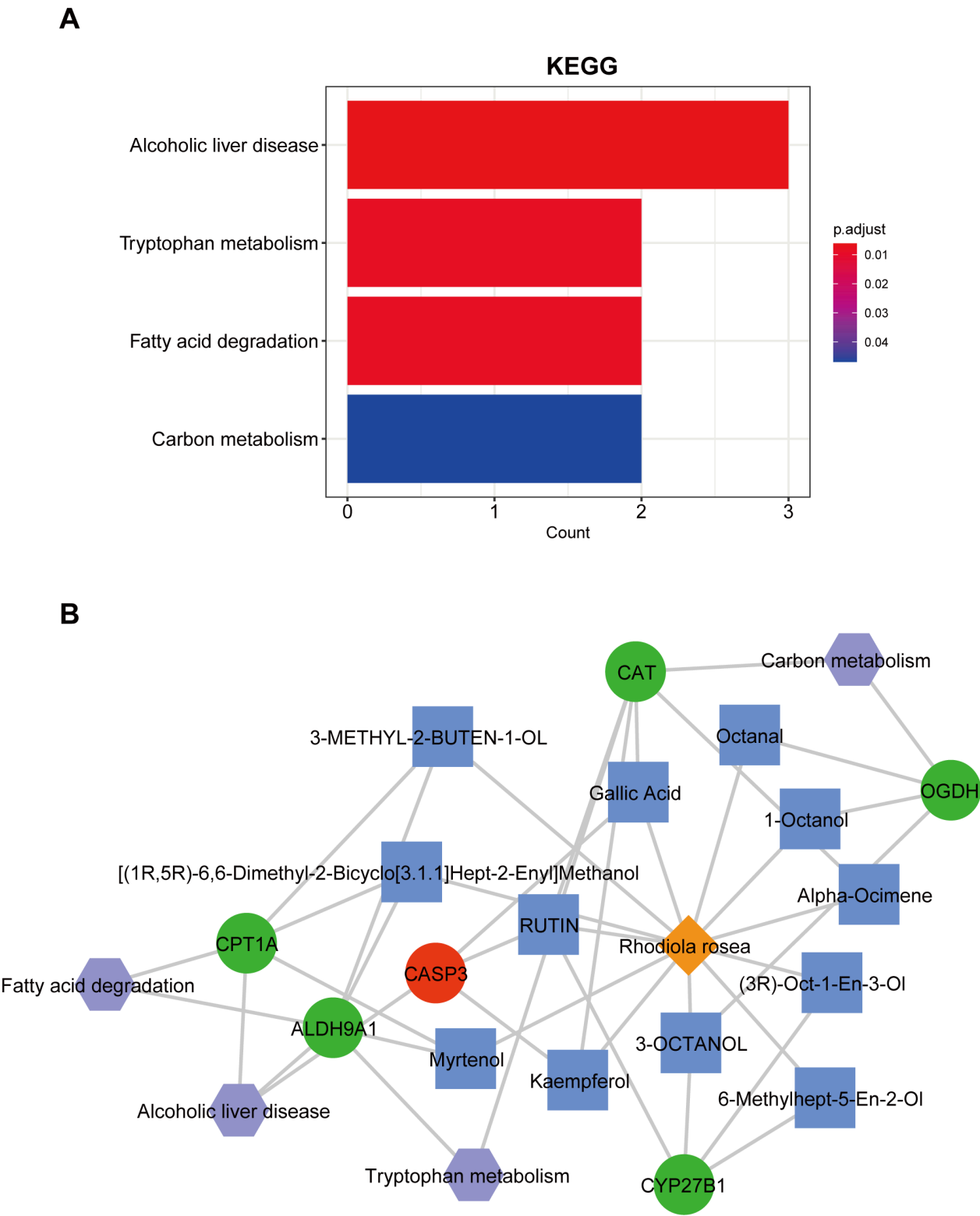


Figure 5

RR-active ingredient-diagnostic biomarker-signaling pathway network construction.

A KEGG pathway analysis of diagnostic biomarkers.

B RR-active ingredient-diagnostic biomarkers-signaling pathway network with yellow diamonds for RR, blue squares for active ingredients, red circles for upregulated genes, green circles for downregulated genes, and purple hexagons for signaling pathways.

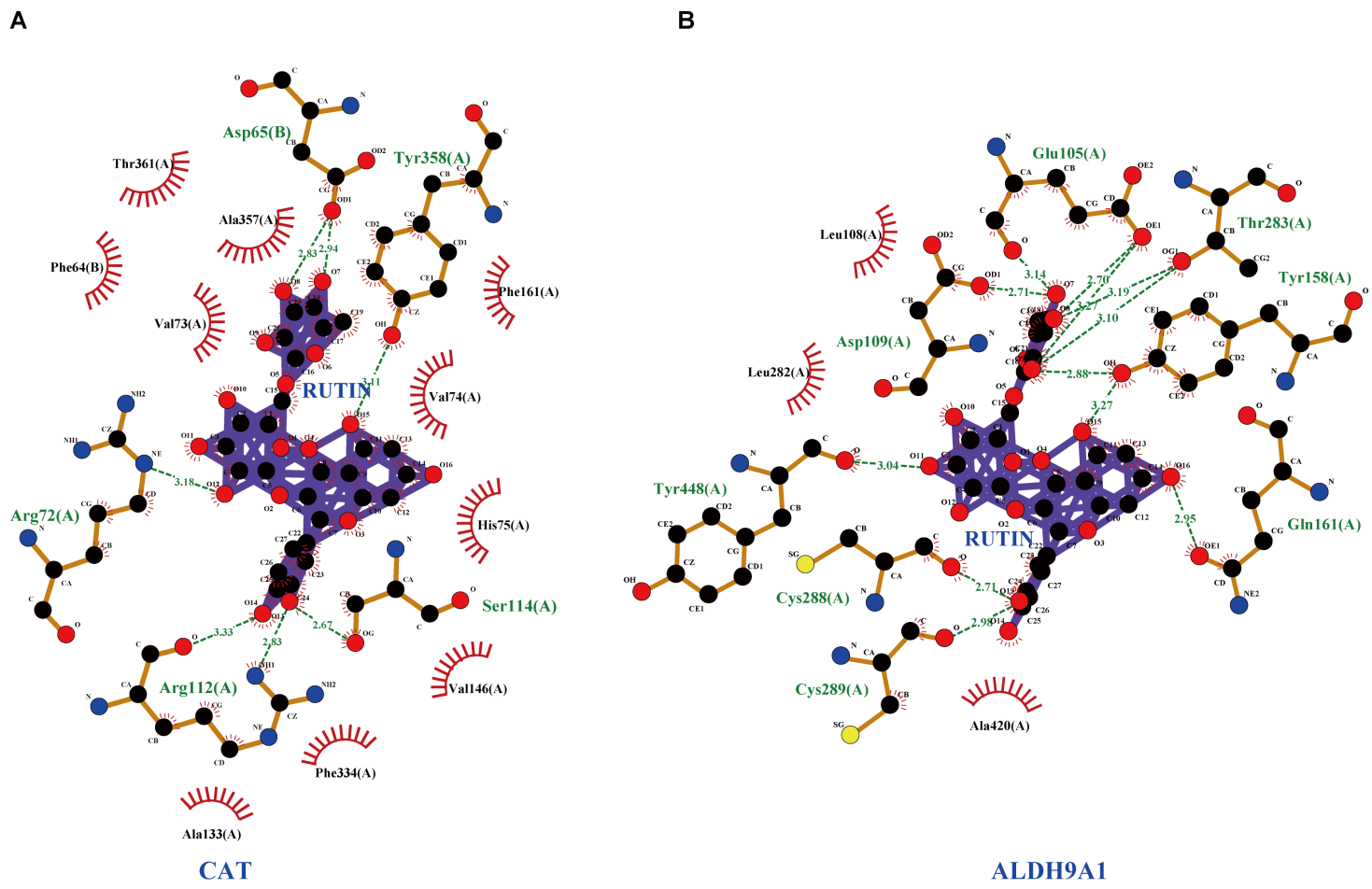


Figure 6
2D diagram of the molecular docking of RUTIN, the core active ingredient of RR, with CAT (A) and ALDH9A1 (B).

Supplementary Files

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

- [SupplementaryTable1.xlsx](#)
- [SupplementaryTable2.xlsx](#)