Network Pharmacology-based Analysis on Treatment of Diabetic Retinopathy by Salidroside

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Research Article

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Abstract

Objective: To study the mechanism of salidroside in the treatment of diabetic retina injury.

Methods: The animal model of diabetic retinal injury was established, salidroside treatment group was treated with salidroside, and then OCT detection, water maze detection, heat map difference analysis, molecular docking, PPI protein interaction, GO and KEGG analysis were used to study the protective mechanism of salidroside against diabetic retinal injury.

Results: OCT and water maze results showed that the learning and cognitive functions of rats in the DM group and sham group were significant on day 1 and 5 after salidroside treatment. Venny intersection map was made for the data screened by proteomic analysis, and heat map was made for the three intersection genes. It was found that Nsmce1, Inpp5f and Vcan genes were all up-regulated in the diabetes group. By establishing molecular docking between the intersection gene and salidroside, Vcan did not docking with salidroside ligand, and BOTH Nsmce1 and Inpp5f could establish stable molecular binding mode with salidroside. Through keyword search in PubMed, the associated gene combination and intersection genes were finally obtained, and PPI protein interaction was analyzed. The genes with mutual relationship were analyzed by GO enrichment. It was found that response to molecule of Bacterial Origin is the most important BP. The most important CC process is raft and cytokine receptor binding. KEGG analysis found that the most important signaling pathways were Lipid and atherosclerosis.

Conclusion: Salidroside has certain therapeutic effect on diabetic retina injury.

Introduction

Diabetic retinopathy (DR) is a neurovascular disease caused by diabetes mellitus (DM) that impairs vision. It is the cause of irreversible blindness in developed countries and in majority of working-age adults, and its prevalence increases with age and the elderly population (Kempen, O'Colmain et al. 2004, Monteiro, Santos et al. 2015). It has been found that almost all patients with type 1 diabetes mellitus (T1DM) and nearly half of patients with type 2 diabetes mellitus (T2DM) develop DR, leading to retinal damage and, in severe cases, vision loss (Fong, Aiello et al. 2004). DR in the setting of persistent hyperglycemia has been reported to lead to penetrating hemorrhages, microaneurysms, cotton wool, and retinal microvascular abnormalities, and may result in vision loss, color weakness, and night vision problems (Liu, Hu et al. 2011, Lee, Byeon et al. 2020, Wang and Zhang 2021). Among them, hyperglycemia is a main factor affecting the progression of DR, which can lead to apoptosis of peripheral retinal vascular cells and abnormal proliferation of retinal vascular endothelial cells, thus seriously affecting retinal vascular function and leading to a series of pathological changes of retinal cells (Min, Kim et al. 2020, Yu, Zhang et al. 2021). In the pathogenesis of DR, retinal vascular leakage, retinal ischemic angiogenesis, endothelial dysfunction, inflammation and oxidative stress (OS) are its pathological features (Lu, Zhang et al. 2020). Although the underlying cause of DR is chronic hyperglycemia, much remains unknown about the exact pathogenesis of the disease (El-Asrar 2012).

Salidroside (molecular formula: $C_{14}H_{20}O_7$, molecular weight: 300.12) is one of the active components of *Rhodiola Rosea L.* extracted from various natural plants and has been used as an original component in the adaptation of Chinese medicine. It has anti-aging, antioxidant anti-aging, immunity-enhancing, radiation-protective properties, protective effect on the nervous and cardiovascular system, and therapeutic effects on tumors and inflammatory diseases (Chiang, Chen et al. 2015, Zhu, Chen et al. 2016, Zhang, Xie et al. 2021). According to findings of researches, salidroside have a hypoglycemic effect on DM and a beneficial protective effect on vascular function in DM (Ma, Wang et al. 2017). It was also found that salidroside could protect retinal endothelial cells from hydrogen peroxide-induced damage by regulating apoptosis and oxidative status (Shi, Wang et al. 2015). However, the therapeutic effect of salidroside on DR is still unclear and further studies are needed.

Therefore, the present study was aimed to investigate the therapeutic effect of salidroside on treatment of DR using bioinformatics, molecular docking, and animal experiments.

Materials And Methods

Establishment of DM rat model and drug therapy

A total of 30 Sprague-Dawley (SD) rats (weighing 180-200g，male) were provided from the Department of Experimental Animals of Kunming Medical University (No. SCXK (Yunnan) K2020-0004) and were randomly divided into Sham group (n=10), DM group (n=10), and salidroside group (SAL group, n=10). The rats in DM and SAL groups were intraperitoneally injected with streptozotocin (STZ) (Shanghai Yuanye Bio-Technology Co., Ltd., China) to establish the DM rats model, and the successful establishment of DM rat model was considered if the blood glucose value was ≥16.7 mol/L when the tail vein blood was taken after 72 hours for determination. After successful modeling, the rats in SAL group were given orally with salidroside (dissolved with normal saline, 100mg/kg/day) for 6 weeks continuously, and the rats in Sham group and DM group were given normal saline (100 mg/kg/day) for 4 weeks continuously. All procedures were performed in accordance with the guidelines and approval of the Ethics Committee of the Kunming Medical University，Animal ethical approval number is KMMU20220854.

Optical coherence tomography (OCT)

OCT is a non-invasive, high-resolution optical imaging technique that produces cross-sectional images of objects in real time, two-dimensional images in space in real time (Yao, Son et al. 2018). In this study, OCT（SPECTRALIS OCT, Heidelberg, Germany） measurements were performed on rats in the Sham group, DM group and SAL group. Rats were anesthetized by intraperitoneal injection of 10g/L pentobarbital sodium (0.6ml/100g), the pupils were dilated with tropicamide phenylephrine eye drops（the pupils were dilated with Compound Topicamide eye drops and the corneas were kept moist with drops of hydroxy glucan The manufacturer of compound Topicamide eye drops is Shentian Pharmaceutical (China) Co., LTD. ） and the corneas were kept moist with drops of

hydroxy glucan eye drops（Hydroxyglucan eye drops, made by Alcon Research LLC）. Then placed in front of a corneal contact lens, the angle of light was then adjusted for fundus photography and OCT examination. All images were acquired at one optic disc interval, and the retinal thickness was measured by ImageJ software.

Morris water maze

The Morris water maze test was used to measure learning and cognitive function in rats and to assess hippocampal-dependent spatial learning and memory (Mifflin, Winslow et al. 2021), and was performed at 1 day (d), 3 d, and 5 d after modeling. A 10 cm diameter platform was placed in a black circular pool at a temperature of approximately 22°C and curtains were used to block light around the pool. The pool was divided into four quadrants on the computer and an automatic camera system was connected to the computer was place above the pool. The rats were put into the pool and the number of times the rats crossed the platform, the platform delay and the total distance of the rats were recorded by the SMART 3.0 Tracking System (Harvard Bioscience (Shanghai) Co., Ltd.).

Proteomics analysis

After 6 weeks of salidroside treatment, the rats were anesthetized with intraperitoneal injection of 3% sodium pentobarbital (0.3ml/100g), and the tissues of hippocampus, cortex, lung and retina were collected and stored at -80°C for proteomics analysis. The proteomics analysis of samples were performed by Shanghai Luming Biotechnology Co., Ltd. The four-dimensional data-independent acquisition (4D-DIA) labeling was used for quantitative proteomic analysis. Total proteins in the sample were extracted and part of samples were determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and protein concentration, while the other part of the samples were digested by trypsin, enzymatically digested for peptides, and the samples were identified by liquid chromatography tandem mass spectrometry (LC-MS/MS).

Venny diagram

Use Venny2.1 database (https://bioinfogp.cnb.csic.es/tools/venny/), use omics data diabetes astragalus armour glycosides in the treatment group and control group for venny intersection. List1 and List2 are named short for Sham and SAL-DR（Diabetic retinopathy group） names respectively, change the image to color and download.

heat map

Organize the data and draw the tables as required, The table is divided into three parts, The first part is between berberine (D) and SHAM (C) related Accession, ProteinDescriptions, Gene Name, MW [KDa], Qvalue, Cscore, D1-2, D5-2, D7-2, C3-2, C4-2, C5-2, P-value, log2 (FC), and FC; The second part is the related data of rhodiola rosine (B) and diabetic retinal injury (E), The required data type is the same as that in Part 1; The third part summarizes the genetic data related to SHAM (C), rhotinine (B) and diabetic retinal injury (E).Then in the wechat website, the website is: http://www.bioinformatics.com.cn/, import the data, and then generate a heat map.

Molecular Docking

The 3D structure of salidroside was obtained from the PubChem database (https://pubchem.ncbi.nlm.nih.gov), and the structures of key proteins of salidroside screened from omics data were obtained from the Protein Data Bank (PDB) (https://www.rcsb.org). The target protein structure was dehydrated with PyMOL software to separate the ligand and receptor. With the target protein as the receptor and the active substance as the ligand, the active site of molecular docking was determined according to the coordinate of the ligand in the target protein complex. Molecular docking is performed using AutoDock Vina and the binding conformation with the lowest free binding energy was selected.

Protein-protein interaction (PPI) network analysis

Using String database (https://string-db.org/), we summarized the genes related to diabetic retinal injury found on PubMed and the genes screened by omics into the List Of Names in String database. Select Homo Sapiens from the "Organism" box, search for the protein interaction map, export and download the map. The string table obtained through PPI interaction was sorted out, and the node1 and Node2 items were duplicated respectively, so as to obtain the remaining genes with mutual relationship.

Biological function enrichment analysis

The genes screened from PPI network analysis were analyzed by Gene Ontology (GO) (http://www.geneontology.org) and Kyoto Encyclopedia of Genes and Genomics (KEGG) (https://www.kegg.jp/kegg/pathway.html) analyses using R 3.6.0 (http://www.r-project.org). It was required to pre-install R packages such as RSQLite, clusterProfiler, org.hs. eg, DOSE, enrichPlot, GGploT2, Colorspace, Stringi, and PathView. The signaling pathway map, bar plot and bubble plot for GO and KEGG analyses were plotted using DB, enrichment plot and ggplot2. The top 10 biological process (BP), cellular component (CC), and molecular function (MF) of GO enrichment analysis were reorganized and presented in a circular plot.

Results

Morphological changes of fundus

As shown, there was no morphological change in the Sham group, and the thickness of retinal pigment epithelial (RPE) outer nucleus and intima boundary-core layer in the DM group was significantly thinner than in the Sham group. It was shown that the thickness of RPE outer nucleus, intima boundary-core layer and external morphology layer was thickened in SAL group, compared with Sham and DM groups. **(Figure 1)**

Results of Morris water maze

We examined the learning and cognitive function of rats from the water maze platform, sham, DM and SAL at 1,3 and 5 days.We performed a one-way ANOVA and repeated measures ANOVA in the SPSS software, After finding that the rats underwent diabetic modage, The DM group from day 1 to day 5, when compared to the sham group, Rat learning and cognitive function are both meaningful, P <0.001. Based to the repeated measures ANOVA, sphericity test, Results for P> 0.050, Satisfying the spherical assumption, If the spherical test results shall prevail, Within-subject effect tests showed P=0.001 for time, make sense; In the inter-subject effect tests, the group, A P <0.001 for the intercept, This indicates that there are certain differences between the different treatment groups.Figure 2Table 1

Table 1 1,3, and 5 days rats platform latency

Variables	DF	SS	MF	F	P
Intergroup error	2	38575.5	19287.7	116.869	P<0.001
Time	21	3465.78	165.037		
Time*Intervence	2	1984.48	992.242	8.83713	P=0.001
Repeated measurement	4	958.406	239.602	2.13395	P=0.093
error	42	4715.8	112.281		

Shared genes of salidroside and DR

Venny diagram was used to analyze 130 genes involved in the treatment of diabetic retinal injury by salidroside and 248 genes in the diabetic retinal control group. Three common targets were obtained by Venny analysis, which were Nsmce1, Inpp5f and Vcan.**Figure 3, Table 2**

There were 3 key genes of shared genes between the salidroside and diabetic retinopathy were obtained, including the Nsmce1, Inpp5f and Vcan.

Table 2. The raw data of proteomics analysis.

Omics raw data											
Accession	**ProteinDescriptions**	**Gene Name**	**MW [KDa]**	**Qvalue**	**Cscore**	**D1-2**	**D5-2**	**D7-2**	**C3-2**	**C4-2**	**C**
A0A0G2JSX1	Non-structural maintenance of chromosomes element 1 homolog	Nsmce1	33.30919	0.000604	0.737861	-1.61671	-1.55057	-1.31588	1.070106	0.611183	0
D3ZKG7	Inositol polyphosphate-5-phosphatase F	Inpp5f	127.2608	7.92E-10	0.76665	0.303086	1.380455	0.123877	-0.45179	-1.36482	-2
D4A8Y6	Versican core protein	Vcan	332.3625	2.22E-58	0.828678	0.687897	0.703911	0.746899	-0.53866	-0.95319	-(

Note: C: Sham group. D: SAL group, rats with diabetes mellitus were treated with salidroside. E: DR group, Diabetic retina injury rats.

Comparison of expression of key genes

The expression level of the three shared genes, Nsmce1, Inpp5f and Vcan, were performed using heat map **(Figure 4)**. It was shown that the expression of Nsmce1, Inpp5f and Vcan genes were up-regulated in the DR group; the expression of Vcan was down-regulated but Nsmce1 was up-regulated in Sham group; the expression of Nsmce1 and Inpp5f were down-regulated in SAL group.

Red indicates up-regulation of gene expression, and blue indicates down-regulation of gene expression. (B: SAL group, ats with diabetes mellitus were treated with salidroside; C: Sham group; E: DR group, Diabetic retina injury rats.)

Molecular Docking

As shown, the Nsmce1 and Inpp5f genes had a good binding activity with salidroside, but no relevant results were found between the Vcan and salidroside **(Figure 5)**.

PubMed search for genes

Enter the keyword "diabetic retinal injury" in PubMed, search the articles in the past year, and record the reported related genes. There are 343 genes in total, which are combined with the 3 intersection genes we made in Venny, and there are 346 genes in total.**Table 3**

Table 3. Gene list of DR and salidroside

Gene list of DR and salidroside				
Ghrelin	IL-1 β	p-ERK	TAK-242	Co-IP
ROS	mRNA	4-HNE	HMGB-1	LPS
ARPE-19	Lmo4	WIF1	TRX1	P38 MAP
IL-1B	microRNA	Wnt	PGC-1α	FASN
IL-18	miR-139-5p	pdx1	NRF1	NFAT2
NLRP3	lncRNA H19	MAP1LC3	TFAM	p-NF-κB
VEGF	TGF-β	SQSTM1	ERK	TRPC6
RPE	EndMT	BECN1	AMPK	FC-DenseNet
RGC	IL-1β	DAPI	MEK	MA-FC-DenseNet
Muller	IL-17A-KO	EdU	MEK1	ATG4D
SOD	IL-17A	ERG	MEK2	miR-125b-5p
TUNEL	IL-17RA	FITC	circRNA	Wnt β
TNF-α	IL-17RA-shRNA	CTRP3	TXNIP	RT-qPCR
IL-6	IL-23	Claudin-5	circADAM9	GLP1R
MCP-1	SIRT1	Occludin	CARM1	S1PR2
MDA	AGE-RAGE	CRP	KLF3	JTE-013
T2DM	CUR	INS-1	Trx-R	hRVECs
GHSR	INS	c-jun	FGF-1	PFK-2
IL17A	CUR + INS	Akt	p38 MAPK	OGG1
RMC	IL-17	TLR9	8-OHdG	RGC-5
IL17RA	ADGRE1	MTOR	ICAM-1	FoxOs
Th17	C57BL6	EMT	cJun	SMP30
Act1	CCL2	YY1	JNK	Akt
TRAF6	CD45	VEGFA	ERK2	GSK-3β
IKK	CXCL1	Snail1	HRMECs	RPE-EMT
NFκB	DAMPs	Smurf2	LC3	PEI-CeO□
EAAT1	DBA/2J	LOX	Pro	HA-CeO□
Ins2	HSPGs	CaMKII	PRR	STK25
USA	IL6	SH-SY5Y	Gyp17	RGCs
CA	PCO	Bax	NF-κB	CCK-8
Gibco	PDGF	Bcl-2	SERCA2a	Caspase-3
MD	PDR	HSP	PP1	p53
Abcam	PVD	MAPKs	AGEs	LC3-II
ZO1	PVR	hRPEs	PKC	LC3-I
GFAP	TG	HIF-1*α*	syndcan-1	p-p53
GS	TGF1	COX-2	CD44	p53
SYTO13	TM	VEGF-A	syndecan-3	CASP8
PI	Th1	FGF2	RRMECs	CASP9
Nrf2	Th2	miR-140-3p	syndecan-2	p38αMAPK
AGE	αSMA	circsLC16A12	syndecan-4	c-Jun N
RAGE	mTOR	RUNX1	glypican-1	JNK1
ECM	miR-15b-5p	RNS	glypican-3	ERK1
Keap1	circ_001209	ML-SA1	miR-5195-3p	SGK1

NeH2	Glu	MCOLN1	miR-124-3p	PAC
HO1	DR	TRPML1	CCK-8	Hsa-miR-3184-3p
Nox2	IS	rMC1	G3BP2	hsa-miR-24-3p
LC3B-II.	IL-17	PGC1α	p38MAPK	hsa-miR-197-3p
p62	NFKB	SOD3	Irs2-KO	BMP4
PI3K	STAT-3	CCN1	Irs2	MiR-340-5p
p-AKT	MCP-1	TLR2	TH	miR-18a-3p
p-mTOR	JNK	DPP	miR-195	VEGFR2
REDOX	HRPC	GLP	ChIP	VEGF121
Col12a1	RBY79	SGLT	NCAM	eVEGF-38
Chad	SO-RB50	eGFR	HO-1	eVEGF-53
Bgn	TNF	RIPK1	L6H21	MAPK
PPP1CA	5-LOX	RIPK3	QRT-PCR	Rho GTPase
YAP	IL-1	TNFR1	SIRT2	CDC42
STZ	IL-8	IFNR	SIRT3	ROCK
HG	iNOS	TLR	SIRT4	VEGF165
Gln	FGF	MLKL	SIRT5	RIPK3
mTORC1	IGF-1	SGLT2i	SIRT6	circEhmt1
NLR	GAPDH	Cx43	SIRT7	NFIA
GCL	GSH	TGFβ1	LKB1	SA-β-gal
caspase-1	CCN1	P2X7R	TLR4	P21
CGA	GLP-1RA	ATP	MD2	M1
HREC	GLP-1	NRK-52E	NOX4	AMPK
APRE19	TCM	pCD5	NADPH	SIRT1
M2 ipRGCs	Keap1-Nrf2-ARE	BCX	NAD	NADH
M3 ipRGCs	GMFB	GAP43	Inpp5f	Vcan
Nsmce1				

PPI interaction relationship

The PPI network of 346 genes was presented in **Figure 6**, and 203 genes were found to be interrelated as shown in **Table 4**.

Table 4. Related genes in the PPI network

Related genes in the PPI network				
AGER	TLR4	GPC1	PPP1CA	PRRT2
AKT1	KEAP1	GPC3	VEGFA	PTGS2
AKT1S1	GAPDH	GSTP1	IL1B	PVR
ALOX5	MAPK14	GTF2H1	NLRP3	RIPK1
APC	MAPK3	HMOX1	IL33	RIPK3
ATG16L1	PIK3CA	HSP90AA1	RIPK1	RUNX1
ATG4D	TP53	IL17A	TJP1	SDC2
BAX	SOD1	IL17RA	OCLN	SDC3
BCL10	CASP3	IL18	TNFRSF1A	SDC4
BCL2	NOS2	IL1B	RIPK3	SIRT1
BECN1	CASP9	IL23A	MLKL	SIRT7
BGN	HSP90AA1	IL6	MAP2K7	SNAI1
BMP4	MTOR	INS	TNF	SOD1
CARKD	BECN1	IRF6	CXCL1	SQSTM1
CARM1	MCL1	IRS2	IL6	STK11
CASP1	JUN	JUN	CXCL8	STUB1
CASP3	CYBB	KDR	FOXM1	TFAM
CASP8	SOD3	KEAP1	FGF2	TLR2
CASP9	AKT1S1	LY96	ICAM1	TLR4
CCL2	INS	MAP1LC3A	PTPRC	TNF
CCNA2	BCL2	MAP2K1	DOCK7	TNFRSF1A
CD180	CDC42	MAP2K2	KDR	TP53
CD44	TRAF6	MAP2K7	RUNX1	TRAF3IP2
CDC42	IL18	MAPK1	GATB	TRAF6
CHRM1	PTGS2	MAPK14	IL23A	TXN
CLDN5	MAP1LC3A	MAPK3	IL17A	SNAI1
CRP	SIRT1	MAPK8	MAP2K1	NFATC1
CXCL1	MAPK8	MCL1	TFAM	NRP1
CXCL8	CASP8	MLKL	GSS	NEIL2
CYBB	MAPK1	MTOR	IRS2	S1PR2
EARS2	PRKAB1	NEIL2	GHRL	PRRT2
EGFR	EGFR	NFATC1	GHSR	TH
FGF1	SQSTM1	NLRP3	STUB1	YY1
FGF2	TLR2	NLRX1	STK11	OGG1
FOXM1	GPC1	NOS2	SGK1	TXN
GABPA	VCAN	NOX4	IL17RA	TXNIP
GAPDH	LY96	NRF1	TRAF3IP2	SIRT7
GCLC	SDC2	NRP1	TLR9	PVRL1
GH1	GPC3	OCLN	MAP2K2	SIRT2
GHRL	SDC3	PIK3CA	LMO4	TBX21
GJA1	SDC4	PRKAB1	PDX1	

GO analysis

The results of biological function enrichment analysis of salidroside on the treatment of DR were presented in the **Figure 7**.

The top 10 BP involved in GO enrichment analysis were as follows: response to molecule of bacterial origin, activation of protein kinase activity, response to oxidative stress, response to lipopolysaccharide, cellular response to biotic stimulus, cellular response to chemical stress, cellular response to oxidative stress, cellular response to molecule of bacterial origin, cellular response to lipopolysaccharide, and reactive oxygen species metabolic process **(Figure 7A)**. The top 10 CC involved in GO enrichment analysis included raft of membrane, membrane microdomain, membrane region, Golgi lumen, focal adhesion, cell-substrate junction, lysosomal lumen, vacuolar lumen, vesicle lumen, and organelle outer membrane **(Figure 7B)**. The top 10 MF involved in GO enrichment analysis were as follows: cytokine receptor binding, protein serine/threonine/tyrosine kinase activity, protein serine/threonine kinase activity, the MAP Kinase activity, ubiquitin protein ligase binding, ubiquitin-like protein ligase binding, cytokine activity, receptor ligand activity, signaling receptor activator activity, and glycosaminoglycan binding **(Figure 7C)**. The circular plot of GO analysis was shown in **Figure 7D**.

1. Top 20 biological processes (BP) involved in GO analysis. **B.** Top 20 cellular components (CC) involved in GO analysis . **C.** Top 20 molecular function (MF) involved in GO analysis. **D.** The circular diagram of BP, CC, and MF involved in GO analysis.
2. KEGG **analysis**
3. the top 10 signaling pathways involved in the salidroside on the treatment of DR were as follows: Lipid and atherosclerosis, Salmonella infection, Fluid shear stress and atherosclerosis, AGE-RAGE signaling pathway in diabetic complications, NOD-like receptor signaling pathway, Shigellosis, Kaposi sarcoma-associated herpesvirus infection, TNF signaling pathway, Toll-like receptor signaling pathway, and Hepatitis **(Figure 8A, Table 5)**.According to The results in Figure 8B, the first 20 KEGG pathways set the corresponding acting genes for each pathway, and the pathways were classified. TNF Signaling Pathway was classified in the Environmental Information Processing category. The pathways of Organismal Systems are Nod-like receptor signaling Pathway, Toll-like receptor signaling Pathway and IL-17 signaling Pathway, The pathways for Human Diseases include Lipid and atherosclerosis, Salmonella infection, Fluid shear stress and atherosclerosis, and age-rage Signaling pathway in diabetic claus, Shigellosis, Kaposi sarcoma-associated herpesvirus infection, Hepatitis B, Yersinia infection, Pathogenic Escherichia coli infection, Influenza A, Chagas disease, Pertussis, non-alcoholic fatty liver Disease, Proteoglycans in cancer, Tuberculosis, Human cytomegalovirus Infection.

Table 5. The top 10 KEGG signaling pathways

ID	Description	pvalue	p.adjust	qvalue	Count
hsa05417	Lipid and atherosclerosis	1.57E-26	3.50E-24	1.04E-24	34
hsa05132	Salmonella infection	1.60E-25	1.79E-23	5.32E-24	35
hsa05418	Fluid shear stress and atherosclerosis	6.25E-21	4.64E-19	1.38E-19	25
hsa04933	AGE-RAGE signaling pathway in diabetic complications	1.67E-20	9.29E-19	2.76E-19	22
hsa04621	NOD-like receptor signaling pathway	3.77E-20	1.68E-18	5.00E-19	27
hsa05131	Shigellosis	6.06E-20	2.25E-18	6.69E-19	30
hsa05167	Kaposi sarcoma-associated herpesvirus infection	1.57E-19	4.99E-18	1.48E-18	27
hsa04668	TNF signaling pathway	2.34E-19	6.52E-18	1.94E-18	22
hsa04620	Toll-like receptor signaling pathway	9.00E-19	2.23E-17	6.63E-18	21
hsa05161	Hepatitis B	4.71E-18	1.05E-16	3.12E-17	24

Discussion

In this study, we established the DM（diabetes） rats model to investigate the effect of salidroside on the treatment of DM. The results showed that the learning and cognitive functions of rats in the DM and SAL groups was poorer than in the Sham group at 1d, 3 d, and 5 d after modeling, and the thickness of retinal pigment epithelial (RPE) was thinner in DM and Sham groups but thickened in SAL group. Additionally, three genes were screened out by 4D-DIA labeled quantitative proteomic analysis, including Nsmce1, Inpp5f and Vcan. The results showed Nsmce1, Inpp5f and Vcan genes were upregulated in DR(Diabetic retinal injury) group, Nsmce1 and Inpp5f formed stable molecules binding to salidroside but Vcan. The results of biological function enrichment analysis showed that the most important BP, CC, and MF was response to molecule of bacterial origin, raft, and cytokine receptor binding, respectively, and the most important signaling pathways were lipid and atherosclerosis.

DM is a metabolic disease mainly caused by abnormal insulin secretion and characterized by chronic hyperglycemia, in which long-term hyperglycemia can lead to DR and blindness, seriously affecting the quality of life of patients (Wang, Lan et al. 2019).

We sorted out the proteomic data and drew the Venny intersection map, obtaining three intersecting genes: Nsmce1, Inpp5f and Vcan. Nsmce1 gene is a nonstructural maintenance homolog of chromosomal element 1 (NSE1), which plays an important role in maintaining genomic integrity, DNA damage response and DNA repair (Gong, Wang et al. 2020). It has been found that Inpp5f expression is coordinated by insulin, glucose and lipid levels in STZ and high-fat diet (HFD) induced diabetic mouse models(Bai, Zhang et al. 2016). DR is also affected by insulin, blood sugar and lipid levels. Vcan, or Versican for short, interacts with inflammatory cells via hyaluronic acid either indirectly or directly via CD44, p-selectin glycoprotein ligand-1 (PSGL-1), and Toll-like receptors (TLRs) present on the surface of both immune and non-immune cells. These interactions activate signaling pathways that promote the synthesis and secretion of inflammatory cytokines such as TNFα, IL-6, and NFκB. Versican also influences inflammation by interacting with various growth factors and

cytokines involved in regulating inflammation, thereby affecting its bioavailability and bioactivity. Versican is produced by a variety of cell types involved in inflammatory processes(Wight, Kang et al. 2020). In summary, Versican, as a component of ECM, affects immunity and inflammation by regulating the transport and activation of immune cells. Versican is emerging as a potential target for controlling inflammation in many different diseases(Islam and Watanabe 2020). The differential expression of heat map showed that Nsmce1, Inpp5f and Vcan genes were up-regulated in the diabetes group. In the SHAM group, Vcan was down-regulated, Nsmce1 was up-regulated, Inpp5F was down-regulated in C5-2, and the rest were up-regulated. In the salidroside treatment group, Nsmce1 and Inpp5f were down-regulated, Vcan was down-regulated in B2-2 and B1-2, and up-regulated in B5-2.

Molecular docking

Molecular docking of the three intersection genes with rhocidine found that Inpp5 and Nsmce1 were able to form a stable molecular binding pattern with rhocidine, while Vcan was not found in PDB.Inpp5 was found to be associated with retinal disease according to the Genecards query.The Nsmce1 gene is a non-structural maintenance homolog of chromosomal element 1 (NSE1), which is mainly involved in the maintenance of genome integrity, DNA damage response and DNA repair, and has a strong association with the treatment of retinal diseases

PPI interaction between proteomic genes and genes queried in PubMed

In this paper, PPI interaction was conducted on urine disease retinal injury and the protein relationship between salidroside and SHAM, which was found on PubMed. The top ten closely related genes were AGER and TLR4. AKT1, KEAP1; AKT1 and GAPDH; AKT1 and MAPK14; AKT1, MAPK3; AKT1 and PIK3CA; AKT1, TP53; AKT1, SOD1; AKT1, CASP3; AKT1, NOS2. These closely related genes may be the key genes in the treatment of diabetic retinal injury by salidroside.

Biological function enrichment analysis

In GO analysis, the most important BP is response to molecule of Bacterial Origin. Diabetes patients are often complicated with other complications, so the body resistance is low, bacterial infection is easy to occur, and the blood flow of the lesion site will be slow, the blood supply of organs and tissues will also be reduced, but also hypoproteinemia, malnutrition, bacteria are easy to invade, resulting in various infections. Once the infection occurs, the metabolic disorder will be aggravated, and all kinds of complications caused by the disease will become more complex, or even delayed, endangering the safety of patients. The most important CC process is membrane raft. Raft is a heterogeneous and dynamic domain in which lipids are tightly packed and rich in cholesterol, sphinolipids and other proteins such as various cell signaling proteins. Therefore, membrane rafts play an important role in cellular signaling pathways. And because they are rich in cholesterol, the raft domain is less mobile than other domains, which may stimulate signaling pathways(Li, Zhang et al. 2018). Sphingomyelin synthase (SMS) is the rate-limiting enzyme of sphingomyelin, and sphingomyelin is an indispensable component of lipid rafts. SMS2 is highly expressed in brain cells and is involved in the formation of sphingomyelin in the central nervous system. When SMS2 is deficient in organisms, sphingomyelin content in the plasma membrane is reduced, which may disrupt the formation of functional membrane rafts, which play an important role in cellular signaling pathways. More importantly, SMS2 deficiency results in a decrease in the abundance of LPS-stimulated TLR4 / MD2 complexes on the surface of macrophages to exert anti-inflammatory effects (Xue, Yu et al. 2019). Cytokine receptor binding is the most important MF process. Cytokines work by binding to specific extracellular receptors. In addition, cytokines play a crucial role in the regulation of immune homeostasis(Moraga, Spangler et al. 2014).

The first ten signaling pathways of KEGG were analyzed. The first pathway is Lipid and atherosclerosis. Both type 1 and type 2 diabetes have been proved to be accelerated the independent risk factors for the development of atherosclerosis, including diabetes and closely related to the pathogenesis of atherosclerosis, the connection of diabetes and atherosclerosis known pathological mechanism including dyslipidemia, associated with an increase in the AGE of high blood sugar, OS and inflammation (Poznyak, Grechko et al. 2020). The second channel is Salmonella infection. Diabetic retinal injury is associated with hyperglycemia, which promotes the growth and reproduction of bacteria, thus increasing the possibility of infection, which also leads to rebound hyperglycemia. Thus, the interaction between infection and hyperglycemia leads to the progression and aggravation of these diseases. Hyperglycemia impairs immune tolerance and accelerates the onset and progression of bacterial infections. Salmonella infection significantly increased the number of neutrophils in intestinal LP, with marked inflammation (Zhang, Wang et al. 2019). It has been reported that type 1 diabetes can be prevented and reversed by oral administration of msbB mutants of Salmonella (Cobb, Rawson et al. 2021). The third pathway is Fluid shear stress and atherosclerosis. Fluid shear stress (FSS) within the physiological range can stabilize the vessel, but prolonged low or high flow results in inward or outward remodeling of the vessel wall, respectively. Arterial regions with bifurcation, curvature, or valves have weak flow with complex directional changes that predispose them to atherosclerotic lesions. Occlusion due to atherosclerosis can also result in very high flow in the stenosis area and low flow downstream, thus affecting disease progression. Atherosclerosis may be considered as an ineffective remodeling of blood vessels that occurs in the interfered shear region in an inflammatory environment, while diabetic retinal injury leads to angiogenesis, neovascularization, and vascular inflammation (Baeyens, Bandyopadhyay et al. 2016). The fourth pathway is age-rage signaling pathway in diabetic complications. Age-rage signaling Pathway is one of the fibrosis signaling pathways. AGE/RAGE activation stimulates the secretion of many pro-fibrotic growth factors, promotes the increase of collagen deposition, leads to tissue fibrosis, and increases the expression of RAGE (Zhao, Randive et al. 2014). AGE/RAGE mediated vascular calcification as a key area of diabetes complications, AGE/RAGE signaling has been shown to increase OS by activating NOX-1 and decreasing SOD-1 expression, thereby promoting diabetes-mediated vascular calcification(Kay, Simpson et al. 2016). OS plays an important role in the pathogenesis of diabetic vascular complications. It is well known that the accumulation of advanced glycation end products (AGEs) and the activation of AGEs receptors (RAGE) induce sustained OS in vascular tissue (Wang, Zhang et al. 2019). Diabetic nephropathy is also a common complication of diabetes, and the binding of AGE receptor (RAGE) with its ligand causes OS and chronic inflammation in renal tissue, ultimately leading to renal function loss (Sanajou, Ghorbani Haghjo et al. 2018). The fifth pathway is nod-like receptor signaling pathway. Fibrosis is the ultimate common pathway for inflammatory diseases of various organs. Inflammasome as innate immune receptor plays an important role in the progression of fibrosis. There are four main members of inflammasome, such as NOd-like receptor protein 1 (NLRP1), Nod-like receptor protein 3 (NLRP3), and Nod-like receptor C4 (NLRC4). (Zhang, Chen et al. 2021). Nod-like receptors, NLRP3, have been reported to be STZ-induced DR in rats Increased vitreous expression in

patients with retinopathy. These results suggest that Nod-like receptors are particularly important for the development of DR and may be an important factor in retinal cell death and retinal function loss. The sixth passage was Shigellosis. Shigellosis is a clinical syndrome caused by shigellosis invading the epithelium of the terminal ileum, colon and rectum. Shigella multiplies within the colon epithelial cells, causing cell death and lateral diffusion to infect and kill adjacent epithelial cells, causing mucosal ulcers, inflammation and bleeding. Treatment of shigellosis inflammatory response can alleviate the inflammatory response of DR (Niyogi 2005). The seventh pathway is Kaposi sarcoma-associated Herpesvirus infection. Kaposi's sarcoma-associated herpes virus is the cause of several malignant tumors of endothelial and B cell origin (Fröhlich and Grundhoff 2020). KSHV infection is associated with certain types of inflammation. In addition, KSHV hijacks and blocks the host's immune response by using, for example, extracellular vesicles (evs), which carry a variety of molecules that can be transferred from cell to cell and represent an important mode of cell-to-cell communication (Barrett, Dai et al. 2021). Studies have found that people with diabetes are often prone to pathogen infection and tumor progression. High glucose significantly increased the cleavage and reactivation of KSHV in latent infection cells, among which, high glucose activated transcription factor AP1 was the key to the cleavage and reactivation of KSHV. High glucose enhances the susceptibility of various target cells to KSHV infection, especially in endothelial and epithelial cells, where levels of specific cell receptors that KSHV enters, including integrin α3β1 and xCT/CD98, are elevated under high glucose, which is associated with increased cell susceptibility to infection (Chang, Yang et al. 2017). The eighth pathway is TNF Signaling Pathway. Tumor necrosis factor (TNF), also known as TNFα, is a tumor suppressor and pro-inflammatory mediator, and participates in systemic inflammatory response, which is one of the cytokines responsible for the acute phase response. By inhibiting TNF-α, DR can be reduced. The ninth pathway is toll-like receptor signaling pathway. Toll-like receptors (TLR), protective immune sentinels, are transmembrane pattern recognition receptors known for their role in innate immunity, used to detect and defend against microbial pathogens. TLRs guide immune and developmental programs by activating downstream signaling pathways, usually through activation of the NF-κB pathway. Toll-like receptor involvement in post-induced mitochondrial DNA (mtDNA) synthesis is critical for NLRP3 signaling, which has been reported to be an important factor leading to retinal cell death and loss of retinal function. The tenth pathway is Hepatitis B. In hbV-infected adults, diabetes is associated with progression of serious liver outcomes, including cirrhosis, HCC, and death, and diabetic retinal injury is a subset of diabetes. There is increasing evidence that adult populations, particularly those with DM, may benefit from HBV vaccination (Younossi, Kochems et al. 2017).

Conclusions

In this study, we explored the key genes, pathways, BP, CC and MF, and Inpp5 and Nsmce1 are the related effects of rhotinine in the treatment of diabetic retinal injury.

Declarations

Acknowledgement:

Translational study of microrNA-target gene regulatory network in stroke and acute brain injury. Major Science and Technology Project of Sichuan Province (in the field of social development), Project No. 2020YFS0043, fund 1 million, period 2020.1-2021.12, principal: Wang Tinghua

Ethical approval

All procedures were performed in accordance with the guidelines and approval of the Ethics Committee of the Kunming Medical University. Approved by the Animal Experiment Ethics Review Committee of Kunming Medical University, the approval number is KMMU20220854

Consent to participate:

No human studies are involved

Publicly agree:

I declare that all authors agree to publish.

Acknowledgement: Translational study of microrNA-target gene regulatory network in stroke and acute brain injury. Major Science and Technology Project of Sichuan Province (in the field of social development), Project No. 2020YFS0043, fund 1 million, period 2020.1-2021.12, principal: Wang Tinghua

Author contribution statement:

WTH and GXL conceived and designed the study. SLL and CJL conducted experiments,LN analyzed data and wrote manuscripts. WTH and GXL conceived and designed the study. SLL and CJL conducted experiments,LN analyzed data and wrote manuscripts. All authors have read and approved the manuscript. The authors state that all data were generated internally and no paper mills were used.

Conflict of Interest: There is no Conflict of interest in this study.

Availability of data and materials:

I declare that the data and materials contained in this manuscript have not been published elsewhere and are available.

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Figures

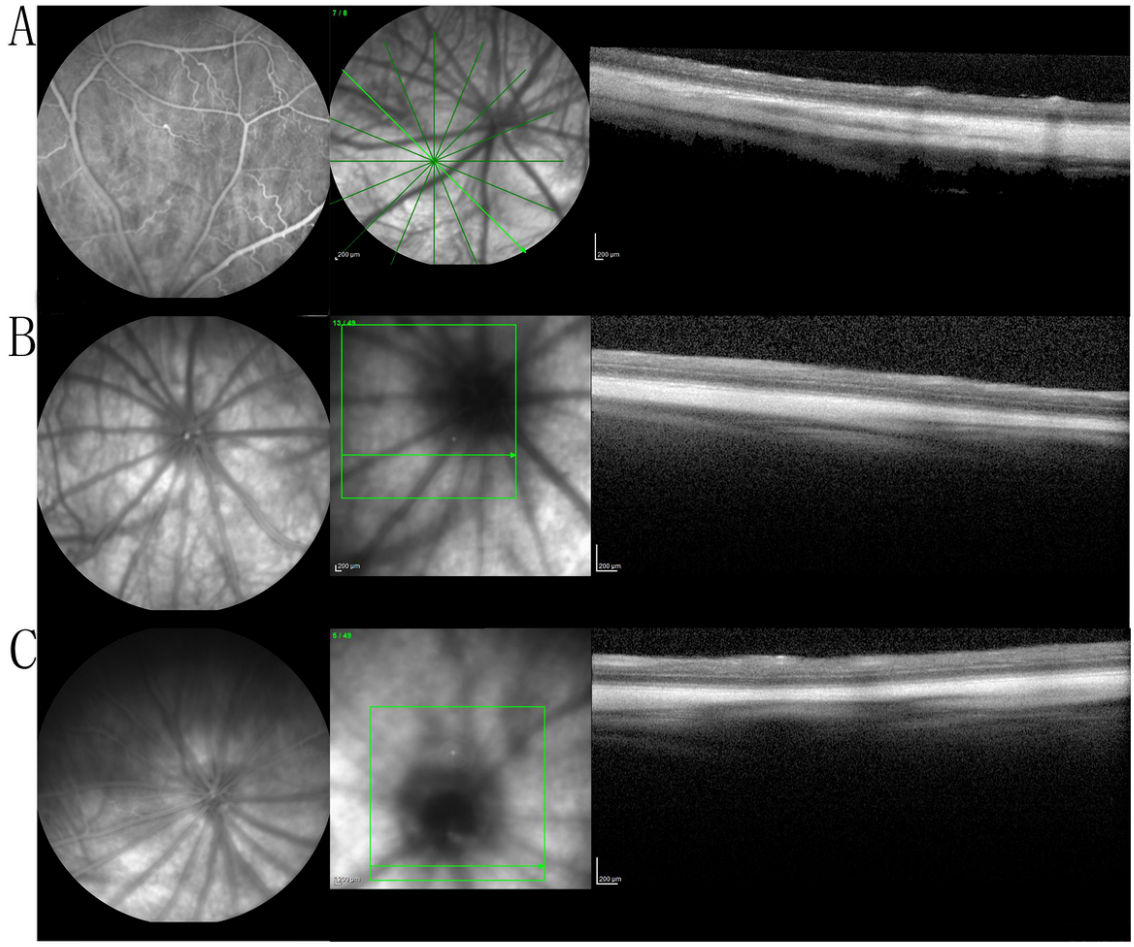

Figure 1

Images of OCT fundus in Sham, DM, and SAL groups.

A. The OCT images of Sham group. **B.** The OCT images of DM group. **C.** The OCT images of SAL group. (DM group, rats with diabetes mellitus; SAL group, rats with diabetes mellitus were treated with salidroside.)

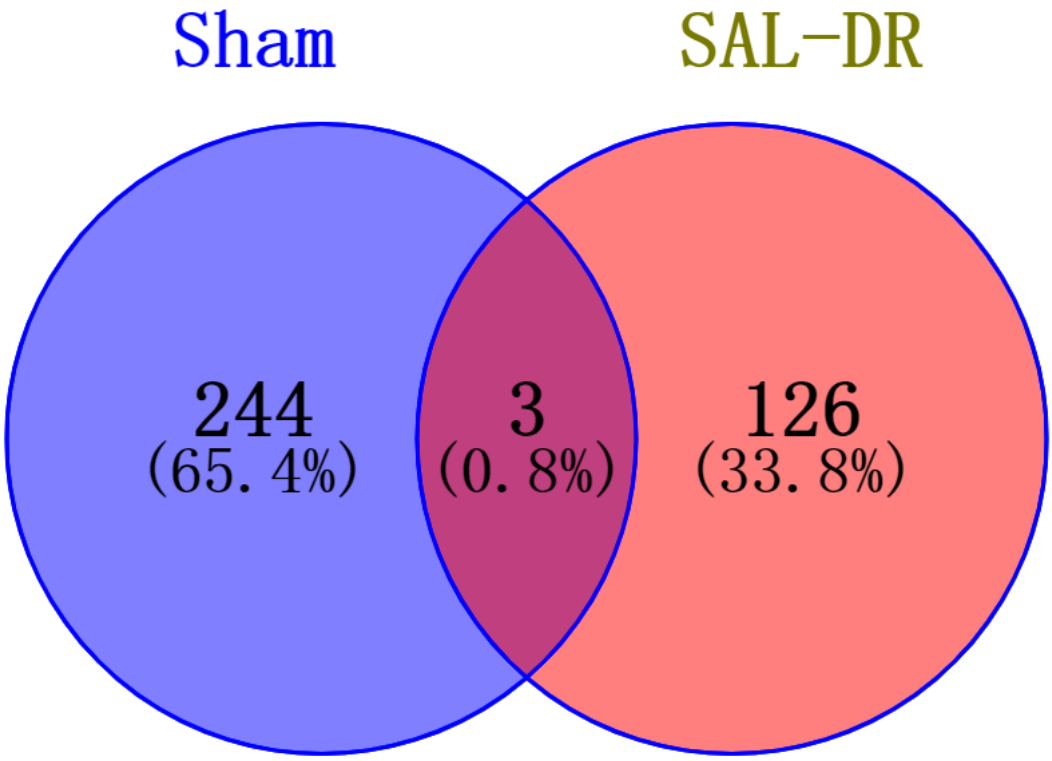

Figure 2

Comparison of Latency to targets of rats in each group at 1 d, 3 d, and 5 d after modeling.

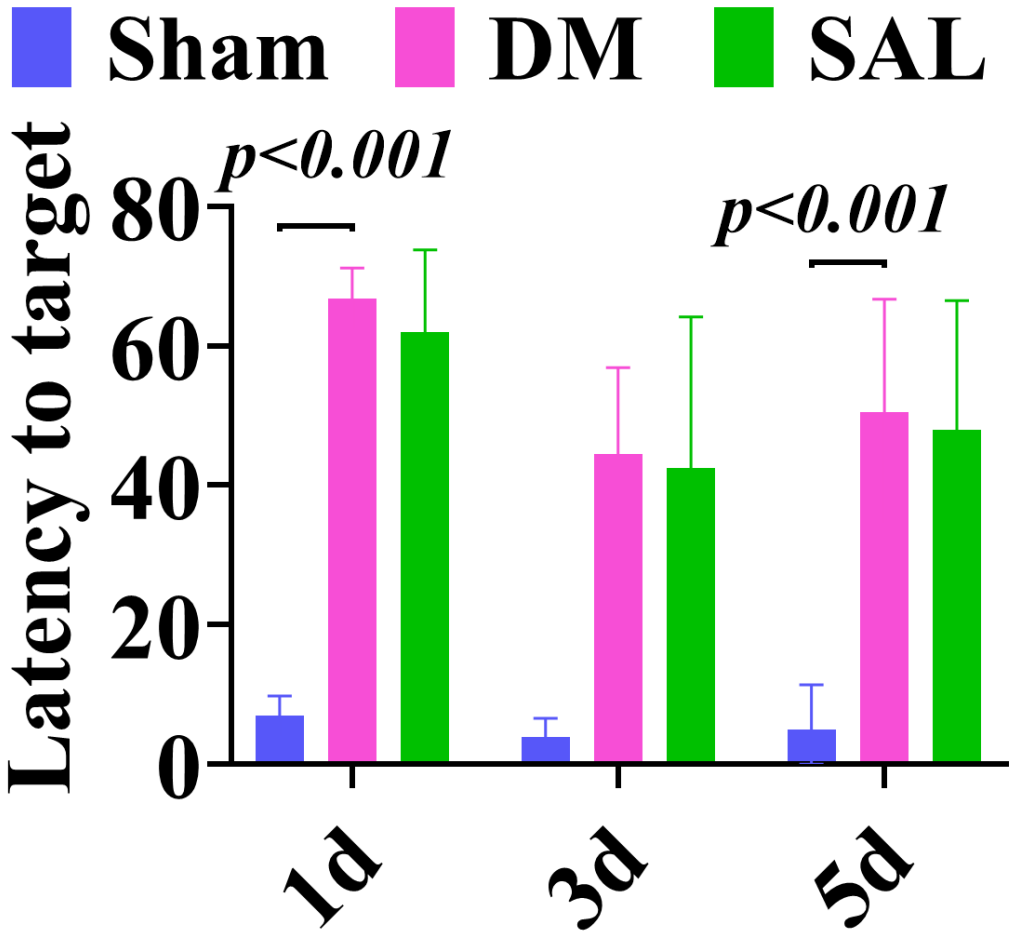

Figure 3

Venn diagram of shared genes between the salidroside and diabetic retinopathy.

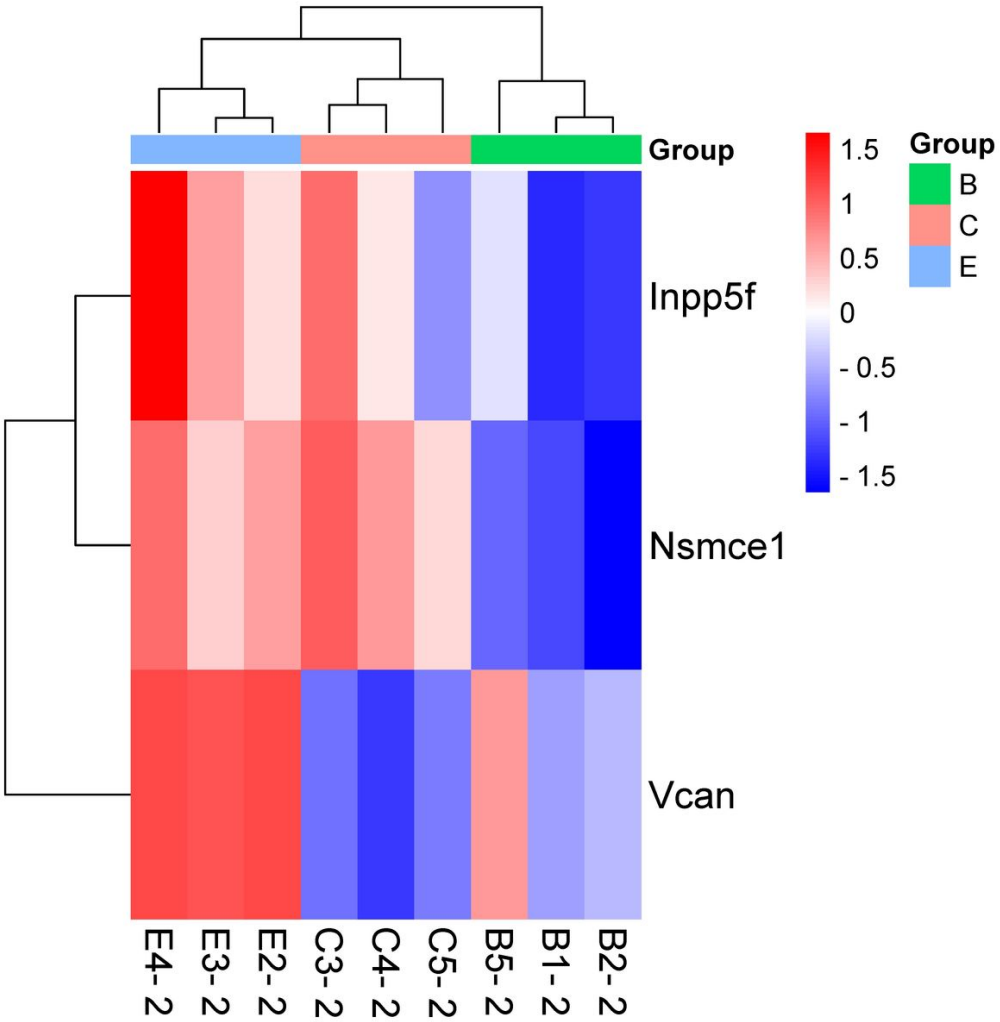

Figure 4

Expression level of Inpp5f, Nsmce1, and Vcan in each group.

Figure 5

Molecular docking of Nsmce1 and Inpp5f with salidroside.

A. Molecular docking of Inpp5f with salidroside. B. Molecular docking of Nsmce1with salidroside.

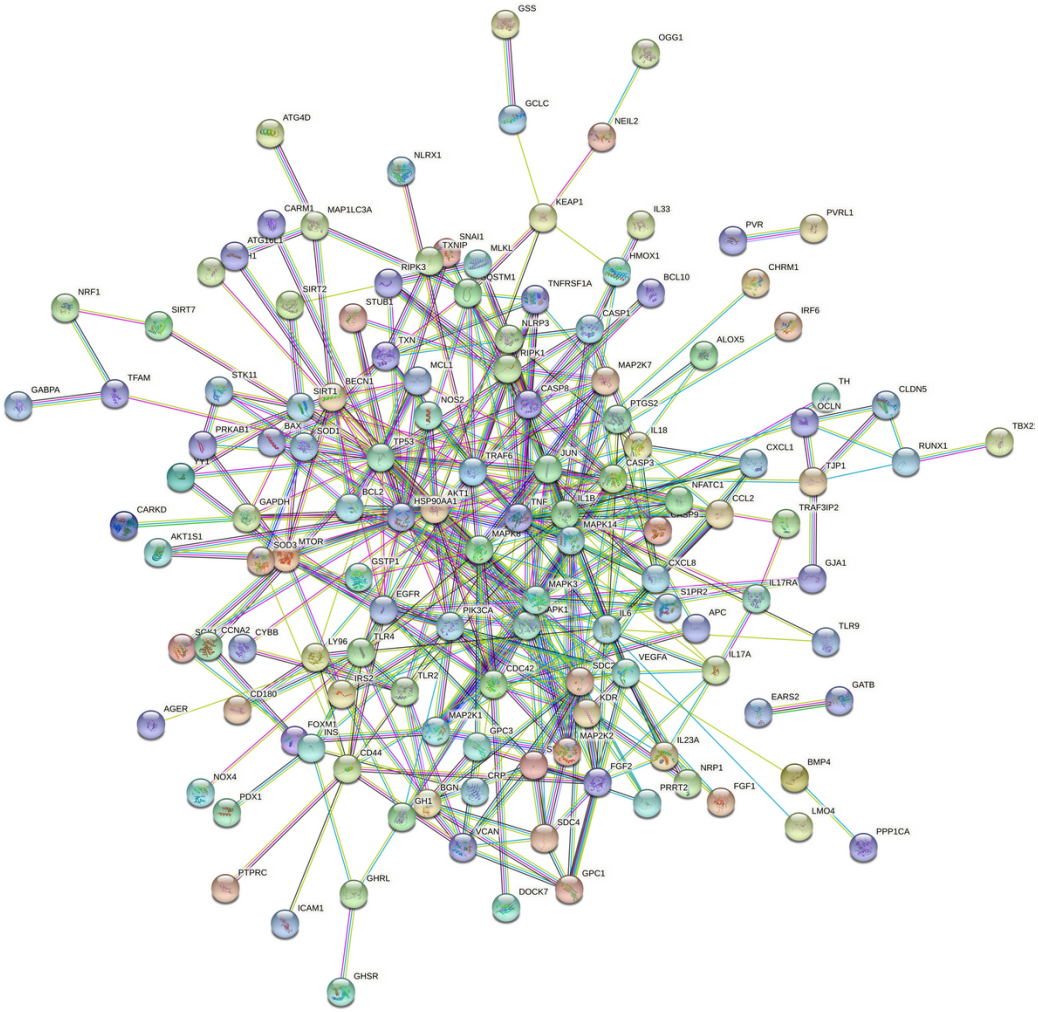

Figure 6

Protein-protein interaction network of genes.

Figure 7

GO analysis.

A. Top 20 biological processes (BP) involved in GO analysis. B. Top 20 cellular components (CC) involved in GO analysis . **C.** Top 20 molecular function (MF) involved in GO analysis. **D.** The circular diagram of BP, CC, and MF involved in GO analysis.

Figure 8

KEGG signaling pathway diagram