Research Square

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Based on network pharmacology and molecular docking technology, to explore the molecular mechanism of Sedum sarmentosum affecting atherosclerosis by interfering with MMP-2 related signal pathway

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Abstract

By matching 333 Sedum sarmentosum targets with 2072 atheromatosis targets, 175 overlapping targets were screened, and MMP-2 was determined as one of the core targets. Gene functions are mainly involved in lipid metabolism, energy pathways, signal transduction, and so on. PI3K-Akt signaling pathway, Ras signaling pathway, Arachidonic acid metabolism, Rap1 signaling pathway, etc. may be the key pathways for Sedum sarmentosum treatment of atheromatosis. Sedum sarmentosum has great potential to be developed as a drug and targets various genes and pathways to play a role in the treatment of atheromatosis.

Full Text

Atherosclerosis (AS) is a chronic inflammatory disease of the vascular wall, and it can cause vascular endothelial cell dysfunction, smooth muscle proliferation and migration, and activate macrophages. It is one of the most common potential causes of coronary atherosclerotic heart disease, carotid artery disease and peripheral artery disease, and its main causes are lipid metabolism disorders and immune dysfunction [1-2].Modern studies have revealed that its pathogenesis is closely related to endothelial cell injury, increase of adhesion factors, migration of macrophages, foam cell formation, and smooth muscle cell proliferation [3]. Although there are many in-depth studies in the field of prevention and treatment of AS, it is still one of the main causes of human death [4-5]. The current mainstream strategy for the treatment of AS is statin therapy, but it often causes adverse reactions such as liver injury and myalgia [6]. Therefore, it is of great significance to research and develop new drugs for the treatment of AS.

The medicinal use of natural plants has spread all over the world. Currently, it has been proved that about 70,000 species of plants have medicinal value [7]. Sedum sarmentosum is the dry whole grass of Sedum sarmentosum. In ancient China, Sedum sarmentosum was commonly used to treat jaundice and hepatitis [8]. Modern pharmacological studies have found that Sedum sarmentosum has unique advantages in the treatment of liver diseases, and has been widely used in clinical treatment of viral hepatitis, fatty liver disease, drug-induced liver injury, etc. However, the current research on Sedum sarmentosum is mostly focused on liver disease. There are still few studies on Sedum sarmentosum in the treatment of AS, and the specific mechanism is still unclear.

MMP-2(matrix metalloproteinase2，MMP-2) is closely related to AS. The lesions of AS-affected arteries begin from the vascular endothelium [9]. Once being stimulated by risk factors such as smoking, hypertension, inflammation, unhealthy eating habits, insulin resistance, etc., the surface of vascular endothelial cells can express adhesion molecules. Adhesion molecules combine with monocytes to enter the vascular intima, and under the action of ECM, they are transformed into macrophages, which absorbs fat granules, thereby releasing a variety of growth factors and inflammatory factors to jointly induce the production of MMPs. Matrixmetalloproteinases (MMPs) are a group of structurally and functionally related zinc ion-dependent endopeptidases. MMPs have the ability to degrade various basement membranes; they are a group of important extracellular matrix (ECM) degrading enzymes in the human

body; a zinc ion-dependent proteolytic enzyme that plays a key role in tumor cell-mediated ECM degradation. Exposure to lipids and oxygenated LDL-induced monocytes-macrophages infiltrate massively in the shoulder of the plaque fibrous cap and produce MMPs [10]. MMPs disrupt the dynamic balance of ECM synthesis and degradation. At the same time, excessive degradation of plaque fibrous cap matrix and excessive mechanical tension result in increased plaque fibrous cap instability and easy rupture. MMPs play an important role in regulating the process of atherosclerotic lesions [11], and their content and activity are closely related to the increase and decrease of plaque area [12]. Some studies have also revealed that the growth of unstable plaques is caused by a combination of factors, including a complex cholesterol infiltration, inflammatory cell infiltration, and vascular smooth muscle cell migration. The role of MMPs on endothelial and vascular smooth muscle cell migration in the initiation and progression of atherosclerosis has been demonstrated, thus increasing the inhibitory effects of these proteases in plasma [13]. MMP-2, as a kind of MMPs, is particularly related to plaque instability [14]. MMP-2 is involved in unstable plaque formation, ECM remodeling, and cell infiltration or migration. The dysregulation of ECM metabolism affects vascular remodeling, thereby leading to vascular remodeling. When AS occurs, local hypoxia, oxidized low-density lipoprotein, and local plaque-produced factors (tumor necrosis factor, interleukin, etc.) can induce endothelial cells and smooth muscle cells to secrete MMP-2 [15-16]. MMP-2 will degrade the basement membrane of AS plaques, thereby decreasing the endothelial cell defense function and damaging the vascular wall. Vascular smooth muscle cells can form unstable plaques in the intima area, and MMP-2 will degrade the ECM in the intima area, thereby accelerating the unstable plaques [17]. Decreased synthesis and increased degradation of ECM are the main internal causes of plaque rupture. And the degradation of ECM by matrix metalloproteinase 2 (MMP-2) is an important factor in the instability of atherosclerotic plaques. [18]. MMP-2 promotes the degradation of arterial ECM and the fibrous cap of atherosclerotic plaques, thus resulting in the thinning of the fibrous cap of atherosclerotic plaques, plaque instability and rupture [19-20]. In addition, the mechanical tension of the vascular wall can increase the mRNA expression of MMP-2 and the release of its zymogen through the mediation of reduced nicotinamide adenine dinucleotide phosphate [21]. The changes in pressure and shear stress on the side of the vessel wall are important reasons for the secretion of MMP-2 [22].

Network pharmacology is a new pharmacological research method that integrates chemoinformatics, bioinformatics, biological networks, network analysis and traditional pharmacology [23]. According to network pharmacology, drugs do not only act on one target, but produce effects through the interaction of multiple targets, which coincides with the idea of traditional Chinese medicine (TCM). Therefore, analyzing the pharmacological effects of active components of TCM through network pharmacology can provide great help in this field [24], and it is of great significance to predict the mechanism of action of TCM with multiple components, multiple targets, and multiple pathways [25]. In this study, network pharmacology and molecular docking methods were adopted to study the molecular mechanism of Sedum sarmentosum's intervention in AS, and provide reference for its clinical medication and drug development.

1. Materials And Methods(Fig. 1)

1.1 Materials

1.1.1 Database

TCMSP database (http://tcmspw.com/tcmsp.php), Swiss Target Prediction database (http://www.swisstargetprediction.ch/), Disgenet database (https://www.disgenet.org/), Genecards database (https://www.genecards.org/), Drugbank database (https://go.drugbank.com/), STRING database (https://string-db.org/), Uniprot database (https://go.drugbank.com/) www.uniprot.org/), Venny2.1 online software mapping tool platform (https://bioinfogp.cnb.csic.es/tools/venny/), Cytoscape3.7.2 software, R3.6.1 software, etc.

1.2 Methods

1.2.1 Screening of effective and active ingredients of Sedum sarmentosum

The ingredients of Sedum sarmentosum were searched in the TCMSP database as potential active ingredients.

1.2.2 Screening of common drug-disease targets

The structure of the above components was obtained using the TCMSP database and imported into the Swiss Target Prediction database, and then the targets with a prediction score greater than 0 were taken as drug targets. At the same time, the targets of related components were obtained from TCMSP, and the target names were standardized and unified using the Uniprot database. With "atheromatosis" as the keyword, Disgenet, Genecards, and Drugbank databases were used to obtain disease targets. Drug targets and disease targets were input on the Venny2.1 online software mapping tool platform, Venn diagrams were drawn, and common drug-disease targets were obtained after the intersection of the two.

1.2.3 Construction and analysis of TCM-component-target-disease network

Cytoscape 3.7.2 software was used to constructe a "drug-ingredient-target-disease" network diagram. The Network Analyzer was used to analyze the main active ingredients of Sedum sarmentosum, and perform topology analysis on network graphs. The Degree value indicates the number of association between the component and the target: The larger the Degree value is, the more important the component is.

1.2.4 Construct a protein-related interaction network diagram (PPI)

The above 175 common targets were entered into the STRING database for retrieval. The protein species was set to "Homo sapiens" and the minimum interaction threshold was 0.4. The network relationship data of target interaction were obtained and imported into Cytoscape software. A protein interaction network diagram was drawn, where the size, color, and shade of nodes represent the magnitude of the Degree value.

1.2.5 Core target screening based on topology analysis:

The PPI network was imported into Cystoscap 3.7.2. Topology analysis was performed by Network Analyzer. By sorting by Degree, the genes whose degree value is greater than the average score were selected as core targets. The top 30 targets were plotted as bar graphs using R 3.6.1.

1.2.6 Core target screening based on cluster analysis

The constructed PPI network was imported into Cytoscape 3.7.2, and the MCODE module was used to analyze gene clusters and screen core targets.

1.2.7 GO functional enrichment analysis and KEGG pathway enrichment analysis of core targets

Based on R software, Bioconductor bioinformatics software package was used, with P value < 0.05 and Q value < 0.05, to carry out GO and KEGG functional enrichment analysis of key target genes, and output the results in the form of bubble charts.

1.2.8 Active ingredient-core target molecular docking for the Sedum sarmentosum treatment of AS

According to the small molecule MOL ID, the 3D structure of the small molecule mol2 format was downloaded from the TCMSP database, namely the ligand small molecule. The key target proteins, namely receptor proteins, were downloaded from the PDB (http://www.rcsb.org/) database. AutoDock Vina1.1.2 was used for docking, and the best-binding small molecules for each protein were selected and visualized using Pymol software (https://pymol.org/2/).

2. Results

2.1 Sedum sarmentosum Effective Active Ingredients

In the TCMSP database, OB≥30% and DL≥0.18 were set, the active ingredients of Sedum sarmentosum were screened, and a total of 5 potential active ingredients were obtained (Table. 1).

2.2 Screening of common drug-disease targets

A total of 333 drug targets were screened using the Swiss Target Prediction database and the TCMSP database. With "atheromatosis" as the key word, the databases of Disgenet, Genecards and Drugbank were searched, and 2072 disease targets were obtained after deduplication. Totally, 333 drug targets and 2072 disease targets were input on the Venny2.1 online software mapping tool platform, and a Venn diagram was drawn (Fig. 2A). After the intersection of the two, 175 common drug-disease targets were obtained (Table 2).

2.3 Construction and analysis of TCM-component-target-disease network

The 5 potential active ingredients and 175 common drug-disease targets in Sedum sarmentosum were input into Cytoscape software, and a network diagram of "drug-ingredient-target-disease" interaction was drawn (Fig. 2B). The purple in the figure represents the drug, the green is the 5 active ingredients in Sedum sarmentosum, the blue marks the 175 common targets, and the red refers to the disease (Fig. 2C). Network Analyzer was used to perform topology analysis on network graphs. The Degree value indicates the association between the component and the target: The larger the Degree value is, the more important the component is. The degrees of active ingredients from high to low are as follows: DFV (degree=53), isorhamnetin (degree=46), luteolin (degree=44), quercetin (degree=44), and beta-sitosterol (degree=26). These ingredients may be the key active ingredients of Sedum sarmentosum in the treatment of AS.

2.4 Construction of the PPI network

The PPI network was imported into Cystoscap 3.7.2, and the topological analysis was performed through the NetworkAnalyzer tool. Degree was used for sorting, and the genes whose Degree value was greater than the average score were selected as the core targets AKT1, VEGFA, PTGS2, SRC, EGFR, MMP9, ESR1, PIK3CA, CYP3A4 , AR, KDR, PPARG, MMP-2, IGF1R, SERPINE1, MPO, APP, F2, PLG, CYP2C9, CYP19A1, ALOX5, Sedum sarmentosumG2, AHR, PTGES, HNF4A, PGF, GSK3B, MCL1, PTGS1 (Fig. 2D).

2.5 Core target screening based on cluster analysis

The constructed PPI network was imported into Cytoscape 3.7.2, and the MCODE module was used to analyze the gene clusters and screen core targets. A total of 6 gene clusters and 6 core genes were obtained, and the core genes were FOS, CASP3, PTGS1, PIK3CB, ACACA, and PLAT (Fig. 2E).

2.6 GO functional enrichment analysis and KEGG pathway enrichment analysis of key targets

The 175 common targets were run by R language and analyzed by GO to select three parts, namely biological process, cellular components and molecular functions. The GO results showed that the intersection gene set was enriched to 2300 biological processes, mainly including cellular response to peptide，steroid metabolic process，regulation of inflammatory response，epithelial cell proliferation，response to peptide hormone，regulation of small molecule metabolic process，cellular response to peptide hormone stimulus，fatty acid metabolic process，response to steroid hormone，positive regulation of response to external stimulus，regulation of MAP kinase activity，lipid localization，regulation of lipid metabolic process，gland development，response to oxidative stress，leukocyte migration，cellular response to steroid hormone stimulus，protein kinase B signaling，lipid transport and so on (Fig.3A).

The intersection gene set was enriched to 38 cell components in the expression process, mainly involving vesicle lumen，transcription factor complex，cytoplasmic vesicle lumen，transferase complex, transferring phosphorus-containing groups，secretory granule lumen，nuclear envelope，membrane raft，membrane microdomain，membrane region，RNA polymerase II transcription factor complex，nuclear transcription factor complex，extrinsic component of membrane，protein kinase complex，platelet alpha granule lumen，platelet alpha granule，mast cell granule，phosphatidylinositol 3-kinase complex and so on (Fig.3B).The

intersection gene set was enriched to 203 processes related to molecular functions, mainly including nuclear receptor activity、transcription factor activity, direct ligand regulated sequence-specific DNA binding、steroid hormone receptor activity、heme binding、tetrapyrrole binding、protein serine/threonine kinase activity、endopeptidase activity、steroid binding、iron ion binding、hormone binding、protein tyrosine kinase activity、RNA polymerase II transcription factor binding、oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen、transcription coactivator activity、serine-type endopeptidase activity and so on (Fig.3C).A total of 172 KEGG pathways were obtained after running the 175 common targets through the R language, and the top 20 results formed a bar graph of KEGG function enrichment. Padjust represents the significance of enrichment: The redder the color is, the higher the significance is, mainly including PI3K-Akt signaling pathway, Ras signaling pathway, Arachidonic acid metabolism, Rap1 signaling pathway, etc. (Fig. 3D).

2.7 Sedum sarmentosum Active ingredient-core target molecular docking for the treatment of AS

Combined with the literature analysis and the above results, it is obvious that MMP-2 plays an important role in the two networks, so it can be speculated that it is the core target of Sedum sarmentosum in the treatment of AS. The five active ingredients in Sedum sarmentosum were molecularly docked with MMP-2 respectively. The docking results illustrated that the active ingredients of Sedum sarmentosum had good docking activity with the core targets. Among them, the best combination with MMP-2 was beta-sitosterol (Fig.4A). The binding energy of β-sitosterol to MMP-2 protein was -8.0kcal/mol, which proved that it had good binding effects. Beta-sitosterol interacted with MMP-2 protein, mainly through the formation of hydrogen bonds and hydrophobic forces, and formed hydrogen bonds with Thr56 (A), the length of which is 3.17 Å; it had hydrophobic effects with Phe57 (A), Met97 (A) , Leu190(A), Gly189(A), Arg98(A), Asp370(A), Pro43(A), Lys44(A), Asp370(A), Gln52(A), Leu49(A), Asp185(A)), and Tyr53 (A). Isorhamnetin also had better docking (Fig. 4B). The binding energy of isorhamnetin to MMP-2 protein was -8.2kcal/mol, which revealed that it had good binding effects. Isorhamnetin interacted with MMP-2 protein, mainly through the formation of hydrogen bonds and hydrophobic forces, and formed hydrogen bonds with Gln480 (A), the length of which is 3.03 Å; It had hydrophobic interactions with Pro527 (A), Glu525 (A) , Glu530 (A), Gly483 (A), Ala526 (A), Ser575 (A), Asn573 (A), Asp622 (A), and Ala479 (A). The binding energy of luteolin to MMP-2 protein was -8.2kcal/mol, which indicated that it had good binding effects. luteolin interacted with MMP-2 protein, mainly through the formation of hydrogen bonds and hydrophobic forces, and formed hydrogen bonds with Gln52 (A) and His193 (A). The lengths of the hydrogen bonds were 2.95Å, 3.22Å, 3.10Å, and 2.82Å; It had hydrophobic interaction with Leu190(A), Thr56(A), Asn111(A), Tyr182(A), Pro105(A), Gly103(A), Pro183(A), and Asp185(A) (Fig. 4C). The binding energy of quercetin to MMP-2 protein was -8.7kcal/mol, which proved that it had good binding effects. Quercetin interacted with MMP-2 protein, mainly through the formation of hydrogen bonds and hydrophobic forces, and formed hydrogen bonds with Asp622 (A), Asn573 (A), and Lys578 (A), and the lengths of the hydrogen bonds were 3.09 Å and 3.17 Å, and 2.94Å, respectively. It has hydrophobic effects with Ser575 (A), Phe572 (A), Trp574(A), Glu530(A), Pro527(A), Glu525(A), Gln480(A), Ala479(A), and Val621(A) (Fig. 4D). The binding energy of DFV to MMP-2 protein was -8.4kcal/mol, which demonstrated that it had good binding effects. DFV interacted with the MMP-2 protein, mainly through the formation of

hydrogen bonds and hydrophobic forces, and formed hydrogen bonds with Gln480 (A) and Asn573 (A), and the lengths of the hydrogen bonds were 2.84 Å and 3.19 Å, respectively; It has hydrophobic interactions with Ala479 (A), Asp622 (A), Trp574 (A), Ala526 (A), Pro527 (A), Glu530 (A), and Glu525 (A) (Fig. 4E). The detailed docking scores are listed in Table 3.

3. Discussion

According to the comprehensive analysis of key targets, KEGG, GO enrichment analysis, and molecular docking, Sedum sarmentosum may act on the key targets of MMP-2 through the active ingredients of Isorhamnetin, beta-sitosterol, luteolin, quercetin and DFV, regulate PI3K- Akt signaling pathway, Ras signaling pathway, Arachidonic acid metabolism, Rap1 signaling pathway, etc., and produce pharmacological effects of regulating lipid metabolism, regulating blood coagulation, protecting blood vessels, promoting angiogenesis, and inhibiting the formation of AS plaques, so as to play a role in the treatment of AS (Fig. 5).

(1) PI3K-akt and AS

Akt plays a major role in the pathogenesis of vascular remodeling. It can increase cell survival through Akt signaling. In AS, Akt protects endothelial cells by increasing the proliferation and survival of vascular smooth muscle cells [26].The PI3K/Akt/mTOR signaling pathway is widely involved in cell differentiation and growth as an important regulator. Some studies have confirmed that thispathway is closely related to atherosclerotic disease [27-28]. Selective inhibitionof Akt/mTOR signaling pathway can inhibit the progression of AS by activating macrophages, thereby improving the stability of atherosclerotic plaques [29]. The PI3K/Akt signaling pathway can regulate the important cellular biological basis of AS pathogenesis, such as vascular endothelium, vascular smooth muscle cells, platelets, and macrophages [30]. Kaempferol can alleviate ox-LDL-induced apoptosis of human umbilical vein endothelial cells by up-regulating autophagy through the PI3K/Akt/mTOR pathway [31]. Macrophages are the "key driver" of atherosclerosis development, and it affects the plaque stability and atherosclerotic outcome [32]. Macrophages are regulated by multiple signaling pathways, among which the PI3K/Akt signaling pathway plays an important role in the survival, proliferation and migration of macrophages [33-34]. PI3K is involved in the transformation of macrophages to foam cells. The inhibition of PI3K reduces modified LDL and lipid pinocytosis [35-37]. The inhibition of the PI3K-Akt pathway can significantly down-regulate the expression of lipoprotein lipase (LPL) in macrophages, reduce lipid uptake by macrophages and the level of intracellular lipid accumulation, and delay cell foaming, with anti-AS progression [38]].

(2) Ras, Rap1 and AS

The two axes of RAS, namely ACE/AngII/AT1R axis and ACE2/Ang-(1-7)/Mas axis, play key regulatory roles in AS, hypertension, and aneurysm occurrence, development and formation. Among them, AngII can increase blood pressure and increase the incidence of atherosclerosis. Ang-(1-7)/Mas pathway can effectively reduce the occurrence of atherosclerosis, play antagonistic effects on AngII, and play the role of vasodilation, anti-proliferation, anti-inflammatory and anti-fibrosis. The activation of the ACE2Ang-(1-

7)/Mas axis reduces inflammatory cell function. The inflammatory development process of AS can be alleviated by reducing the expression of various inflammatory factors. Because Ang-(1-7) is not resistant to proteolytic enzymes and has a limited role in clinical applications, the activator with the same efficacy highlights its important medical value. The currently known CGEN-856S and AVE0991 can activate the Ang-(1-7)/Mas pathway, while A779 and D-Pro-angiotensin-(1-7) are Ang-(1-7) inhibitors. AR244555 is an inverse agonist of the Mas receptor, and it plays an important role in the study of RAS. When Ras binds to GTP protein and is activated, it can activate the MAPK cascade reaction, enter the nucleus to participate in the phosphorylation modification of other kinases or transcription factors, and induce gene transcription related to cell cycle progression or other cellular processes. Monocytes invade the arterial intima and differentiate into macrophages and begin to absorb lipids to form foam cells. It was found that the MAPK cascade can promote the formation of foam cells [39]. In most cases, it does not regulate cell function alone, but interacts with other signaling pathways (such as PI3K/Akt/mTor, Wnt/b-catenin pathway) to regulate inflammation, cell adhesion and other processes in the process of atherosclerosis. Rapl, a member of the RAS superfamily, is a class of proteins associated with cell adhesion, with unique ability to trigger cell polarization, enhance cell motility, and contribute to adhesion interactions with antigen-presenting cells and vascular endothelial cells [40].

(3) Arachidonic acid and AS

When cells are stimulated, arachidonic acid is decomposed into free form under the action of phospholipase and released into the cell fluid, and then hundreds of biologically active metabolites are formed under the action of a series of metabolic enzymes. At least three types of enzymes are known to be involved in the metabolism of arachidonic acid, including cyclooxygenase (COX), lipoxygenase (LOX) and cytochrome P450 (cytochromeP450, CYP). Arachidonic acid is metabolized under the action of COX to generate prostaglandin G, which in turn generates prostaglandin H:, and uses this as a substrate to generate other types of prostaglandin products, including prostacyclin (PGI), prostaglandin E: (PGE) :), prostaglandin D2 (PGD:), thromboxane A2 (thromboxane A, TXA) and so on. The specific type of prostaglandins produced is related to the type and state of cells. The activities of PGI and TXA have opposite effects. Under normal physiological conditions, the levels of TXA and PGI in circulating blood are in a relatively balanced state, which is one of the important factors to maintain smooth blood circulation. If the TXA/PGI level is out of balance, a variety of inflammatory factors will be released, which would damage the blood vessel wall, induce platelet aggregation, and initiate the formation of AS [41]. In human AS plaques, MMP-2 and MMP-9 play a major role, and macrophages can produce MMP-2 and MMP9 through a PGE/cAMP-dependent pathway [42].

(4) MMP-2 and PI3K-akt

PI3K/AKT/FoxO1 is the upstream regulatory signal of MMPs, and it has been confirmed that PI3K/AKT/FoxO1 can regulate the expression of MMPs in various cells [43-44].

(5) MMP-2 and Ras

Activated Ras clearly induced CDCP1, whereas CDCP1 knockdown or inhibition of CDCP1 phosphorylation by Src-directed treatment abrogated activated Ras-induced anoikis resistance, migration, and invasion. In a Ras-induced invasion model, it has been found that the activation of MMP-2 and secretion of MMP9 is regulated by the induction of phosphorylated CDCP1 [45].

4. Conclusion

Network pharmacology was used to predict the potential mechanism of Sedum sarmentosum for the treatment of AS and its potential for drug development. The mechanism of Sedum sarmentosum treatment of AS may be related to the core gene expression of its active ingredients, including Isorhamnetin, beta-sitosterol, luteolin, quercetin and DFV intervention MMP-2. The signaling pathways involved in this process mainly include PI3K-Akt signaling pathway, Ras signaling pathway, Arachidonic acid metabolism, and Rap1 signaling pathway. These results may help guide further researches on the targets of Sedum sarmentosum in the treatment of AS. Network pharmacology has the potential to identify compounds from TCM and develop them into drugs and compound-related target genes. However, databases used in network pharmacology may not recruit all known or all potential targets to address difficulties in data acquisition. If the development of databases are more comprehensive and available, the way in which targets related to compounds or diseases are obtained using network pharmacology will be more accurate. Most importantly, due to limited funding, the experiments could not be further verified, so the predicted target genes and therapeutic pathways still need further clinical and experimental verification.

Declarations

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Authors' contributions

Zhe Liu, Qintong Yang and Yudong Fang planned and designed the study.

Data collection was done by Zhe Liu, and Zhe Liu analyzed the data, and Qintong Yang and Yudong Fang interpreted the data. Zhe Liu and Yudong Fang were the lead-authors in writing the draft and revising the manuscript. Yudong Fang were responsible for critical review and revision of the manuscript for important intellectual content and final approval of the version to be published. All authors read and approved the final manuscript.

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The data that support the findings of this study are available.

Ethics approval and consent to participate

Not applicable

Consent for publication

All authors have seen and approved the manuscript and have contributed signifcantly to the paper and agree with its submission.

Competing interests

The authors have disclosed no potential competing interest, fnancial or otherwise.

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Tables

Table 1.

Mol ID	Molecule name	OB (%)	DL
MOL001792	DFV	32.76	0.18
MOL000354	isorhamnetin	49.6	0.31
MOL000358	beta-sitosterol	36.91	0.75
MOL000006	luteolin	36.16	0.25
MOL000098	quercetin	46.43	0.28

Table 2 is available in the Supplementary Files section.

Table 3.

Molecule ID	Molecule name	Affinity(kcal/mol)
MOL000006	luteolin	-8.3
MOL000098	quercetin	-8.2
MOL000354	isorhamnetin	-8.2
MOL000358	beta-sitosterol	-8.5
MOL001792	DFV	-8.4

Figures

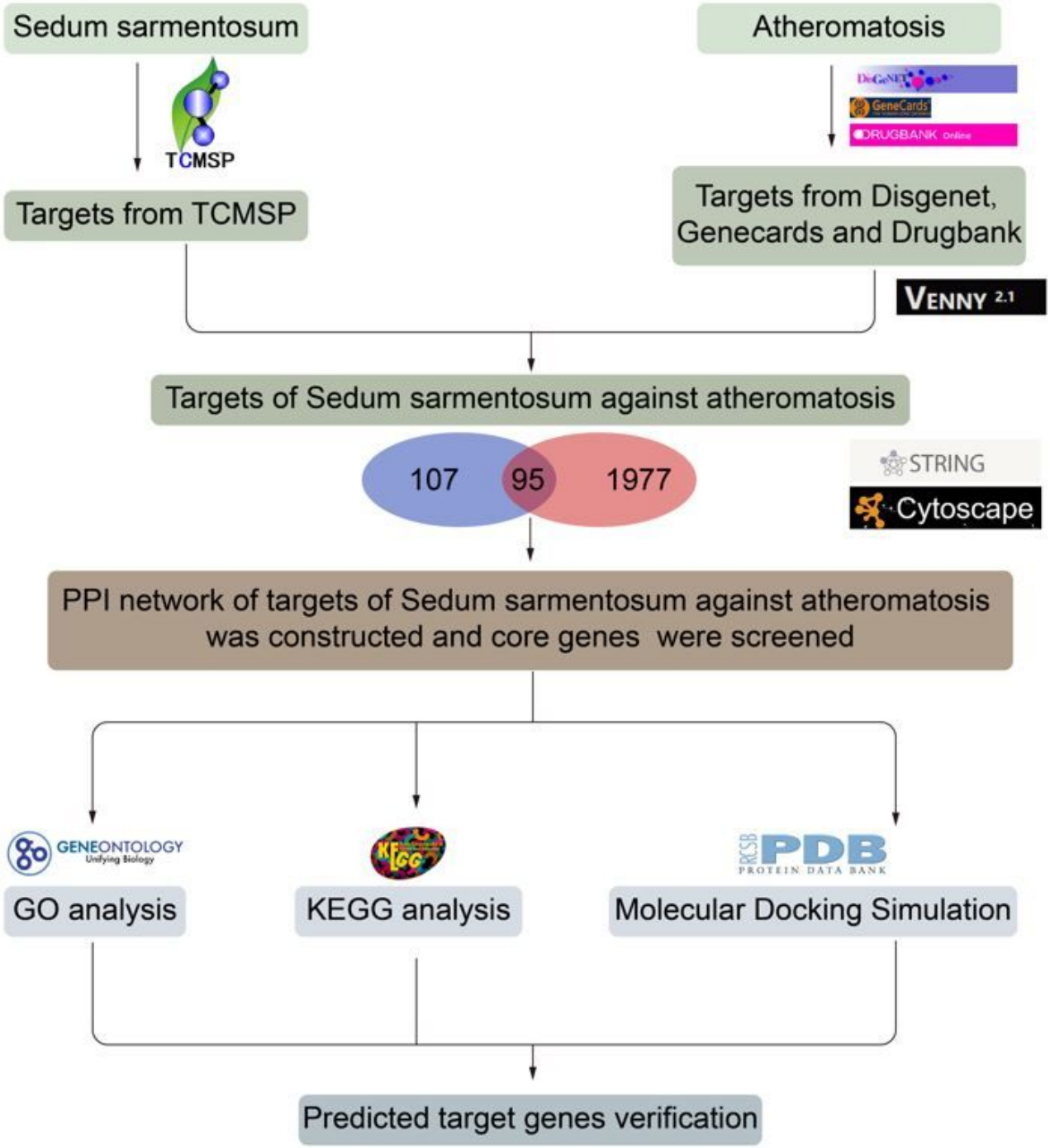

Figure 1

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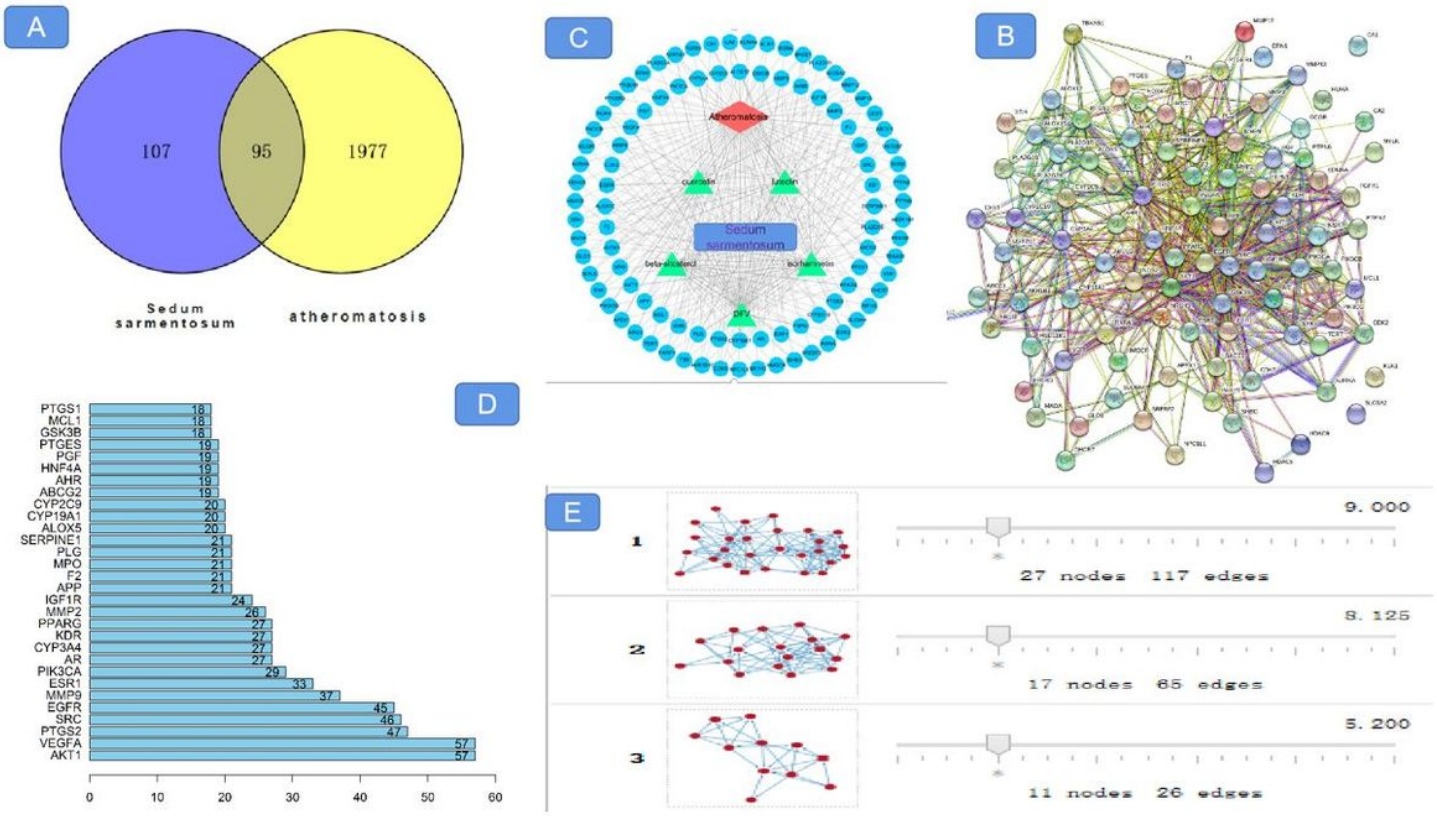

Figure 2

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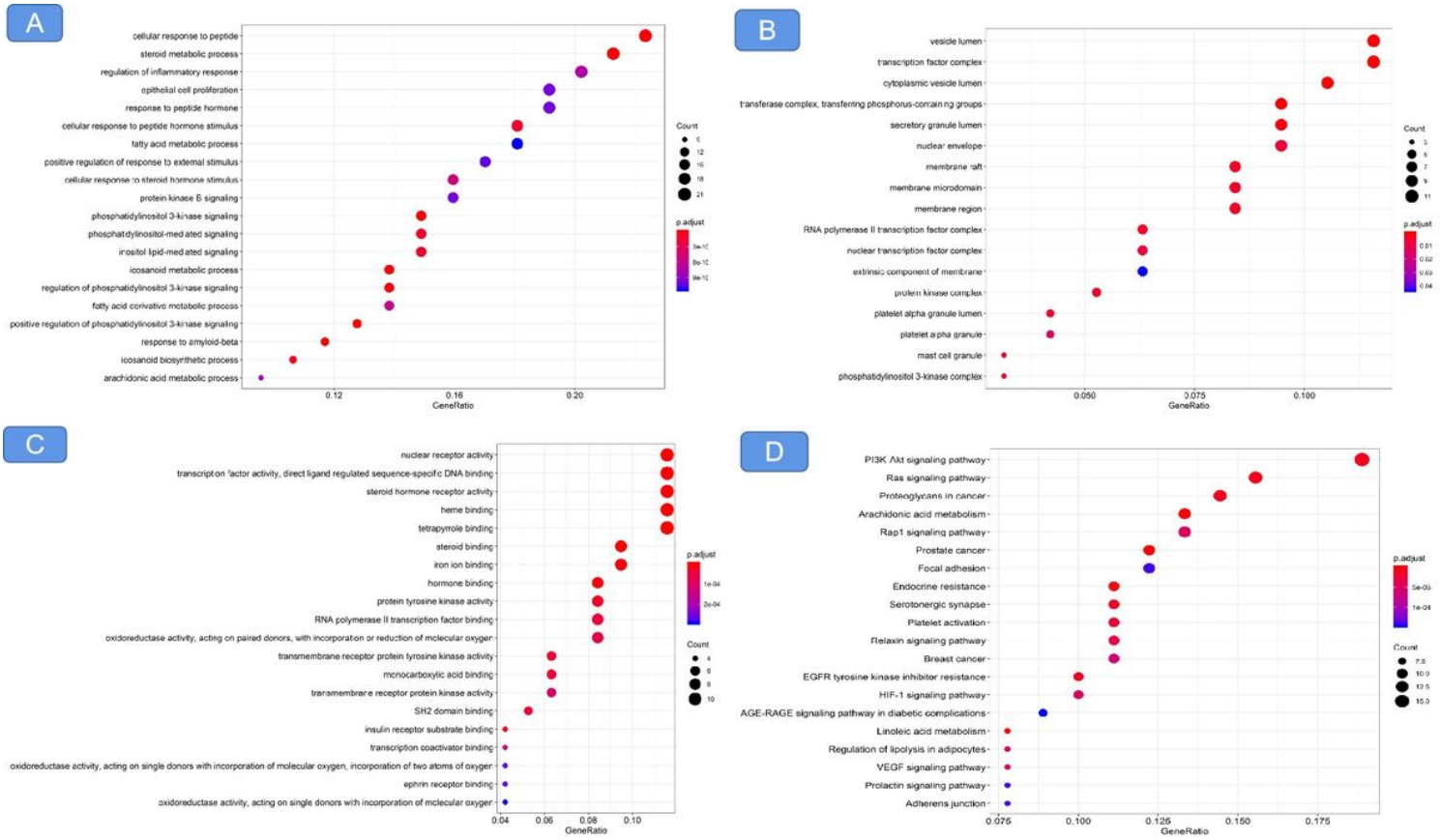

Figure 3

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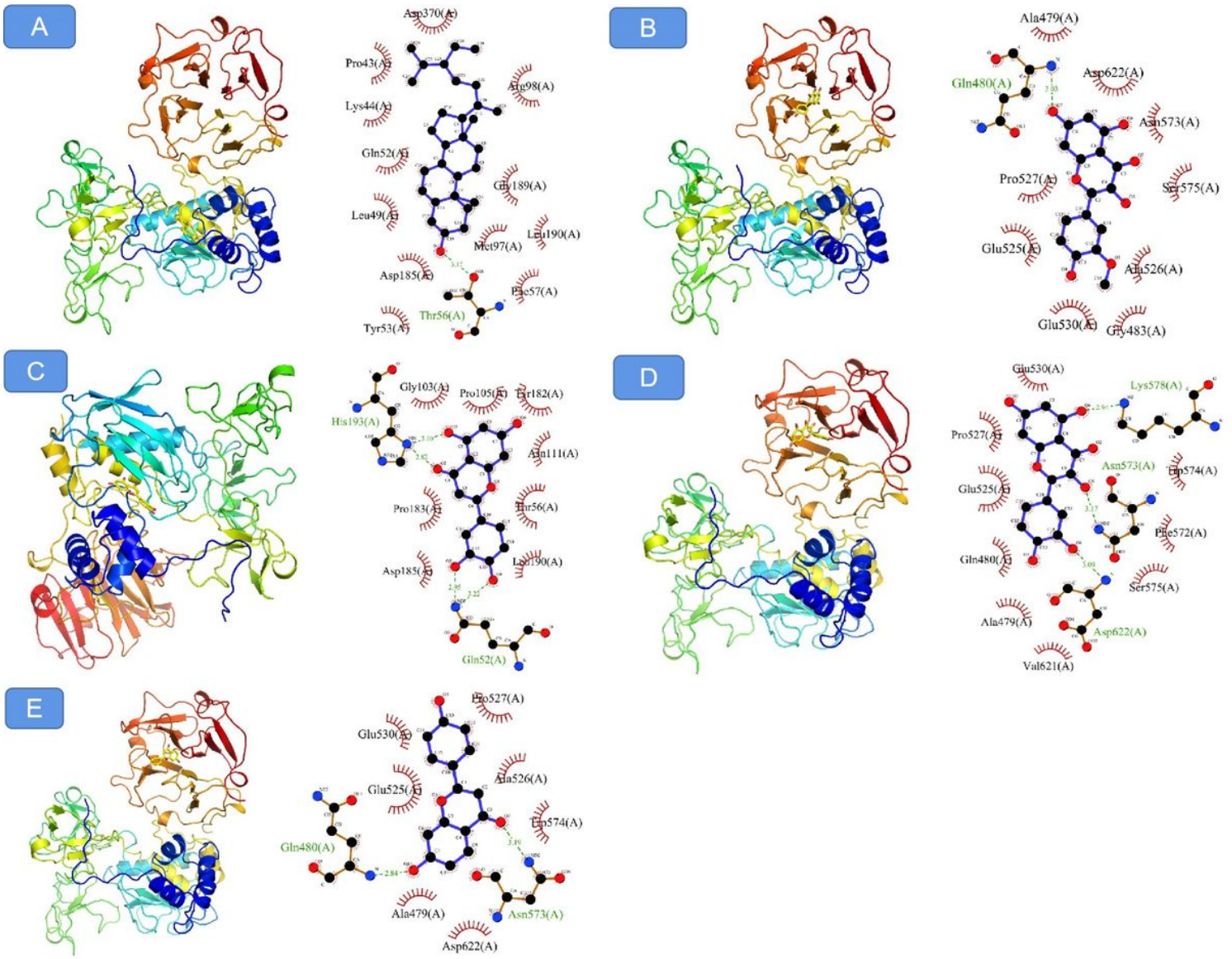

Figure 4

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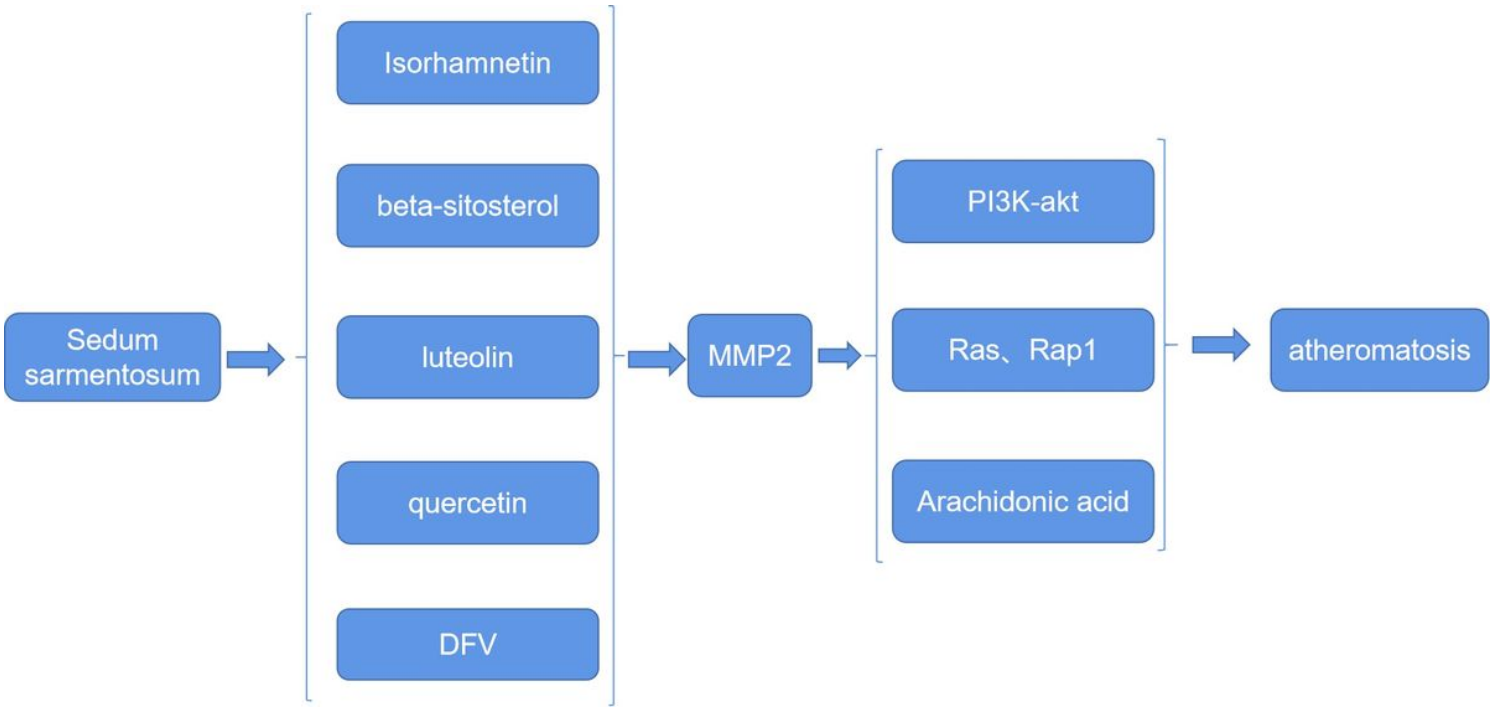

Figure 5

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