European Review for Medical and Pharmacological Sciences 2023; 27: 1774-1792

Modular characteristics and mechanism of action of herbs for vascular calcification treatment in Chinese medicine: a data mining and network pharmacology-based identification

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Abstract. – OBJECTIVE: The aim of this study was to investigate the modular characteristics and mechanism of action of Chinese herbs for vascular calcification (VC) treatment.

MATERIALS AND METHODS: Network pharmacology coupled with literature data mining was utilized to assess the Chinese herbal clinical performance as well as its similarity, characteristics, ingredient, target, and Gene Ontology (GO) analysis, Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment, and network construction.

RESULTS: The top 15 medications from the literature, according to the usage, and 190 active chemicals, 183 common targets between medication and VC-related targets were weeded out. Analysis of the relationships between the active ingredients, pharmacological targets, and signaling pathways helped to clearly define the therapeutic effect of Traditional Chinese Medicine (TCM). Importantly, we discovered seven most hub proteins (AKT1, CTNNB1, TNF, EGFR, TP53, JUN and IL-6) and two of the herbs' most fundamental ingredients (Formononetin and Luteolin) in TCM-mediated VC suppression. Mechanistically, the metabolic pathways [AGE-RAGE pathway, interleukin-17 (IL-17) pathway, and p53 pathway] as well as smooth muscle adaptation (functional remodeling) and oxidoreductase activity (redox homeostasis modulating) are also crucially implicated.

CONCLUSIONS: Our work, accomplished by network pharmacology and data mining, increases our understanding of TCM in VC therapy and may offer insightful information for future drug discovery investigations.

Key Words:
Vascular calcification, Traditional Chinese medicine, Chinese herbs, Pharmacological mechanism, Medication rule, Network pharmacology.

Introduction

Vascular calcification (VC), characterized by calcium-phosphate complex deposition in the vasculature, is an independent risk factor for both severe adverse cardiac events and cardiovascular disorders (CVD)[1]. Recent decades have shown VC is not a passive, degenerative process but an actively regulated, cell-mediated process in patients with CVD caused by multiple basic diseases such as diabetes, hypertension, dyslipidemia, and atherosclerosis[2]. Patients' demands for VC prevention or treatment were not addressed despite the clinical interventions targeting cellular mechanisms of VC including phosphate binders, vitamin K, calcimimetics, vitamin D, and bisphosphonates[3-6]. While Traditional Chinese Medicine (TCM) practitioners and researchers[7] have amassed a large collection of prescriptions for the therapy of VC, during the course of their extensive clinical work.

TCM served as a beneficial practical experience for contemporary clinical practice and fundamental medical research as a priceless intangible cultural legacy of China. TCM has evolved over more than two thousand years into a comprehensive and distinctive system that includes diagnosis and prognosis and is crucial in the prevention and treatment of human ailments. A data mining strategy can discover effective drugs for illness therapy[8,9]. In the meantime, it can examine changes in formula patterns created from a knowledge graph, the frequency of herbal drugs, and drug ontology.

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TCM's "multi-ingredient, multi-target, and multi-pathway" method offers an original, potent, and targeted approach for illness prevention and treatment in contrast to single-target or single-pathway drugs. However, the basic workings of Chinese herbs have not yet been fully understood because of the complexity of their components and the challenges in studying the several goals of Chinese herbs. Network pharmacology, a developing TCM prescriptions research technique, may predict numerous bioactive substances as well as reveal many drug targets and pathways based on the interplay of "disease-target-drug-ingredient[10-12]".

In order to find the Chinese prescriptions and learn more about the important herbs for the treatment of VC, data mining was used in this study. The complicated relationship between herbs and VC was then demonstrated using a network pharmacology method. In order to further confirm the binding between the candidate components and significant proteins, protein-chemical interaction (PCI) was employed to evaluate the bioactive components and therapeutic mechanisms of Chinese herbs used in VC therapy. We anticipated that the findings would contribute to our understanding of the genesis of VC and shed light on the biological basis of the druggable targets, assisting in the creation of more effective VC therapeutic interventions.

Materials and Methods

Collection of Chinese Prescription and Herbs for VC Treatment

Figure 1 shows our workflow diagram for this investigation. Sources were obtained from China National Knowledge Internet (CNKI) (available at: http://www.cnki.net), Wanfang Database (available at: http://www.wanfangdata.com.cn/index.html), China Science and Technology Journal Database (VIP: available at: http://www.cqvip.com), and PubMed (available at: https://www.ncbi.nlm.nih.gov), Web of Science (Clarivate) databases *via* using the terms "vascular calcification" "calcification" "VC" and "traditional Chinese Medicine" and "Chinese herb" updated to August 18,2022. Furthermore, Endnote (version x9.3.3), Zotero (version 6.0.10), and Excel (version 2019) tools were used to analyze and collect pertinent data. Except for repeated and unsatisfactory items, a total of 105 papers were examined further. The following are the criteria for data inclusion and exclusion: (1) Inclusion criteria were primary randomized controlled clinic studies for treating vascular calcification with Traditional Chinese Medicine, considerable therapeutic efficacy with TCM, and explicit herb names in prescriptions. (2) The exclusion criteria included any studies that did not fulfill the inclusion requirements, such as animal tests, case reports, reviews, incomplete herb compositions, identical prescription work. Two different researchers processed prescription collections while another researcher summarized information to verify correctness and dependability. When the same compound drug was reported in multiple publications, only one was included.

Name Standardization and Analysis of Collected Herbs

A total of 107 single herbs were extracted from the ten screened prescriptions, and their names were unified to official names based on the People's Republic of China Pharmacopoeia (2020 edition) and Traditional Chinese Medicine "13th Five-Year Plan" textbook, as well as the Chinese Clinical Medicine Dictionary. Unless otherwise mentioned in China Pharmacopoeia, processing methods of herbs were neglected, such as "Honey-made-HuangQi" dubbing for "HuangQi" and "Wine-made-DaHuang" dubbing for "DaHuang". The frequency, family, genus, categorization, effectiveness, characteristics, flavor, and channel tropism were all investigated and summarized. A total of 16 herbs were chosen for further investigation based on their frequency of use.

Active Ingredients Screening of Therapeutic Herbs

TCM Systems Pharmacology Database (available at: http://lsp.nwu.edu.cn/tcmsp.php), TCMID Database (available at: https://ngdc.cncb.ac.cn/databasecommons/database/id/437), and ETCM (available at: http://www.nrc.ac.cn:9090/ETCM/) were used to search the bioactive elements of herbs. On the basis of the absorption, distribution, metabolism, and excretion (ADME) properties of the herbs in the body, the oral bioavailability (OB) of 30% and the drug-likeness (DL) of 0.18 were set as additional filters. Some Chinese herbs, such as MuLi, were not further investigated in this study due to a lack of bug species in the TCMSP database. The chemical structure was also acquired from the NCBI PubChem database (available at: https://www.ncbi.nlm.nih.gov/).

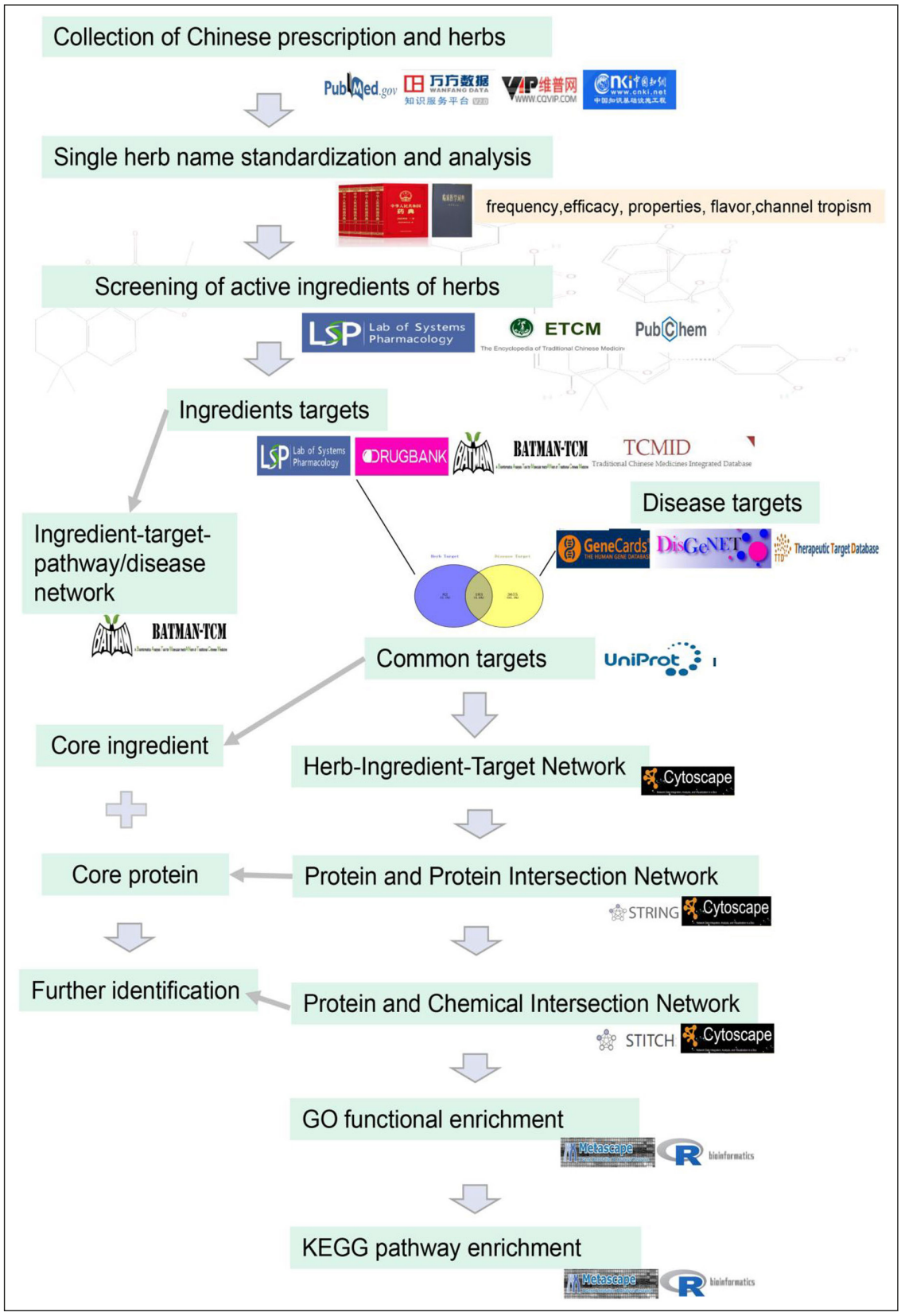


Figure 1. The workflow, database, software of this work. The icon next to each step entry represents the database or software used for this work.

Potential Targets Prediction of Active Ingredients

The putative targets for each component were obtained by combining data from the TCMSP (available at: http://lsp.nwu.edu.cn/tcmsp.php), DrugBank (available at: https://go.drugbank.com/), and BATMAN-TCM (available at: http://bionet.ncpsb.org.cn/batman-tcm/index.php/Home/Index/index) databases.

Related Targets Collection of Disease

GeneCard (available at: https://www.genecards.org/), OMIM (available at: http://www.omim.org/), DisGeNET (available at: http://www.disgenet.org/), and Therapeutic Target Database (TTD; available at: http://db.idrblab.net/ttd/) databases were used to forecast VC-related targets.

Common Targets Acquisition Among Ingredients-Targets and Disease-Targets

The common targets across drug-targets and VC-related-targets were obtained by taking intersection after eliminating duplication, illustrated with Venn diagrams, and then mapped to the UniProt Database (available at: https://www.uniprot.org/) to standardize.

Network Construction of Herbs-Ingredients-Common Targets

We then used f (vlookup) in Excel to find the related substances and herbs based on common targets. Following that, the common targets, associated ingredients, and herbs were loaded into Cytoscape 3.8.2 software to create a network of herbs-components-common targets. Furthermore, the most prevalent herb compounds and the components associated with the most common targets were identified as the most plausible core elements for VC therapy.

GO and KEGG Enrichment Analysis of Common Targets

The Metascape (available at: https://metascape.org/gp/index.html#/main/step1) database was used for Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways and Gene Ontology (GO) enrichment analyses. To represent gene properties, three types of GO keywords were defined: biological process (BP), molecular function (MF), and cellular component (CC). To depict the interacting pathway of proteins, the KEGG (available at: http://www.genome.jp/kegg/) database was employed. The images were created using Bioinformatics (available at: https://www.bioinformatics.com.cn/) and R Software version 3.4.0 (available at: https://www.r-project.org).

Network Construction of Protein-Protein Interaction (PPI) and Protein-Chemical Interaction (PCI)

Protein-protein interaction (PPI) networks were gathered from the STRING database version 11.5 (available at: https://www.string-db.org/) to gain a better knowledge of how those common proteins interact with one another. Furthermore, the Protein-chemical interaction (PCI) network was retrieved from the STITCH database version 5.0 (available at: http://stitch.embl.de/cgi/input.pl?UserId=IS4zrGBSE1vJ&sessionId=Zr13lb0cc17a&input). STRING database conducts further analysis *via* the column "multiple protein" on the left, whereas STITCH database does more analysis *via* the column "Item by name" or "Chemical structure" on the left. Both databases' homepage organisms were chosen to be "Homo sapiens". In the STRING database, an interaction scores higher than 0.9 was defined as moderate confidence. The maximum number of interactors was set at no more than 20 in STITCH. Following the acquisition of raw data from two databases, the Cytoscape 3.8.0 software (available at: http://cytoscape.org/) was used to visualize networks. The Molecular Complex Detection (MCODE) plugin and the Cytohubba plugin in the Cytoscape program were used to perform the crucial network and hub gene of biological networks, respectively.

Molecular Docking Between Core Therapeutic Ingredients and Proteins

AutoDock 1.5.6 (available at: https://autodock.scripps.edu/) is a suite of automated docking tools, and it is designed to predict how small molecules, such as substrates or drug candidates, bind to a receptor of a known 3D structure. Based on the above screening results, we determined the proteins (AKT1 and CASP3) and molecule ligands (Formononetin and Luteolin) for docking. The 2D structures of the active compounds were obtained from TCMSP, and the 3D structures of the core targets were gained by RSCB PDB database (available at: https://www.rcsb.org/). The structures of compounds and targets were introduced into AutoDockTools for adding hydrogen, deleting water and other operations, then, we carried out the molecular docking to analyze the binding energy of the receptors and ligands. Finally, the docking results were visualized using PyMOL 2.5.0 (available at: https://pymol.org/2/).

Statistical Analysis

A value of $p<0.05$ was considered statistically significant in the bioinformatics analysis.

Results

The Details and Characteristics of Herbs for VC Treatment

Data mining in the literature assists us in obtaining clinical herbs for VC therapy. A total of 1,431 papers on Chinese medicine in VC therapy were obtained: 1,275 from Chinese databases (CNKI, WanFang, and VIP) and 173 from English databases (PubMed and Web of Science database). Among those, 202 pieces of literature were duplicates, 126 were clinical studies, 282 were experimental research (235 were *in vitro*, 39 were *in vivo*, and 8 were both *in vitro* and *in vivo*), and 434 were case or review studies. Others were 387 pieces of literature. As a consequence, 14 clinical randomized controlled trials papers were utilized for additional data mining[13-22].

A total of ten medical prescriptions were chosen from the screened literature, including those for "Jian-Pi-Bu-Shen-Tong-Luo-Fang", "Wen-Yang-Xie-Zhuo-Fang", and "Tong-Lin-Tang", among others. 56 different Chinese herbs were obtained through prescriptions and single herbs, and the top 15 herbs (frequency>20%) in clinical use were used for subsequent analysis (details on Table I).

Furthermore, the interpretation of Chinese herb features such as effects, qualities, flavor, and channel tropism are the greatest approach to immediately comprehend a Chinese herb. We discovered that the frequency of action of those 15 herbs, such as "Tonifying Qi herbs", "Remove blood stasis and promote blood circulation", and "Astringency herb", had a larger proportion in VC therapy in this study (Figure 2A).

The herbal properties were predominantly warm (Figure 2B and 2C), the flavor was Gan, and the channel tropism was liver and spleen (Figure 2D).

These findings indicate that 15 Chinese herbs were often utilized in VC therapeutic research, and that these herbs mostly served the roles of "tonifying qi and activating blood circulation". The basic characteristic of these herbs was gan-warm herb of channel tropism of the liver-spleen.

Table I. The details of top 15 of herbs (frequency > 20%).

Chinese name	English name	Family	Genus	Frequency (%)	Components	Candidate components
HuangQi	Astragali Radix	*Fabaceae* Lindl	Astragalus aaronii (Eig) Zohary	80	87	16
FuLing	Poria	Polyporaceae	Poria cocos (Schw.) Wolf	70	34	14
DaHuang	Rhei Radix et Rhizoma	*Polygonaceae*	Rheum officinale Baill.	60	92	13
DangGui	Angelicae Sinensis Radix	*Apiaceae* Lindl.	Angelica sinensis (Oliv.) Diels	50	125	2
DanShen	Salviae Miltiorrhizae Radix et Rhizoma	*Lamiaceae* Martinov	Salvia miltiorrhiza Bunge	40	202	52
ChuanXiong	Rhizoma Chuanxiong	*Apiaceae*	Ligusticum chuanxiong Hort.	40	189	6
BaiZhu	Atractylodis Macrocephalae Rhizoma	Composite	Atractylodes macrocephala Koidz.	40	55	5
DuZhong	Eucommiae Cortex	*Eucommiaceae*	Eucommia ulmoides Oliv	40	28	16
ShanYao	Rhizoma Dioscoreae	*Dioscoreaceae*	Dioscorea oppositifolia L.	30	16	16
HongHua	Carthami Flos	*Asteraceae* Bercht. and J.Presl	Carthamus tinctorius L.	30	22	16
MoHanLian	Herba Ecliptae	Composite	Eclipta prostrata L.	20	10	10
ShanZhuYu	Corni Fructus	Cornaceae	Cornus officinalis Sieb. et Zucc.	20	20	16
FuZi	Aconiti Lateralis Radix Praeparata	*Ranunculaceae*	Aconitum carmichaelii Debx.	20	21	18
DangShen	Radix Codonopsis Tubulosae	*Campanulaceae*	Codonopsis pilosula (Franch.) Nannf.	20	21	19
GeGen	Puerariae lobatae radix	*Leguminosae*	Pueraria lobata (Willd.) Ohwi	20	4	4

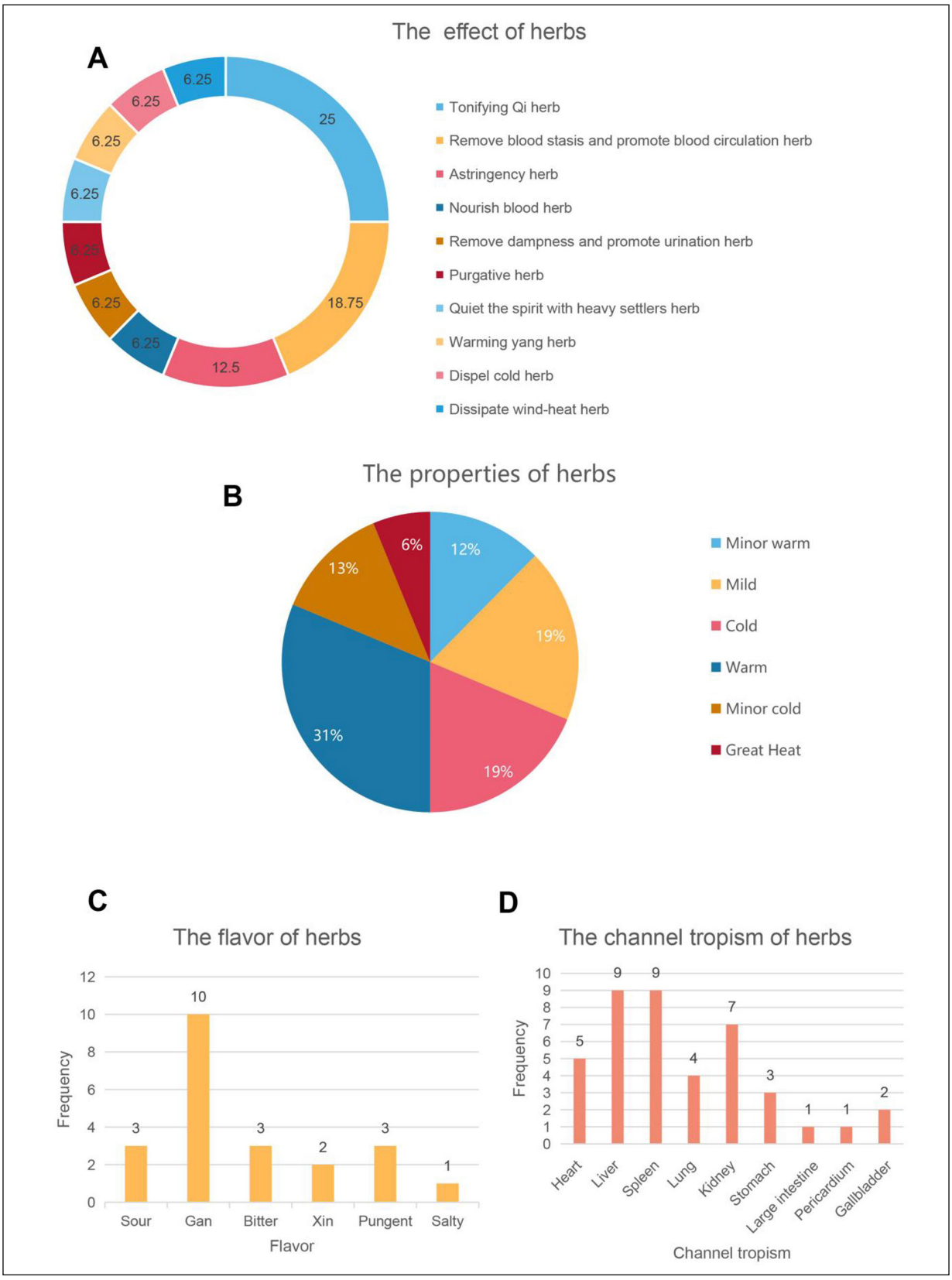
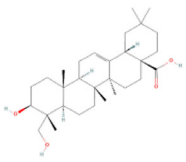
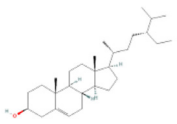
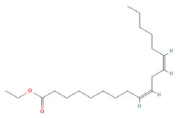
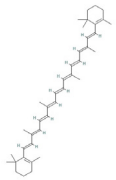
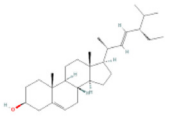
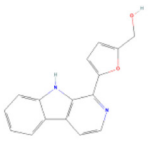


Figure 2. The effects, properties, flavor, and channel tropism of herbs. **A**, The effect of herbs. **B**, The properties of herbs. **C**, The flavor of herbs. **D**, The channel tropism of herbs.

The Potential Active Ingredients of Therapeutic Herbs

Chinese herbs are known for their complex composition[23]. Several databases were utilized to uncover the fundamental components. As a result, 1,682 herbal components were gathered from the TCMSP DrugBank database and BATMAN-TCM. Among those, 190 putative plant constituents were identified for further investigation since they met all criteria "OB ≥ 30 %, DL ≥ 0.18, and HL ≥ 4" (**Supplementary Table I**) and Table II exhibits sections of them at random that are connected to more than one plant as examples.

Table II. The part of candidate ingredients.

MOL-ID	Component Name	OB (%)	DL	HL	Molecular formula	Compound CID	Structure	Related Herbs
MOL000296	Hederagenin	36.91	0.75	5.35	C30H48O4	73299		HuangQi, FuLing
MOL000358	Beta-sitosterol	36.91	0.75	5.36	C29H50O	222284		DaHuang, DangGui, DuZhong, HongHua, ShanZhuYu GeGen
MOL001494	Mandenol	42	0.19	5.39	C20H36O2	5282184		ChuanXiong ShanZhuYu
MOL002773	Beta-carotene	37.18	0.58	4.36	C40H56	5280489		DuZhong HongHua
MOL000449	Stigmasterol	43.83	0.76	5.57	C29H48O	5280794		DangGui, HongHua, ShanZhuYu, DangShen
MOL002140	Perlolyrine	65.95	0.27	12.62	C16H12N2O2	160179		ChuanXiong DangShen

The Candidate Targets of Active Ingredients

Following the removal of duplication, a total of 265 ingredient targets were found in this study (**Supplementary Table II and III**). Then, we constructed the active ingredients-targets network by Cytoscape 3.8.0 software. The results in Figure 3 show that the blue nodes in the middle represents the targets, and the purple nodes around them represents the active ingredients. The results indict that herb worked with multi-target and multi-ingredients. Further exploration is necessary to get most central targets or ingredients.

The Common Targets and Herbs- Ingredients-Common Targets Network

We searched for the illness target after discovering the medication target. After removing duplicates, nearly 3,858 VC-related targets were obtained from the GeneCards database, OMIM database, TTD database, DisGeNET database, and DrugBank database (**Supplementary Table III**). The Venn diagram revealed 183 overlapping targets across medication targets and disease targets (Figure 4A, **Supplementary Table III**).

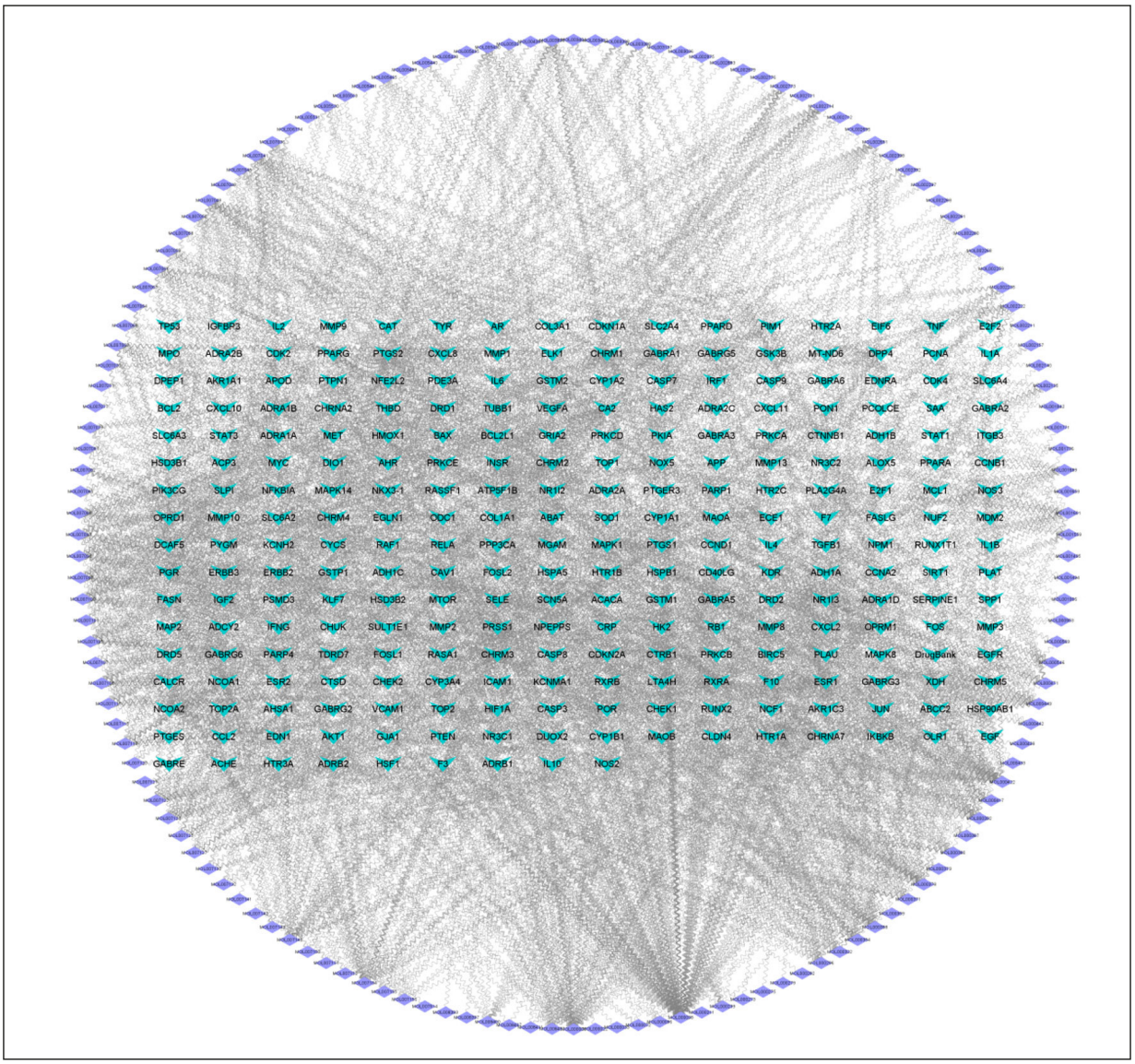


Figure 3. The active ingredients-targets network: the blue nodes in the middle represents the targets, the purple nodes around them represents the active ingredients.

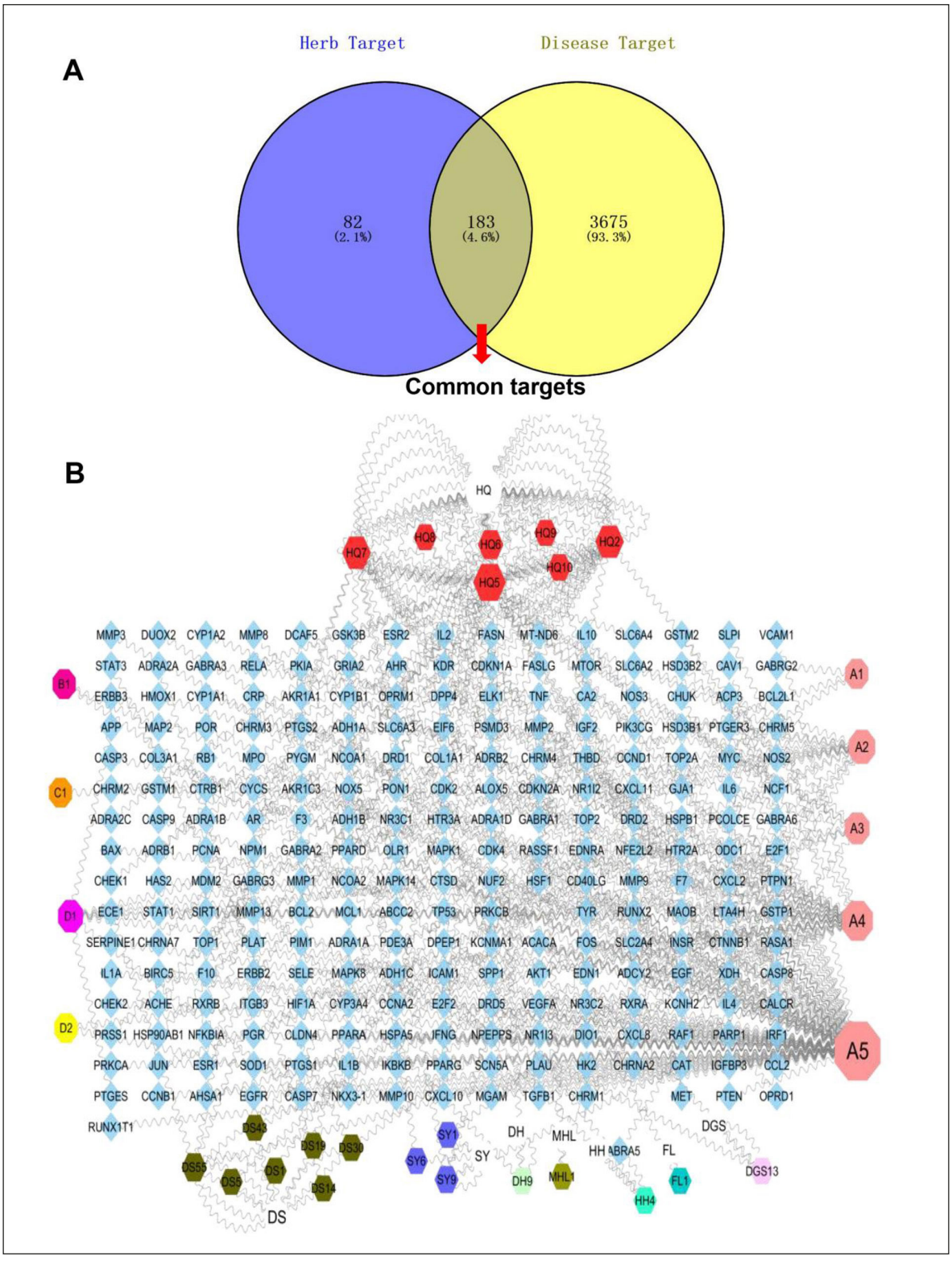


Figure 4. Herb-ingredient-common target network. **A**, Venn diagram of common targets between VC-related targets and drug targets. **B**, herb-ingredient-common target network. White circles at the top and bottom of the figure represent single herb, hexagons at the top and bottom of the figure with different colors represent the unique components of each herb, octagons at the left and right of the figure with different colors represent the common components of several drugs, and light blue in the middle represents the common target. The number after the letter has no special meaning, such as DGS13, DH9, just a kind of mark of the ingredients.

To be more specific, in this paper, the 183 overlapping targets were referred to as "common targets", and most of the ensuing research was focused on them. To begin, the matching components and herbs were discovered using those common targets. Of those common targets, 31 components and 8 herbs were linked (Figure 4B). Seven components derived from HuangQi (HQ), seven components from DanShen (DS), three from ShanYao (SY), one from DaHuang (DH), one from HongHua (HH), one from MoHanLian (MHL), one from FuLing (FL), one from DangShen (DGS), and nine components from multiple herbs (A1-A5, B1, C1, C2, D1). White circles at the top and bottom of Figure 4B represent single herbs, hexagons with different colors at the top and bottom of Figure 4B represent the unique components of each herb, octagons with different colors at the left and right of Figure 4B represent the common components of several drugs, and light blue in the middle represents the common targets. Table III also displays the same components of multiple herbs. Interestingly, quercetin, which is also a component of four medicines used to treat VC, is linked to 143 common targets. Although Beta-sitosterol has just related 36 common targets, it is the most frequent component of six herbs for VC therapy. As a result, Mairin, Hederagenin, Formononetin, Kaempferol, quercetin, Beta-sitosterol, Luteolin, Mandenol and beta-sitosterol are considered promising substances for VC therapy and will be studied further.

The Promising Therapeutic Mechanism from GO and KEGG Enrichment Analysis

The medicinal targets and pathways of herbs were the focus of subsequent investigation. GO and KEGG pathway enrichment of common targets was done to further find prospective targets and pathways in the treatment of VC using Chinese herbs. There were 203 KEGG pathways and 2,630 GO terms that were enhanced (**Supplementary Table IV and V**). Figure 5A depicts the top 20 KEGG pathways, demonstrating that numerous pathways, such as "AGE-RAGE signaling pathway in diabetic complications", "interleukin-17 (IL-17) signaling pathway", and "p53 signaling pathway", may be implicated in the therapeutic process of VC. Furthermore, Figure 5B shows the top ten GO terms from each of the three categories, indicating that these targets were relevant to the physiological and pathological processes of vascular disease, such as smooth muscle adaption and oxidoreductase activity.

All in all, the GO and KEGG enrichment analysis indicated that smooth muscle adaptation and oxidoreductase activity are the key two functional modules that underpin the mechanism of action of herbs in the treatment of VC, and "the AGE-RAGE signaling pathway in diabetic complications, the IL-17 signaling pathway, and the p53 signaling pathway" are the critical complex bio-pathway network.

The Core Active Ingredients and Proteins from PPI and PCI Network Analysis

Although 183 common targets associated to both herbs and VC have been discovered, and their probable activities and pathways have been validated, the most core therapeutic targets and ingredients, and the interaction between proteins, as well as among ingredients and proteins, remains unknown. Thus, PPI network and PCI network were constructed by Cytoscape software.

Table III. The most common component related to multiple herbs for VC treatment.

Symbol	Mol ID	Ingredient	Common target number	Herbs
A1	MOL000211	Mairin	1	HuangQi, DuZhong
A2	MOL000296	Hederagenin	22	HuangQi, FuLing
A3	MOL000392	Formononetin	34	HuangQi, GeGen
A4	MOL000422	Kaempferol	56	HuangQi, DuZhong, HongHua
A5	MOL000098	Quercetin	143	HuangQi, DuZhong, HongHua, MoHanLian
B1	MOL000358	Beta-sitosterol	36	DaHuang, DangGui, DuZhong, HongHua, ShanZhuYu, GeGen
D1	MOL000006	Luteolin	55	ChuanXiong,ShanZhuYu
C1	MOL001494	Mandenol	3	DanShen,HongHua, MoHanLian, DangShen
D2	MOL002773	Beta-carotene	19	DuZhong, HongHua

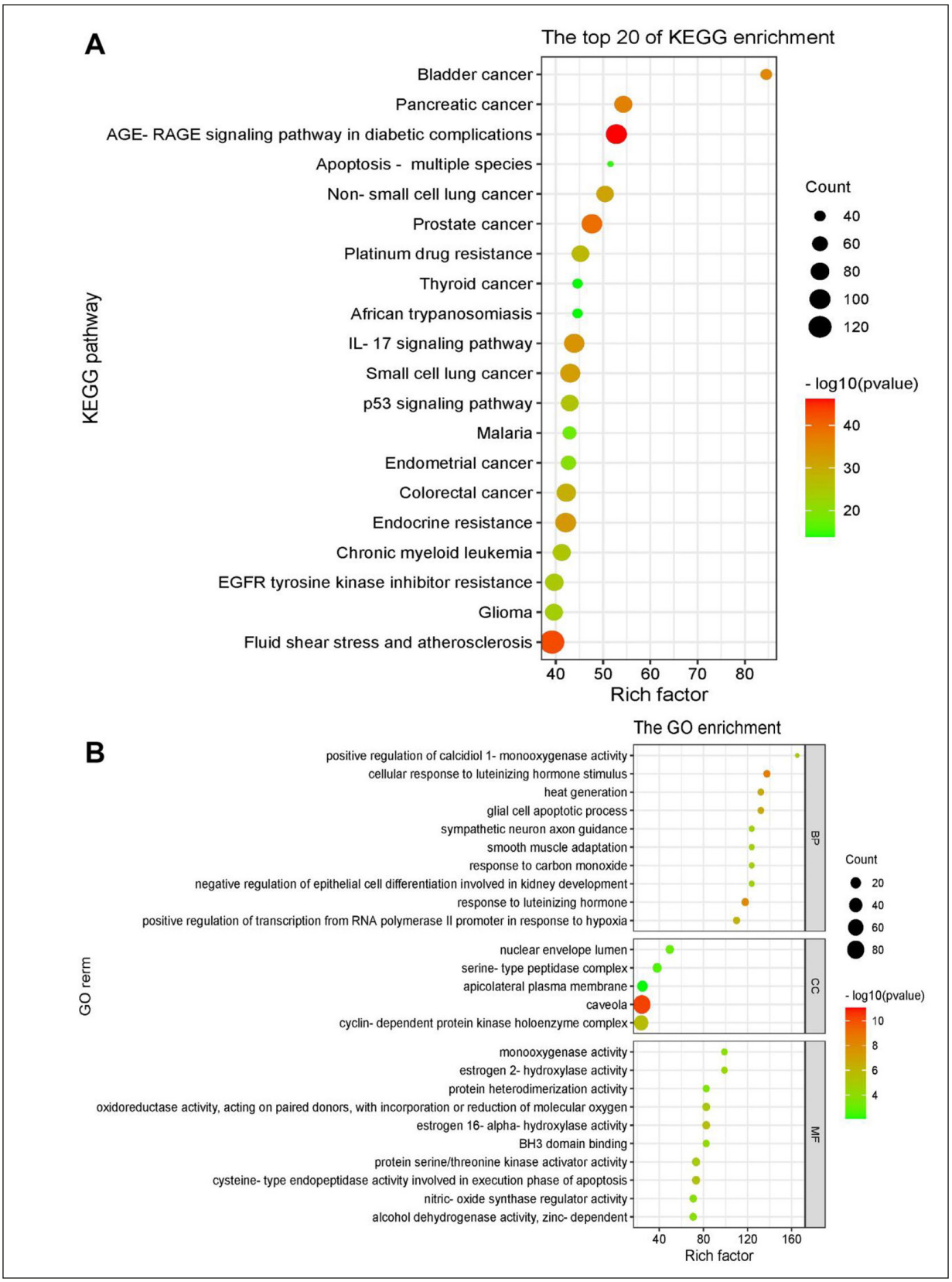


Figure 5. The GO and KEGG enrichment of common targets. **A**, The top 20 of KEGG pathway. **B**, The top 10 GO term of each of three categories.

PPI network

PPI serves critical functions in a variety of biological processes. Most proteins carry out their functions by interacting with a large number of other proteins. Degree Centrality (DC), Closeness Centrality (CC), and Betweenness Centrality (BC) are prominent tools for analyzing network structure. These common proteins have intricate interactions in the PPI network in our experiment (Figure 6A). We first computed the BC, CC, and DC values for all proteins (**Supplementary Table VI**), and then sorted them independently. We next screened the top 100 (DC>34, BC>40.22, CC>0.53), top 50 (DC>63, BC>136.64, CC>0.59), and top 10 protein targets (DC>106, BC>633, CC>0.7). Notably, we specifically ranked DC value, CC value, and BC value individually, then chose the top 100, 50, and 10 of each item for intersection, accordingly. The intersectional proteins among the top proteins in each item individually, were ultimately counted as the top hub proteins. As a result, a total of 83 proteins were obtained, which were not only the top 100 proteins in BC terms, but also the top 100 proteins in DC items and the top 100 proteins in CC items (Figure 6B). A total of 39 proteins were obtained, which were not only the top 50 proteins in BC terms, but also the top 50 proteins in DC items and the top 50 proteins in CC items (Figure 6C). Also, a total of 7 proteins including AKT1, CTNNB1, TNF, EGFR, TP53, JUN and IL-6 were acquired, which were not only the top 10 proteins in BC terms, but also the top 10 proteins in DC items and the top 10 proteins in CC items (Figure 6D). Thus, the seven proteins were determined to be the most core therapeutic targets for VC therapy.

PCI network

Following that, the core ingredients obtained above including Mairin, Hederagenin, Formononetin, Kaempferol, quercetin, Beta-sitosterol, Luteolin, Mandenol and Beta-carotene were put into the STITCH database to confirm the interaction between protein and chemical. We only looked at 10 proteins that interacted with those chemical constituents in this study. As a consequence, beta-sitosterol interacted with SREBF2, ABCG8, ABCG5, APOE, DHCR24, CASP3, SREBF1, ABCB11, ICAM1 and CYP7A1 (Figure 7A), among those proteins, CASP3 and ICAM1 were also the top 50 hub proteins. Luteolin interacted with MAPK8, MMP9, CASP3, JUN, FOS, CDK2, EGFR, SMAD2, CCNA2 and AKT1 which all were the top 50 hub proteins excepted SMAD2, CDK2 and CCNA2 (Figure 7B). Beta-carotene interacted with BCMO1, BCO2, RBP2, SCARB1, LOX, FN1, TMPRSS11D, NR1I2, UQCRFS1 and CYP1A1 (Figure 7C). Quercetin was interacted with MCL1, CYP1B1, HCK, PIM1, SLC2A2, CYP2C8, CYP1A1, ATP5B, HIBCH, STK17B (Figure 7D). Kaempferol interacted with CDK1, NR1I2, UGT3A1, CYP1B1, RPS6KA3, AHR, UGT1A3, UGT1A8, UGT1A7 and UGT1A9 (Figure 7E). Formononetin interacted with PDE4A, UGT1A8, UGT1A7, UGT1A9, UGT1A10, UGT1A1, CYP1A1, CASP8, CASP9, CASP3 (Figure 7F) and CASP8, CASP9 and CASP3 were the top 50 hub proteins. Besides, zero proteins were predicted interacting with Mairin and Mandenol. Only one protein, RNASE3, interacted with Hederagenin (Figure 7G).

In summary, among those core ingredients, Formononetin and Luteolin were regarded as the most core two ingredients, as they can interact with more hub proteins.

The Binding Capability and Binding Locations Among Core Therapeutic Ingredients and Proteins from Molecular Docking

As described above, the AKT1, CTNNB1, TNF, EGFR, TP53, JUN and IL-6 were considered as the most core seven hub proteins, and Formononetin and Luteolin were regarded as the most central two ingredients. To further explain the binding capability and binding locations among those core proteins and ingredients, molecular docking was performed.

In this work, among the core seven hub proteins, we only selected AKT1 to do the molecular docking. In addition, among the top 50 hub proteins, CASP3 were also selected to do the molecular docking. Two core target proteins, including AKT1 (PDB ID: 6HHF) and CASP3 (PDB ID: 1RHQ), were conducted to do the molecular docking with two key bioactive compounds that consisted of Formononetin (MOL ID: MOL000392) and Luteolin (MOL ID: MOL000006). The molecular docking results displayed that all the protein-ligand groups showed binding energy < -6 kcal/mol, which average binding energy were < -6.94 kcal/mol, the binding energy of AKT1-Formononetin was -7.32 kcal/mol (Figure 8A), the binding energy of CASP3-Formononetin was -6.38 kcal/mol (Figure 8B), the binding energy of AKT1 and Luteolin was -7.43 kJ/mol (Figure 8C), and the binding energy of CASP3-Luteolin was -6.63 kcal/mol (Figure 8D), suggesting that the binding between the core targets proteins and the key bioactive compounds was strong.

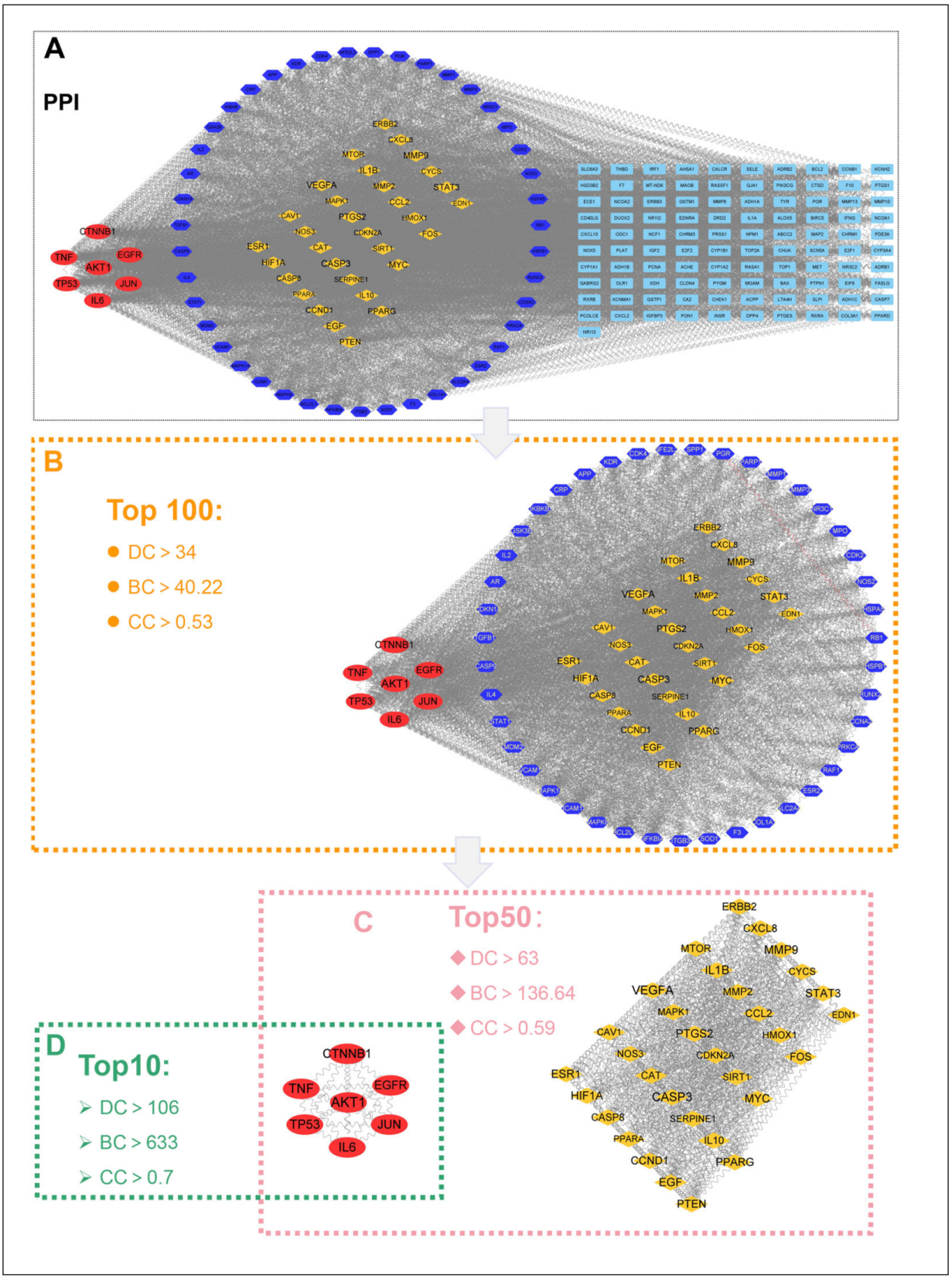


Figure 6. PPI network and hub proteins. **A**, PPI network. **B**, The top 100 hub proteins ranked by BC, CC, and DC item. **C**, The top 50 hub proteins ranked by BC, CC, and DC item. **D**, The top hub 10 proteins ranked by BC, CC, and DC item.

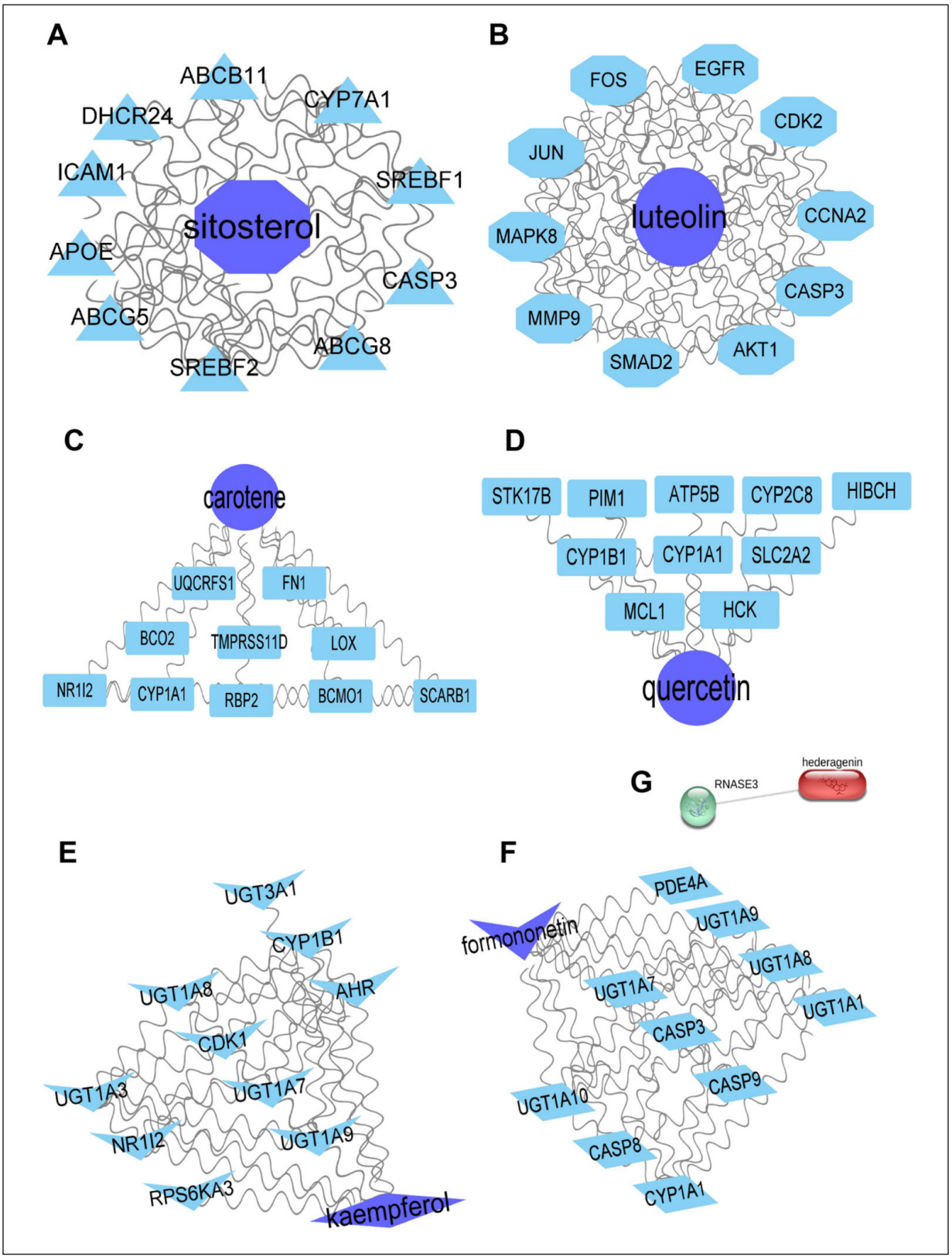


Figure 7. PCI network. **A**, (Beta-) sitosterol and protein interaction network. **B**, Luteolin and protein interaction network. **C**, (Beta-)carotene and protein interaction network. **D**, Quercetin and protein interaction network. **E**, Kaempferol and protein interaction network. **F**, Formononetin and protein interaction network. **G**, Hederagenin and protein interaction network.

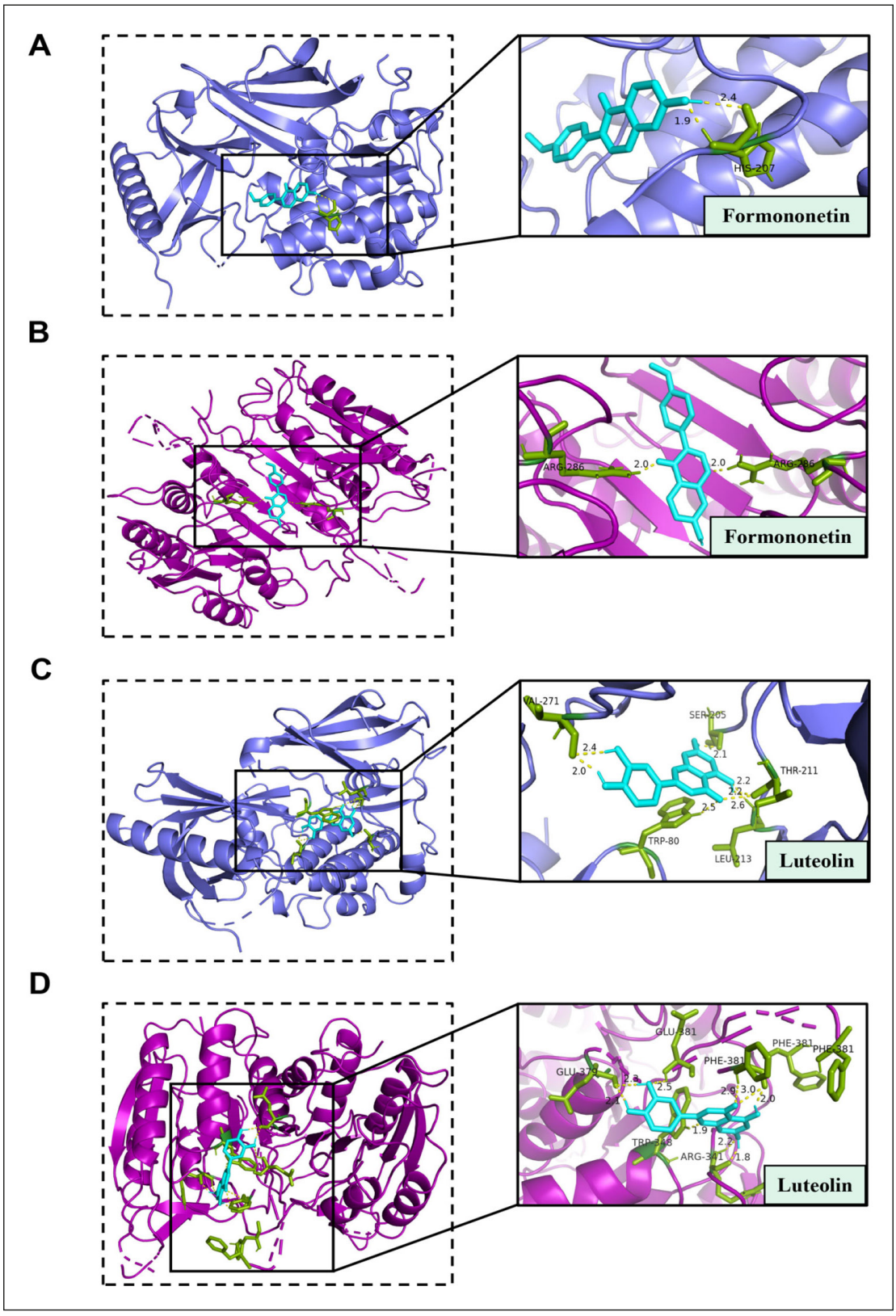


Figure 8. The detail of molecular docking. **A**, AKT1-Formononetin (-7.32 kcal/mol). **B**, CASP3-Formononetin (-6.38 kcal/mol). **C**, AKT1 and Luteolin (-7.43 kJ/mol). **D**, CASP3-Luteolin (-6.63 kcal/mol).

Moreover, our results showed that the binding energies of AKT1 and the two molecules were the best, (-7.32 and -7.43 respectively), suggesting that they may be the best choice for future experimental verification. At last, we displayed the 3D docking conditions between core two target proteins and two key bioactive compounds in Figure 8.

Discussion

Vascular calcification (VC) is a distinct predictor of adverse cardiovascular events, and its complex pathological processes are similar to those of bone formation in many aspects. Despite the considerable mortality of arterial calcification in patients with metabolic disorders, there are no established therapies[22]. Throughout its vast medical practice, TCM has evolved a distinct medical system that includes prevention, diagnosis, and treatment. Compounding Chinese medicine produces clinical benefits by focusing on certain targets or components. Thanks to the advancement of Chinese herb pharmacology, several approaches are now accessible to explore the delicate interaction between the chemical system of the TCM prescription and the biological system of the human organism. This study attempted to explain the "multi-ingredient, multi-target, and multi-pathway" properties of Chinese herbs as well as the molecular pathogenesis of VC using data mining and network pharmacology. There were two possible outcomes: (1) As prospective possibilities for VC-related ingredients and targets, seven of the most critical proteins (AKT1, CTNNB1, TNF, EGFR, TP53, JUN and IL-6) and two of the herbs' most fundamental chemicals (Formononetin and Luteolin) have been found. Furthermore, the systematic administration of those active components may provide useful information on prospective combination therapy for the treatment of VC. (2) The two functional modules that underpin the mechanism of action of Chinese herbs in the treatment of VC are smooth muscle adaptation and oxidoreductase activity. This complex bio-pathway network has three signaling pathways that are critical to its function: the AGE-RAGE signaling pathway in diabetic complications, the IL-17 signaling pathway, and the p53 signaling pathway.

Numerous investigations[1-3] conducted in recent decades have demonstrated that vascular calcification in both the intimal and medial layers is an active, tightly controlled process that is primarily driven by the vascular smooth muscle cell (VSMCs). Besides, the internal elastic lamina is thought to be the location of microcalcifications (calcification in the atherosclerotic neointima), which are believed to be triggered by apoptotic smooth muscle cells or matrix vesicles released by these cells[23]. Smooth muscle cells reprogramming and differentiation to an osteoblast-like phenotype, as well as the deposition of calcifying matrix vesicles produced by smooth muscle cells in the arterial wall, are linked to the pathophysiology of vascular intimal and medial calcification[24]. Smooth muscle cells apoptosis, and disorders of calcium-phosphate homeostasis that may develop as a result of disturbed hormonal regulation of the system, all contribute to the facilitation of VC processes[1,25]. In a physiological situation, smooth muscle (SM) myocytes produce a wide range of contractile proteins, such as SM a-actin (SMaA), SM-22a, SM myosin heavy chains SM-1 and SM-2, calponin, and smoothelin[2]. They also proliferate slowly, are functionally contractile, and respond to signals like acetylcholine and norepinephrine[24-26]. However, SM cells exhibit phenotypic plasticity and are not terminally differentiated like other myocytes. When exposed to local signals like damage, smooth muscle cells can change their phenotype[26]. They are able to downregulate contractile proteins, boost proliferation, and modify the extracellular matrix (ECM) to aid in migration. The emergence of calcifying vesicles, down-regulation of mineralization inhibitory molecules, and the creation of a calcification-prone matrix are the hallmarks of the transition from the contractile to the osteo/chondrogenic phenotype[27]. Loss of SM cells biomarkers (SM22a and SM a-actin) and an increase in osteochondrogenic signals [Runx2, SP7, osteopontin, osteocalcin, alkaline phosphatase (ALP), Sox9, Type II and X collagen (Col II and Col X)] are both associated with this phenotype[24]. It is significant that many of these characteristics are pathogenic and contribute to the development of vascular disease[25]. Therefore, one of the key approaches for the therapy of VC involves targeting the plasticity of smooth muscle cells. Fortunately, the present research revealed that the main mechanism of Chinese herbs in treating VC is by specifically connecting smooth muscle adaptation (functional remodeling).

The role of phenotypic transformation of SMCs in vascular calcification has been discussed above. This highly controlled cellular phenotypic switch can be triggered by abnormal mineral homeostasis, and hyperglycemia with advanced

glycation endproduct (AGE) accumulation, which coincide with the clinical features of the high-risk population for developing VC[28]. While AGE accumulation are sources of oxygen species (ROS) abnormal increase in calcified VSMCs, vascular calcification is significantly influenced by oxidative stress induced by the excessive generation of ROS[29]. A vicious cycle of ROS accumulation and increased apoptosis can also result from mitochondrial injury, which can be accompanied by further ROS leakage from the inner mitochondrial membrane[30]. Inflammation that follows cytokine release driven on by oxidative stress can assist in laying the groundwork for the development of VC. Given the significance of ROS in the pathophysiology of VC, antioxidants can be a promising therapeutic strategy for treating VC[28,29]. As in this study, the main mechanism of Chinese herbs in treating VC is involved in oxidoreductase activity (redox homeostasis modulating).

According to the literature, VC is a metabolic disorder[31]. The pathways (the AGE-RAGE signaling pathway in diabetic complications, the IL-17 signaling pathway) found in this study are all metabolic pathways, which are closely related to vascular calcification. As mentioned above, AGEs are mainly produced by hyperglycemia. Advanced glycation end-products receptor (RAGE or AGER), a member of the immunoglobulin superfamily and a pattern recognition receptor, is the primary AGE receptor[32]. VC, one of the complications of diabetes, can be triggered by AGE/RAGE signaling pathway through activating a series of intracellular signaling pathways, such as nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and protein kinase C[33].The IL-17 is an extremely customizable pro-inflammatory cytokine crucial for a range of processes, including host defense, tissue repair, and the pathogenesis of inflammatory process[34]. This cytokine has a significant impact on inflammation, but it also plays an important role in regulating cellular and organismal metabolism[35]. Indeed, both the physiological and the pathogenic elements of IL-17 responses incorporate metabolic control. As a result, it is anticipated that targeting IL-17 would open up new therapeutic possibilities for a variety of metabolic disorders[36]. A number of stress signals, including DNA damage, oxidative stress, and activated oncogenes, cause p53 to become active[37]. We have already explored the significance of high glucose-induced oxidative stress in vascular calcification. As a gatekeeper, the transcription factor p53 controls a wide range of genes to preserve regular cell processes. P53 expression is increased in pathological cardiovascular conditions in both patient samples and animal models. In vascular disease, p53 is essential for carrying out pathological processes and preserving physiological function[38]. Additionally, research[39] has shown that controlling the p53 signaling pathway helps preventing vascular calcification, and aids in the treatment of VC.

Conclusions

A network pharmacology and data mining approach were used to uncover the biochemical foundation and underlying processes of a Chinese herb in the therapy of VC. A total of 190 active compounds and 183 common targets across medication and VC, were obtained. In addition, we discovered seven most hub proteins (AKT1, CTNNB1, TNF, EGFR, TP53, JUN and IL-6) and two of the herbs' most fundamental ingredients (Formononetin and Luteolin) in TCM-mediated VC suppression, and five critical therapeutic pathways (smooth muscle adaption, oxidoreductase activity, AGE-RAGE, IL-17, and p53) that are linked to Chinese herb therapy of VC. It is worth noting that more experimental confirmation of the aforementioned projected outcomes is necessary in the future for their clinical translational potential.

Availability of Data and Materials

The original contributions presented in the study are included in the article/Supplementary Material; further inquiries can be directed to the corresponding author.

Authors' Contributions

Chan Yang and Chunguang Xie conceived and designed the research; Chan Yang, Xi Peng, Guocheng Rao and Hanyu Liu contributed to data collection and analysis. Chunguang Xie and Ziyan Xie were responsible for data analysis. Xinqiong Li, Lian Du, and Qiangfei Yang supervised the project, designed experiments, and provided vital advice. Chan Yang, Ziyan Xie and Qiangfei Yang wrote the manuscript. All the authors read and approved the final manuscript, and all data were generated in-house.

Funding

This work was financially supported by National Natural Science Foundation of China (Grant Number: 81774302 and 82004341), Sichuan Science and Technology Program (Grant Number: 2020JDTD0022) and China Postdoctoral Science Foundation (Grant Number: 2022M712286).

Acknowledgments

All of the authors like to express their gratitude to their respective universities and institutions for the technical aid and valuable assistance offered by the appropriate departments with this research project.

References

1) Villa-Bellosta R. Vascular Calcification: Key Roles of Phosphate and Pyrophosphate. Int J Mol Sci 2021; 22: 259-270.
2) Abbasian N. Vascular Calcification Mechanisms: Updates and Renewed Insight into Signaling Pathways Involved in High Phosphate-Mediated Vascular Smooth Muscle Cell Calcification. Biomedicines 2021; 9: 23.
3) Quaglino D, Boraldi F, Lofaro FD. The biology of vascular calcification. Int Rev Cell Mol Biol 2020; 354: 261-353.
4) Xu C, Smith ER, Tiong MK, Ruderman I, Toussaint ND. Interventions To Attenuate Vascular Calcification Progression in Chronic Kidney Disease: A Systematic Review of Clinical Trials. J Am Soc Nephrol 2022; 33: 1011-1032.
5) Vossen LM, Kroon AA, Schurgers LJ, de Leeuw PW. Pharmacological and Nutritional Modulation of Vascular Calcification. Nutrients 2019; 12: 100.
6) Hildebrand S, Cunningham J. Is there a role for bisphosphonates in vascular calcification in chronic kidney disease? Bone 2021; 142: 115751.
7) Y S, C J. Research progress of Chinese medicine intervention in vascular calcification in CKD. Western Chinese Medicine 2020; 33: 150-155
8) Sun JH, Sun F, Yan B, Li JY, Xin DL. Data mining and systematic pharmacology to reveal the mechanisms of traditional Chinese medicine in Mycoplasma pneumoniae pneumonia treatment. Biomed Pharmacother 2020; 125: 109900.
9) Zheng W, Wu J, Gu J, Weng H, Wang J, Wang T, Liang X, Cao L. Modular Characteristics and Mechanism of Action of Herbs for Endometriosis Treatment in Chinese Medicine: A Data Mining and Network Pharmacology-Based Identification. Front Pharmacol 2020; 11: 147.
10) Zhang R, Zhu X, Bai H, Ning K. Network Pharmacology Databases for Traditional Chinese Medicine: Review and Assessment. Front Pharmacol 2019; 10: 123.
11) Li S, Zhang B. Traditional Chinese medicine network pharmacology: theory, methodology and application. Chin J Nat Med 2013; 11: 110-120.
12) Wang X, Wang ZY, Zheng JH, Li S. TCM network pharmacology: A new trend towards combining computational, experimental and clinical approaches. Chin J Nat Med 2021; 19: 1-11.
13) Wu H, Li C, Li F, Gu S. Study on the effect and mechanism of Jianpi Bushen Tongluo prescription in the treatment of senile chronic kidney disease. Journal of Medical Forum 2021; 42: 125-128. Available at: https://kns.cnki.net/kcms2/article/abstract?v=3uoqIhG8C44YLTlOAiTRKibYlV5Vjs7iy_Rpms2pqwbFRRUtoUImHZsJwxzcjPps__fOXv9LuIA-JwORu_RSA7zakZrXixqu&uniplatform=NZKPT.
14) Zhong J, Tang N, Shi W, Huang X, Zhao N, Liu C. Effect of Wenyang Tongluo Xiezhuo prescription on vascular calcification in maintenance hemodialysis patients. J Tradit Chin Med 2016; 57: 588-591.
15) Chen J. Effect of Tonglin decoction assisted hemodialysis in the treatment of uremia and its effect on the expression of vascular calcification factors BMP-2 and OPG. Modern diagnosis and treatment 2018; 29: 3207-3208. Available at: https://kns.cnki.net/kcms2/article/abstract?v=3uoqIhG8C44YLTlOAiTRKibYlV5Vjs7iLik5jEcCl09uHa3oBxtWoAmZfam6ifll-p0FxRLC_00ZiPdauXV1lllkQ0G5qoXD&uniplatform=NZKPT.
16) Fan Z. Clinical observation of Quyu Huazhuo Decoction in the treatment of CKD-iv wet turbidity and blood stasis syndrome with vascular calcification. Heilongjiang University of Traditional Chinese Medicine, 2017. Available at: https://kns.cnki.net/kcms2/article/abstract?v=3uoqIhG8C475KOm_zrgu4lQARvep2SAkVtq-vp-8Qbjqyhl E-4l1YnljL_-Ts5MqNyCU0HbnNRMXfK9u6l5syVbD-5k4kra-&uniplatform=NZKPT.
17) Zhou M. Effect of Yiqi Huoxue Tongluo method on fetoglobulin-A and osteocalcin in patients with chronic kidney disease stage 3-5 non-dialysis. Anhui University of Chinese Medicine, 2015. Available at: https://kns.cnki.net/kcms2/article/abstract?v=3uoqIhG8C475KOm_zrgu4lQARvep2SAk6nr4r5tSd-_pTaPGgq4znPXSL4RXicnO79b4Vg0jMTt5BxwM9t6rYZjJv-CC0Fqd&uniplatform=NZKPT.
18) Xiong J, Yang Q, Li N, Zhang P. Effect of self-designed Guben Quzhuo prescription on hyperphosphatemia in maintenance hemodialysis patients. Guangxi Medical 2022; 44: 521-525+538.

Available at: https://kns.cnki.net/kcms2/article/abstract?v=3uoqIhG8C44YLTlOAiTRKibYlV5Vjs7iJTKGjg9uTdeTsOI_ra5_XR1OmJO5UFmufE0MCOlFTN1SeERwJUBCP-S_uirFybU7&uniplatform=NZKPT.

19) Ma X. Effect of Chenshi Shenfu decoction on patients with chronic kidney disease (CKD3a) with spleen-kidney qi deficiency and dampness and stasis and its effect on serum fetubulin Av. Fujian University of Traditional Chinese Medicine, 2020. Available at: https://kns.cnki.net/kcms2/article/abstract?v=3uoqIhG8C475KOm_zrgu4lQARvep2SAkHr3ADhkADnVu66WViDP_3POzTTcGA1vx93LVhfIFxzQJO8q5N0JZ2pEcNSzVB26a&uniplatform=NZKPT.
20) Ma Y. Effect of Yishen Lixue Xiezhuo decoction on CKD-MBD and its clinical evaluation. Shandong University of Traditional Chinese Medicine, 2019. Available at: https://kns.cnki.net/kcms2/article/abstract?v=3uoqIhG8C475KOm_zrgu4lQARvep2SAkyRJRH-nhEQBuKg4okgcHYmU-

ladSbE130HsgGdUvqzLXbEWimm9nDrMKDHthizIC5&uniplatform=NZKPT.

21) Ge H, Liu X, Zhai L. Effect of Baoshen decoction on renal function index and vascular calcification in uremia patients undergoing high throughput hemodialysis. World Journal of Integrated Traditional Chinese and Western Medicine 2021; 16: 1491-1494+1499. Available at: https://kns.cnki.net/kcms2/article/abstract?v=3uoqIhG8C44YLTlOAiTRKibYlV5Vjs7iy_Rpms2pqwbFRRUtoUImHeKeYxBOlS14t9HpRyCYFgWZvcwhRE5lUtSkctGpmVkB&uniplatform=NZKPT.
22) Hu S, Wang D, Zhang R, Cao Y, Jin H, Mao Y, Wei L, Ren K, Zhang X, Wang Y. Effect of Ronghuang granule on serum FGF23, FGFRs and Klotho proteins in non-dialyzed CKD-MBD patients with kidney deficiency and damp-heat syndrome. Nan Fang Yi Ke Da Xue Xue Bao 2018; 38: 1427-1432.
23) WANG X-Q, ZOU X-R, ZHANG YC. From "Kidneys Govern Bones" to Chronic Kidney Disease, Diabetes Mellitus, and Metabolic Bone Disorder: A Crosstalk between Traditional Chinese Medicine and Modern Science. Evid Based Complement Alternat Med 2016; 2016: 4370263.
24) Ghosh S, Luo D, He W, Chen J, Su X, Huang H. Diabetes and calcification: The potential role of anti-diabetic drugs on vascular calcification regression. Pharmacol Res 2020; 158: 104861.
25) Leopold JA. Vascular calcification: Mechanisms of vascular smooth muscle cell calcification. Trends Cardiovasc Med 2015; 25: 267-274.
26) Durham AL, Speer MY, Scatena M, Giachelli CM, Shanahan CM. Role of smooth muscle cells in vascular calcification: implications in atherosclerosis and arterial stiffness. Cardiovasc Res 2018; 114: 590-600.
27) Shi J, Yang Y, Cheng A, Xu G, He F. Metabolism of vascular smooth muscle cells in vascular diseases. Am J Physiol Heart Circ Physiol 2020; 319: H613-H631.
28) Allahverdian S, Chaabane C, Boukais K, Francis GA, Bochaton-Piallat ML. Smooth muscle cell fate and plasticity in atherosclerosis. Cardiovasc Res 2018; 114: 540-550.
29) Shi N, Mei X, Chen SY. Smooth Muscle Cells in Vascular Remodeling. Arterioscler Thromb Vasc Biol 2019; 39: e247-e252.
30) Chao CT, Yeh HY, Tsai YT, Chuang PH, Yuan TH, Huang JW, Chen HW. Natural and non-natural antioxidative compounds: potential candidates for treatment of vascular calcification. Cell Death Discov 2019; 5: 145.
31) Hu CT, Shao YD, Liu YZ, Xiao X, Cheng ZB, Qu SL, Huang L, Zhang C. Oxidative stress in vascular calcification. Clin Chim Acta 2021; 519: 101-110.
32) Nguyen NT, Nguyen TT, Park KS. Oxidative Stress Related to Plasmalemmal and Mitochondrial Phosphate Transporters in Vascular Calcification. Antioxidants (Basel) 2022; 11: 494.
33) Demer LL, Tintut Y. Inflammatory, metabolic, and genetic mechanisms of vascular calcification. Arterioscler Thromb Vasc Biol 2014; 34: 715-723.
34) Shen CY, Lu CH, Wu CH, Li KJ, Kuo YM, Hsieh SC, Yu CL. The Development of Maillard Reaction, and Advanced Glycation End Product (AGE)-Receptor for AGE (RAGE) Signaling Inhibitors as Novel Therapeutic Strategies for Patients with AGE-Related Diseases. Molecules 2020; 25: 5591.
35) Kay AM, Simpson CL, Stewart JA. The Role of AGE/RAGE Signaling in Diabetes-Mediated Vascular Calcification. J Diabetes Res 2016; 2016: 6809703.
36) Li X, Bechara R, Zhao J, McGeachy MJ, Gaffen SL. IL-17 receptor-based signaling and implications for disease. Nat Immunol 2019; 20: 1594-1602.
37) Bechara R, McGeachy MJ, Gaffen SL. The metabolism-modulating activity of IL-17 signaling in health and disease. J Exp Med 2021; 218: e20202191.
38) Wang Z, Tan J, Lei L, Sun W, Wu Y, Ding P, Chen L. The positive effects of secreting cytokines IL-17 and IFN-γ on the early-stage differentiation and negative effects on the calcification of primary osteoblasts in vitro. Int Immunopharmacol 2018; 57: 1-10.
39) Harris SL, Levine AJ. The p53 pathway: positive and negative feedback loops. Oncogene 2005; 24: 2899-2908.
40) Men H, Cai H, Cheng Q, Zhou W, Wang X, Huang S, Zheng Y, Cai L. The regulatory roles of p53 in cardiovascular health and disease. Cell Mol Life Sci 2021; 78: 2001-2018.
41) Liu L, Zeng P, Yang X, Duan Y, Zhang W, Ma C, Zhang X, Yang S, Li X, Yang J, Liang Y, Han H, Zhu Y, Han J, Chen Y. Inhibition of Vascular Calcification. Arterioscler Thromb Vasc Biol 2018; 38: 2382-2395.