Network pharmacology study of vaccarin for the prevention and treatment of postmenopausal osteoporosis

Mengying Bao
Guangxi Medical University https://orcid.org/0000-0003-0258-1059
Yan Dai
The Second Afficiated Hospital of Guangxi Medical University
Xiaojun Chen
Guangzhou University of Chinese Medicine
Shijie Liao
Guangxi Medical University First Affiliated Hospital
Wenyu Feng
Guangxi Medical University First Affiliated Hospital
Chengsen Lin
Guangxi Medical University First Affiliated Hospital
Yu Ye
The Second Affiliated Hospital of Guangxi Medical University
Zengnan Mo
Guangxi Medical University
Yun Liu (liuyun200450250@sina.com)
Guangxi Medical University First Affiliated Hospital https://orcid.org/0000-0002-7745-1083

Research

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Abstract

Background: As the main active ingredient of Semen Vaccariae, vaccarin is a flavonoid glycoside useful for the prevention and treatment of numerous diseases. Our previous study found that vaccarin can reduce osteolysis-induced titanium by inhibiting osteoclast formation. However, the issue of whether vaccarin can prevent and treat postmenopausal osteoporosis remains unclear.

Method: In this study, we explored the mechanism of action of vaccarin for the prevention of postmenopausal osteoporosis via a network pharmacological approach. We identified the intersecting targets of osteoporosis-related genes retrieved from multiple disease target databases, as well as targets of potential action of vaccarin retrieved from drug-related databases. We then used the intersectional targets to establish a protein-protein interaction (PPI) network. Finally, we performed bioinformatics analysis to enrich Gene Ontology (GO) biological processes and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways.

Results A total of 28 cross targets of vaccarin and osteoporosis were identified. PPI network analysis identified six target proteins, namely, IL-6, TNF, VEGFA, HSP90AA1, CREB1, and IL-2, which may be the key targets of vaccarin against osteoporosis. The 28 intersectional targets were mainly involved in 23 biological processes, such as regulation of apoptosis, positive regulation of neovascularization, and angiogenesis, whereas KEGG enrichment analysis revealed that they were primarily related to 22 different signaling pathways, such as PI3K/Akt pathway, cancer pathway, hepatitis B pathway, and tuberculosis pathway.

Conclusion: We used a network pharmacology approach to predict the key targets of vaccarin for the prevention of osteoporosis from a systems perspective. We determined that the signaling pathways were chiefly engaged in different pathological processes affecting differentiation and apoptosis of bone rebuilding cells, endocrine metabolic disorders, inflammatory responses, and other disease interactions. This study provides a theoretical basis and therapeutic ideas for the treatment of postmenopausal osteoporosis and offers promising directions for further research on the regulatory mechanism of vaccarin.

1. Introduction

Semen Vaccariae, the dried ripe seeds of the *Vaccaria hispanica* (Mill.) Rauschert, is a traditional Chinese herbal medicine that has been used in China for thousands of years[1].. It is bitter with a neutral pH; it is distributed to the liver and stomach; and it has the effect of activating blood circulation, decreasing lower breast swelling, and promoting diuresis and lactogenesis[2]. This herbal medicine is mainly used in the clinical treatment of lactating difficulties in postpartum women, breast carbuncle swelling and pain, menstrual disorder, gonorrhea astringent pain, and other diseases[1, 3]. Modern technology can isolate various types of compounds from the extract of Semen Vaccariae, such as cyclic peptides, triterpenes, saponins, and flavonol glycosides[4].

As the main active ingredient of Semen Vaccariae, vaccarin is a flavonoid glycoside and a phytoestrogen[5]. Previous studies on vaccarin largely focused on its extraction and separation, drug metabolism, pharmacological mechanism, cell signaling, and other aspects. Numerous studies have confirmed the effectiveness of vaccarin in the prevention and treatment of many diseases, such as in reducing endothelial cell damage[6, 7], promoting angiogenesis[8], inhibiting inflammation, preventing atherosclerosis[9], promoting wound healing[10, 11], improving insulin resistance[12], and preventing renal hypertension and cardiovascular remodeling[13]. Through extensive basic experiments, Professor Qiu Liying's team found that vaccarin can prevent and treat diseases via different targets and signaling pathways. Another study reported that vaccarin can reverse kidney damage from hypertension by reducing inflammation, oxidative stress, and fibrosis[13].

In our previous study, we found that vaccarin can effectively prevent titanium particle-induced pathological osteolysis in vivo by blocking the MAPK and NF-κβ signaling pathways[14]. In terms of pathogenesis, the titanium particle-induced osteolysis model, a model that mimics the common clinical condition of periprosthetic aseptic loosening, induced the loss of periprosthetic bone mass through wear particle-mediated aseptic inflammation. Postmenopausal osteoporosis is primarily due to a sharp decline in estrogen, leading to metabolic disorders caused by bone loss [15]. In this manner, postmenopausal osteoporosis is similar to titanium particle-induced osteolysis in terms of pathogenesis. Nevertheless, these two conditions have striking differences. Thus, the issue of whether vaccarin can also prevent and treat postmenopausal osteolysis is not yet clear.

Various methods, such as osteoclast formation experiments and luciferase gene reporting experiments on the NFAT or NF-κβ signaling pathway, are available for screening compounds or herbal monomers that are effective in the prevention and treatment of osteoporosis. However, a large number of drugs in the early stage must be bought, and constructing luciferase gene cell lines and purchasing reagents are time-consuming and expensive. Therefore, a simple, rapid, and economical screening method for selecting effective drugs for a particular disease must be developed.

The concept of "network pharmacology" was first proposed by the British scholar Andrew L. Hopkins in 2007[16]. This concept is based on the similarity between drugs and drugs in structure, efficacy, and other aspects to predict the potential target genes of the active pharmaceutical ingredients. By combining the multiple interactions of target molecules and biological effector molecules, the efficacy of drugs and the biological functions and signaling pathways involved in drug treatment can be predicted. Compared with Western medicine, traditional Chinese herbal medicine has the advantages of strong plant origin, easy access, and low toxicity, and thus it enjoys high trustworthiness among the general public. However, it also has shortcomings, such as complex compositions and unknown mechanisms of action, which are the primary reasons that hinder the recognition and further promotion of Chinese herbal medicine worldwide. The research and development model of Chinese herbal medicine is different from that of Western medicine. The herbal active monomer is the main effective part of the herbal compound, and this monomer often acts as a disease-modifying agent by regulating multiple targets and pathways. The emerging field of network pharmacology largely focuses on multitarget and multipathway action

characteristics of the active monomers of Chinese herbal medicines. Through this approach, the potential mechanism of action of the active monomers at the gene and protein levels can be revealed. This approach has bridged the modernization and internationalization of traditional Chinese medicine and addressed the challenges in research on Chinese herbal medicines.

Research on osteoporosis via network pharmacology is still in its initial stage and mainly focuses on the compounds of Chinese herbal medicines. Rhizoma drymariae, Er-xian decotion, and Sinomenii Caulis are folk Chinese herbs with good clinical effects commonly used for the treatment of osteoporosis and with relevant network pharmacology studies[17–19]. By contrast, the network pharmacology of Semen Vaccariae and its active ingredient vaccarin for the treatment of osteoporosis is lacking. This study conducted bioinformatics analysis via a drug component–target gene–disease pathway network to explore the pharmacological mechanisms of vaccarin in relation to osteoporosis and provide a theoretical basis for further study of vaccarin for osteoporosis prevention and clinical translational applications.

2. Material And Methods

2.1 Study design

Genes associated with osteoporosis were searched from multiple disease target databases, whereas the potential targets of vaccarin were retrieved from drug-related databases. The cross targets between the genes and the potential targets were then identified. Multiple public databases were consulted to analyze the cross-target networks and enrichment pathways to explore the major signaling pathways of vaccarin and its molecular networks involved in the prevention and treatment of osteoporosis. The workflow is shown in Fig. 1.

2.2 Prediction of vaccarin targets

SwissTargetPrediction (http://www.swisstargetprediction.ch/), SEA (http://sea.bkslab.org/search/), and TargetNet (http://targetnet.scbdd.com/) were used to retrieve the potential targets of vaccarin with the “human” species setting, and then duplicate data were eliminated to obtain the final potential targets of vaccarin.

2.3 Mining of osteoporosis targets

Comparative Toxicogen-omics Database (http://ctdbase.org/), Genecards (http://genecards.org/), Therapeutic Target Database (http://db.idrblab.net/ttd/), OMIM (https://www.omim.org/), and PharmGKB (https://www.pharmgkb.org/) were searched for information on osteoporosis-related genes and target proteins.

2.4 Establishing a network of protein-protein interactions (PPI)

Drug–disease crossover genes were screened and imported into the String database (http://string-db.org/). The species was defined as “human” to construct the vaccarin for osteoporosis protein–protein

interaction (PPI) network. Finally, network node and edge information were entered into Cytoscape3.6.0 for PPI network visualization.

2.5 Bioinformatics annotation and pathway analysis of crossover targets

Metascape database (http://metascape.org/gp/index.html#/main/step1) [20] and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways[21] (https://www.genome.jp/kegg/pathway.html) were utilized to retrieve information about the functional annotation of the drug–disease crossover genes and extract the typical pathways highly associated with these proteins. The relevant pathways were then filtered with P-value < 0.05 and mapped using the R3.2.5 software.

3. Results

3.1 Analysis of crossover targets

The SEA, Swiss, and TargetNet databases were used to retrieve 56, 15, and 20 possible targets for vaccarin, respectively. Only 76 genes remained after exclusion of duplicate data were excluded. The osteoporosis targets were searched in the Comparative Toxicogenomics, Genecards, Therapeutic target OMIM, and PharmGKB databases, leaving a total of 3601 target proteins after eliminating duplicate data. The set of drug targets and disease target proteins and their relationships were represented in a closed-loop form at fixed positions to obtain Venn diagrams, as shown in Fig. 2, and 28 intersecting target proteins were found, with specific target genes, target proteins, associated databases and scores shown in Table 1.

Table 1
Analysis of common targets between vaccarin and osteoporosis

ID	Target genes	Target proteins	Database and scores
1	CREB1	Cyclic AMP-responsive element-binding protein 1	SEA(111.407)
2	FGF1	Fibroblast growth factor 1	SEA(77.350)
3	FASN	Fatty acid synthase	SEA(77.350)
4	TYR	Tyrosinase	SEA(65.708)
5	P4HB	Protein disulfide-isomerase	SEA(60.050
6	NRAS	GTPase Nras	SEA(54.040)
7	PTPRS	Receptor-type tyrosine-protein phosphatase S	SEA(50.396)
8	FGF2	Fibroblast growth factor 2	SEA(26.341)
9	CYP1B1	Cytochrome P450 1B1	SEA(23.156)
10	VEGFA	Vascular endothelial growth factor A	SEA(19.070)
11	LGALS3	Galectin-3	SEA(18.630)
12	ERAP1	Endoplasmic reticulum aminopeptidase 1	SEA(14.186)
13	ALPI	Intestinal-type alkaline phosphatase	SEA(11.618)
14	IL6	Interleukin-6	SEA(10.691)
15	ABCB1	Multidrug resistance protein 1	SEA(10.059)
16	ADRA2A	Alpha-2a adrenergic receptor	SWISS(0.0499)
17	CA2	Carbonic anhydrase II	SWISS(0.0499)
18	TNF	TNF-alpha	SWISS(0.0499)
19	IL2	Interleukin-2	SEA(51.286) SWISS(0.0499)
20	XDH	Xanthine dehydrogenase/oxidase	SEA(36.443) SWISS(0.0499)
21	ACHE	Acetylcholinesterase	SEA(28.721) SWISS(0.0499)
22	NMUR2	Neuromedin-U receptor 2	SEA (14.777) SWISS(0.0499)
23	DNMT1	DNA (cytosine-5)-methyltransferase 1	TargetNet(0.999)

ID	Target genes	Target proteins	Database and scores
24	PTGS1	Prostaglandin G/H synthase 1	TargetNet(0.991)
25	BCL2A1	Bcl-2-related protein A1	TargetNet(0.987)
26	CYP1A2	Cytochrome P450 1B1	TargetNet(0.822)
27	HSP90AA1	Heat shock protein HSP 90-alpha	Targetnet(0.757)
28	NOS2	Niric oxide synthase, inducible	Targetnet(0.384)

3.2 PPI network analysis

The Cytoscape software was used to map the PPI network of 28 potential targets (Fig. 3A). PTPRS and ERAP1 were excluded as the results showed they were isolated. Further analysis of the remaining 26 targets revealed that the PPI contained 26 nodes and 83 edges (Fig. 3B). Among these nodes, the degree values of IL-6, TNF, VEGFA, HSP90AA1, CREB1, IL2, FGF2, ABCB1, NRAS, TYR, NOS2, and ACHE were greater than the median (5). They were suggested to play an important role in the anti-osteoporosis activity of vaccarin. Moreover, the degree values of IL-6, TNF, VEGFA, HSP90AA1, CREB1, and IL-2 were more than twice the median value; hence, they could be the key targets of vaccarin against osteoporosis (Table 2).

Table 2
Protein-protein interaction at common targets of vaccarin and osteoporosis

No.	Potential target	Degree	Closeness centrality
1	IL6	18	0.75757576
2	TNF	14	0.65789474
3	VEGFA	14	0.65789474
4	HSP90AA1	12	0.625
5	CREB1	11	0.625
6	IL2	10	0.58139535
7	FGF2	9	0.56818182
8	ABCB1	9	0.54347826
9	NRAS	8	0.52083333
10	TYR	7	0.49019608
11	NOS2	6	0.5
12	ACHE	6	0.54347826
13	FGF1	5	0.49019608
14	LGALS3	5	0.5
15	DNMT1	5	0.52083333
16	PTGS1	5	0.49019608
17	CYP1B1	4	0.48076923
18	XDH	3	0.46296296
19	CYP1A2	3	0.37313433
20	ADRA2A	3	0.41666667
21	BCL2A1	3	0.46296296
22	P4HB	2	0.45454545
23	NMUR2	1	0.29761905
24	CA2	1	0.34722222
25	ALPI	1	0.35714286
26	FASN	1	0.43859649

3.3 GO and KEGG pathway enrichment analyses

GO annotation revealed that the expressed 28 potential targets were mainly associated with 23 different biological processes, including positive regulation of the apoptotic process, lung development, positive regulation of sprouting angiogenesis, epithelial cell proliferation involved in salivary gland morphogenesis, and nitric oxide biosynthetic process (Fig. 4). This result indicated that vaccarin could regulate multiple biological processes to prevent and treat osteoporosis.

The intersection targets were found to interact with 22 KEGG pathways mainly involving the PI3K/Akt signaling pathway (eight targets), pathways in cancer (seven targets), HTLV-1 infection (five targets), microRNA in cancer (five targets), Chagas disease (American trypanosomiasis) (four targets), hepatitis B (four targets), and tuberculosis (four targets) (Fig. 5A). These pathways were then cataloged into four groups: (1) affecting the differentiation and apoptosis of bone reconstruction-related cells (5 pathways), as represented by the PI3K/Akt and the Rap1 signaling pathways; (2) the estrogen signaling pathway correcting endocrine metabolic disorder (1 pathway); (3) inhibiting inflammatory response (2 pathways), as represented by the NOD-like receptor signaling pathway; and (4) 14 other disease signaling pathways, as represented by the cancer-signaling pathway (Fig. 5B). PI3K/Akt is the signaling pathway with the largest number of targets, including NRAS, IL6, HSP90AA1, CREB1, VEGFA, FGF1, FGF2, and IL2. Further analysis of the PI3K/Akt pathway in the KEGG database revealed that it might be closely related to osteoclast protein synthesis, proliferation, survival, cell cycle, and differentiation (Fig. 6). This analysis suggested that vaccarin may play a synergistic role in the prevention and treatment of osteoporosis by modulating multiple targets and pathways.

4. Discussion

The emerging field of network pharmacology can apply systematic research on the multitarget and multipathway characteristics of the compounds or monomers of Chinese herbal medicines and reveal them at the molecular level. Therefore, network pharmacology, a systems biology-based approach, provides great convenience and saves valuable time for researchers in conducting drug screening and validating disease-signaling pathways.

Previous studies reported that vaccarin has a wide range of biological effects, such as the induction of FGF2 expression and the promotion of angiogenesis [22]. In terms of clinical translational applications, wound dressings containing vaccarin can inhibit inflammatory cell infiltration [8] and effectively promote endothelial cell migration and wound healing [11]. However, the issue of whether vaccarin can prevent and treat osteoporosis by influencing these biological processes is not yet clear.

Six target proteins, namely, IL-6, TNF, VEGFA, HSP90AA1, CREB1, and IL-2, found in the PPI network analysis were considered to be the most critical targets for pharmacological intervention in osteoporosis. The occurrence of osteoporosis is a very complex pathological process that may involve several key

target proteins. The monomers of Chinese herbal medicines, unlike small-molecule inhibition, often exert their therapeutic effects by affecting multiple targets. The biggest advantage of conducting a PPI network analysis is that the proteins with the greatest effect on gene interactions at the intersecting targets can be rapidly calculated, which in turn provides clear targets for drug intervention. Among the aforementioned key target proteins, IL-6, TNF, and IL-2 are important members of the inflammatory factor family, and they have been found to play critical roles in the differentiation of osteoclast precursor cells to mature osteoclasts[23]. VEGFA is a member of the vascular endothelial growth factor family, HSP90AA1 is an important member of the heat shock protein family [24], and CREB1 belongs to the important family of leucine zippers of DNA-binding proteins[25].

GO annotation analysis suggested that vaccarin might influence the pathogenesis of osteoporosis through 23 different biological processes, including regulation of apoptosis, angiogenesis, endothelial cell migration, lipolysis metabolism, insulin secretion, and cellular response to hydrogen peroxide. Several studies have proved that vaccarin plays a role in the treatment of other diseases by influencing the aforementioned biological processes. For instance, high glucose-induced endothelial cell injury can be reversed by downregulating the expression level of the apoptotic protein caspase 3[26]. Fibroblast growth factor receptor 1 activation can promote angiogenesis [19]. Disorders of glycolipid metabolism can be corrected by improving insulin resistance [11]. Increasing the activity of superoxide dismutase, catalase, and glutathione peroxidase in vivo can reduce the damage caused by H_2O_2 and oxidative stress to endothelial cells[9].

Notably, GO annotation analysis revealed positive regulation of osteoclast differentiation, which was inconsistent with the findings of previous studies that vaccarin negatively regulates osteoclast differentiation. This inconsistency may be attributed to the fact that network pharmacology can only predict the biological process in which the drug is involved, and the positive or negative regulation by the drug must be experimentally confirmed. Moreover, other biological processes in which vaccarin may be involved were identified, but extensive basic experiments are warranted to confirm them. Research on the potential signaling pathways of vaccarin in the treatment of osteoporosis mainly focused on the following four different pathological processes.

Effects on osteogenic cell differentiation and apoptosis pathways

Osteoblast apoptosis plays a crucial role in the pathogenesis of postmenopausal osteoporosis[27]. Both osteoblasts and osteoclasts are engaged in the process of bone reconstruction, and any influence on either side may break the balance inside the bone. PI3K/Akt is a signaling pathway closely related to cell proliferation and apoptosis, and PI3K/Akt signaling activation can reportedly promote osteoblast apoptosis [28]. Rap1 and Ras, which belong to the small GTPase family, are upstream signaling molecules of the MAPK signal and can be activated by various extracellular signals, such as growth factors, receptor tyrosine kinases, and Ca2+. Experimental studies have proved that Rap1 can promote osteoblast proliferation and differentiation by influencing the MAPK signaling pathway[29], whereas Ras activation can increase cyclinDI expression to promote osteoblast proliferation [30]. HIF-1α is also

involved in osteoclast formation and can effectively prevent the occurrence of osteoporosis by inhibiting its expression [31].

Internal secretion metabolic disorder

Postmenopausal osteoporosis is a common endocrine metabolism disorder. As the ovaries stop producing estrogen and serum estrogen levels drop precipitously after menopause, the postmenopausal female experiences a state of high inflammation and oxidative stress. This situation leads to osteoclast formation promotion, osteoblast proliferation inhibition, and steady state of bone rebuilding disruption[15].

Inflammatory response

Numerous inflammatory factors and inflammation-related signaling pathways are involved in the pathogenesis of osteoporosis. NOD-like receptors have been found to activate cysteine proteases, upregulate the secretion of the inflammatory factor IL-1B, and exacerbate inflammation-induced pathological bone loss[32].

Pathways associated with other diseases

The present KEGG pathway enrichment analysis revealed that rheumatoid arthritis, prostate cancer, tuberculosis, and amoebiasis may interact with osteoporosis. Overactive osteoclasts in advanced rheumatoid arthritis cause bone resorption, resulting in severe destruction of cartilage and subchondral bone[33]. Bone metastasis, which is different from general malignant tumor metastasis, is most common in advanced prostate cancer. Bone tuberculosis often occurs in bones or joints that bear heavy weights and are thus prone to damage, with the greatest damage being joint deformity and nerve compression due to bone destruction. Both of these disease processes are closely related to osteoclast-induced bone resorption. Thus, signaling pathways associated with rheumatoid arthritis, prostate cancer, tuberculosis, and other diseases may also be targets for vaccarin in the treatment of osteoporosis.

5. Conclusion

Through network pharmacology, we predicted that IL-6, TNF, VEGFA, HSP90AA1, CREB1, and IL2 might be the key targets of vaccarin against osteoporosis. The potential mechanisms of action of vaccarin against osteoporosis were based on various biological functions, such as regulation of apoptosis, positive regulation of neovascularization, negative regulation of lipolysis metabolic process, and positive regulation of osteoclast differentiation. KEGG pathway enrichment analysis revealed that vaccarin mainly treats osteoporosis by affecting the differentiation and apoptosis of bone rebuilding cells, correcting endocrine metabolic disorders, inhibiting inflammatory responses, and exerting therapeutic effects through interactions with other diseases. On the basis of these results, we propose that the antiosteoporosis activity of vaccarin is mainly based on the direct or indirect modulation of the

aforementioned potential targets and pathways. These results provide a new therapeutic approach for the treatment of postmenopausal osteoporosis.

Abbreviations

PPI: Protein-Protein Interaction; GO: Gene ontology; KEGG: Kyoto encyclopedia of genes and genomes

Declarations

Authors' contributions

YL, XJC and SJL designed the research. WYF and CSL performed the research, YL and XJC analyzed the data, MYB, YD and YL wrote the manuscript. All authors participated in the preparation of the manuscript. All authors read and approved the final manuscript.

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Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

Not applicable.

Competing interests

All authors declare that they have no competing interests.

Author details

[1] Center for Genomic and Personalized Medicine, Guangxi Medical University, Nanning 530021, Guangxi, China;

[2] Guangxi key Laboratory for Genomic and Personalized Medicine, Nanning 530021, Guangxi, China;

[3]Guangxi Collaborative Innovation Center for Genomic and Personalized Medicine, Nanning 530021, Guangxi, China;

[4] Guangxi Key Laboratory of Colleges and Universities, Nanning 530021, Guangxi, China;

[5] First Clinical Medical College, Guangzhou University of Chinese Medicine, Guangzhou 510400, Guangdong, China;

[6] Departments of Orthopedics, The First Affiliated Hospital of Guangxi Medical University, Nanning 530021, Guangxi, China;

University, Nanning 530021, Guangxi, China;

[7] The Second Affiliated Hospital of Guangxi Medical University, Nanning 530000, Guangxi, China;

[8] Institute of Urology and Nephrology, First Affiliated Hospital of Guangxi Medical University, Nanning 530021, Guangxi, China

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Figures

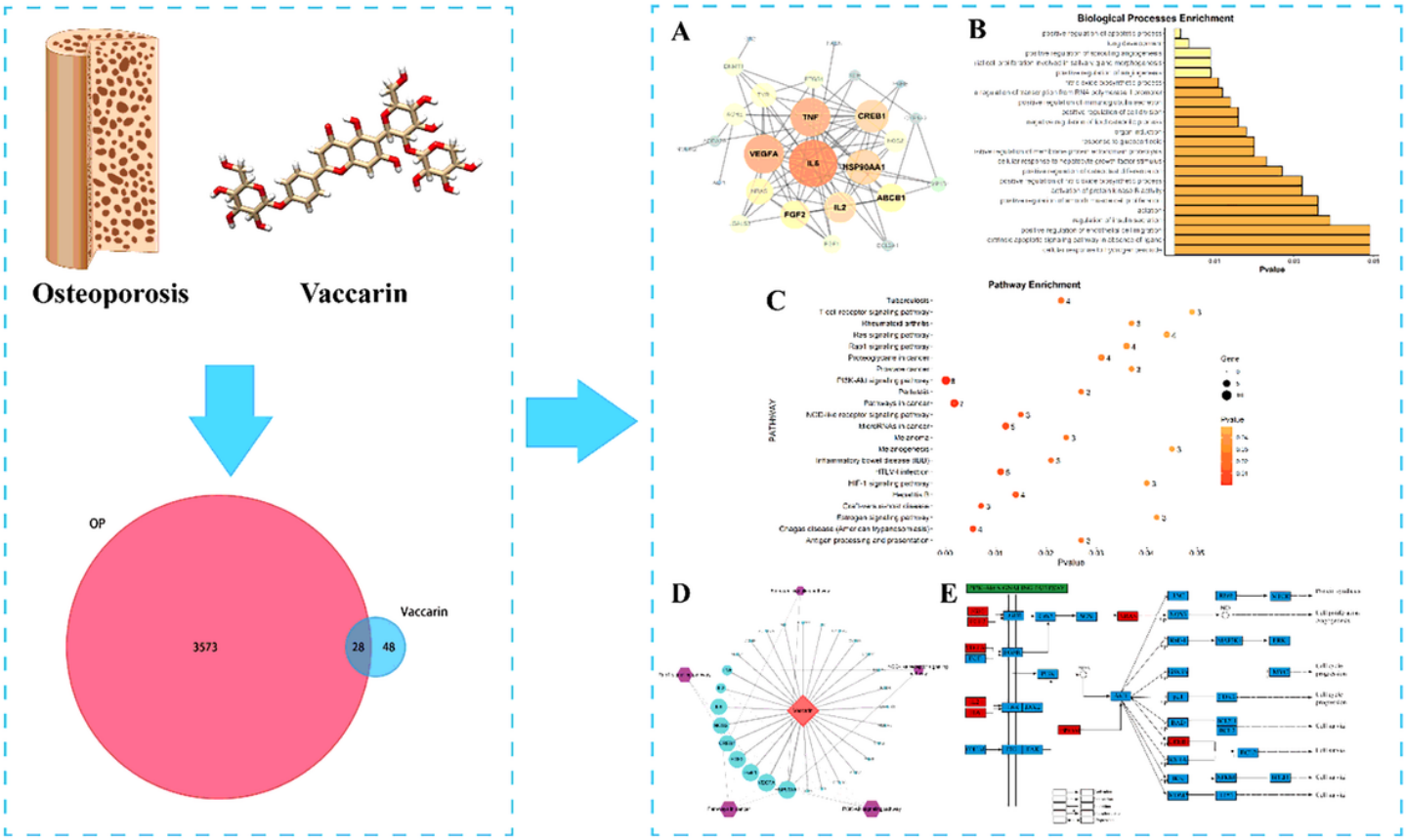

Figure 1

Flow chart of network pharmacology study on intervention of vaccarin in osteoporosis

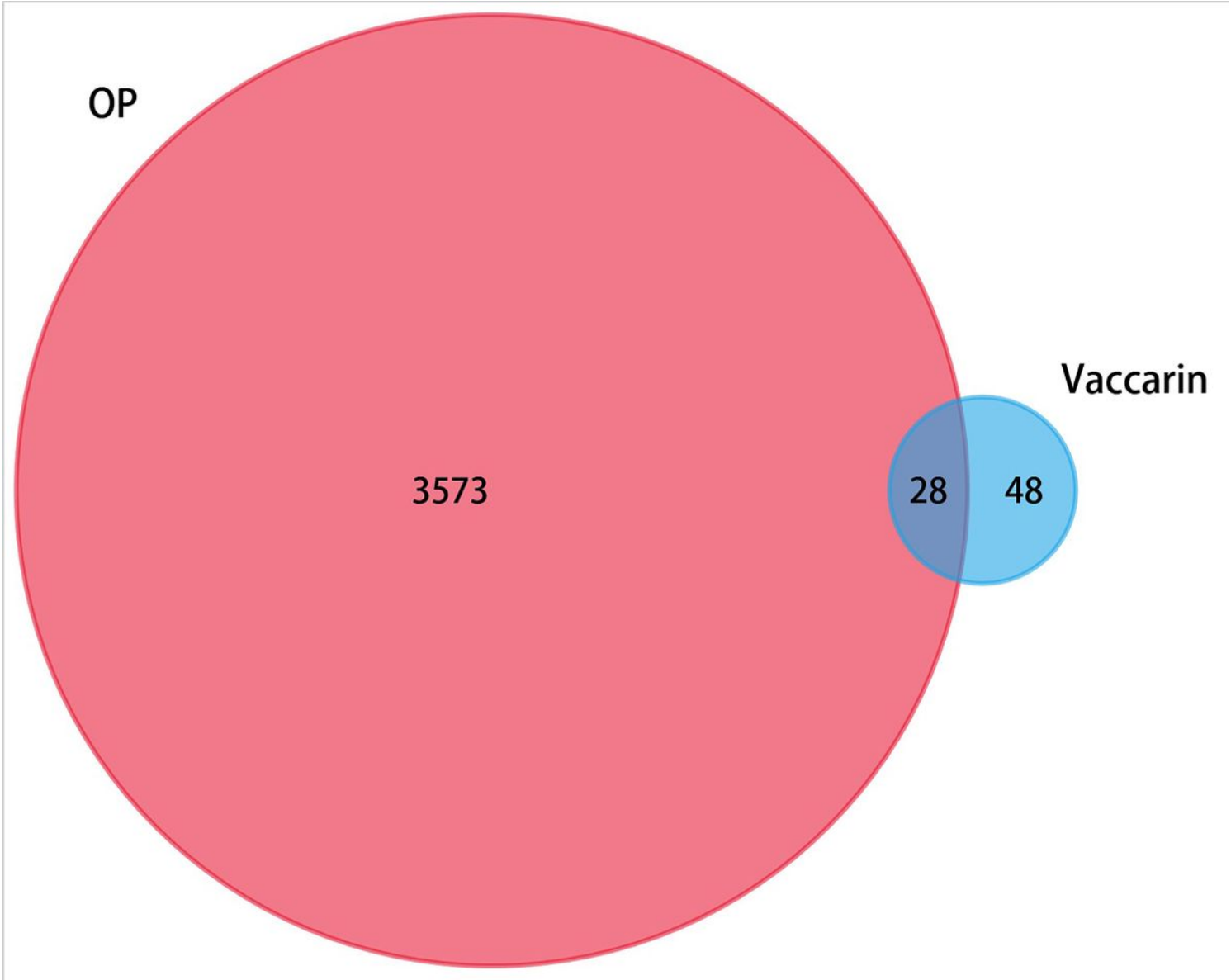

Figure 2

Venn diagram of the target genes of vaccarin and osteoporosis.

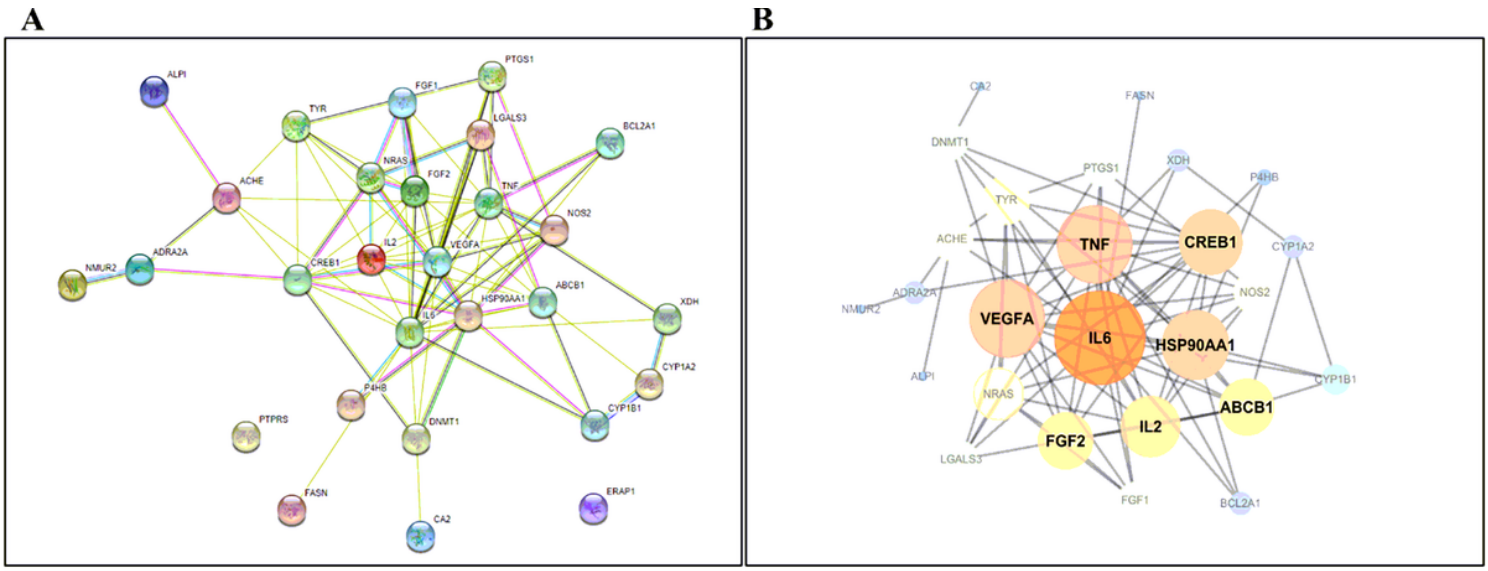

Figure 3

The protein interaction network of the combined action targets of vaccarin and osteoporosis. (A) PPI network of 28 targets. (B) PPI network after exclusion of isolated targets PTPRS, ERAP1.

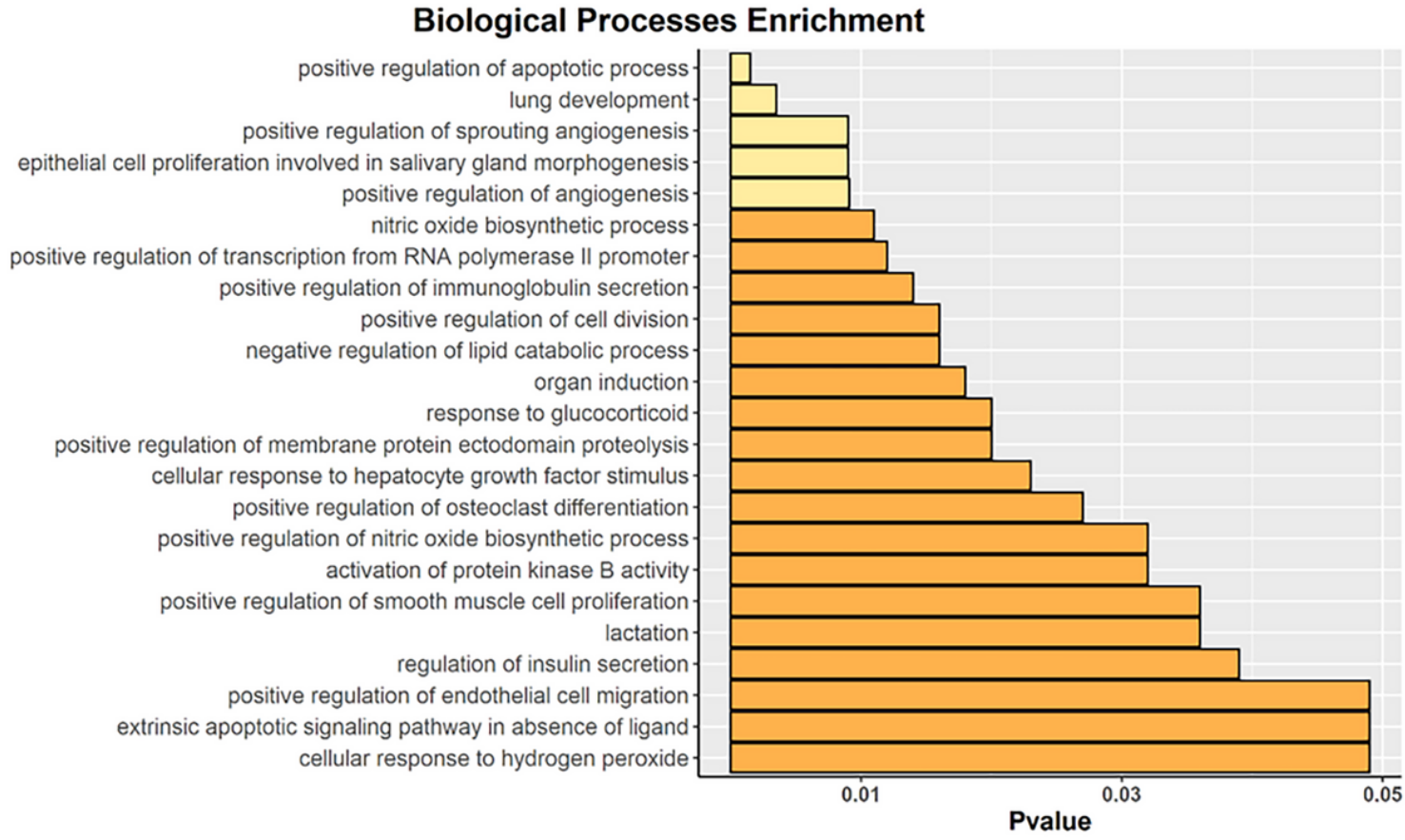

Figure 4

Biological process results of gene ontology annotations of intersection target proteins.

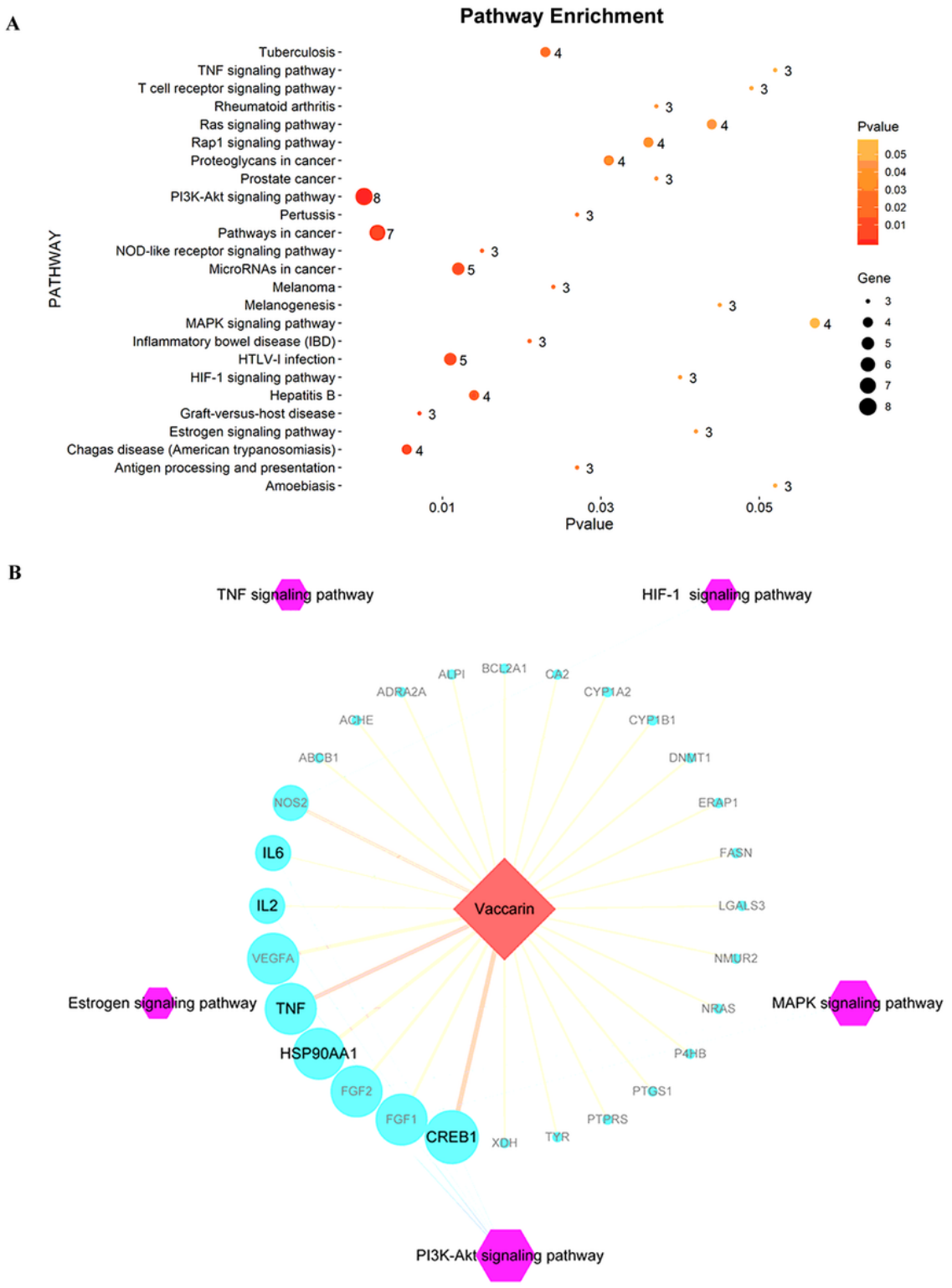

Figure 5

KEGG pathway enrichment analysis of the intersection targets of vaccarin and osteoporosis. (A) KEGG pathway enrichment analysis bubble map of intersecting targets. (B) Mapping of intersecting targets in the PI3K/Akt, cancer pathway, estrogen pathway, Rap1 pathway, and NOD-like receptor pathway.

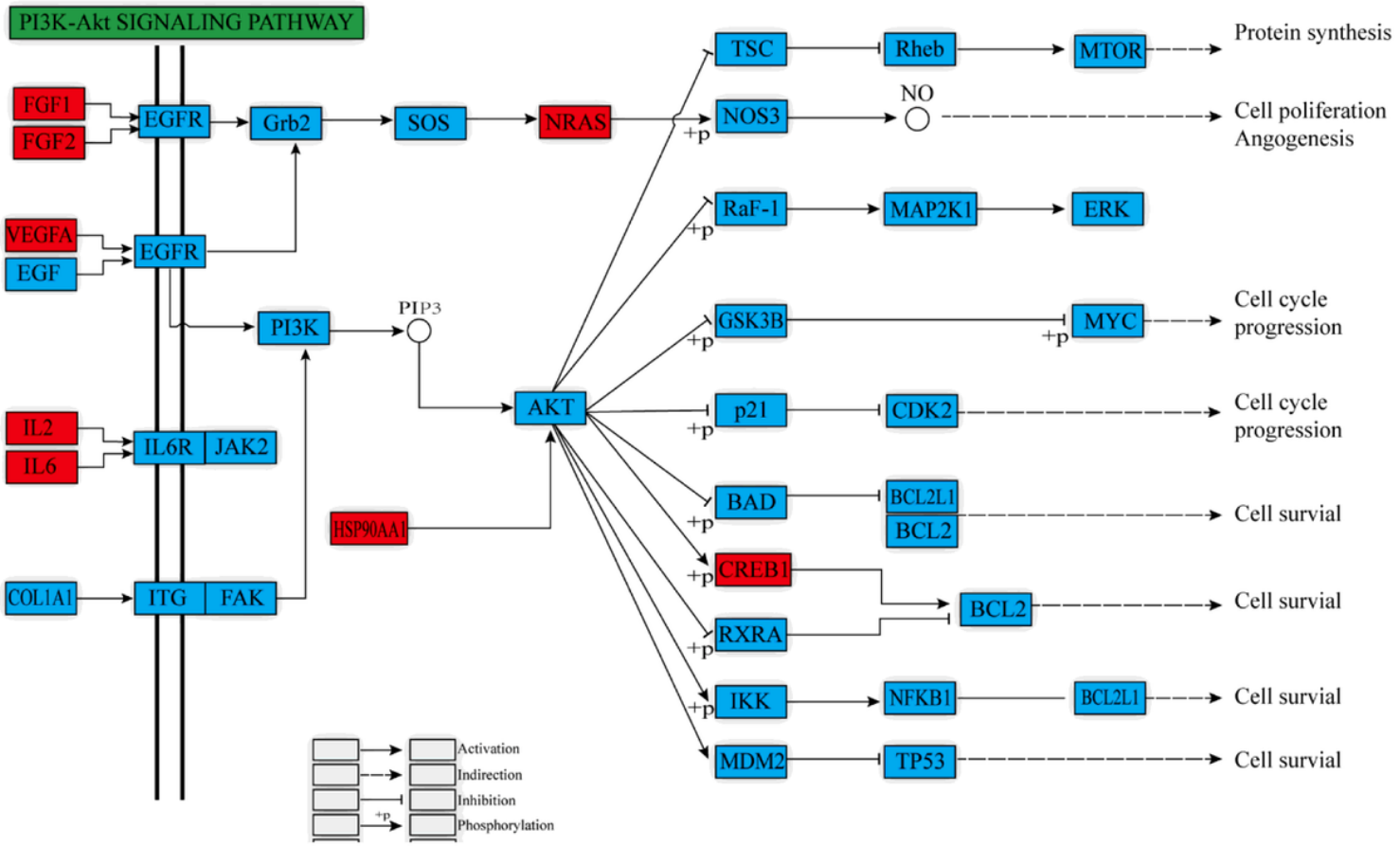

Figure 6

PI3K/Akt target-pathway network. The red nodes represent the hub genes, the green nodes represent other genes and the blue nodes are the genes of this pathway