Network pharmacology prediction and molecular docking-based strategy to explore the potential mechanism of RSG and Phellodendron against HPV infection

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Research Article

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Abstract

Background: Human papillomavirus (HPV) is a double-stranded circular DNA virus that mainly infects the human epidermis and mucosal squamous epithelium. We aimed to explore the mechanism of the Rhizoma Smilacis Glabrae (RSG) and Phellodendron compound in the treatment of HPV by network pharmacology and molecular docking.

Methods: Potential targets and active compounds of RSG and Phellodendron, as well as HPV-related targets, were retrieved from public databases. First, the key targets were obtained by intersection of the differentially expression genes (DEGs) between HPV (+)and HPV (-)tissue samples from GSE3292 dataset and top 10 proteins from protein-protein interaction (PPI) network according to Degree. Gene set enrichment analysis (GSEA) of key targets was analyzed. Subsequently, immune microenvironmentwas explored by CIBERSORT algorithm. The competing endogenouse RNA (ceRNA) network of key targets was created. Lastly, molecular docking was performed to predict the binding activityof active ingredients with key targets.

Results: A total of 37 bioactive ingredients of RSG and Phellodendron, and 59 RSG and Phellodendron-HPV-related targets were screened. GSEA showed (ESR1, EGFR and PTEN) were associated with PI3K-AKT signaling pathway. Immune infiltration analysis showed that CD8 T cells were found more abundant in the HPV (+) groups. Moreover, lncRNA-miRNA-mRNA network containing 20 nodes (3 key targets, 13 miRNAs and 4 lncRNAs) and 29 edges was constructed. Finally, molecular docking suggested that quercetin combined well with EGFR and PTEN, and palmatine had the strong affinity with ESR1.

Conclusion: 3 key targets (*ESR1*, *EGFR* and *PTEN*) and 2 bioactive ingredients (quercetin and palmatine) were successfully identified for RSG and Phellodendron against HPV, which provided theoretical basis for the clinical application of traditional Chinese medicine compound and the molecular mechanism of human papillomavirus infection.

1. Introduction

Human papillomavirus (HPV) is a common pathogen of female genital tract and skin mucosa with high host specificity[1], and more than 80% of women have been infected with HPV at least once in their lifetime while human as its only host[2]. The genomes of 450 HPV species have been isolated[3], divided into five genera. To date, the HPV vaccine remains the primary means of preventing HPV infection and treatment[4]. HPV vaccines include therapeutic vaccines and prophylactic vaccines[5]. The development and application of prophylactic vaccines can effectively reduce the rate of HPV infection at the source. Therapeutic vaccines include peptide or protein-based vaccines, recombinant DNA vaccines with viral vectors, recombinant vaccines with bacterial vectors and DNA vaccines, etc, they are aimed at generating cell-mediated immunity rather than neutralising antibodies[6]. However, there are no specific chemical drugs for HPV infection so far, and treatment relies mainly on improving the body's own immunity[7][8][9]. Therefore, early detection and development of new therapeutic strategies are urgently needed to improve the survival rate of HPV patients.

RSG (Rhizoma Smilacis Glabrae) is a perennial evergreen climbing shrub in the lily family, and its dried rhizomes are used as medicine, which contain saponins, tannins, resins and other chemical components. Studies have shown that RSG has practical value in the preventing of liver cancer, and clinically RSG can prevent leptospirosis, treat syphilis, measles and acute and chronic nephritis[10]. Phellodendron is a broad-leaved tree belonging to the family Rutaceae. It is used as a medicine by its bark, and contains various chemical components such as palmatine hydrochloride[11], berberine hydrochloride[12]. The therapeutic effects of Phellodendron are mainly focused on inflammation[13],

endocrine[14] (corticosterone, insulin, testosterone, beta-endorphin) and other aspects[15]. Currently, the therapeutic value of RSG and Phellodendron in HPV has not been reported.

In this study, we used public databases to identify potential therapeutic targets in HPV, as well as the active chemical components and potential targets of RSG and Phellodendron, to establish a pharmacological network of HPV and RSG and Phellodendron. The core active compounds corresponding to the key targets were predicted by molecular docking, providing a theoretical basis for improving the current statuation of HPV patients and the selection of therapeutic strategies.

2. Materials And Methods

2.1 Data source

The targets and active components of Rhizoma Smilacis Glabrae (RSG) and Phellodendron were retrieved from the Traditional Chinese Medicine Systems Pharmacology (TCMSP) database (http://tcmspw.com/tcmsp.php).

The Human Papilloma Virus (HPV)-related targets were searched in the DisGeNET Database (https://www.disgenet.org/home/), the Therapeutic Target Database (TTD, http://db.idrblab.net/ttd/), the Online Mendelian Inheritance in Man database (OMIM, https://www.omim.org/), and the Human Gene Database (Genecards, https://www.genecards.org/) with the keyword of ‘HPV’.

The head and neck squamous cell carcinoma (HNSCC)-related data were obtained from the Gene Expression Omnibus (GEO) database (http://www.ncbi.nlm.nih.gov/geo). The mRNA expression profile of GSE3292 dataset contained 8 HPV (+) tissue samples and 28 HPV (-) tissue samples. The long non-coding RNA (lncRNA) expression profile of GSE190222 dataset included 3 HPV (+) tissue samples and 3 HPV (-) tissue samples. The microRNA (miRNA) expression profile of GSE190223 dataset contained 3 HPV (+) tissue samples and 3 HPV (-) tissue samples.

2.2 Screening and functional enrichment analysis of candidate targets

Drugs (RSG and Phellodendron) targets and HPV-related targets were intersected to obtain candidate targets of drugs in the treatment of HPV. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses were carried out to further explore the functions and pathways of candidate targets based on R packages ‘clusterProfiler’ package (version 4.4.4)[16].

2.3 Network construction

Data of candidate targets, bioactive compounds, disease, herbs, functions or signaling pathways were imported into the Cytoscape 3.8.2 software[17]. After being polished, two networks were obtained as follows: targets-functions-pathways network and compounds-herbs-targets-disease network of treatment with RSG and Phellodendron for HPV.

2.4 Protein-protein interaction (PPI) network construction

To explore the linkage between candidate targets, PPI analysis was performed by submitting candidate targets of HPV to the STRING database (https://string-db.org/).

2.5 Acquisition of key targets

First, the differentially expression genes (DEGs) between HPV (+) and HPV (-) tissue samples from GSE3292 dataset were screened by ‘limma’ package (version 3.52.2) (p_ value < 0.05)[18]. Volcano plot of DEGs was made by R package

'ggplot2' (version 3.3.6)[19]. The intersection of DEGs with TOP 10 proteins from PPI network according to Degree was shown by Venn diagram to capture key targets.

2.6 Single-gene gene set enrichment analysis (GSEA)

Single-geneGSEA was carried out to explore the molecular mechanism of key targets in disease using 'clusterProfiler' R package (version 4.4.4)[20] with |NES|>1, adjust $p < 0.05$ and q_value < 0.2 based on KEGG gene sets.

The R package 'pathview' (version 1.36.0)[21] was used to visualize the enriched pathways.

2.7 Competing endogenouse RNA (ceRNA) network construction

The miRNAs associated with key mRNAs were screened by miRWalk (http://mirwalk.umm.uni-heidelberg.de/), and the predicted results were intersected with the differentially expressed miRNAs from GSE190223 dataset contained 3 HPV (+) tissue samples and 3 HPV (-) tissue samples, which yielded the miRNA-mRNA pairs. In addition, the lncRNAs were predicted by miRNAs in the miRNA-mRNA pairs, and the predicted results were intersected with the differentially expressed lncRNAs from GSE190222 dataset included 3 HPV (+) tissue samples and 3 HPV (-) tissue samples, which yielded the lncRNA-miRNA pairs. Finally, the two pair groups were matched to construct an 'lncRNA-miRNA-mRNA' network.

2.8 Molecular docking

We downloaded the PDB format file of the 3D structure of the targets in the RCSB PDB database (https://www.rcsb.org/). The target proteins and their active ingredient of the ligand with the highest OB score were selected for molecular docking by AutoDock Vina (version 1.1.2)[22]. The binding activity was evaluated by the lowest binding efficiency.

3. Results

3.1 Targets acquisition for HPV and drugs (RSG and Phellodendron)

A total of 74 components of RSG and 140 components of Phellodendron were obtained by TCMSP database. According to oral bioavailability and drug-likeness threshold (OB $\geq$ 30% and DL $\geq$ 0.18), the active ingredients of the components were screened. Among them, 15 active ingredients of RSG and 37 active ingredients of Phellodendron were acquired, as shown in **Table 1**. Among the 52 active ingredients predicted 232 drug targets.

Table 1 active ingredients of RSG and Phellodendron

Molecule name	OB %	DL	Herb	Molecule name	OB %	DL	Herb
4,7-Dihydroxy-5-methoxyl-6-methyl-8-formyl-flavan	37.03	0.28	RSG	kihadanin A	31.60	0.70	Phellodendron
Neoastilbin	40.54	0.74	RSG	niloticin	41.41	0.82	Phellodendron
Enhydrin	40.56	0.74	RSG	rutaecarpine	40.30	0.60	Phellodendron
(2R,3R)-2-(3,5-dihydroxyphenyl)-3,5,7-trihydroxychroman-4-one	63.17	0.27	RSG	Skimmianin	40.14	0.20	Phellodendron
(-)-taxifolin	60.51	0.27	RSG	Chelerythrine	34.18	0.78	Phellodendron
beta-sitosterol	36.91	0.75	RSG and Phellodendron	Worenine	45.83	0.87	Phellodendron
sitosterol	36.91	0.75	RSG	Cavidine	35.64	0.81	Phellodendron
naringenin	59.29	0.21	RSG	Candletoxin A	31.81	0.69	Phellodendron
Stigmasterol	43.83	0.76	RSG and Phellodendron	Hericenone H	39.00	0.63	Phellodendron
isoengelitin	34.65	0.70	RSG	Hispidone	36.18	0.83	Phellodendron
astilbin	36.46	0.74	RSG	Magnograndiolide	63.71	0.19	Phellodendron
taxifolin	57.84	0.27	RSG	Palmidin A	35.36	0.65	Phellodendron
cis-Dihydroquercetin	66.44	0.27	RSG	palmatine	64.60	0.65	Phellodendron
diosgenin	80.88	0.81	RSG	Fumarine	59.26	0.83	Phellodendron
quercetin	46.43	0.28	RSG and Phellodendron	Isocorypalmine	35.77	0.59	Phellodendron
berberine	36.86	0.78	Phellodendron	phellamurin_qt	56.60	0.39	Phellodendron
coptisine	30.67	0.86	Phellodendron	(S)-Canadine	53.83	0.77	Phellodendron
Kihadalactone A	34.21	0.82	Phellodendron	poriferast-5-en-3beta-ol	36.91	0.75	Phellodendron
Obacunone	43.29	0.77	Phellodendron	berberrubine	35.74	0.73	Phellodendron
Phellavin_qt	35.86	0.44	Phellodendron	campesterol	37.58	0.71	Phellodendron
delta 7-stigmastenol	37.42	0.75	Phellodendron	dihydroniloticin	36.43	0.82	Phellodendron
Phellopterin	40.19	0.28	Phellodendron	melianone	40.53	0.78	Phellodendron
Dehydrotanshinone II A	43.76	0.40	Phellodendron	phellochin	35.41	0.82	Phellodendron
delta7-Dehydrosophoramine	54.45	0.25	Phellodendron	thalifendine	44.41	0.73	Phellodendron
dihydroniloticin	36.43	0.81	Phellodendron				

Via the keyword ‘HPV’, 29 disease targets were screened with ‘Relevance score ≥ 7’ in Genecards database. Meanwhile, 429 disease targets in DisGeNET database, 60 targets in OMIM database, and 5 targets in TTD database were predicted. The four results were combined to obtain 490 targets (**Fig.1**).

3.2 Enrichment analysis of candidate targets of drugs in the treatment of HPV

First, 59 candidate targets of drugs in the treatment of HPV were obtained by intersection of 490 HPV-related targets and 232 drug targets (**Fig.2A**).

Then, the biological processes and signaling pathways of candidate targets were analyzed by GO and KEGG analyses**(Fig.2B**). The results showed that biological processes of response to xenobiotic stimulus, cellular response to chemical stress, gland development, response to oxidative stress, pathways of extrinsic apoptotic signaling pathway and human papilloma virus infection were associated with 59 candidate targets.

3.3 Network construction of candidate targets of drugs in the treatment of HPV

The association between 59 targets, 37 active ingredients of drugs, HPV and two drugs (RSG and Phellodendron) was visualized by compounds-herbs-targets-disease network containing 99 nodes and 171 edges (**Fig.3A**). The association between 59 targets, 30 GO entries and 30 KEGG pathways was visualized by targets-functions-pathways network containing 116 nodes and 809 edges (**Fig.3B**). Furthermore, to elucidate the interrelationships of 59 targets, PPI network containing 59 nodes and 821 edges was constructed (**Fig.3C**).

3.4 Identification and GSEA of key targets

First, a total of 3,576 DEGs were screened from HPV (+) and HPV (-) samples in GSE3292 dataset, including 1,852 up-regulated genes and 1,724 down-regulated genes in HPV (+) samples. Then, 3 key targets (*ESR1*, *EGFR* and *PTEN*) were obtained by intersection of 3,576 DEGs and TOP 10 proteins derived from PPI network according to Degree (**Fig.4A**).

To explore biological functions and pathways of key targets, GSEA was performed. The results showed that *ESR1* was mainly enriched in pathways of Olfactory transduction, Salmonella infection and Neuroactive ligand-receptor interaction, *EGFR* was associated with pathways of Herpes simplex virus 1 infection, Protein processing in endoplasmic reticulum and Autoimmune thyroid disease, and *PTEN* was mainly enriched in pathways such as Parkinson disease, Amyotrophic lateral sclerosis and Huntington disease, as shown in **Fig.4B**. Moreover, the 3 key targets were co-enriched in autophagy-related PI3K-Akt signaling pathway.

3.5 The ceRNA network construction

First, a total of 58 differentially expressed miRNAs were obtained from HPV (+) and HPV (-) samples in GSE190223 dataset, including 31 up-regulated miRNAs and 27 down-regulated miRNAs in HPV (+) groups. 3 key targets were input in miRWalk database, 1533 miRNAs were predicted. Three key targets were up-regulated in the HPV (+) group, so we took the intersection of 27 down-regulated miRNAs and 1533 miRNAs to obtain 13 targeted miRNAs (**Fig.5A**).

Next, a sum of 132 differentially expressed lncRNAs were acquired from HPV (+) and HPV (-) samples in GSE190222 dataset, including 63 up-regulated lncRNAs and 69 down-regulated lncRNAs in HPV (+) groups . 13 targeted miRNAs were input in starbase database, 662 lncRNAs were obtained. 4 targeted lncRNAs were identified by overlapping 63 up-regulated lncRNAs and 662 lncRNAs(**Fig.5B**).

Finally, lncRNA-miRNA-mRNA network containing 20 nodes and 29 edges was constructed (**Fig.5C**), in which *PTEN* and *ESR1* were regulated by STX18-AS1 through has-miR-409-3p, and ASB16-AS1 might regulate *EGFR* through has-miR-214-3p.

3.6 Molecular docking of key targets

The active components (quercetin and palmatine) were molecularly docked by Auto Dock Vina. The molecular docking results showed that the binding ability of quercetin to EGFR(**Fig.6A**) and PTEN(**Fig.6B**) was strong, and ESR1 had the strong affinity with palmatine(**Fig.6C**).

4. Discussion

HPV viruses are DNA viruses and most HPV infections (about 90%) clear within 6-18 months of infection without any clinical signs or symptoms[23]. However, 10-20% of infections persist and increase the risk of the infection leading to precancerous or malignant disease, with part of infections eventually progressing to invasive cervical cancer[24]. Tertiary treatment of HPV includes primary prevention, secondary prevention, tertiary prevention, and palliative care[25]. Among these, primary prevention is HPV vaccination which is the most effective methods of preventing HPV-related cancers. However, due to the low vaccination rate of HPV vaccine, the incidence of HPV-related cancers remains high[26][27]. The standard HPV treatments include radiation therapy, chemotherapy, and surgical excision, still these treatments have limitations include immunotherapy[28]. Secondary prevention for screening and treatment of precancerous lesions caused by HPV is an effective method to improve the development and prognosis of HPV-related cancers. Chinese medicine, as a traditional Chinese treatment method, has a vigorous vitality and clinical usefulness. In clinical practice, we found that RSG and Phellodendron together can effectively improve various discomfort symptoms caused by HPV infection. Therefore, in this paper, we predict the pathway of HPV infection intervention by RSG and Phellodendron through network pharmacology in order to provide a new direction for clinical treatment.

Frist, we obtained 52 active ingredients associated with RSG and Phellodendron for the treatment of HPV infection based on the network pharmacology, including beta-sitosterol, quercetin, palmatine, etc. Beta –sitosterol, one of the most abundant naturally occurring phytosterols, has been experimentally demonstrated to exhibit multiple pharmacological properties, such as anti-diabetic[29], anti-anxiety[30], antiatherogenic[31], antimicrobial activity[32] and so on. Quercetin is a bioactive natural compound built upon the flavon structure nC6(ring A)-C3(ring C)-C6(ring B)[33], it has great potential for the treatment of diabetes, cardiovascular diseases, etc[34][35][36]. Palmatine, a natural isoquinoline alkaloid, is able to inhibition of oxidative stress and apoptosis[37], regulating gut microbiota[38], anti-inflammatory function[39] and more. And most of those compositions have been shown to have anti-cancer effects[40][41][42][43][44][45], but the role in HPV treatment is still unclear. Also, candidate targets were analyzed, it shows that the herbs may work against HPV via different biological processes and KEGG pathways.

After getting 59 candidate targets from HPV-related targets and drug targets, the target genes associated with HPV infection were obtained by PPI network analysis and the further screened against differentially expressed genes of HPV (+) and HPV (-) to obtain three key targets, namely ESR1, EGFR, and PTEN. Hypermethylation of CpG islands in the gene promoter region silences tumor suppressor genes[46], a common feature of human cancers. EGFR (Epidermal growth factor receptor) is one of the ErbB family receptors, and is activated upon binding to its ligand. Activated EGFR activates PI3K's and converts PIP2 to PIP3. Meanwhile, cytoplasmic molecules such as Akt are localized to the plasma membrane and activated by linking to PIP3 through its N-terminal PH structural domain, followed by phosphorylation of mTOR[47]. EGFR, an HPV-associated cancer marker, is highly expressed in more than 90% of HNSCC, and more than half of HPV-negative HNSCC show EGFR amplification or PI3K pathway activation. Studies have found that pEGFR and its upstream and downstream protein activation were negatively correlated with HPV+ cancer survival, that EGFR was regulated by HPV E7 protein, and down-regulation of phosphorylated EGFR to inhibit EGFR-related pathway could induce a better prognosis[48]. And it was found that HPV (+) infected was associated with EGFR expression only, and that the higher the tumor grade, the more significant the high expression of EGFR. As the negative upstream regulators of mTOR[49], PTEN inhibit the production of mTOR, negatively regulates the PI3K/AKT/mTOR pathway[50] , then PTEN reverse the

production of PIP3[51].Therefore, PTEN expression is mostly down-regulated or absent in HPV-related cancers[52]. Some reports have shown no significant difference in the expression of mTOR, pPTEN, and PTEN between HPV (+) and HPV (-) patients. Meanwhile, other studies have found that 75% of HPV-associated penile cancer patients have a statistically significant decrease in PTEN expression[53]. We can speculate that decreased or absent expression of PTEN as a cancer predictor is prevalent in HPV-associated cancers. The role of ESR1 in HPV-associated infections is unclear, but some studies have shown that ESR1 appears to be highly expressed in HPV-associated cancers. Pap smear analysis of patients with cervical cancer caused by HPV infection showed hypermethylated ESR1 expression in 64% of patients with cervical cancer; another study also showed hypermethylated ESR1 expression in 73% of patients[54]. The role played by ESR1 in HPV infection needs to be further investigated, and perhaps be monitored as a new predictor of HPV.

We performed GSEA analysis of the above three genes and enriched to multiple pathways. In particular, all three targets were closely related to PI3K-Akt signaling pathway. Studies have shown that PI3K-Akt signaling pathway plays an important role in a variety of cancers[55]. PI3K activity is controlled by positive upstream regulators, including epidermal growth factor (EGF), sonic hedgehog and insulin-like growth factor (IGF)-1, then antagonized by phosphatase and tensin homolog (PTEN), glycogen synthase kinase 3β and transcription factor HB9. After PTEN activation, Akt is activated by binding to it. Activated Akt fulfills various biological functions, including activating cAMP-response element binding protein, inhibiting p27, activating phosphatidylinositol 3-phosphate (PtdIns3 ps or PI3P) and activating mammalian target of rapamycin (mTOR)[56], then affects many fundamental aspects of cell biology by promoting cell survival, growth, proliferation, migration and energy metabolism[57][58]. Mutations in the PI3K-Akt signaling pathway are very common in HPV (+) HNSCC[59]. During carcinogenesis[60] excessive activation of the EGFR/PI3K/Akt/mTOR cascade is frequently observed. It leads to uncontrolled cell growth, angiogenesis, metastatic potential and therapeutic resistance. There are several experimental and preclinical data suggesting that HPV disrupts the EGFR/PI3K/Akt/mTOR pathway through gene mutations and alterations in the expression of pathway component proteins[43][61].

To further clarify the pathways of action of the three genes mentioned above, we performed RNA analysis and constructed a ceRNA network. It is hypothesized that PTEN and ESR1 are regulated by STX18-AS1 via has-miR-409-3p and ASB16-AS1 may regulate EGFR via has-miR-214-3p. Then, the pathway of HPV treatment with Phellodendron and RSG was further traced.

In order to verify whether RSG and Phellodendron can be closely associated with the three genes enriched, we performed molecular docking validation. The results showed that quercetin had a stronger binding ability with EGFR and PTEN, and ESR1 had a stronger affinity with palmatine. This suggests that the use of RSG and Phellodendron may affect the expression of the above three genes.

In summary, there are 37 active ingredients in RSG and Phellodendron that are associated with HPV infection, of which quercetin and palmatine may be the most critical components. The use of RSG and Phellodendron may stimulate the transcription of ASB16-AS1 and STX18-AS1 to regulate the expression of EGFR, PTEN and ESR1 by affecting has-miR-214-3p and has-miR-409-3p, respectively, and thus intervene in HPV infection through PI3K-Akt signaling pathway, meanwhile, lots of biological processes and KEGG pathways may also participate in this process, such as cellular response to chemical stress, gland development, response to oxidative stress, pathways of extrinsic apoptotic signaling pathway, human papilloma virus infection, ect.

This study explored the network pharmacology of RSG and Phellodendron in the treatment of human papillomavirus infection for the first time, providing a theoretical basis for the clinical application of RSG and Phellodendron, and suggesting that ESR1 may have a potential role in the diagnosis of HPV infection. This study only predicted the mechanism of RSG and Phellodendron for HPV treatment by network pharmacology, which has certain limitations. In the

next step, we will design animal experiments based on the results of this study to further focus on the effects of RSG and Phellodendron on EGFR, PTEN and ESR1.

Declarations

Ethics approval and consent to participate

As this study does not involve animal and patient experiments, the ethical approval and consent to participate are not applicable.

Consent for publication

Not applicable.

Authors' contributions

XL and LSS conducted the study and wrote the manuscript. LHX and SWW designed the study and revised the paper. ZRH controlled the language editing. All authors read and approved the final manuscript.

Availability of data and materials

The data used to support the findings of this study are available from the corresponding author upon reasonable request.

Declaration of competing interest

The authors declare that there are no conflicts of interest.

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Figures

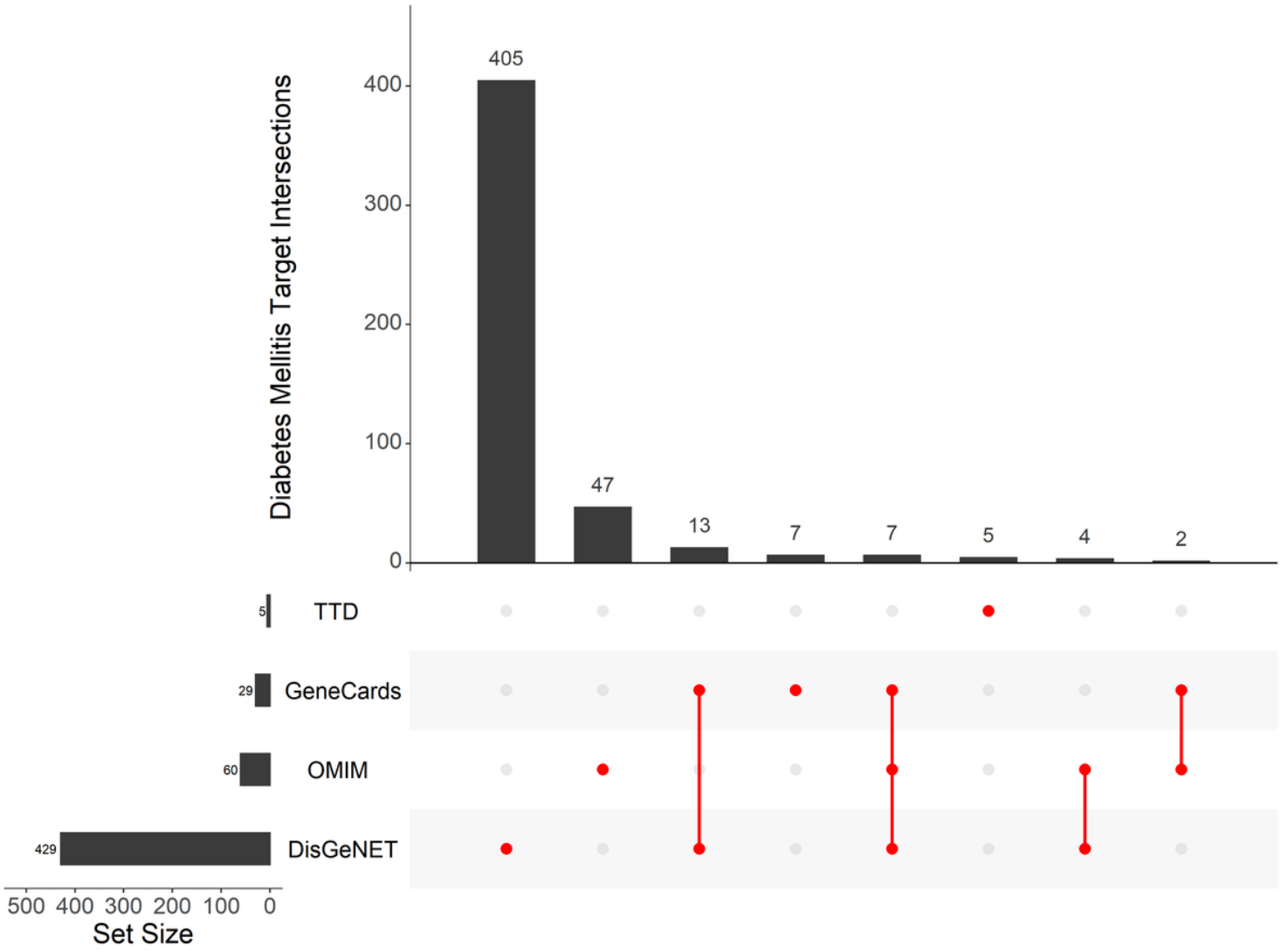

Figure 1

HPV targets

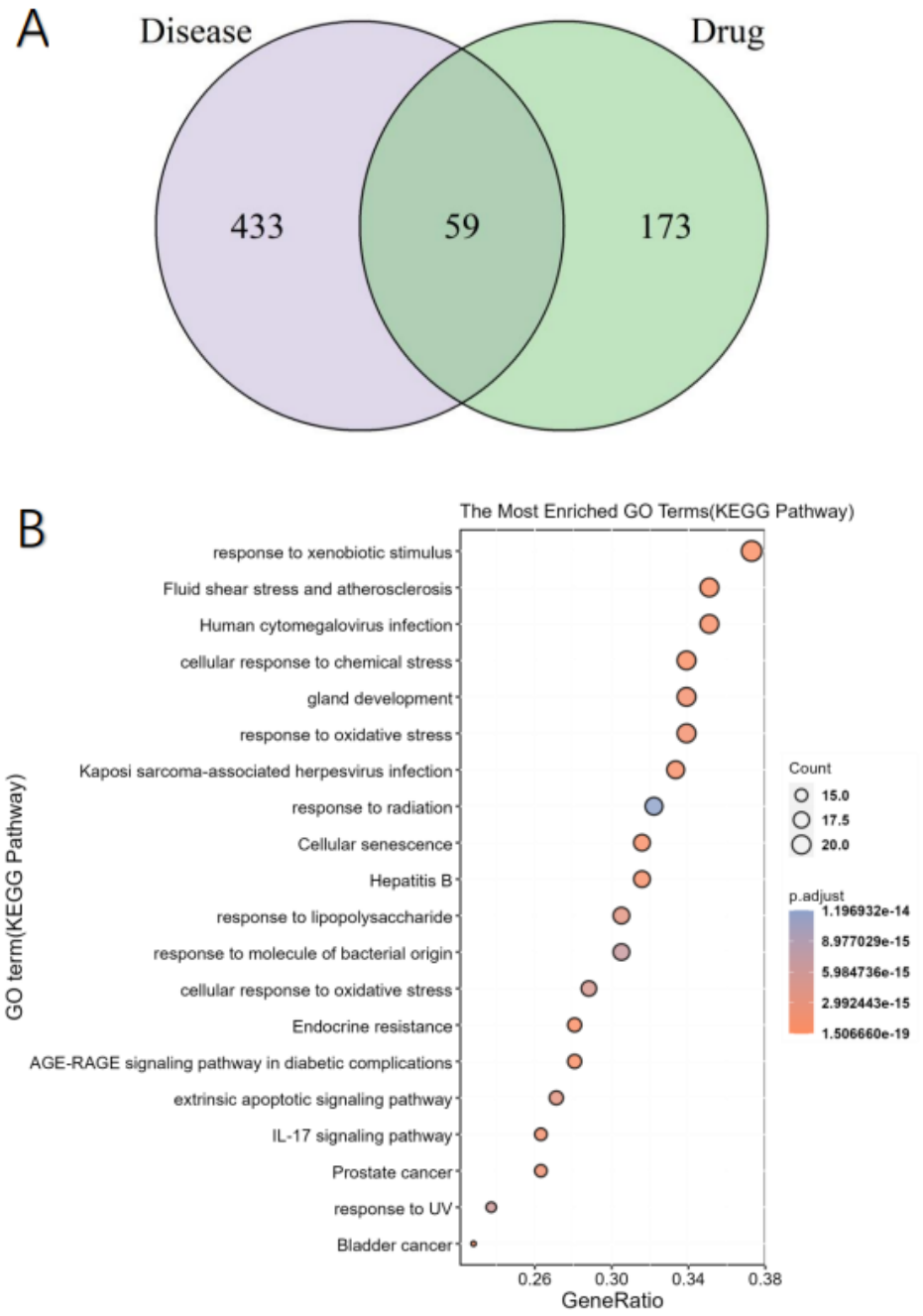

Figure 2

Enrichment analysis of candidate targets of drugs in the treatment of HPV. (A) Venn diagram of the target of herbs and the target of HPV. (B) GO and KEGG analyses of 59 targets.

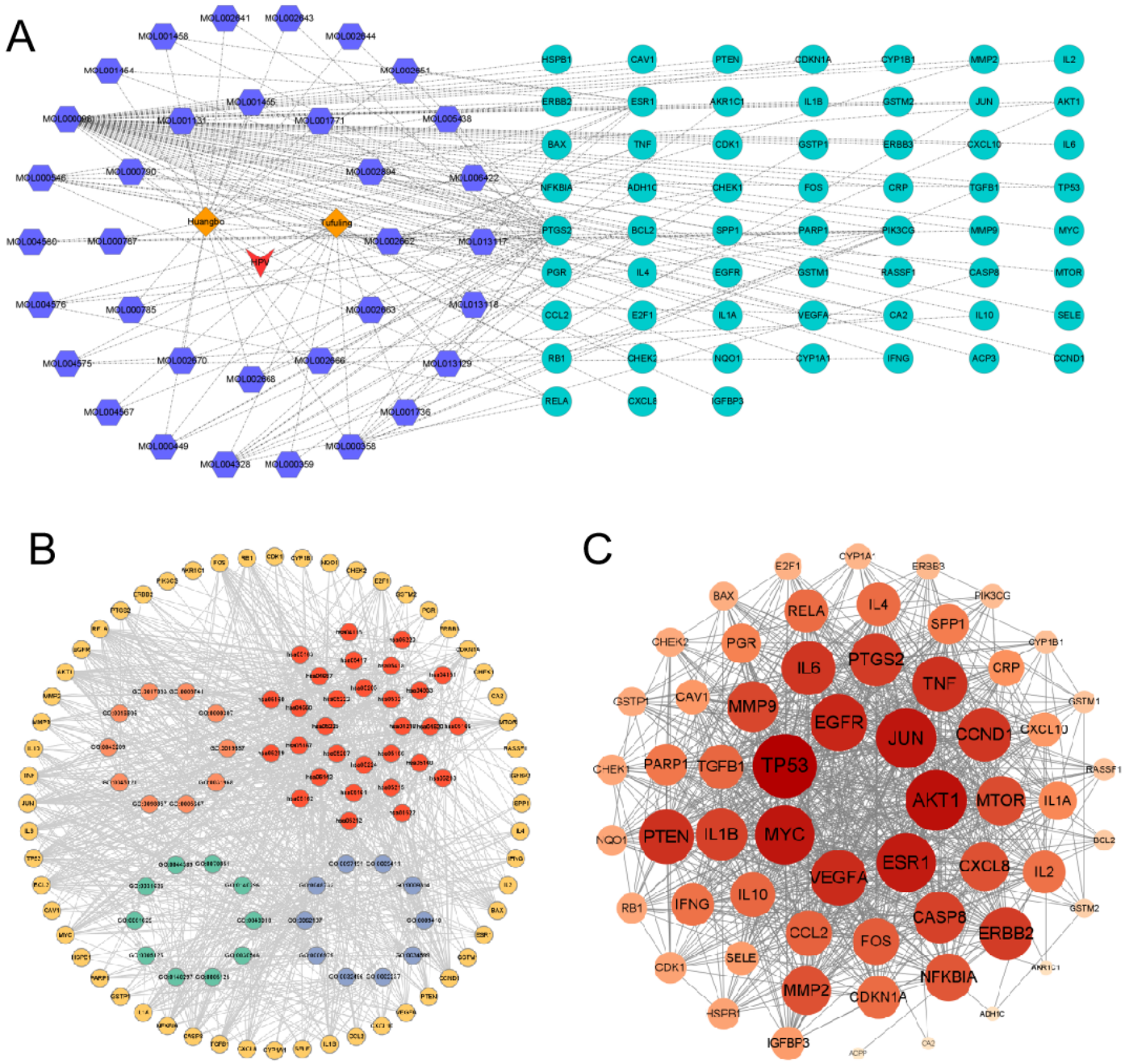

Figure 3

Network construction of candidate targets of drugs in the treatment of HPV. (A) Active ingredient-herb-target-disease network network. In that, 2 orange ellipses represent herbs, 37 blue ellipses represent active ingredient, 59 blue-green ellipses represent targets, 1 red ellipses represent disease. (B) Pathway-target network. In that, 59 orange ellipses represent targets, 30 red ellipses represent KEGG analyses, the rest of ellipses represent GO analyses. (C) PPI network of 59 targets. In that, 59 nodes represent 59 targets, the darker the color, the higher the degree of connection.

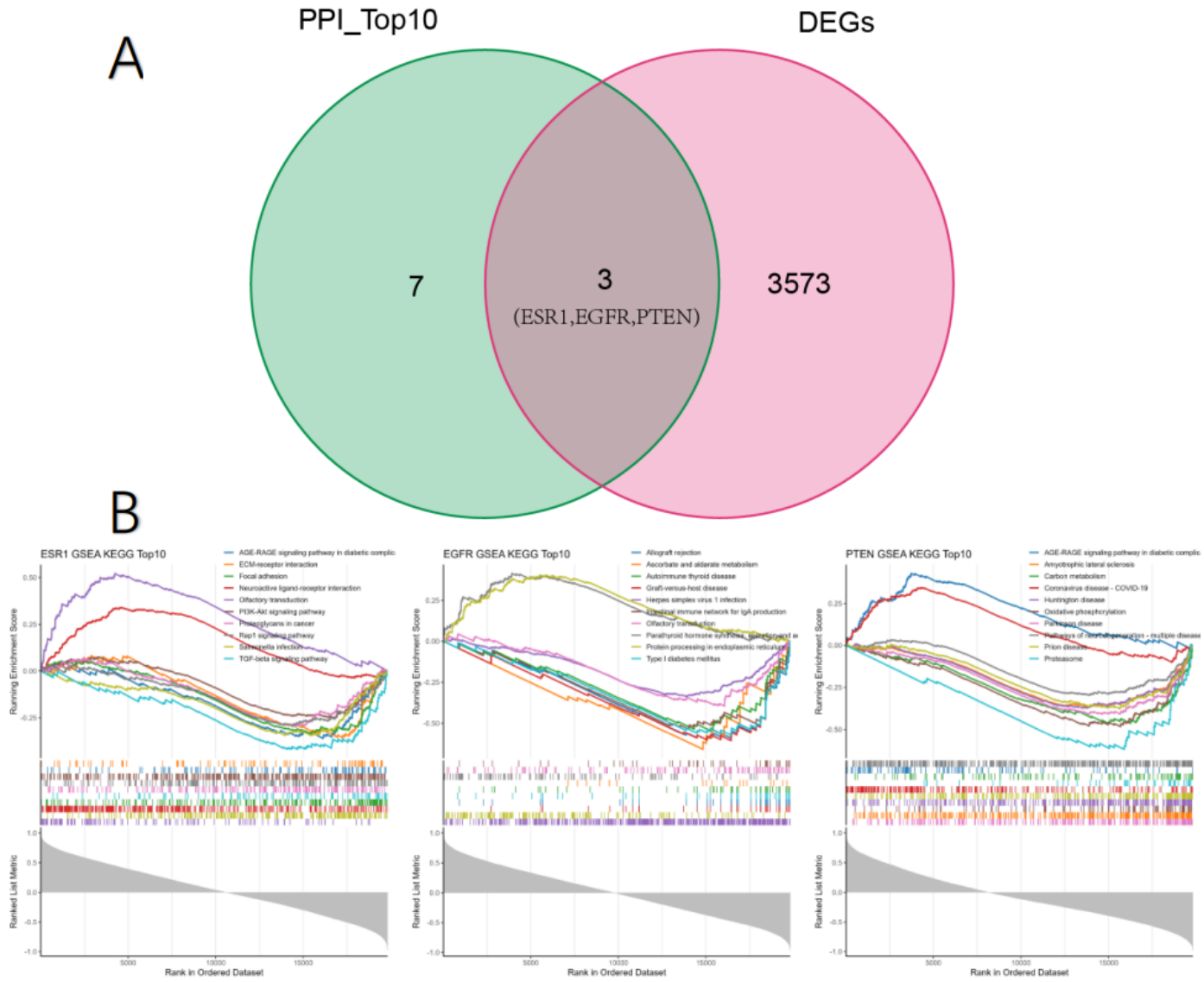

Figure 4

Identification and GSEA of key targets. (A) Venn diagram of DEGs and ten ptoteins of PPI. (B) The results of GSEA.

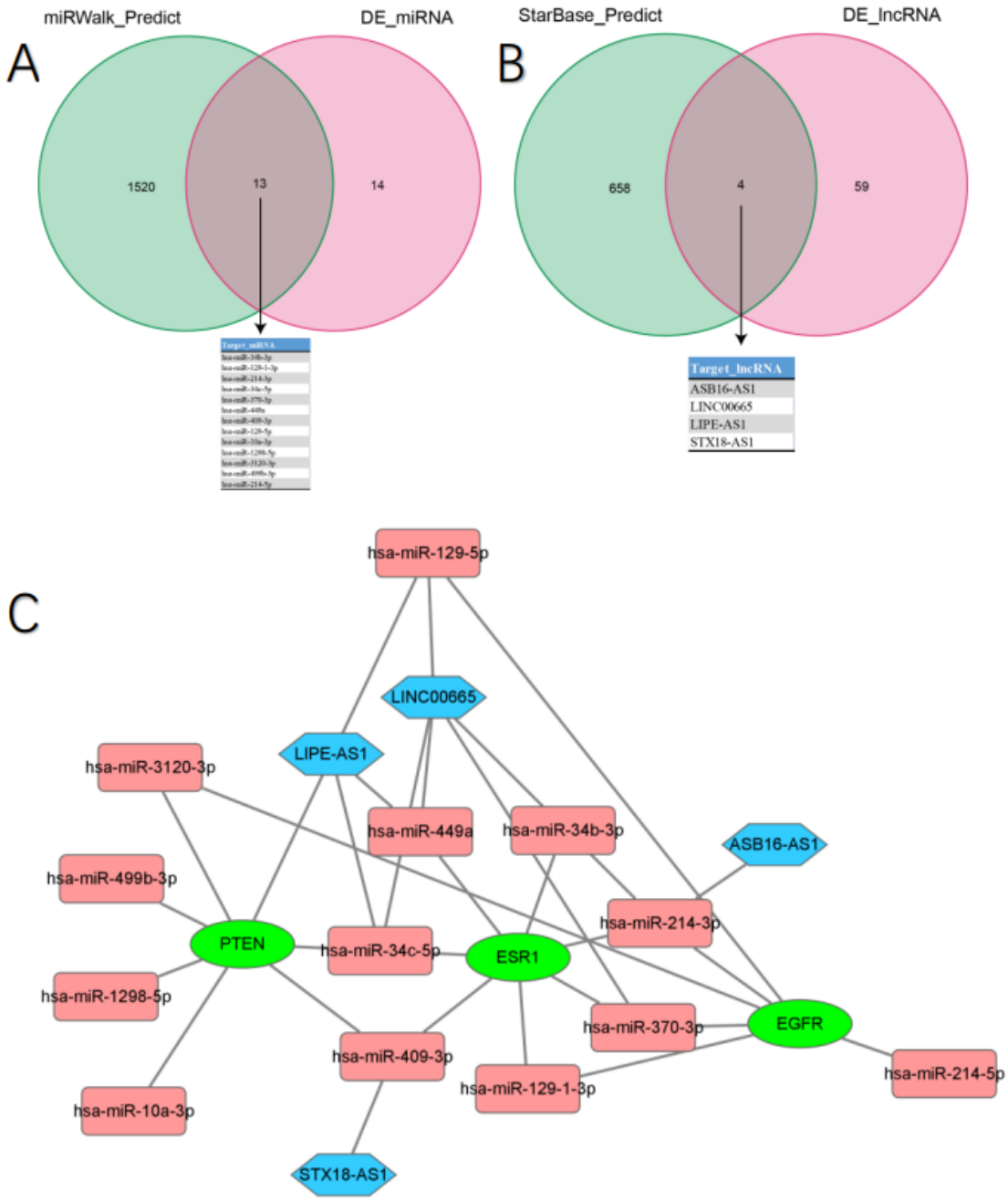

Figure 5

The ceRNA network construction. (A) 13 targeted miRNAs. (B) 4 targeted lncRNAs. (C) The lncRNA-miRNA-mRNA network.

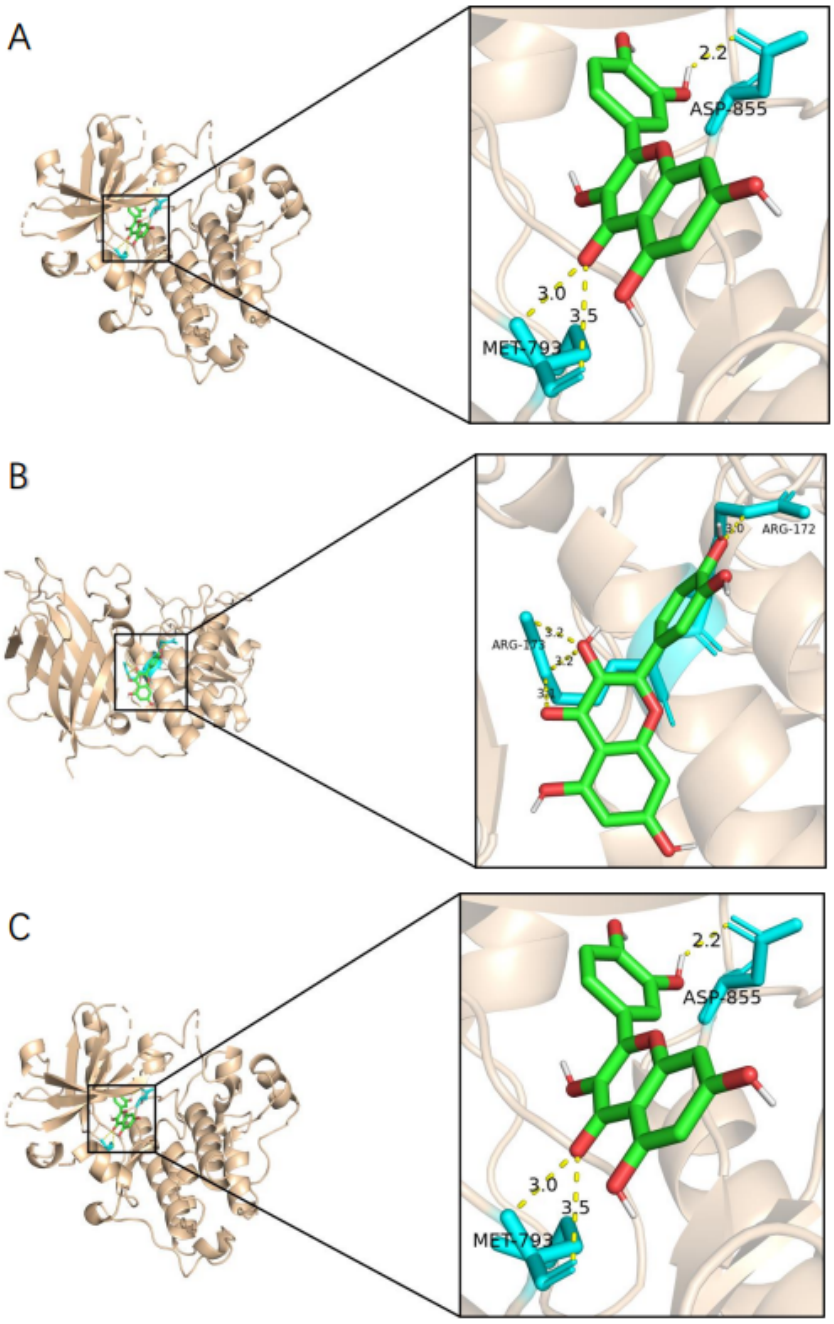

Figure 6

Molecular docking results. (A)The result of quercetin-EGFR. (B)The result of quercetin-PTEN. (C)The result of ESR1-palmatine