Network pharmacology-based investigation of potential targets of Triptonodiol acting on Non-Small-Cell Lung Cancer

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Research Article

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Abstract

Background

COE is a very promising anti-tumor drug candidate extracted from the Chinese herbal, Celastrus orbiculatus Thunb, and many related studies are underway. Methods: To explore the mechanism of Triptonodiol for lung cancer treatment, we used network pharmacology and molecular docking, and ultimately protein validation. Gene ontology (GO) analysis and Kyoto Encyclopedia of Gene and Genome (KEGG) pathway enrichment analysis were performed through David database. molecular docking was performed using PyMoL2.3.0 and AutoDock Vina software. After screening, the major targets of Triptonodiol were identified for the treatment of lung cancer. Target networks were established and PPI network topology was analyzed, Then KEGG pathway enrichment analysis was performed. Useful proteins were screened by survival analysis and Western Bolt analysis was performed. Results: Triptonodiol may regulate cell proliferation, drug resistance, MTOR, etc. by acting on GSK3B, AKT1, PIK3CA, HSP90AA1, MTOR glucose metabolism and other processes. KEGG pathway enrichment analysis showed that these targets were associated with tumor, PI3K signaling, ERBB signaling, insulin resistance, calcium signaling, etc. Molecular docking showed that the target protein GSK has good binding activity to the main active component of Triptonodiol. The results of cellular studies showed that Triptonodiol significantly reduced GSK3B levels in lung cancer cells H1299 and A549. Conclusion: The cellular level studies combined with network pharmacology and molecular docking approaches provide new ideas for the development and therapeutic application of Triptonodiol, and identified it as a potential GSK inhibitor.

1. Introduction

With the change in living habits and the increase in global emissions, lung cancer has become the most common cancer in terms of incidence and mortality worldwide. Traditional Chinese medicine has a long history of application in China and play an important role in against tumors. Materia Medica records, Celastrus orbiculatus Thunb is an important Chinese medicinal plant that has been used for centuries for anti-inflammatory, anti-tumor, analgesic, and treatment of various diseases. Our team has extracted its anti-tumor ingredients, and the related extraction process and usage have been awarded the Chinese national invention patent. We first identified a significant inhibitory effect of COE on non-small cell lung cancer and conducted extensive studies. Triptonodiol, a diterpenoid component of COE, has antitumor activity. However, its molecular mechanism remains unknown. Therefore, this study used network pharmacology combined with molecular docking to predict whether Triptonodiol could act on lung cancer targets. Western bolt experiments were performed to validate the true pharmacological activity of the predicted target and the prognostic value of the target in the clinic were analyzed by TCGA data. This study provides the first systematic study of the action and mechanism of Triptonodiol, and provides an experimental basis for the application of Triptonodiol as an antitumor drug candidate.

2. Methods

2.1 Collection of Triptonodiol and lung cancer related genes

The molecule structure of Triptonodiol was portrayed using PubChem[14] database (https://pubchem.ncbi.nlm.nih.gov/) and the SDF format was preserved. The structural information of Triptonodiol was imported into Swiss Target Prediction database (http://www.swisstargetprediction.ch/) [7] and PharmMapper database (http://www.lilab-ecust.cn/). pharmmapper/) to obtain potential targets for Triptonodiol. The GeneCards database (https://www.genecards.org/) and DISGENT database (https://www.disgenet.org/home/) and OMIM database (https://omim.org/) were searched by the keywords "lung cancer" to obtain lung cancer-related genes, and the results of multiple database information searches were integrated to construct a lung cancer-related gene information database.

2.2 Prediction of Triptonodiol's anti-lung cancer targets

Using the Venny 2.1 (https://bioinfogp.cnb.csic.es/tools/venny/) online too, the potential targets of Triptonodiol were compared with the relevant genes of lung cancer. Venn diagrams were drawn, and the intersection of the two was the potential therapeutic target of Triptonodiol against lung cancer

2.3 Construction of protein-protein interaction (PPI) network and its analysis

The crossing point of potential Triptonodiol targets and lung cancer-related qualities was imported into STRING database[31] (https://string-db.org, Version 11.0), "Organism" was set as "Homo sapiens", set the "minimum required interaction score" to 0.400, avoided the free target qualities, developed the PPI network[25], traded the tsv record of the arrange, and imported the tsv record into Cytoscape (Version 3.9.0) software[4] was utilized to imagine and analyze the PPI network.

2.4 GO enrichment analysis and KEGG enrichment analysis

The important genes of lung cancer and potential helpful targets of Triptonodiol were imported into the DAVID [11] database (https://david.ncifcrf.gov/, Version 8.0)[23], "Identifier" was chosen as " GENE_OFFICIAL_SYMBOL", "species" was characterized as "Homo sapiens", and GO enrichment investigation and KEGG enrichment examination were performed. Both GO and KEGG improvement investigation were performed with the screening condition of $P < 0.05$[12], and the three aspects of biological process (BP), cellular ingredients (CC) and molecular function (MF) of the significant targets were examined, and the bar graphs of enrichment analysis were drawn.

2.5 Target analysis of Triptonodiol for lung cancer

The KEGG PATHWAY Database (https://www.genome.jp/kegg/pathway.html) was used to search for signaling pathways and annotate the genes in the pathways. The predicted Triptonodiol anti-lung cancer targets were compared with the pathway-related genes, and the intersection was taken to identify the Triptonodiol anti-lung cancer enzymatic targets.

2.6 Molecular docking

We performed atomic docking examination. 2D structures of the dynamic compound was downloaded from the PubChem database, and their 2D structures were optimized utilizing Chem3D. The 3D maps of the core proteins were retrieved and downloaded from the PDB database. The target proteins were dewatered, and the original ligands were removed by PyMOL software. Molecular docking was performed using Autodock visualization software[34]. The target proteins were dewatered, and the first ligands were evacuated by PyMOL program. Atomic docking was performed utilizing Autodock visualization program

2.7 Analysis of Gene Prognosis of Drug-Targets.

Survival investigation was performed on the EGPIA2 site (http://gepia2.cancer-pku.cn/#index) by entering the target genes of interest and selecting tissue samples for lung adenocarcinoma (LUAD) and lung squamous carcinoma (LUSC). 50% of Cutoff-High and 50% of Cutoff-Low each. The TCGA database contains expression and clinical information on 33 sorts of tumors.

2.8 Western blotting

After COE treatment of NSCLC cells for 24 hours, the cells were collected and lysed with RIPA buffer, and total cellular protein was extracted, followed by quantification using the BCA kit. Cell lysates were mixed with loading buffer and subjected to vertical gel electrophoresis on SDS-PAGE, followed by horizontal electrophoresis to transfer the proteins to PVDF (Milipore) membranes. PVDF membranes were blocked with buffer containing 10 mmol/L Tris, 150 mmol/L NaCl, 0.1%Tween-20 and 5%skimmed milk, then incubated with primary antibody at 4°C overnight (usually 14 hours), followed by incubation with a cognate secondary antibody coupled with horseradish peroxidase for 1 hour at room temperature. Internal reference was controlled by GAPDH. Immunoreactive bands were detected using an enhanced chemiluminescence gel imaging system (Bio-Rad).

3. Results

3.1. Screening of the Potential Targets of Triptonodiol in Treating lung cancer

The 2D structure of Triptonodiol was acquired from the pubchem database (Fig. 1) and entered into the SwissTargetPrediction website, which yielded a total of 101 potential targets. Genes related with lung cancer were mapped as volcanoes which identified from the GEO database, of which 1815 genes were upregulated, and 2276 genes were downregulated (Fig. 2). Based on the screened components and their targets, the intersection of drug and disease targets (Fig. 3) was taken and a total of 88 genes was obtained, which will be used as potential targets of Triptonodiol for lung cancer treatment in subsequent network construction and pathway enrichment analysis.

3.2. The core target of Triptonodiol in the treatment of lung cancer

The potential targets of Triptonodiol for lung cancer were entered into the STRING database with 88 nodes to obtain the PPI network (Fig. 4). the node with Degree > 2 times the Avg. number of neighbors (Degree > 14.41) is regarded as the key node in the network, then, 10 key proteins were screened out (Fig. 5). Among them, AKT1 is the highest scoring one with degree values of 24. These four genes all play a central role in PI3K/AKT or PI3K/MTOR, Another of the highest scores is HSP90AA1, PIK3CA and MTOR, with the degree values of 21, 16 and 15, respectively. The above results suggest that Triptonodiol is likely to be able to effectively regulate PI3K-related pathways and has important potential efficacy in the treatment of lung cancer.

3.3. GO and KEGG Enrichment Analysis

GO and KEGG pathway enrichment analysis of the identified core targets of Triptonodiol for lung cancer by DAVID software. Cellular composition and molecular functional analysis of the candidate targets were performed based on biological processes. As shown in the Fig. 6, GO terms that are exceedingly enriched in biological processes, cellular components, and molecular functions incorporate cellular signaling, protein phosphorylation, and protein kinase activity. Then, the target genes was analyzed by KEGG enrichment investigation, and the 20 signaling pathways with the most elevated enhancement scores were positioned(Fig. 7). We found that the signaling pathway with the highest score for cancer and the third highest ranked signaling pathway was PI3K/AKT. These comes about show that Triptonodiol could be a promising antitumor medicate.

3.4. Analysis of Gene Prognosis of Drug-Targets and Molecular docking

Genetic prognostic analysis of drug-targets was performed using the TCGA tumor database, which contains expression and clinical data for 33 tumors. We show 10 core genes screened for prognostic significance in lung cancer patients, containing GSK3B, HSP90AA1, PRKCZ, ESR1. Among them, GSK3B

has important effects on both lung adenocarcinoma and squamous lung cancer patients (Fig. 8). Therefore, the molecular docking simulations of Triptonodiol and GSK3B was performed. An absolute value of free binding energy greater than 4.2 prompts binding activity, greater than 5.0 prompts good binding activity, and greater than 7.0 prompts strong binding activity [10]. The absolute value of free binding energy for Triptonodiol and GSK3B was 6.35, indicating good binding activity (Fig. 9).

3.5.Western bolting

Network pharmacology can significantly improve the hit rate of drug target screening, but it is still a virtual screening tool. To finally confirm the biological activity of Triptonodiol against GSK3B, the protein level of GSK3B was detected by Western bolt. Excitingly, the protein levels of GSK3B were significantly downregulated after 24 hours treatment of non-small cell lung cancer H1299 and A549 with the indicated concentrations of Triptonodiol(Fig. 10). GSK3B is an effector downstream of PI3K/AKT that plays an important role in tumor proliferation by directly regulating P21 and Cyclin D1. The above results suggest that Triptonodiol is a highly promising antitumor agent that inhibits the biological activity of GSK3B.

4. Discussion

Currently, tumors have ended up one of the most serious diseases endangering human wellbeing. It is evaluated that by 2019, the number of tumor-related deaths will surpass 20 million around the world. The frequency of tumors in China is additionally on the rise year by year[26]. Chinese traditional medicine plays an imperative role in the prevention and treatment of tumors, and its role and mechanism in enhancing the immune function of the body, preventing the multidrug resistance of tumor cells, restraining the division and expansion of tumor cells, and accelerating the apoptosis of tumor cells are gradually revealed. The foundation for its effective application in antitumor has been laid[36]. Be that as it may, the composition of Chinese traditional medications is complex and their active ingredients are still unclear in pharmacology and clinical practice. The isolation of the monomers among them is time-consuming and costly, and the specific target genes or proteins for drug action have not been fully identified. In the past decade, with the fast improvement of high-throughput innovations and bioinformatics, the research of network pharmacology in disease treatment has attracted attention. Based on computer simulation technology, it provides an effective method for component screening and prediction of drug targets, which is a feasible solution to improve the correct rate of drug target prediction[18].

COE (Celastrus orbiculatus Thunb extract) is an ethyl acetate extract of Celastrus orbiculatus Thunb, which is considered to be a very promising antitumor drug. Our group has continuously and thoroughly investigated the antitumor pharmacology of COE and elucidated its antitumor mechanism, the important extraction prepare and utilize have gotten Chinese national invention patent Past considers by our group have appeared that Triptonodiol, a monomer in COE, has antitumor activity. In this study, we further examine the mechanism of activity of Triptonodiol in lung cancer, and eighty-eight key targets were

acquired. In addition, we used PPI to explore data mining and network analysis. We found 10 core targets, which are exceedingly connected with tumor resistance and proliferation.

Drug resistance is a multifactorial process also mediated by a cellular stress response to the tumor microenvironment [33]. Heat shock proteins (HSPs) are highly conserved molecular chaperone proteins that play an important role in cell survival. In stress conditions, heat shock proteins mediate tumor drug resistance through regulation of autophagy[2; 3]. HSP90AA1 is one of the most important heat shock proteins and is considered as a potential molecular target for tumor therapy [28]. In growing cells, high mTORC1 activity promotes biomolecule synthesis while inhibiting autophagy. mTORC1 inhibits autophagy induction by phosphorylation-dependent inhibition of ULK1/2 and VPS34 complexes and strictly regulates autophagy by blocking the overall expression of lysosomal and autophagic genes through TFEB phosphorylation[15]. AKT1/MTOR inhibition of autophagy promotes cell growth by inhibiting catabolism and accelerates tumor growth by promoting the warburg effect. mTORC1 prevents ULK1 activation by AMPK, a key activator of autophagy, by phosphorylating ULK1, which is involved in angiogenesis and EMT (Epithelial-Mesenchymal Transition) processes through autophagy regulation and promotes tumor metastasis. AKT is able to increase LC3-II levels and autophagic flux through the reduction of autophosphorylation [5; 35]. Not only that, AKT1 is also able to regulate cell proliferation through PIK3CA and GSK3B, by regulating cyclin D1 protein hydrolysis and subcellular localization[6]. GSK-3 is display in all eukaryotes and could be a broadly expressed and exceedingly conserved serine/threonine protein kinase that can be inactivated by p70S6k through serine 9/21 phosphorylation[27; 29; 30]. EMT can be positively regulated by the Wnt/β-catenin pathway[8], and GSK-3 plays an imperative administrative part in this process. Active GSK-3β can prevent transcription of β-catenin target genes by stimulating the degradation of β-catenin protein and promoting the destruction of nuclear phosphorylated β-catenin[13; 32]. P53, a potent tumor suppressor, exerts antiproliferative effects when faced with stress, including growth arrest and apoptosis[24]. In this study, MDM2 was also a potential target that scored highly, an oncoprotein that inhibit p53[21]. MDM2 promotes proliferation by binding to P53 and blocking its transcriptional activation domain[9]. On the other hand, there is an autoregulatory feedback loop between p53 and MDM2. At the same time, MDM2 can promote the degradation of P53 By binding to p53, even if P53 is in a steady state[9].

At the same time, we carried out KEGG enrichment analysis on the predicted target. The calculated results of KEGG enrichment are in high agreement with those of previous analysis. Triptonodiol is highly correlated with tumor signaling, especially ERBB and PI3K/AKT signaling. ERBB receptors were linked to human cancer pathogenesis approximately thirty years ago. The transmembrane receptor tyrosine kinases (RTKs) of the ERBB family include ERBB1, HER2, ERBB3, ERBB2 and HER4 [1]. ERBB family members play an important role in the occurrence and maintenance of various solid tumors, especially HER2 amplified breast cancer and EGFR mutated lung cancer. This led to the development and widespread implementation of specific ERBB inhibitors as cancer therapies. At the same time, particularly in lung cancer, the mutations of ERBB4 have been identified[22]. The mutation in the gatekeeper residue of EGFR, T790M is the most common mechanism of acquired resistance to EGFR inhibitors in the EGFR mutant lung cancer. At least 50% of biopsies from patients with acquired drug resistance carry the

T790M mutation of EGFR[16; 20]. It is reported that, S492R mutation occurred in EGFR extracellular domain, which can interfere with the binding of cetuximab[17]. In addition to the immune effect of ERBB antibody, most of the activities of many similar drugs are due to the inhibition of downstream signal transduction, especially PI3K/AKT and MEK/ERK[1]. Therefore, although the targeted receptor is completely inhibited, at least one of these key downstream pathways is still maintained, so many cancers are resistant to ERBB inhibitors. This type of drug resistance, also known as "bypass track" resistance, is usually used to describe the drug resistance caused by maintaining these key downstream signal pathways when RTK is fully inhibited[19]. Therefore, the calculation results of Triptonodiol by network pharmacology model have excellent application prospect. Because our results show that Triptonodiol can regulate ERBB signal pathway and PI3K/AKT at the same time, theoretically, it can prevent the occurrence of drug resistance while treating cancer.

In conclusion, we provides a preliminary exploration of the dominant targets and effective pathways of Triptonodiol with the help of network pharmacology and laboratory validation of the protein molecules, confirming the anti-tumor pharmacological effects of Triptonodiol. With the help of network pharmacology, possible targets in Triptonodiol can be further explored, which may become the basis for the development of novel drugs, but more trials are still needed. Meanwhile, this study also further confirmed the antitumor effect of Triptonodiol and further promoted the development of drugs related to the Chinese medicine Celastrus orbiculatus Thunb.

5. Conclusion

Through analyzing the pharmacological mechanism of the compound Triptonodiol in COE, we found that Triptonodiol acts on multiple targets and proteins of multiple signaling pathways. Triptonodiol mainly interferes with the PI3K/AKT/GSK signaling to delays the progression of NSCLC. Since Western Bolt experiments established that Triptonodiol can inhibit the biological activity of GSK in vitro, we next plan to carry out animal pharmacology experiments to observe the effect and mechanism of Triptonodiol for NSCLC treatment in depth and provide more references for its clinical application and development.

Declarations

Author contributions

F.J. and X.C.N. conceived the experiment, S.L.Y. and X.M.J. conducted the experiment, J.Z. and D.F.M analyzed the results. Y.Q.L and F.W provided the laboratory. All authors reviewed the manuscript.

Competing interests

The authors declare no competing interests.

Ethical Approval : All experiments were approved by the Research and Ethics Committee of the Affiliated Hospital of Yangzhou University(Approval No. SYXK( )2022-0044).

Approval of the research protocol by an Institutional Reviewer.

Informed Consent: N/A.

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Availability of data and materials: All experiments are carried out in the Key Laboratory of Syndrome Differentiation and Treatment of Gastric Cancer of the State Administration of Traditional Chinese Medicine. None of the data comes from third parties.

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Figures

Figure 1

The 2D and 3D structures of Triptonodiol

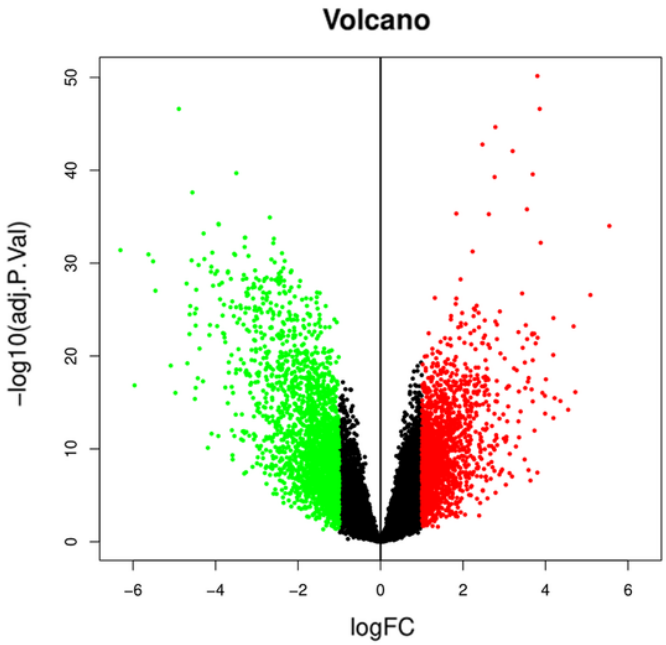

Figure 2

Volcano plot of differentially expressed genes in lung cancer. The horizontal coordinates indicate the fold relationship of gene expression changes and the vertical coordinates indicate the statistical significance of gene expression changes. Red dots represent genes with significantly up-regulated expression and green dots represent genes with significantly down-regulated expression.

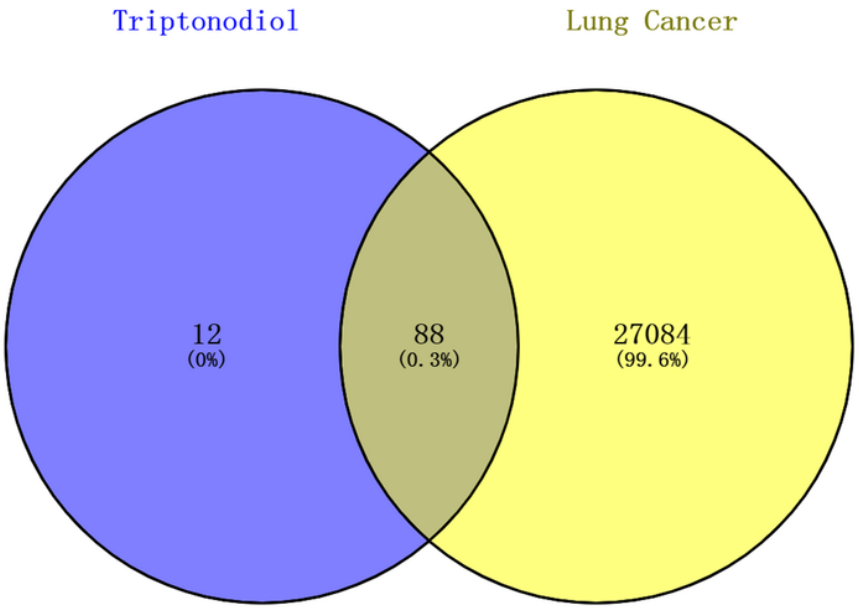

Figure 3

Venn diagram of the targets of the active ingredients of Triptonodiol and the lung cancer-related targets.

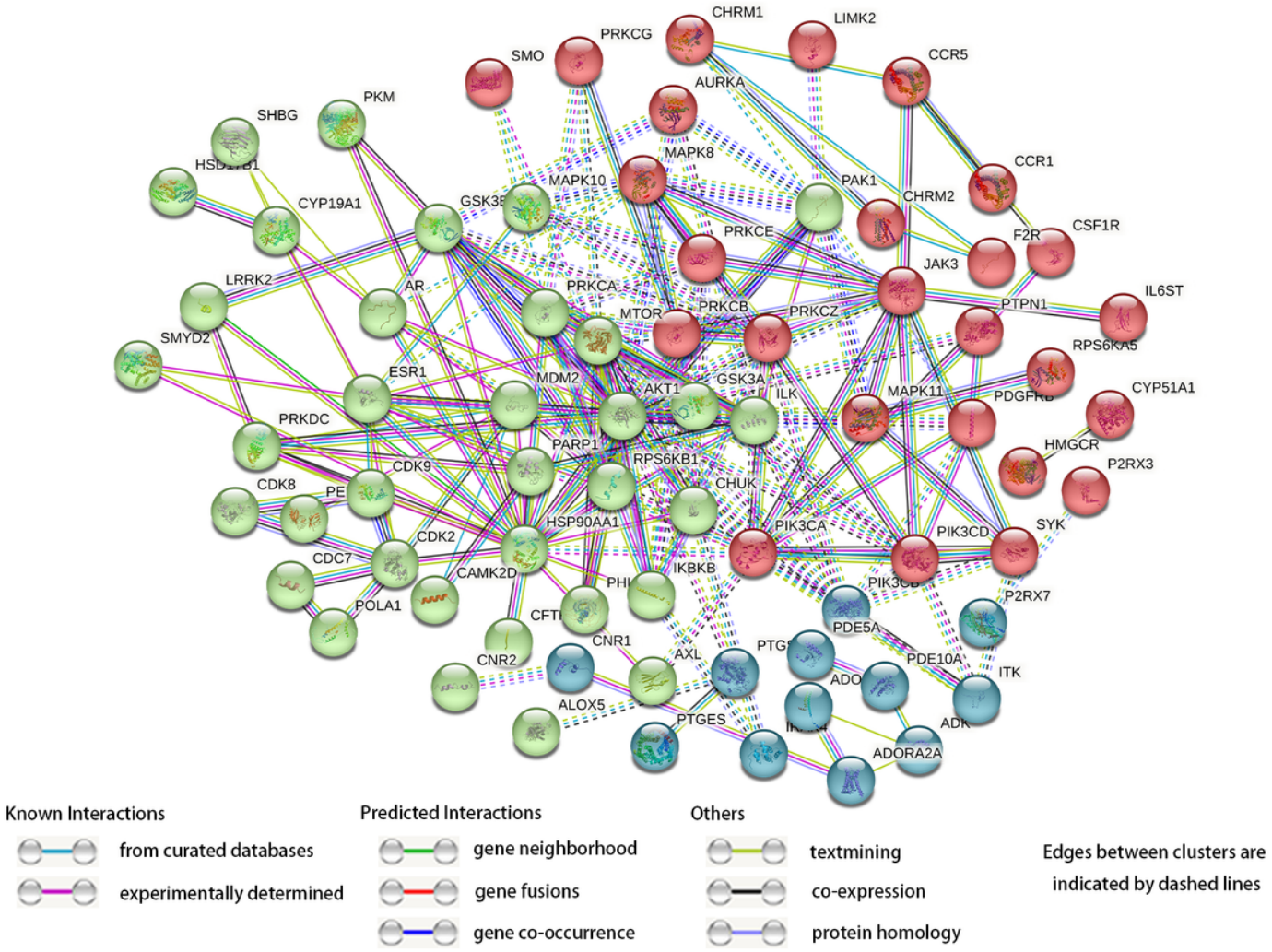

Figure 4

PPI network of the common targets of Triptonodiol and lung cancer.

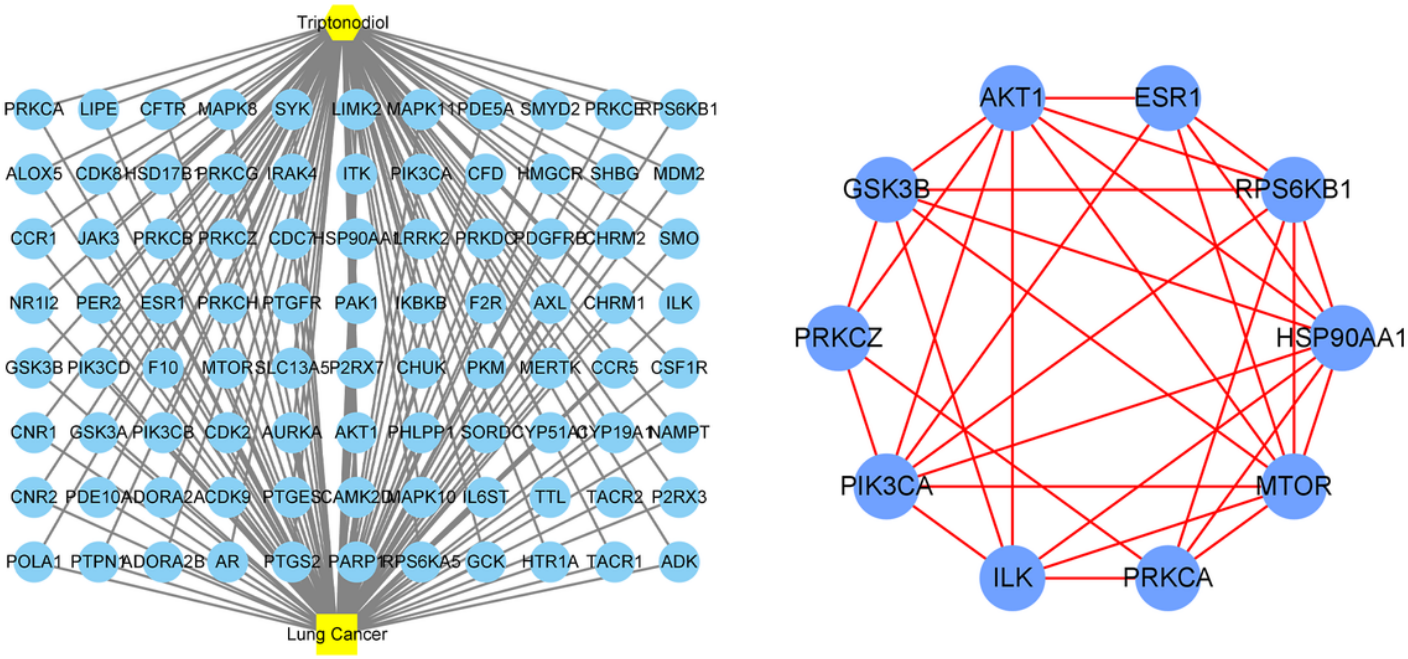

Figure 5

Ingredient-target network of Triptonodiol and the network of the first 10 genes.

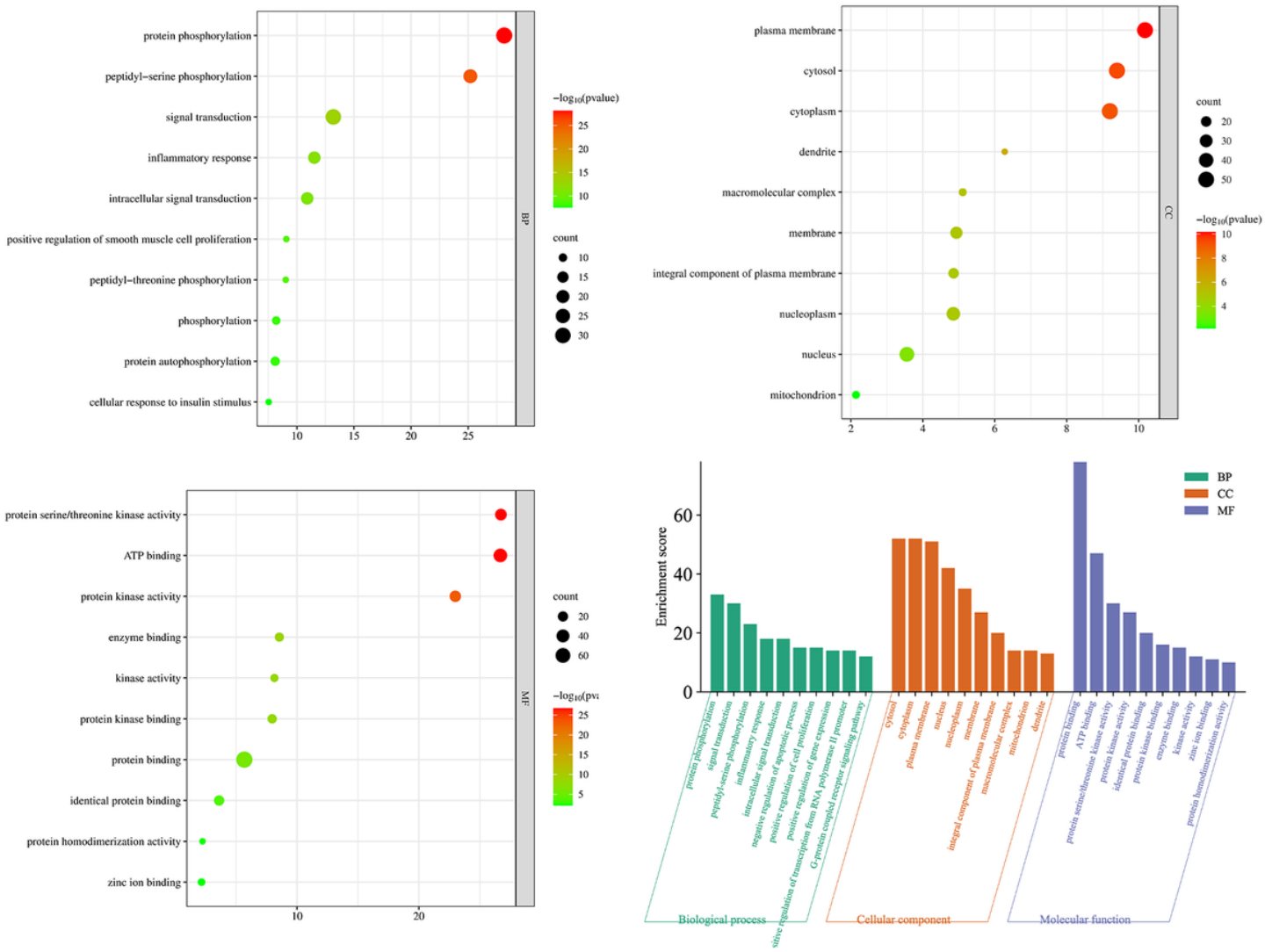

Figure 6

Gene ontology terms of candidate targets of Triptonodiol againstlung cancer. The top 10 GO functional categories with FDR <0.05 were selected.

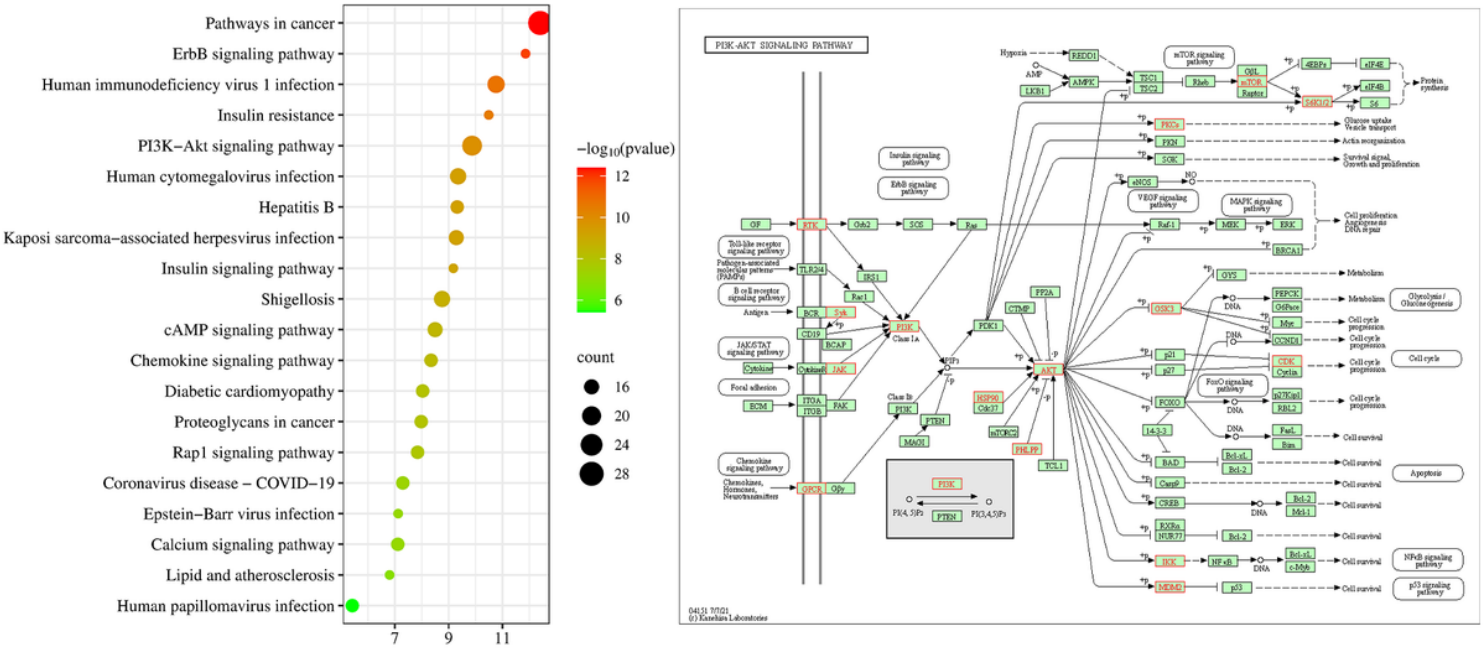

Figure 7

KEGG pathway enrichment of candidate targets of Triptonodiolagainst lung cancer. Pathways that had significant changes of FDR <0.05 were identified. The size of the spot represents a number of genes and color represents FDR value. The most enriched pathways are shown in the PI3K signaling pathway diagram on the right, with the top ten gene targets for screening marked by red boxes.

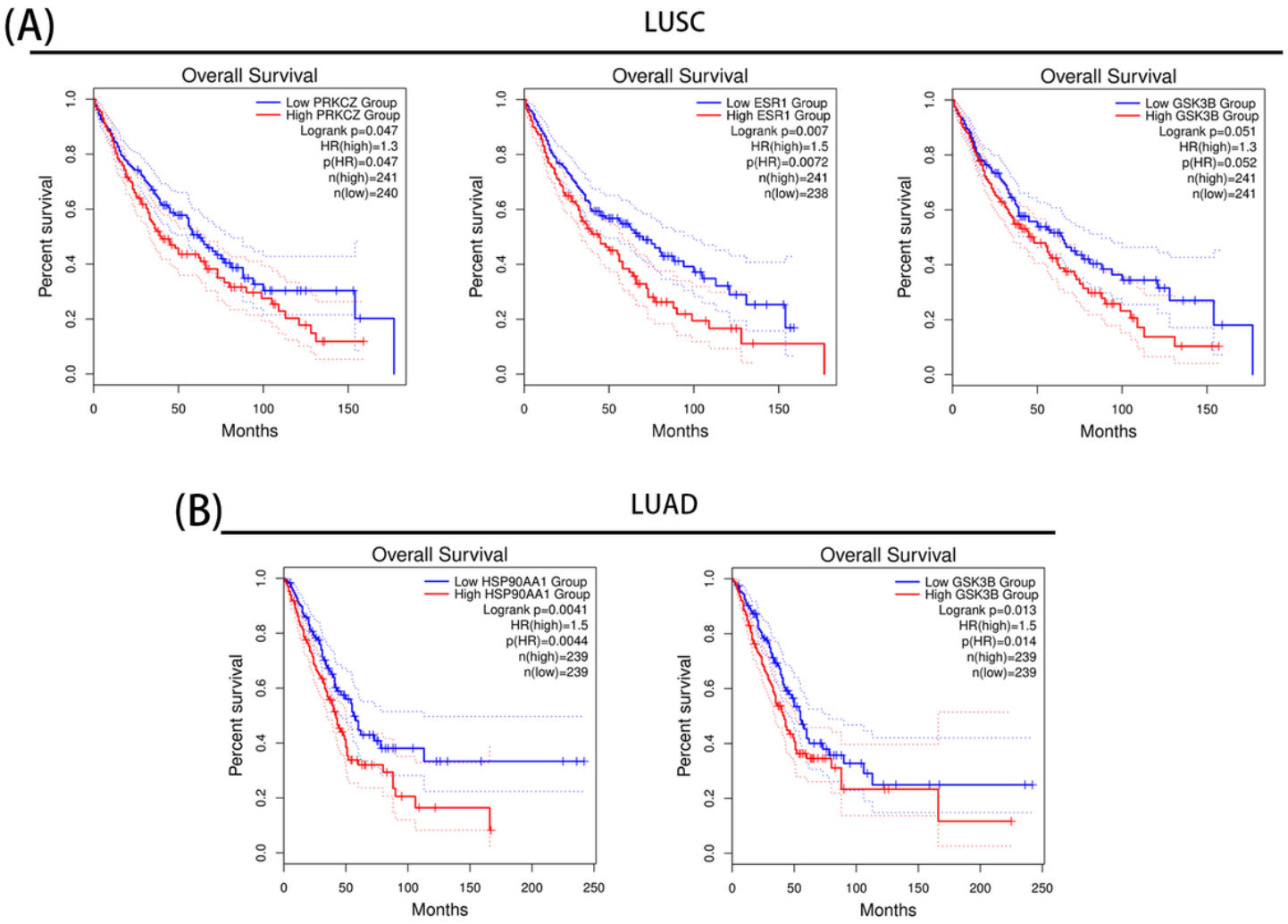

Figure 8

Prognostic curves of four hub genes. The prognostic significance of the hub genes in patients with lung cancer, according to the TCGA database. A: PRKCZ, ESR1, GSK3B in squamous lung cancer. B: HSP90AA1, GSK3B in lung adenocarcinoma.

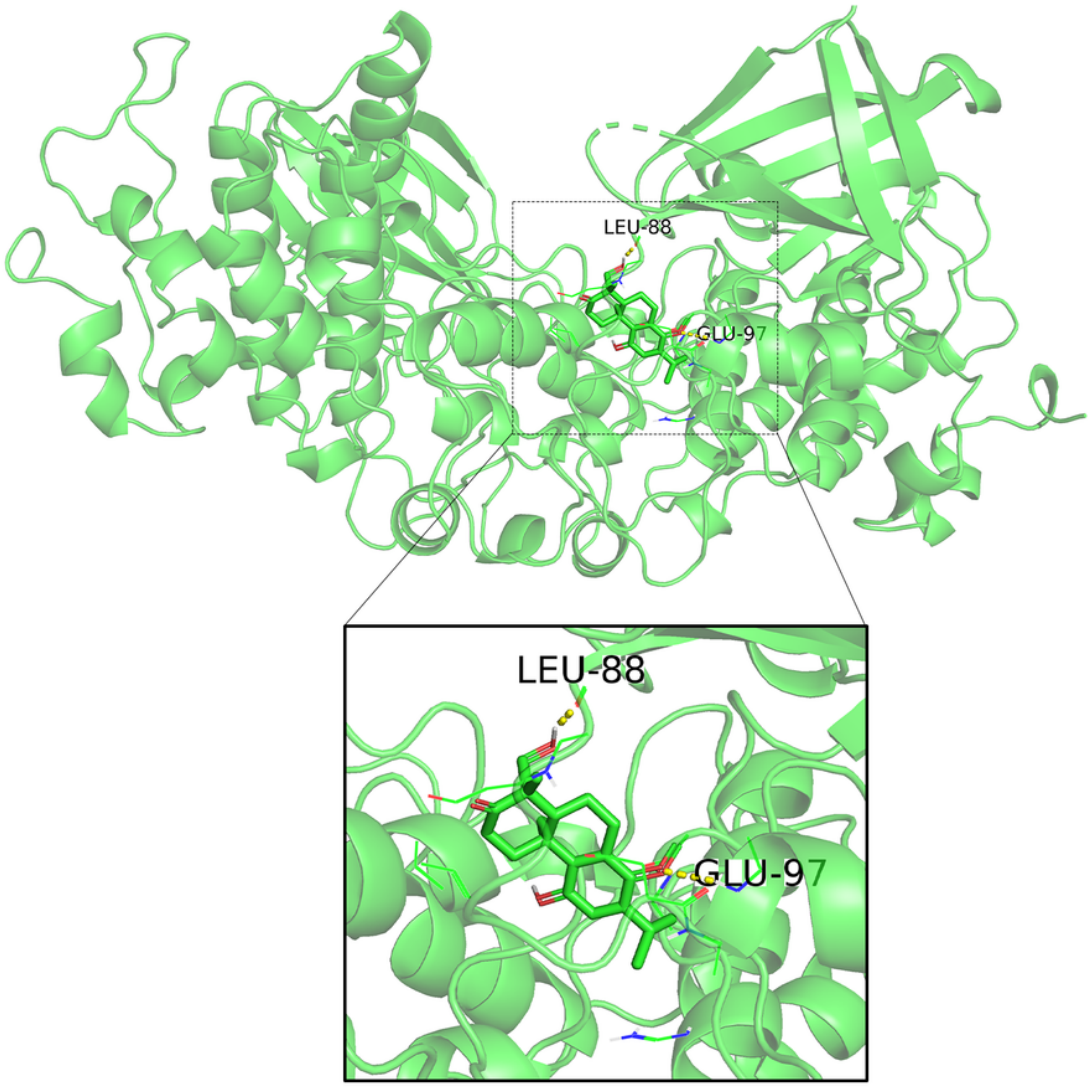

Figure 9

Molecular docking patterns

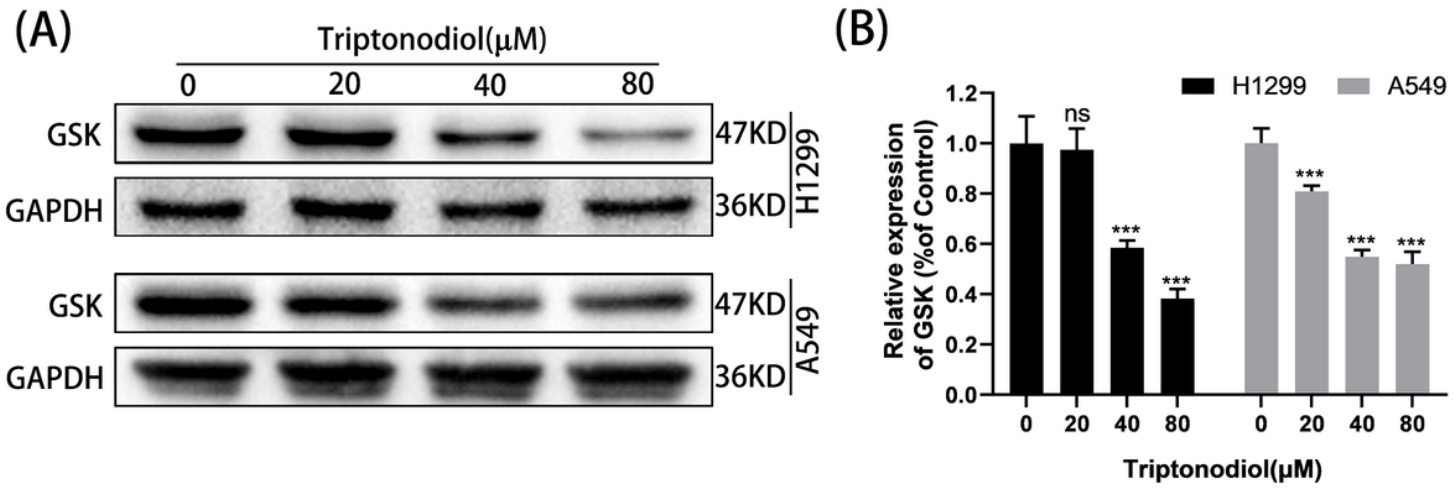

Figure 10

Western bolt. After treatment of Triptonodiol for 24 hours, the protein levels of GSK were significantly downregulated.