

Exploring the mechanism of *Inula japonica* Thunb. against non-small cell lung cancer using a computer-aided drug design approach

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Abstract

In China, *Inula japonica* Thunb. (IJT) is extensively used for the treatment of non-small cell lung cancer (NSCLC). However, further research is required to understand the pharmacological mechanisms of IJT owing to the complicated of the chemicals and targets. To investigate the anti-NSCLC mechanisms of IJT, network pharmacology, molecular docking, and molecular dynamics simulation were used. In network pharmacology, the TCMSP, Swiss Target Prediction, DisGeNet, and therapeutic target database databases were utilized to predict the active ingredients of IJT and their targets for the treatment of NSCLC. The DAVID database was used for gene ontology enrichment and the Kyoto encyclopedia of genes and genomes enrichment analysis of its core NSCLC-related targets. Quercetin and luteolin were selected as major compounds and 23 putative targets of IJT against NSCLC were picked out as the primary hubs. The major targets just modulated the NSCLC-pathway, which included Ras, ERBB, MAPK, PI3K-Akt, calcium, p53, and JAK-STAT signaling sub-pathways. The molecular docking results showed that all major compounds with NSCLC-pathway-relevant targets of IJT exhibited effective binding. Further, molecular dynamics simulations revealed that the luteolin-AKT1 and quercetin-AKT1 complexes possessed a steady state and bound extremely stably during molecular docking. The study demonstrates that the “multi-compounds, multi-targets and multi-pathways” mechanisms of IJT in treating NSCLC are elucidated clearly by network pharmacology, laying the foundation for the subsequent clinical application of traditional Chinese medicine.

Abbreviations: AKT1 = RAC-alpha serine/threonine-protein kinase, ALK = anaplastic lymphoma kinase, CCND1 = cyclin D1, CDKN2A = cyclin-dependent kinase inhibitor 2A, C-T = component-target, EGFR = epidermal growth factor receptor, ERBB2 = receptor tyrosine-protein kinase erbB-2, IJT = *Inula japonica* Thunb., MAPK = mitogen-activated protein kinase, MD = molecular dynamics, NSCLC = Non-small cell lung cancer, PPI = protein-protein interaction, TCM = traditional Chinese medicine, Tp53 = tumor antigen p53.

Keywords: *Inula japonica* Thunb, molecular docking, molecular dynamics simulation, network pharmacology, non-small cell lung cancer

1. Introduction

Lung cancer is a leading cause of cancer mortality around the world and is associated with poor prognosis.^[1] Lung cancer can spill into small cell cancer and non-small cell lung cancer (NSCLC).^[2] Remarkably, between 85% and 90% of lung malignancies are NSCLC.^[3] Tobacco consumption, air pollution, secondhand smoke exposure, occupational and

environmental exposures, and hereditary traits are the well-known pathogenic factors of NSCLC.^[4–6] Chronic cough, bloody phlegm, chest pain, dyspnea, weight loss, metastatic pain, exhaustion, fever, and respiratory distress are among the most common clinical signs of NSCLC, which can have a major negative influence on a person's health or even their life.^[7] Furthermore, due to delayed diagnosis, the majority of patients with terminal-stage NSCLC are not candidates for

This work was carried out with the support of Scientific and technological research projects in Henan Province (No. 222102310374) and Key Research Projects of Henan Higher Education Institutions (No. 23B360007).

The authors have no conflicts of interest to disclose.

All data generated or analyzed during this study are included in this published article [and its supplementary information files].

Our research was based on bioinformatics analysis in which the data's were extracted from public databases, so this study did not require the approval of an ethics committee.

A preprint has previously been published [Huiqin Qian, Bailing Wang et al 2023]. <https://doi.org/10.21203/rs.3.rs-2970066/v1>

Supplemental Digital Content is available for this article.

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How to cite this article: Qian H, Wang B, Zhang L, Li J, Huang X, Liu B, Zhao Y, Wang M. Exploring the mechanism of *Inula japonica* Thunb. against non-small cell lung cancer using a computer-aided drug design approach. *Medicine* 2025;104:51(e46660).

Received: 4 January 2024 / Received in final form: 31 July 2025 / Accepted: 27 October 2025

<http://dx.doi.org/10.1097/MD.0000000000046660>

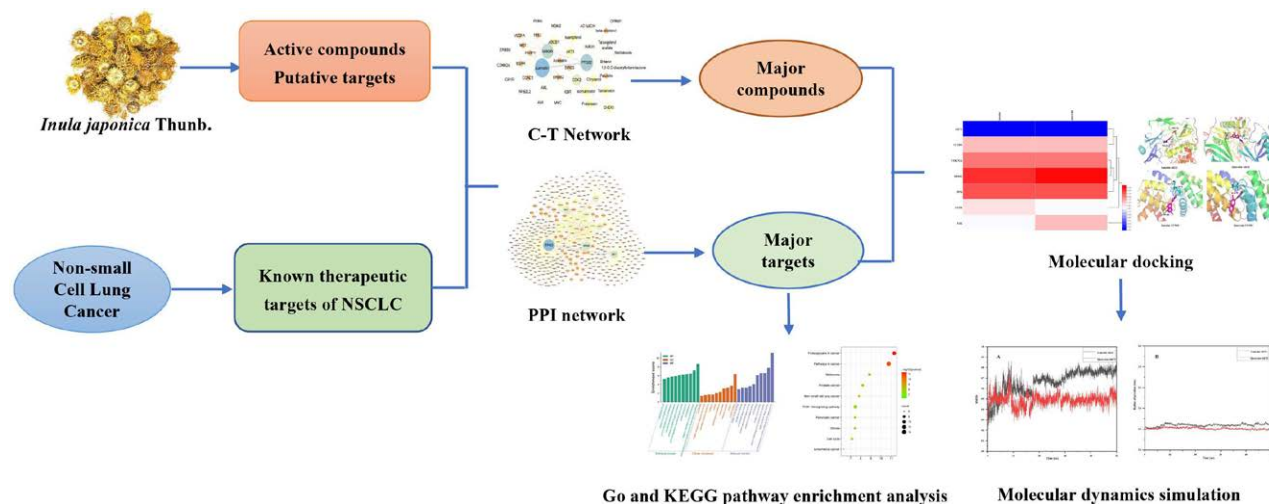


Figure 1. Workflow of the study.

surgery. Chemotherapy, radiation therapy, or targeted medication therapy must thus be used to alleviate the symptoms of patients with advanced NSCLC.^[8,9] Although the therapeutic strategies are somewhat effective, they can also trigger a series of adverse effects, such as fatigue, pain, nausea, vomiting, renal toxicity, reduction of lymphocytes, and myelosuppression.^[10] Therefore, searching for novel strategies against NSCLC is of great therapeutic importance. For patients with NSCLC, traditional Chinese medicine (TCM) in conjunction with chemotherapy is frequently advised. TCM exerts its therapeutic effect by regulating multiple compounds, targets, and pathways.^[11,12] TCM may prolong a patient's survival time in addition to alleviating side effects associated with chemotherapy.^[13] Therefore, TCM provides a promising complementary strategy for patients with NSCLC.

Inula japonica Thunb. (IJT) has expectorant, cough-suppressive, and anti-asthma effects properties.^[14] Polysaccharides of IJT are reported to be antidiabetic chemicals with β -cell-protective and antioxidant stress properties.^[15] The nitric oxide generation of mice macrophages is considerably inhibited by sesquiterpenes extracted from IJT, suggesting that they may have anti-inflammatory and antitumor properties.^[15–18] Furthermore, Japonicone A can suppress the growth of NSCLC cells by modulating mitochondria-mediated pathways.^[19] Essential oils extracted from IJT can enhance the sensitivity of MCF-7/ADR breast cancer cells to doxorubicin by down-regulating ABCB1 expression and reducing lipid raft stability.^[20] Although the antitumor activity of IJT has been investigated, the mechanism by which IJT acts against NSCLC remains unclear.

In the area of tumor prevention and therapy, TCM has accumulated a wealth of clinical knowledge. However, the “multi-component, multi-target, and multi-pathway” effects of TCM necessitate the application of contemporary research methods to clarify their scientific connotations. The transformation of conventional empirical knowledge into contemporary scientific evidence has emerged as a crucial area of study in several health-related domains in recent years. The role of vitamin D as a nutrient in immunomodulation has received long-standing attention, and evidence from high-quality randomized controlled trials and their meta-analyses has gradually increased its adjunctive therapeutic value for certain infectious diseases (such as COVID-19 and tuberculosis).^[21,22] Likewise, in the surgical domain, thorough meta-analysis studies have assessed the effect of bariatric surgery on the prognosis of patients suffering from arthroplasty.^[23] This research demonstrated how crucial contemporary evidence-based medical techniques-particularly RCT and meta-analysis for confirming and measuring the results of

therapies. As a systems biology strategy, network pharmacology can effectively integrate multidisciplinary data from bioinformatics and pharmacology to systematically predict the active ingredients, their targets, and the related signaling pathway networks for TCM compounds and individual drugs before proceeding with confirmatory clinical studies (e.g., randomized controlled trials), which provides important theoretical underpinnings and experimental design hints to comprehend the intricate mechanisms. Therefore, the present study employed network pharmacology, molecular docking and MD simulations to predict the mechanism of IJT against NSCLC, aiming to provide a scientific basis for its subsequent experimental validation and eventual translation into clinical application. A detailed flowchart is shown in Figure 1.

2. Materials and methods

2.1. Collection of candidate compounds from IJT

The TCM systems pharmacology database (TCMSP) is an authoritative platform for TCM systemic pharmacology, documenting the connections between herbal components, targets, and diseases. The system also provides information on the absorption, distribution, metabolism, and excretion (ADME) of each compound. The chemical composition of IJT was retrieved from TCMSP using the keywords “*Inula japonica*.” According to the TCMSP database's recommendations, the $DL \geq 0.18$ criterion is set to ensure that the drug exhibits a particular degree of structural similarity with the recognized active compounds, thereby increasing its potential biological activity. In contrast, the $OB \geq 30\%$ criterion is based on the requirement that the drug be effectively and efficiently absorbed after oral administration. Therefore, active components were defined as those that met the criteria of drug-likeness ($DL \geq 0.18$ and oral bioavailability ($OB \geq 30\%$). The simplified molecular input line entry specification of active ingredients from IJT was acquired from the PubChem database for target prediction.

2.2. Collection of NSCLC-relevant targets of IJT

The Swiss target prediction database is a platform for predicting the target of chemicals based on their similarity to the 2D and 3D structures of recognized compounds. With the species restricted to “Homo sapiens” and a probability >0.5 for target prediction, the simplified molecular input line entry specification

of the active components were imported into the Swiss Target Prediction database. The TCMSP database was also utilized further to identify the potential targets of each active component. Using the UniProt database, all IJT active component targets were ultimately converted into the Official Gene Symbol of *Homo sapiens*. The active ingredient targets of IJT were generated by combining and de-duplicating the search results from the databases above.

The Therapeutic Target Database (TTD) features information on validated therapeutic targets. The DisGeNET database is a platform that covers the relationship between diseases and genes as well as between diseases and variations. Using the keywords “NSCLC,” NSCLC-related targets were identified from the TTD and DisGeNET databases. Duplicate and false-positive genes were then eliminated. The OmicShare platform was used to import the active ingredient targets of IJT and NSCLC-related targets, and the intersection targets of the 2 were IJT targets that were relevant to NSCLC.

2.3. Construction of protein-protein interaction network

High-quality INTERactomes (HINT) is a platform for high-quality protein-protein interactions (PPIs) from 8 interactome resources (BioGRID, MINT, iRefWeb, DIP, Innate, HPRD, MIPS, and PDB). The Search Tool for the Retrieval of Interacting Genes (STRING) database provides recognized and predicted protein interactions derived from integrating experimental data, computationally based predictions, and literature mining. The PPIs were obtained by importing the intersection targets into the HINT and STRING databases. In addition, only data on protein interactions with a confidence level (score >0.55) were chosen. After eliminating duplicate items, all of the PPI data were finally combined. Cytoscape 3.6.0 was used to visualize and analyze the PPI network. One of the topological parameters, degree, describes the number of connections to a node. A higher degree value indicates the greater significance of nodes in the network. Target nodes were generally chosen as kernel targets if their degree values were more than twice median.

2.4. Component-target network construction

The component-target (C-T) network was constructed by linking the active components of IJT to the NSCLC-relevant targets of IJT using Cytoscape software (version 3.6.0). The primary active components of IJT in the treatment of NSCLC were screened using degree values.

2.5. Enrichment analysis

To further clarify the biological functions and significant pathways of NSCLC-related targets in IJT, we applied the database for annotation, visualization, and integrated discovery (DAVID, version 6.8) to perform gene ontology (GO) and Kyoto encyclopedia of genes and genomes (KEGG) pathway enrichment analysis. The enriched GO and KEGG keywords with a *P*-value <0.05 were generally considered statistically significant and retained. SRplot (<https://bioinformatics.com.cn/en>) was used to visualize the top 10 results as a bubble diagram or barplot.

2.6. Molecular docking

To verify the binding affinity of core targets with significant compounds in IJT, we used the Autodock Vina (Version 1.2.0) to implement molecular docking. The precise procedure was as follows: pretreatments for proteins: The 3D structures of major target proteins were downloaded from the Protein Data Bank (PDB) (<https://www.rcsb.org/>). Pymol software was then used to remove water molecules and cocrystallized ligands from the target proteins. The preprocessed proteins were then imported

into Autodock Tools (Version 4.2.6) for further modifications, including hydrogenation, calculation of point charges, and insertion of missing atoms in incomplete residues. The final repaired proteins were saved in PDBQT format. Pretreatments for ingredients: Autodock Tools (Version 4.2.6) additionally fixed the primary ingredients by adding atomic type, charge, and hydrogenation. Docking parameters: Repaired proteins in PDBQT format were uploaded into Autodock Tools (Version 4.2.6) to determine the size of the Grid box. Molecular docking: The modified target proteins and ingredients were entered into Autodock Vina for molecular docking. The values for energy range, exhaustiveness, and num_mod are 3, 50, and 10, respectively. Binding energy was computed to assess the stability and effectiveness of the compound-target complex. It was generally believed that the more stable the docking conformation between the molecule and target protein, the lower the docking energy.

2.7. Molecular dynamics simulation

MD can simulate the molecular motion trajectory of molecules within the organism's environment using a computer. Based on the molecule-protein complexes obtained from the foregoing docking as initial structures, all-atom molecular dynamics simulations were performed using GROMACS (2021.4). We opted for CHARMM 36 as the force field to determine the ligand's stability within the binding site and employed the TIP3P water model. Sobtop software was utilized to optimize the charge of the ligand and generate the files required for molecular dynamics parameterization.^[24] The boundaries of the box have a minimum distance of 1 nm from the atoms of the protein-ligand complex. We added 0.145 mol/L of a neutral sodium chloride solution to simulate the human environment. Before the molecular dynamics simulations started, the system was subjected to energy minimization using 50,000 steps of the most rapid descent method. Subsequently, the system performed 100,000 steps of NVT and NPT equilibration. The state of all environments was equilibrated at the set temperature (310 K) and pressure (101 kPa). Ultimately, the system ran 50 ns of MD simulations at constant temperature (300 K) and constant pressure (101 kPa) with a time step of 2 fs and saved trajectory data every 10 ps. The results of the MD simulations were analyzed and visualized by root mean square deviation (RMSD) and radius of gyration (Rg). RMSD was viewed as a gauge of system stability. Generally, the smaller the average RMSD value, the more stable the system. Rg is a key parameter for describing the spatial conformation and compactness of a molecule or collection of molecules. In molecular dynamics simulations, the larger the Rg value of the system, the more stretched the molecular conformation, indicating that the system is less stable.

3. Results

3.1. Active components of IJT and its corresponding targets

A total of 53 compounds in IJT were acquired from the TCMSP database. 19 compounds of IJT met the OB and DL requirements at >30% and >0.18, respectively. Three compounds were ignored because they lacked comparable targets. Using the target prediction algorithms of TCMSP and Swiss Target Prediction databases, 16 candidate compounds, including 8 flavonoids, 1 triterpenoid, 4 sesquiterpenoids, 2 phytosterols, and 1 organic acid, were identified, which collectively hit 280 targets. The detailed information on the candidate compounds in IJT and their corresponding putative was listed in Table 1 and Table S1, Supplemental Digital Content, <https://links.lww.com/MD/Q972>, respectively.

Table 1
Detailed information about candidate compounds of IJT.

Compound name	PubchemID	Categories	OB%	DL
Inulicin	N/A	Sesquiterpenoid	30.12	0.22
Tamarixetin	5281699	Flavonoid	32.86	0.31
Britanin	5315501	Sesquiterpenoid	33.73	0.41
Chryseriol	5280666	Flavonoid	35.85	0.27
Luteolin	5280759	Flavonoid	36.16	0.25
Melilotoside	5280759	Organic acid	36.85	0.26
beta-sitosterol	222284	Phytosterol	36.91	0.75
1,6-O, O-diacetylbritannilactone	10360513	Sesquiterpenoid	39.03	0.31
Pratensein	5281803	Flavonoid	39.06	0.28
Isoramanone	50716134	Phytosterol	39.97	0.51
kaempferol	5280863	Flavonoid	41.88	0.24
Taraxasterol acetate	13889352	Triterpenoid	43.08	0.74
Quercetin	5280343	Flavonoid	46.43	0.28
Patuletin	5281678	Flavonoid	53.11	0.34
Azaleatin	5281604	Flavonoid	54.28	0.3
AC1L9CIK	442263	Sesquiterpenoid	73.35	0.22

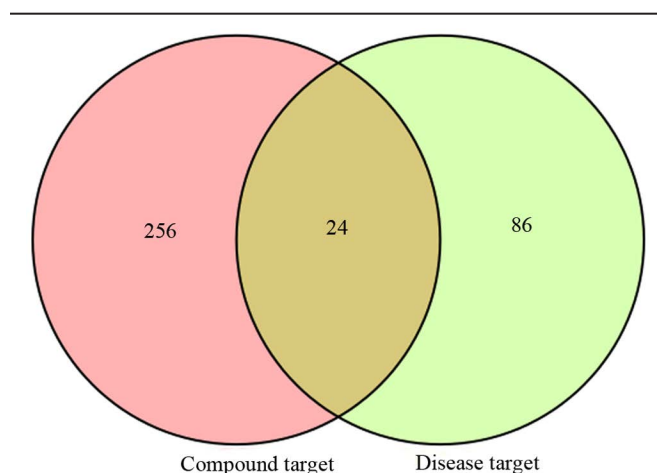


Figure 2. The Venn chart. The yellow part represented the number of compound targets that overlap with the number of disease targets, which was the number of NSCLC-related targets of IJT. IJT = *Inula japonica* Thunb., NSCLC = non-small cell lung cancer.

3.2. NSCLC-related targets of IJT

The TTD and DisGeNET databases contained 63 and 65 NSCLC-related therapeutic targets, respectively. After eliminating duplicates, 110 NSCLC-related targets were identified. The details of NSCLC-relevant targets are depicted in Table S2, Supplemental Digital Content, <https://links.lww.com/MD/Q972>. Furthermore, by significantly overlapping with compound targets and NSCLC-relevant targets, 24 IJT-related targets for NSCLC were identified, which might be potential therapeutic targets of IJT against NSCLC (Table S3, Supplemental Digital Content, <https://links.lww.com/MD/Q972> and Fig. 2).

3.3. C-T network

The C-T network had 60 edges and 40 nodes (16 compounds and 24 NSCLC-related targets of IJT), as shown in Figure 3. Meanwhile, the mean value of the degree was 3.75 (Table S4, Supplemental Digital Content, <https://links.lww.com/MD/Q972>). It signified that each molecule had a minimum correlation of 3.75 targets. Eventually, quercetin and luteolin, with degrees >7.5, were identified as essential active ingredients of IJT in the treatment of NSCLC. Likewise, the median degree value

of the targets was 2.5. It was demonstrated that at least 2.5 chemicals regulate each target. Targets with high degree value were PTGS2 (degree = 16), CDK2 (degree = 7), and CHEK1 (degree = 4). From the above analysis, IJT exhibited synergistic or additive effects on NSCLC through its multi-compound and multi-target properties.

3.4. PPI network for IJT in treating NSCLC

The PPI network comprised 476 nodes and 677 edges (Fig. 4 and Table S5, Supplemental Digital Content, <https://links.lww.com/MD/Q972>). As depicted in Figure 4, the size of the node in the PPI network depended on its degree value. The larger the degree value of a node, the bigger the size of the node was displayed. 23 NSCLC-related targets of IJT had degree values that were twice as high as the average degree (3) of all other nodes, including cellular tumor antigen p53 (TP53), epidermal growth factor receptor (EGFR), cyclin-dependent kinase inhibitor 2A (CDKN2A), G1/S-specific cyclin-D1 (CCND1), Vascular endothelial growth factor A (VEGFA), Myc proto-oncogene protein (MYC), E3 ubiquitin-protein ligase Mdm2 (MDM2), RAC-alpha serine/threonine-protein kinase (AKT1), and receptor tyrosine-protein kinase erbB-2 (ERBB2), etc. As a result, they were classified as candidate hubs. The detailed information is provided in Table S6, Supplemental Digital Content, <https://links.lww.com/MD/Q972>.

3.5. GO and KEGG pathway enrichment analysis

A total of 72 GO enrichment terms that satisfied $P < .05$ were obtained, including 47 biological process (BP) terms, 10 cellular component (CC) terms, and 15 molecular function (MF) terms. Then, the top 10 significant BP, CC, and MF terms were displayed in Figure 5. The terms in the BP category included transmembrane receptor protein tyrosine kinase signaling pathway, negative regulation of the apoptotic process, cell proliferation, and protein sumoylation, among others. CC terms included protein complex, nucleus, receptor complex, and nucleoplasm, etc. In the MF category, there were transmembrane receptor protein tyrosine kinase activity, protein tyrosine kinase activity, receptor signaling protein tyrosine kinase activity, and protein kinase activity, etc.

According to the results of the KEGG pathway enrichment analysis, 23 major targets were mapped onto 33 significant pathways ($P < .05$, Table S7, Supplemental Digital Content, <https://links.lww.com/MD/Q972>). The bubble chart of the

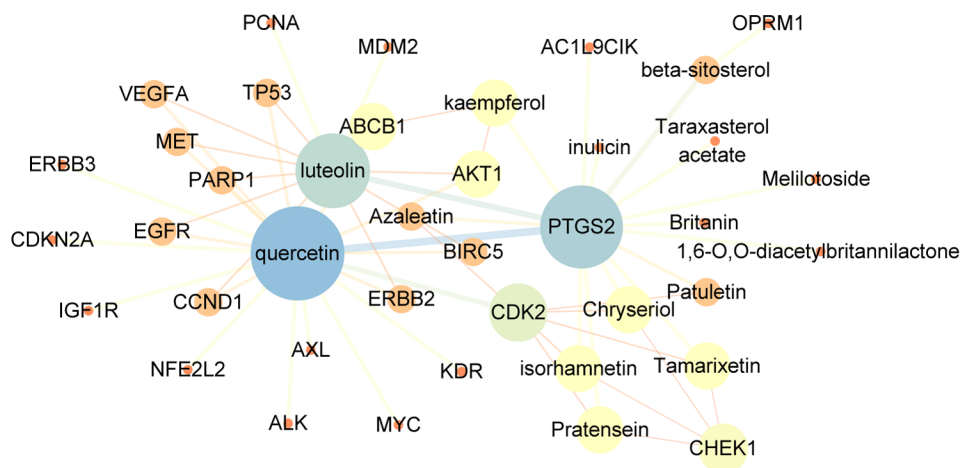


Figure 3. Compound-target network. The bigger the size of the node indicated the larger the degree value of a node.

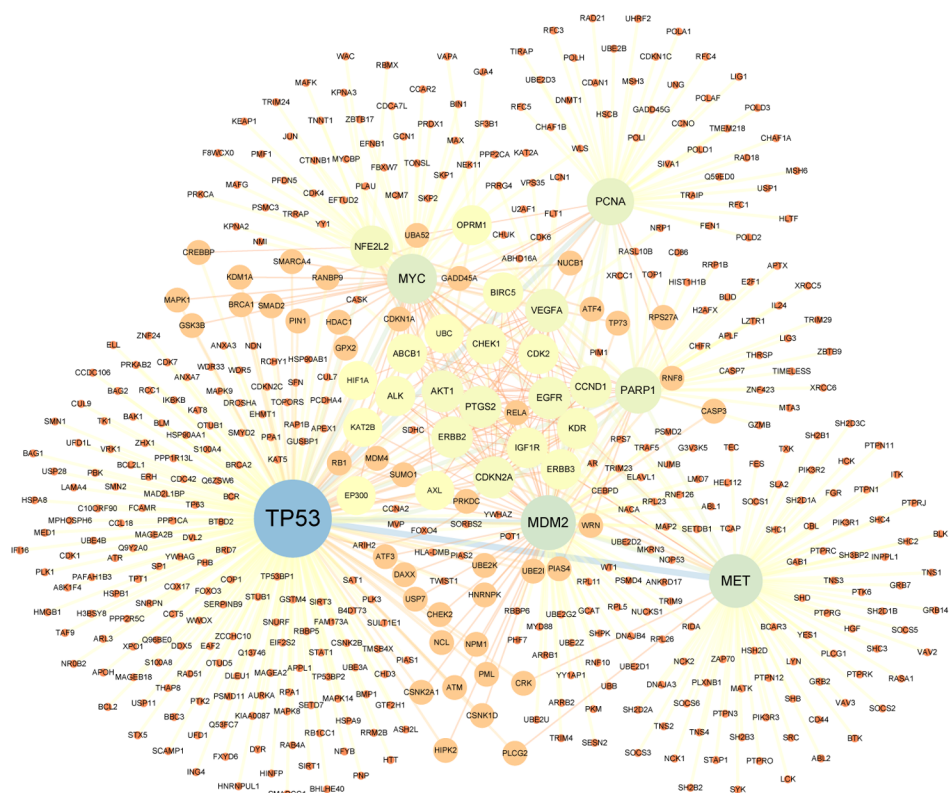


Figure 4. PPI Network. The bigger the size of the node indicated the larger the degree value of a node. PPI = protein-protein interaction.

top 10 KEGG pathways, ordered by significance, is depicted in Figure 6. IJT acted on multiple widespread cancer pathways, incorporating endometrial cancer, pancreatic cancer, glioma, NSCLC, prostate cancer, melanoma, pathways in cancer, proteoglycans in cancer, etc. Besides, IJT could modulate cancer-related pathways, including the cell cycle, PI3K-Akt signaling pathway, FoxO signaling pathway, etc. Therefore, the potential mechanisms of IJT in the treatment of NSCLC might be mainly attributed to its synergistic modulation of the relevant pathways in cancers. Interestingly, the pathway-“NSCLC” had just been included, which enriched TP53, CCND1, ERBB2, CDKN2A, EGFR, ALK, and AKT1. “NSCLC” was considered the significant pathway of IJT in treating NSCLC.

3.6. Molecular docking validation

Molecular docking was used to validate the 2 chemicals (luteolin and quercetin) as ligands and the NSCLC-pathway-modulated targets (TP53, CCND1, ERBB2, CDKN2A, EGFR, ALK, and AKT1) as receptors. In general, activity between the ligand molecule and the receptor protein was generally indicated by a binding energy of less than -6.0 kcal/mol and strong stability by an active compound with a target if the binding energy was less than -7.0 kcal/mol. The results of molecular docking showed that every compound-target complex demonstrated effective docking. The detailed information is described in Table S8, Supplemental Digital Content, <https://links.lww.com/MD/Q972>. The heat map of molecular docking is displayed in Figure 7. Notably,

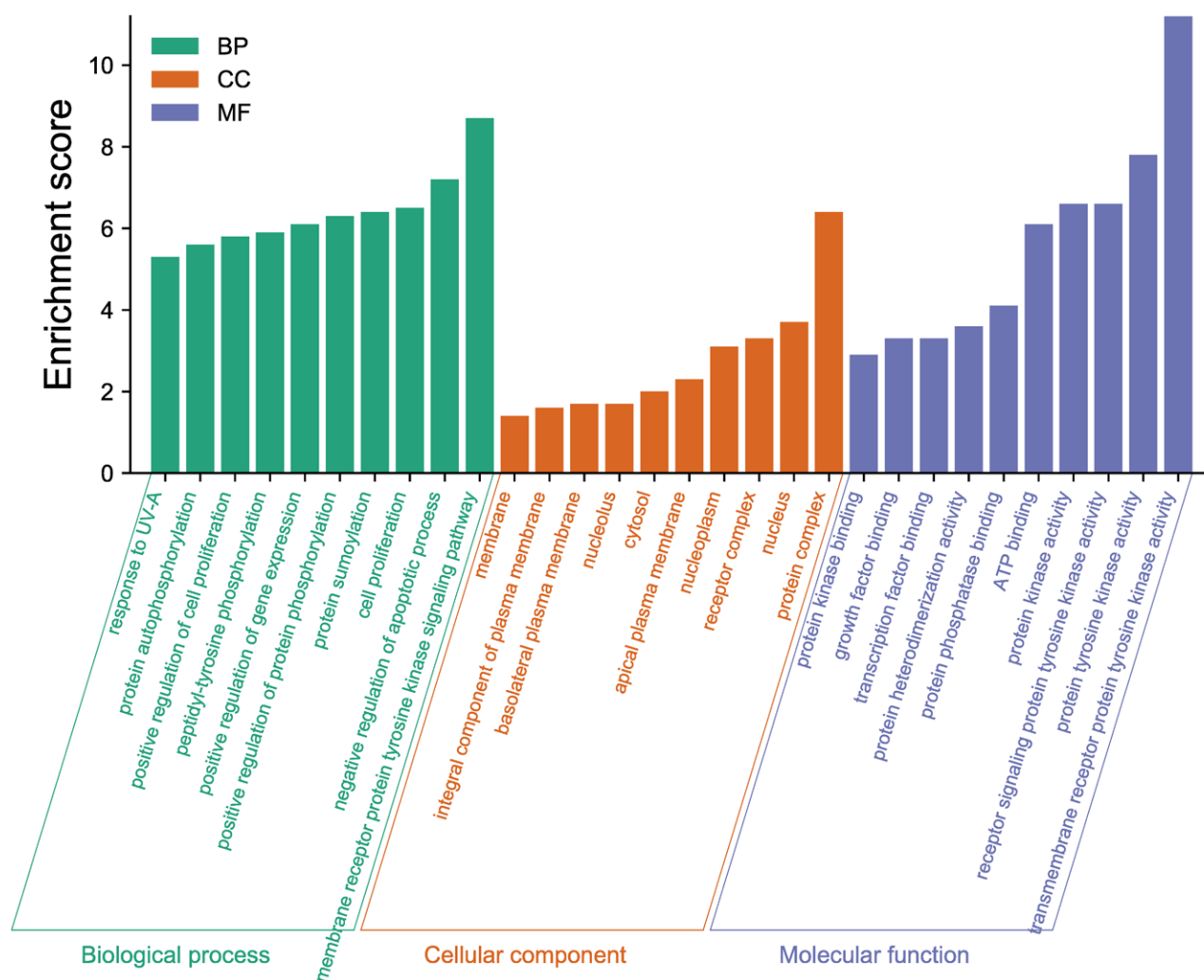


Figure 5. The top 10 significantly enriched terms in BP, CC and MF categories. The x-axis stands for BP, CC and MF categories. The y-axis stands for Log10(P) which is enrichment score of GO terms. BP = biological process, CC = cellular component, GO = gene ontology, MF = molecular function.

8 compound-target complex (57%) demonstrated good binding activity because their binding energy was less than -7 kcal/mol. Especially luteolin with AKT1 (-9.5 kcal/mol), quercetin with AKT1 (-9.4 kcal/mol), quercetin with CCND1 (-7.2 kcal/mol), and luteolin with CCND1 (-7.2 kcal/mol) owned the lower energy binding and displayed more stable conformation (Fig. 8).

3.7. MD simulations

For molecular dynamics simulations, the quercetin-AKT1 and luteolin-AKT1 complexes that were most tightly bound in molecular docking were chosen. RMSD values and Rg values were calculated for the complexes to assess the structural stability of protein-ligand complexes and the tertiary structural stability of protein-ligand binding during MD simulations. From the graph of the RMSD values of MD simulations (Fig. 9A), the RMSD values of the luteolin-AKT1 complex and quercetin-AKT1 complex fluctuated considerably during the initial phase of the simulation (0–20 ns), which indicated that there were noticeable changes in the conformations of the 2 complexes in the MD simulation. The luteolin-AKT1 complex's RMSD values predominantly varied between 0.7589151 and 0.8879525 nm after 20 ns, while the quercetin-AKT1 complex's RMSD values mostly ranged between 0.3397707 and 0.6519626 nm. The RMSD fluctuations were within a reasonable range, which indicated

that the luteolin-AKT1 and quercetin-AKT1 complexes were in a steady state, and the bindings were quite stable.

As seen in the graph of Rg values from MD simulations (Fig. 9B), the RMSD and the magnitude (or degree) of Rg fluctuations were consistent. Compared to the molecular dynamics system generated by quercetin-AKT1 (red dash), luteolin-AKT1 (black dash) demonstrated superior protein hydrophobicity and a more stable tertiary structure in terms of RMSD and Rg. Notably, the 2 complexes exhibited excellent properties and could exist stably without affecting their structures or those of other proteins. Therefore, it was concluded that luteolin and quercetin, which acted on AKT1, might have therapeutic value in the treatment of NSCLC.

4. Discussion

4.1. Analysis of active compounds and their nsclc-relevant targets

Quercetin and luteolin were identified by network pharmacology as the primary potential active ingredients of IJT against NSCLC. Numerous studies on anticancer research have documented the pleiotropic pharmacological properties of these 2 flavonoids. Crucially, the predictive analysis revealed that they might interact with significant targets that are crucial to the pathophysiology of NSCLC, including TP53, CCND1, ERBB2

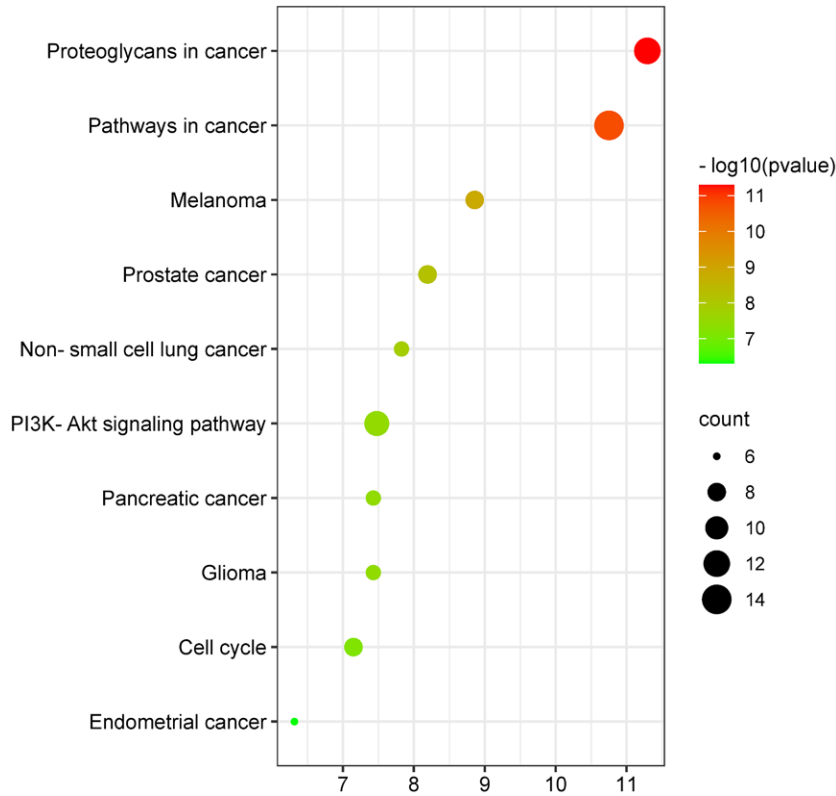


Figure 6. KEGG pathway enrichment analysis of the putative targets of IJT against NSCLC. The x-axis represents the rich factor which referred to proportion of the quantity of targets falling to a pathway to the quantity of all the annotated targets located in the pathway. The y-axis represents expressively enriched KEGG pathways. IJT = *Inula japonica* Thunb., KEGG = Kyoto encyclopedia of genes and genomes, NSCLC = non-small cell lung cancer.

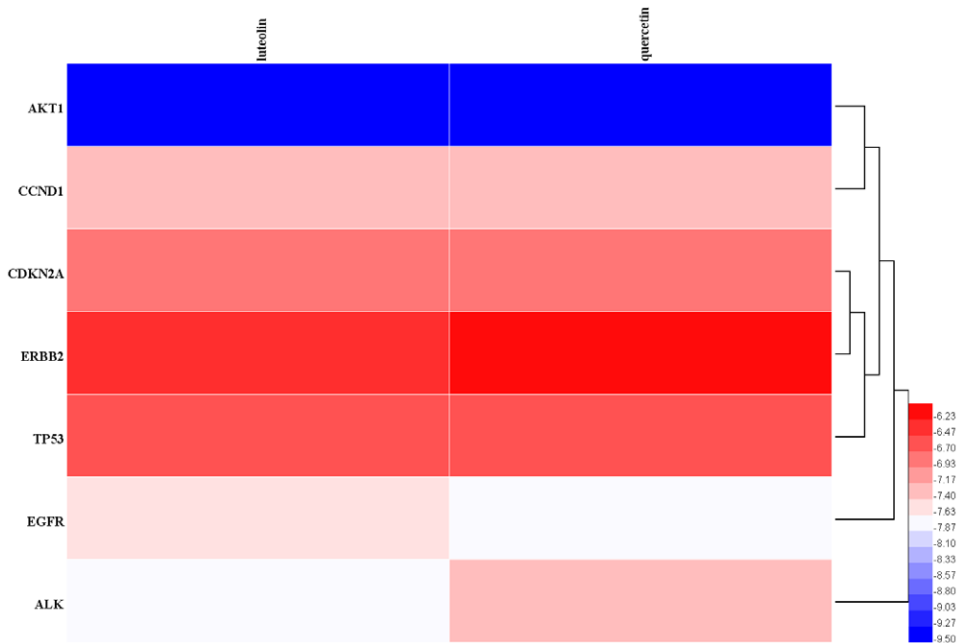


Figure 7. The heat map of molecular docking. Each row showed the docking of different compounds with the same target protein, and each column indicated the docking of different target proteins with the same compound. The average value of binding energy was taken as the base, the binding energy above the average value was labeled in red. The binding energy below the average value was labeled in blue. The color shade indicated the degree of difference in binding energy.

(HER2), CDKN2A, EGFR, ALK, and AKT1. The p53 protein, which regulates cell cycle arrest, DNA repair, senescence, and apoptosis, is encoded by the crucial tumor suppressor gene TP53. More than 50% of NSCLCs have TP53 mutations or

dysfunctions.^[25] A large body of experimental literature has demonstrated that quercetin and luteolin stabilize p53 and enhance its function, suggesting that they may restore the frequently inactivated p53 pathway in NSCLC and induce

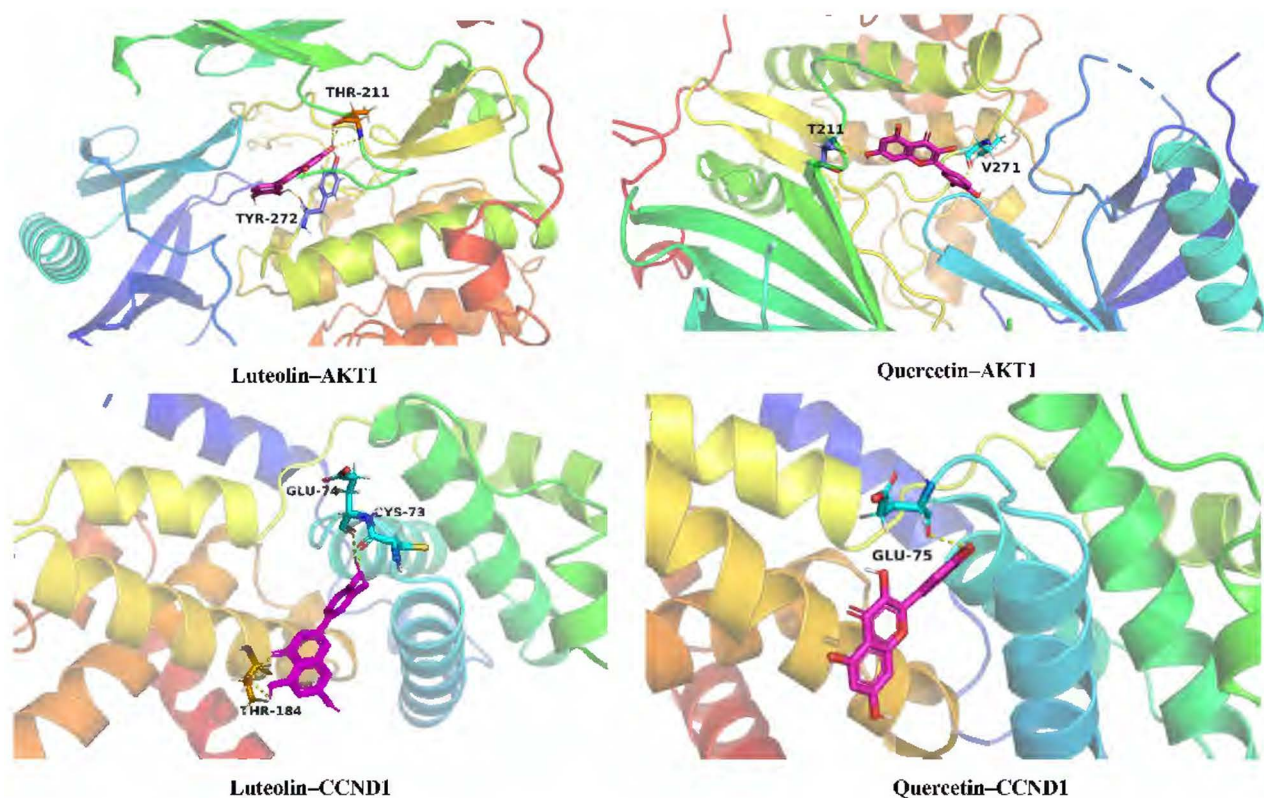


Figure 8. Molecular docking pattern diagram. The binding energy of luteolin with AKT1, quercetin with AKT1, quercetin with CCND1, and luteolin with CCND1 was -9.5 , -9.4 , -7.2 , and -7.2 kcal/mol, respectively. AKT1 = RAC-alpha serine/threonine-protein kinase, CCND1 = cyclin D1.

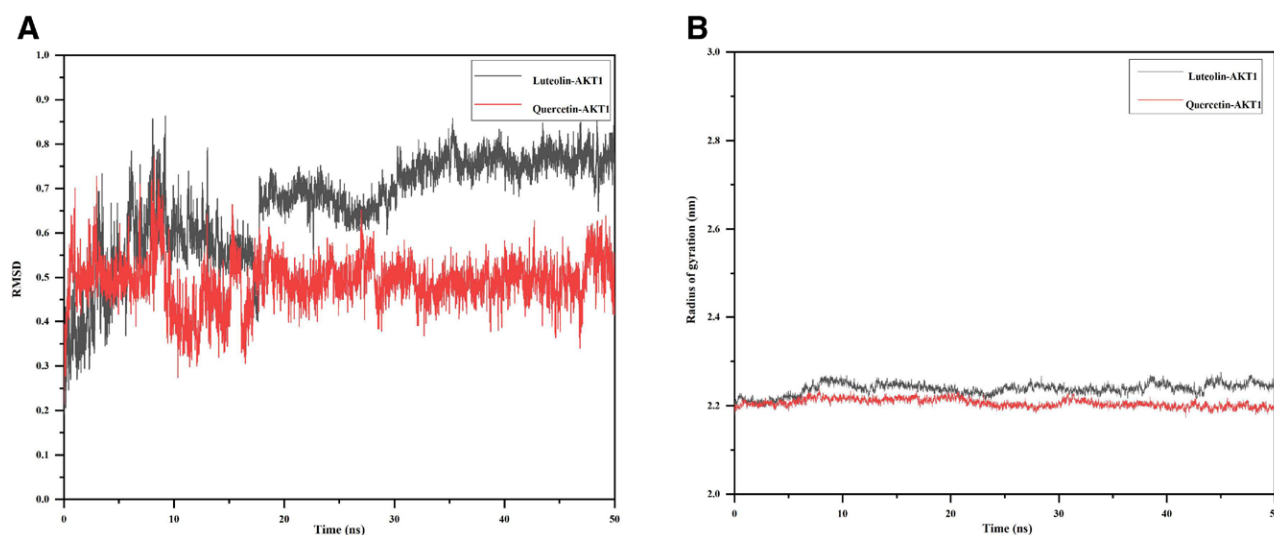


Figure 9. MD simulation. (A) represented the RMSD value of the complex at a given simulation time. (B) indicated the Rg variation of the complex with simulation time. MD = molecular dynamics, RMSD = root mean square deviation.

tumor cell apoptosis and cell cycle arrest.^[26,27] In NSCLC, the overexpression of the oncogene CCND1 promotes abnormal cell proliferation.^[28] Quercetin has been demonstrated to suppress CCND1 expression and induce G1 phase arrest in HepG2 cells.^[29] The prediction that IJT targets CCND1 is strongly consistent with the known pharmacological effects. The p16 protein, encoded by the CDKN2A gene, is a crucial oncogenic component that suppresses the Cyclin D/CDK4/6 complex, thereby blocking the cell cycle.^[30] CDKN2A/p16 is commonly inactivated in NSCLC by deletion, mutation or

methylation.^[31] Despite relatively few specific reports on the direct regulation of p16 by quercetin and luteolin, its predicted targeting of CDKN2A implies that the active ingredient of IJT may be implicated in altering the integrity of the CDK4/6-RB pathway. EGFR, ERBB2 (also known as HER2), and ALK are receptor tyrosine kinases. EGFR mutations are a cardinal driver of NSCLC. Experiments have shown that luteolin dramatically inhibits EGFR phosphorylation and blocks downstream activation of the PI3K/AKT and MAPK/ERK signaling pathways, resulting in NSCLC cell apoptosis.^[32]

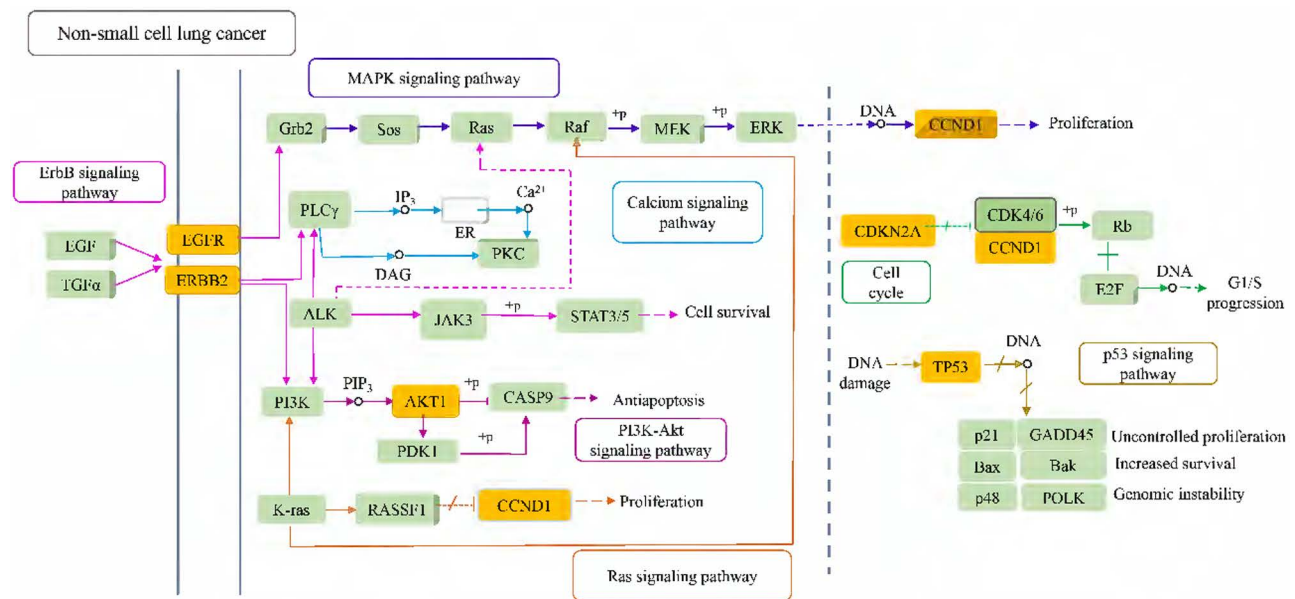


Figure 10. NSCLC-pathway. Yellow rectangles depicted the central NSCLC-associated targets of IJT, whereas green rectangles denoted other targets in the NSCLC-pathway. Arrows and T-arrows delineate respectively activation active and inhibition active, and segments delineate indirectly activation effect or inhibition effect. The original picture came from DAVID database. DAVID = database for annotation, visualization, and integrated discovery, IJT = *Inula japonica* Thunb., NSCLC = non-small cell lung cancer.

According to a recent study, quercetin may enhance the effectiveness of gefitinib by inhibiting glucose-6-phosphate dehydrogenase (G6PD), thereby lowering NADPH levels and promoting the degradation of the EGFR T790M mutation.^[33] Although HER2 abnormalities account for only 2% to 4% of NSCLC, their overexpression may trigger tumor growth and resistance to tyrosine kinase inhibitors.^[34] Quercetin induced apoptosis in HER2-overexpressing breast cancer cells,^[35] indicating that IJT may be useful in treating HER2-overexpressing NSCLC. ALK fusion genes are independent molecular subtypes of NSCLC that commonly occur in young nonsmoking patients.^[36] Quercetin and luteolin might both indirectly affect ALK kinase activity by blocking ALK downstream hub nodes such as AKT and STAT3.^[37–39] Overactivation of AKT1, a key junction of the oncogenic PI3K-AKT-mTOR signaling axis, enhances cell survival, proliferation, metabolism, and treatment resistance in NSCLC.^[40] It is well-established that quercetin inhibits AKT signaling and induces apoptosis in NSCLC cells.^[41] Similarly, luteolin has been demonstrated to inhibit AKT activation and augment chemosensitivity in lung cancer.^[42] Taken together, quercetin and luteolin are predicted to have multi-target modulatory abilities, acting simultaneously on crucial oncogenes (TP53, CDKN2A), significant oncogenic drivers (EGFR, HER2, ALK, CCND1) as well as the core survival kinase (AKT1).

4.2. IJT in treating NSCLC-pathway analysis

KEGG enrichment analysis disclosed that “NSCLC” was the major pathway for IJT in treating NSCLC. Yellow rectangles in Figure 10 represented the core NSCLC-associated targets of IJT, whereas green rectangles indicated other targets in the NSCLC-pathway. “NSCLC” pathway encompasses 7 sub-pathways, including Ras, ERBB, MAPK, PI3K-Akt, calcium, p53 and JAK-STAT signaling sub-pathways. As a crucial receptor of the ErbB family, EGFR forms a homologous dimer when its extracellular domain binds to TGFα. The transmembrane domain undergoes conformational changes, resulting in the autophosphorylation of the intracellular tyrosine kinase domain. Phosphorylated EGFR recruits junctional proteins to activate the Ras→MAPK pathway, which in parallel triggers the PI3K→Akt pathway and

calcium signaling. These pathways then function in concert to mediate cell cycle deregulation, resistance to apoptosis, and angiogenesis through crucial targets (e.g., CCND1, CDKN2A, AKT1).^[43–47] EGFR signaling may indirectly impair p53 pathway function and inhibit its pro-apoptotic effects.^[48] In specific NSCLC subpopulations, the EML4-ALK fusion gene maintains the activation of the JAK-STAT pathway by upregulating anti-apoptotic proteins (such as BCL-2 and MCL-1) through STAT3/5 phosphorylation.^[49,50] In summary, IJT effectively prevents the vicious cycle of NSCLC by co-regulating this signaling network with EGFR as the pivot (Ras/MAPK-PI3K/Akt-calcium pathway), the key oncogenic node (p53), and the immune escape axis (JAK-STAT).

5. Conclusion

The primary active components of IJT, quercetin and luteolin, modulate 23 possible targets to influence several NSCLC signaling pathways, including the Ras, ERBB, PI3K-Akt, etc. Molecular docking and molecular dynamics simulations confirmed that the key components were well bound to the key targets, such as AKT1, and the stability of the complexes was high. The findings provided new insights into the mechanisms of IJT in the treatment of NSCLC. Nonetheless, further experimental validations of the prediction results are required in our future studies.

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