Network Pharmacology-Based Identification of Pharmacological Mechanisms of *Inula japonica* Thunb. on Non-small Cell Lung Cancer

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Research

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Abstract

Background

Inula japonica Thunb. (IJT) is an extensively applied herbal medicine for treating non-small cell lung cancer (NSCLC) due to its anti-asthma, antitussive, and expectorant properties. However, the mechanism of IJT against NSCLC remains to be elucidated.

Methods

Network pharmacology analysis was applied to determine the function mechanism of IJT against NSCLC. Databases were used to collect compounds and their related and known therapeutic targets. The compound–target (C–T) and target–target networks were then constructed to screen the kernel compounds and NSCLC-related targets of IJT. Moreover, the NSCLC-related targets of IJT were input in the DAVID Bioinformatics Resources (version 6.8) for Gene Ontology Biological Processes (GOBP) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis. Finally, the binding affinity of major compounds with the NSCLC-relevant targets of IJT was further verified by molecular docking.

Results

Two active compounds (quercetin and luteolin) and six putative targets (RAC-alpha serine/threonine-protein kinase, G1/S-specific cyclin-D1, cyclin-dependent kinase inhibitor 2A, epidermal growth factor receptor, receptor tyrosine-protein kinase erbB-2, and cellular tumor antigen p53) were screened as the effective compounds and NSCLC-related targets of IJT. GOBP and KEGG enrichment analysis indicated that NSCLC was the major pathway of IJT in the treatment of NSCLC and the mediation of apoptosis, cell cycle, tumor progression, and proliferation of biological processes, including the Ras, ERBB, MAPK, PI3K–Akt, calcium, and p53 signaling pathways. The results of molecular docking validated that 10 of the 12 pairs of compound-target had effective binding.

Conclusions

The mechanisms of IJT against NSCLC through multi-compounds, multi-targets, and multi-pathways were elucidated.

Background

Lung cancer exhibits poor prognosis and is a major cause of death worldwide [1]. Based on the histological profile of the tumor cell, lung cancer can predominantly spilt into small cell cancer and non-small cell lung cancer (NSCLC) [2]. Therefore, NSCLC accounts for approximately 85–90% of lung

cancers [3][4]. NSCLC is caused by tobacco consumption [5], air pollution [6], second-hand smoke exposure, occupational and environmental exposures, and genetic characteristics [7]. The most frequent symptoms of NSCLC include chronic nonproductive cough, blood in the phlegm, thoracalgia, breathlessness, weight loss, metastasis pain, fatigue, fever, and dyspnea [8].

Given the delay in diagnosis, most patients with terminal stage NSCLC are not suitable for surgery. Therefore, the survival and palliation of symptoms of patients with advanced NSCLC may improve with chemotherapy, radiation, and targeted agents [9][10]. However, the therapeutic strategies remain ineffective, which may also trigger a series of adverse effects, such as fatigue, pain, nausea, vomiting, nephrotoxicity, lymphopenia, and myelosuppression [11]. Therefore, new strategies with important clinical significance must be developed to improve the treatment of NSCLC.

Traditional Chinese medicine (TCM), which is characterized by multiple compounds, targets, and pathways, has been widely combined with chemotherapy against NSCLC [12][13][14][15]. TCM can not only relieve chemotherapy-related side effect but also prolong the survival time of patients [16]. TCM provides a promising strategy for patients with NSCLC. *Inula japonica* Thunb. (IJT) is a TCM with anti-asthma, antitussive, and expectorant properties [17]. Polysaccharides are the anti-diabetic compounds in IJT, which can protect β-cells and fight oxidative stress [18]. Sesquiterpenes, isolated from IJT, can significantly display inhibitory outcomes on the nitric oxide production of murine macrophage, indicating that they have potential anti-inflammatory and anti-tumor activities [19][20][21]. Furthermore, Japonicone A can regulate mitochondria-mediated pathways, which suppresses the growth of NSCLC cells [22]. In addition, essential oils extracted from IJT can enhance the sensitivity and absorption of breast cancer cells by regulating the relevant mechanism of ABCB1 expression. This property eventually exhibits the reversal of multidrug resistance [23]. Although the anti-tumor activity of IJT has been studied, the mechanism of IJT on NSCLC must be further investigated.

The traditional research methods focus on the one-compound, one-target, and one-pathway of TCM in treatment of disease, which is difficult to explicit its complex mechanism. Nevertheless, network pharmacology emerged on the principles of network theory and systems biology in recent years can be utilized to probe the comprehensive mechanism of action of drug [24][25]. Network pharmacology can make the connection of drug compound-target and target-diseases clear and conduct their visualization network. Then, the potential mechanisms of drug in the treatment of diseases are systematically expounded through analyzing networks [26]. Thus, the present study applied network pharmacology to predict the mechanism of IJT against NSCLC. A detailed flowchart is shown in Fig. 1.

Methods

Candidate compound collection

The compounds of IJT were acquired from three online database, namely, Traditional Chinese Medicine Systems Pharmacology Database (TCMSP) [27], Traditional Chinese Medicine-Mesh (TCM-Mesh) [28],

and The Encyclopedia of Traditional Chinese Medicine (ETCM) [29]. To screen the potential drug candidate compounds, oral bioavailability (OB) and drug-likeness (DL) were employed in silico ADME models. We set the thresholds of OB and DL at > 30% and > 0.18, respectively. Compounds that satisfied the above criteria were merely reserved. The SMILES of compounds were acquired from the PubChem database.

Targets related to the candidate compounds

To precisely identify the targets correlated with the candidate compounds in IJT, we imported the SMILES format of candidate compound into Swiss Target Prediction [30], with the limitation of "*Homo sapiens*" species. The putative targets were then screened with a high probability (more than 0.50) for the sake of the credibility of targets. In addition, the potential targets of IJT were collected through the TCMSP platform as a supplement. Finally, the corresponding gene names were converted by the UniProt database.

NSCLC-related targets of IJT

The NSCLC-related human genes were obtained from two databases. One was the Therapeutic Target Database [31], which can provide information of known and grouped therapeutic targets. The other was the DisGeNET database [32], which embodies genes and variants linked to human diseases. Finally, the NSCLC-related targets of IJT were obtained by mapping the compound targets of IJT into NSCLC-related targets by Venn, which is a tool of the OmicShare platform.

Protein–protein interaction (PPI)

The NSCLC-related targets of IJT were submitted to the High-quality INTeractomes (HINT) and STRING databases to acquire PPIs. HINT gathered eight interactome resources, namely, BioGRID, MINT, iRefWeb, DIP, IntAct, HPRD, MIPS, and PDB. These resources can acquire high-quality PPIs and update every night [33]. The STRING database furnishes the physical and functional interactions acquired from the integration of experiment data, computation-based prediction, and literature mining [34]. Combined score that can weigh the reliability of PPIs was given by the STRING database. Thus, to construct a background network with advanced confidence edges and ensure that all NSCLC-related targets of IJT were included, we filtered the STRING databases with combined score of more than 0.55. Finally, the different ID types of the proteins acquired by incorporating all the data and deleting duplicates were transformed into UniProt IDs.

Network construction

Two networks were constructed as follows: 1) the compound–target (C–T) network was established for candidate compounds in IJT and their NSCLC-related targets, and 2) the target–target (T–T) network was constructed for NSCLC-related targets in IJT and other NSCLC-related therapeutic targets.

The two networks were visualized using the Cytoscape software (3.6.0). A network analyzer was used to calculate the topological parameters of networks, including "degree," "betweenness," and "closeness."

Degree refers to the quantity of linkage to node *i*. Betweenness represents the quantity of the shortest paths between coupled nodes running through node *i*. Closeness indicates the reciprocal of the sum of distances from node *i* to all other nodes. We then used the three topological parameters to select the major hubs from the network.

Enrichment analysis

To further clarify the biological functions and pathways of NSCLC-related targets in IJT, we applied the DAVID Bioinformatics Resources (version 6.8) to perform Gene Ontology Biological Processes (GOBP) and KEGG pathway enrichment analysis [35]. GOBP terms and KEGG pathways with P values less than 0.05 were enriched. The smaller the P value, the higher the enrichment. The bubble chart of the top 10 KEGG pathways was drawn via the OmicShare platform, which is an online platform for data analysis.

Molecular docking

To verify the binding affinity of targets with candidate compounds in IJT, we used the Autodock tool (version 4.2.6) to implement molecular docking. First, the 3D structures of candidate compounds in IJT were directly acquired from the PubChem database, and the crystal structures of the major targets of NSCLC-related IJT were downloaded from the PDB database. The protein preparation protocol was used before molecular docking, which includes removing unwanted chains, ligands, and water and metal ions; adding hydrogenation and calculating point charges; and inserting missing atoms in incomplete residues. Every protein was considered as a receptor. The receptor had cavities as the active site of proteins, which could be found using Autodock tool. The candidate compounds and modified target proteins were then imported into Autodock tools, and a genetic algorithm was used for molecular docking. Autodock tools could provide 100 predicted binding energies from different docking positions for the pocket of a protein binding every compound. The protein with the lowest binding energy was regarded as the target of IJT with high possibility. In our study, the compound with the target protein was judged as an effective docking if the binding energy was not higher than − 5.0 kcal/mol.

Results

Collection of candidate compounds of IJTand its corresponding targets

A total of 69 compounds in IJT were acquired from TCMSP, TCM-MESH, and ETCM databases after removing duplicates. 20 compounds in IJT accorded with the criteria of OB and DL at >30% and >0.18, respectively. In addition, 4 compounds were disregard owing to having no corresponding targets. Finally, 16 candidate compounds in IJT hit 280 targets altogether via the target prediction systems of TCMSP and SwissTargetPrediction databases . Thereinto, 16 compounds were constituted by 8 flavonoids, 1 triterpenoids, 4 sesquiterpenoids, 2 phytosterols, and 1 organic acid (Table 1).The detailed information of putative targets related to the candidate compounds of IJT is listed in Table S1.

Table 1. Detailed information about candidate compounds of IJT

Collection of NSCLC-related targets of IJT

The quantity of NSCLC-related therapeutic targets from the TTD and DisGeNET databases was 63 and 65, respectively. After the duplicates were removed, 110 NSCLC-related targets were ultimately obtained. The details are depicted in Table S2. A total of 24 NSCLC-related targets of IJT were obtained by overlapping dramatically of compound target and NSCLC-related targets (Table S3 and Figure 2), which might be the potential therapeutic targets of IJT against NSCLC.

C–T network

Extensive biological and pharmacological activities were exerted by TCM via multi-compounds and multi-targets. Hence, we built the C–T network to explore the sophisticated interactions based on the compounds and their related targets on NSCLC at a systems level. As displayed in Figure 3, the C–T network consisted of 40 nodes (16 compounds and 24 NSCLC-related targets of IJT) and 60 edges. Meanwhile, the mean degree value of candidate compounds was 3.75 (Table S4). As previously reported [36], the node is regarded as a major hub if its degree is twice higher than the average degree of all other nodes. Quercetin (degree = 20) and luteolin (degree = 13) possessed degrees greater than 7.5, which became crucial active compounds for IJT. In addition, 10 compounds were corelated with equal to or more than two targets. Similarly, 50% targets hit at least 2 compounds, which demonstrated that IJT exhibited synergistic or additive effects on NSCLC.

T–T network for IJT against NSCLC

To clarify the potential mechanisms of IJT against NSCLC, we built the T–T network for IJT against NSCLC. First, 677 protein-protein interactions of NSCLC-related targets of IJT were obtained from HINT and STRING databases. Among them, 527 were identified from HINT, 168 were obtained from STRING, and 18 were overlapped. Detailed information is listed in Table S5. Furthermore, the linkages of the targets of NSCLC-related IJT, known therapeutic target, and interactional human proteins were visualized by network. A total of 476 nodes and 677 edges were formed in the T–T network (Figure 4). The hub targets measured the significance in the network screened by degree. Nodes with degrees that were twice higher than the average degree (3) of all other nodes were regarded as candidate targets. Consequently, 23 nodes that satisfied the criteria of degrees greater than 6 were encoded as candidate hubs. The detailed information is provided in Table S6.

In order to accurately screen the target of ILT in treatment of NSCLC, the hub of PPI network was constructed with 23 nodes and 167 edges (Figure 5). The major hubs were filtrated with the three topological parameters containing “degree,” “betweenness,” and “closeness.” To ensure that all of the targets of NSCLC-related IJT were contained, the major hubs should satisfy the standard, which was “degree,” “betweenness,” and “closeness” more than 15, 0.02, and 0.8, respectively (Table S7). We ultimately determined nine major hubs for the subsequent GO and KEGG pathway enrichment analysis, including cellular tumor antigen p53 (TP53), epidermal growth factor receptor (EGFR), cyclin-dependent

kinase inhibitor 2A (CDKN2A), G1/S-specific cyclin-D1 (CCND1), VEGFA, MYC, MDM2, RAC-alpha serine/threonine-protein kinase (AKT1), and receptor tyrosine-protein kinase erbB-2 (ERBB2).

GOBP enrichment and KEGG pathway analysis

The GOBP and KEGG pathway enrichment analysis were used to analyze the nine major targets of IJT against NSCLC. A total of 21 GOBP enrichment terms with statistical significance at $P < 0.05$ were obtained, incorporating the positive regulation of protein phosphorylation, cellular response to epidermal growth factor stimulus, response to UV-A, positive regulation of epithelial cell proliferation, cell proliferation, and so on. The top five significantly enriched terms of BP (Figure 6) might be significant biological process of IJT in the treatment of NSCLC.

According to the results of KEGG pathway enrichment analysis, 9 major proteins were mapped onto 32 significant pathways ($P < 0.05$, Table S8). The top 10 KEGG pathways are depicted in Figure 7, which shows that IJT combined significantly with multiple cancer-related pathways in different types of cancers, incorporating bladder cancer, pathways in cancer, endometrial cancer, microRNAs in cancer, NSCLC, glioma, melanoma, chronic myeloid leukemia, prostate cancer, and central carbon metabolism in cancer. Therefore, the potential mechanisms of IJT in the treatment of NSCLC might be mainly attributed to its synergistical modulation on the relevant pathways of cancers. In addition, NSCLC enriched TP53, CCND1, ERBB2, CDKN2A, EGFR, and AKT1 and had minimum p value. Hence, NSCLC was considered the major pathway. TP53, CCND1, ERBB2, CDKN2A, EGFR, and AKT1 were ultamately reconsidered as significant targets.

Molecular docking validation

To verify the credibility of the predicted results, we implemented the docking process to verify the binding affinity of the targets with the compounds of IJT. The two compounds (quercetin and luteolin) and major NSCLC-related targets of IJT (TP53, CCND1, ERBB2, CDKN2A, EGFR, and AKT1) were validated by molecular docking. The results demonstrated that 10 of 12 pairs of C–T had binding efficiencies. The detailed information regarding the results of molecular docking is described in Table S9.

Discussion

ILT signifcantly alleviated the symptoms of NSCLC as a herbal medicine. Hence, From a holistic perspective, we applied network pharmacology to dissect the mechanism of ILT in treating NSCLC. In the study, 16 compounds and 24 NSCLC-related targets of IJT were obtained from multiple databases. After a series of screening, two compounds (quercetin and luteolin) and 6 NCLC-related targets of IJT (TP53, CCND1, ERBB2, CDKN2A, EGFR, and AKT1) were consedered as major compounds and targets. The reaults of molecular docking valided high credibility of the predicted results.

To verify the credibility of the predicted results, we implemented the docking process to verify the binding affinity of the targets with the compounds of IJT. The two compounds (quercetin and luteolin) and six

targets of IJT related to the NSCLC pathway (TP53, CCND1, ERBB2, CDKN2A, EGFR, and AKT1) were validated by molecular docking. The results demonstrated that 10 of 12 pairs of C–T had binding efficiencies. The detailed information regarding the results of molecular docking is described in Table S9.

C-T network disclosed that Quercetin and luteolin considered to play a critical role in treating NSCLC. Quercetin exhibits extremely antiproliferative, proapoptotic, and antimetastatic activities on NSCLC by enhancing cytotoxic efficacy [37], regulating the pathways related to the inhibition of snail-dependent Akt activation and Snail-independent ADAM9 expression [38], mediating BCL2/BAX gene [39], and regulating-down IL-6/STAT-3 signals [40]. Similarity, Luteolin possesses a wide spectra of pharmacological actions, including anti-inflammation [41][42][43], neuroprotective effect [43], protection against nonalcoholic steatohepatitis [44], anti-tumor [45][46], anti-ischemia/reperfusion injury [47], and anti-diabetic cardiomyopathy [48]. A recent pharmacological study presented that luteolin modulates the expression of genes relevant to steroidogenesis, apoptosis, and stress response, such as Fas, Tp53, and PARP. In addition, luteolin contributes to the treatment of NSCLC by decreasing AIM2 mediation [49]. In conclusion, quercetin and luteolin may be the kernel compounds of IJT in the treatment of NSCLC. In addition, Prostaglandin G/H synthase 2 (PTGS2, COX2) and cyclin-dependent kinase 2 (CDK2) targeted 16 and 7 compounds, which play significant pathological roles in inflammation, cancer, and immunoreactivity [50] [51][52]. For example, the overexpression of COX2 can lead to various cancers, such as colorectal, gall bladder, gastric, prostate, breast, lung, endometrial, and skin cancers [53][54][55][56], and plays a significant role in the pathophysiological processes of tumor growth. Prostate cancer cell proliferation, migration, and invasion are regulated by Toll-like receptor 4/cyclooxygenase-2 (COX-2) by activating NF-κB [57]. The level of COX-2 was reduced by targeting the PGE□-mediated activation of β-catenin signaling. Therefore, NSCLC cell migration is inhibited [58]. CDK2 is correlated with the poor outcome of NSCLC. Therefore, CDK2 may be the potential target against NSCLC [59]. To sum up, components and targets of IJT exhibited synergistic or additive effects on NSCLC.

NSCLC pathway was the major pathway that ILT exerted antitumor an NSCLC. As shown in Fig. 8, NSCLC pathway embodies 6 subways, including Ras, ERBB, MAPK, PI3K–Akt, calcium, and p53 signaling pathways. EGFR, a member of ErbB family, is divided into extracellular ligand binding domain, hydrophobic transmembrane domain and intracellular tyrosine kinase domain. EGFR can combine with TGFα, and then form homologous dimer. The autophosphorylation of tyrosine residues in dimer cells triggers a complex continuous molecular interaction and downstream signal cascade reaction, such as MAPK, calcium, Ras, PI3K–Akt signaling pathways. Furthermore, it can induce gene transcription, protein translation, DNA synthesis and promote cell proliferation, migration, adhesion, angiogenesis and apoptosis inhibition. Extensive research indicate that Ras, ERBB, MAPK, PI3K–Akt, calcium can mediate apoptosis, cell cycle, tumor progression, and proliferation of via various cytokines, such as CCND1, ERBB2, CDKN2A, EGFR, and AKT1 [60] [61] [62] [63] [64], In addition, It has been reported that p53 signaling pathways can relate closely to NSCLC [65]. In summary, the mechanisms of IJT in treating NSCLC involved multiple targets and pathways.

Conclusions

In this work, network pharmacology analysis and molecular docking technology were applied to predict the mechanism of IJT on NSCLC. 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.

Abbreviations

ILT: I*nula japonica* Thunb.; NSCLC: non-small cell lung cancer; TCM: Traditional Chinese Medicine; C-T: compound-target; T-T: target-target; PPI: protein–protein interaction; DAVID: Database for Annotation, Visualization and Integrated Discovery; GOBO: Gene Ontology Biological Processes; KEGG: Kyoto Encyclopedia of Genes and Genomes; TCMSP: Traditional Chinese Medicine Systems Pharmacology Database; TCM-Mesh:Traditional Chinese Medicine-Mesh; ETCM: The Encyclopedia of Traditional Chinese Medicine; OB: oral bioavailability; DL: drug-likeness; HINT: High-quality INTeractomes; CCND1: G1/S-specific cyclin-D1; AKT1: RAC-alpha serine/threonine-protein kinase; cyclin-dependent kinase inhibitor 2A, EGFR: epidermal growth factor receptor; ERBB2: receptor tyrosine-protein kinase erbB-2; P53: cellular tumor antigen p53

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

The manuscript is approved by all authors for publication.

Availability of data and materials

The data and materials generated or analyzed during this study are available

from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Q-HQ and W-BL conceived and designed the research methods.L-JX and H-XX collected the data. Z-LY analyzed the data. Q-HQ wrote the paper. All authors read and approved the final manuscript.

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Table

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

Figures

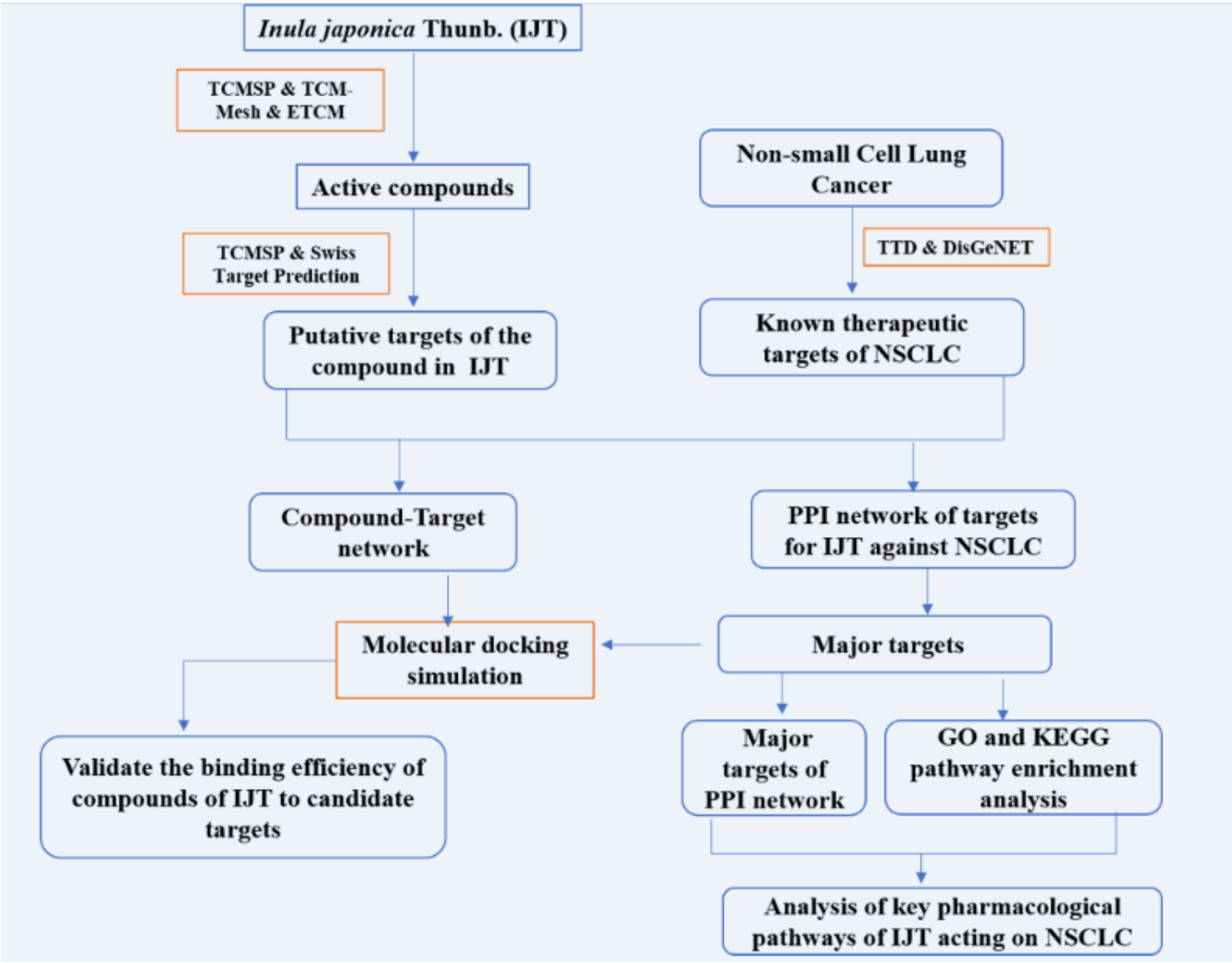

Figure 1

Network pharmacology approach flowchart.

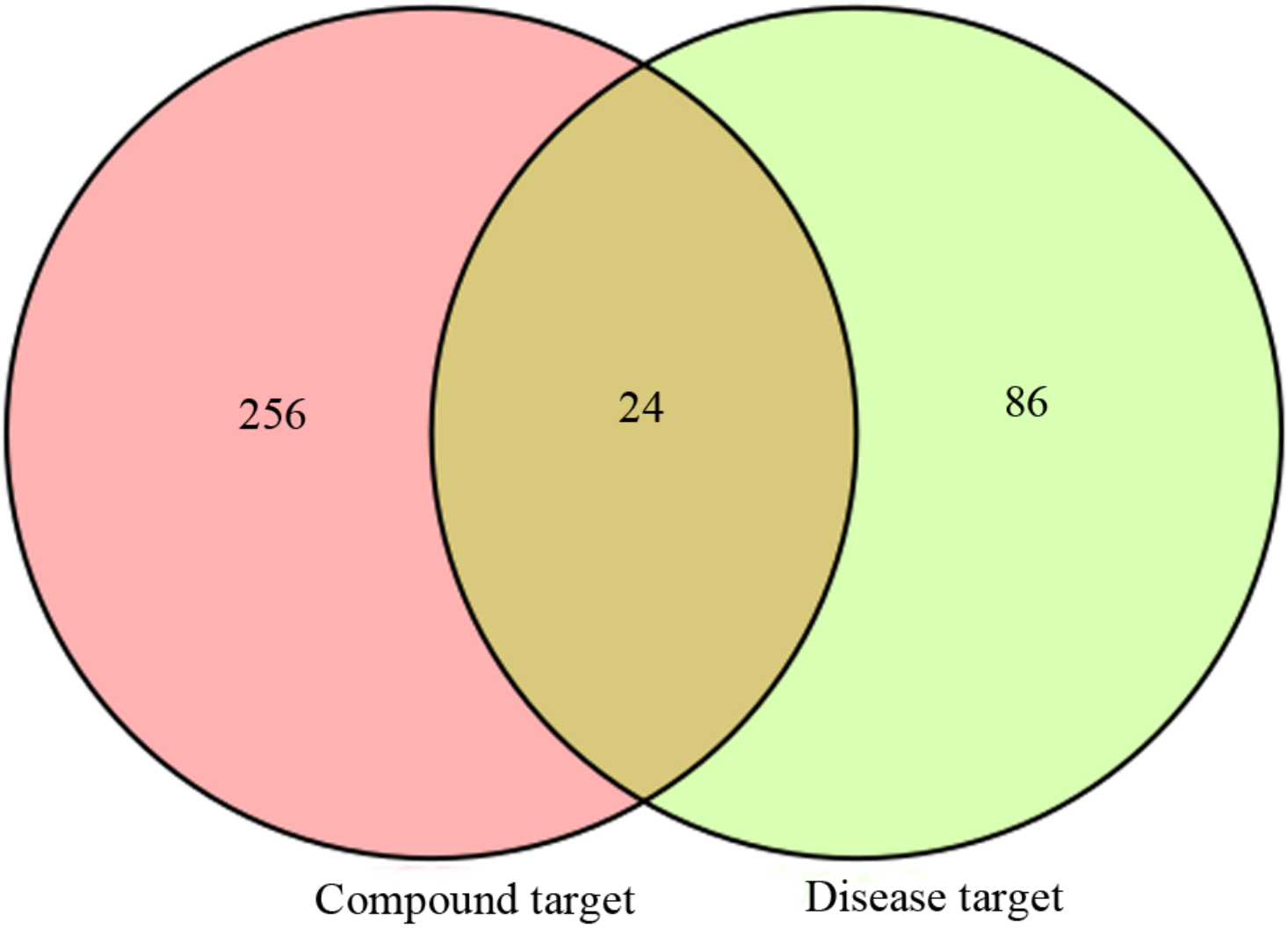

Figure 2

Venn chart of compound and disease targets.

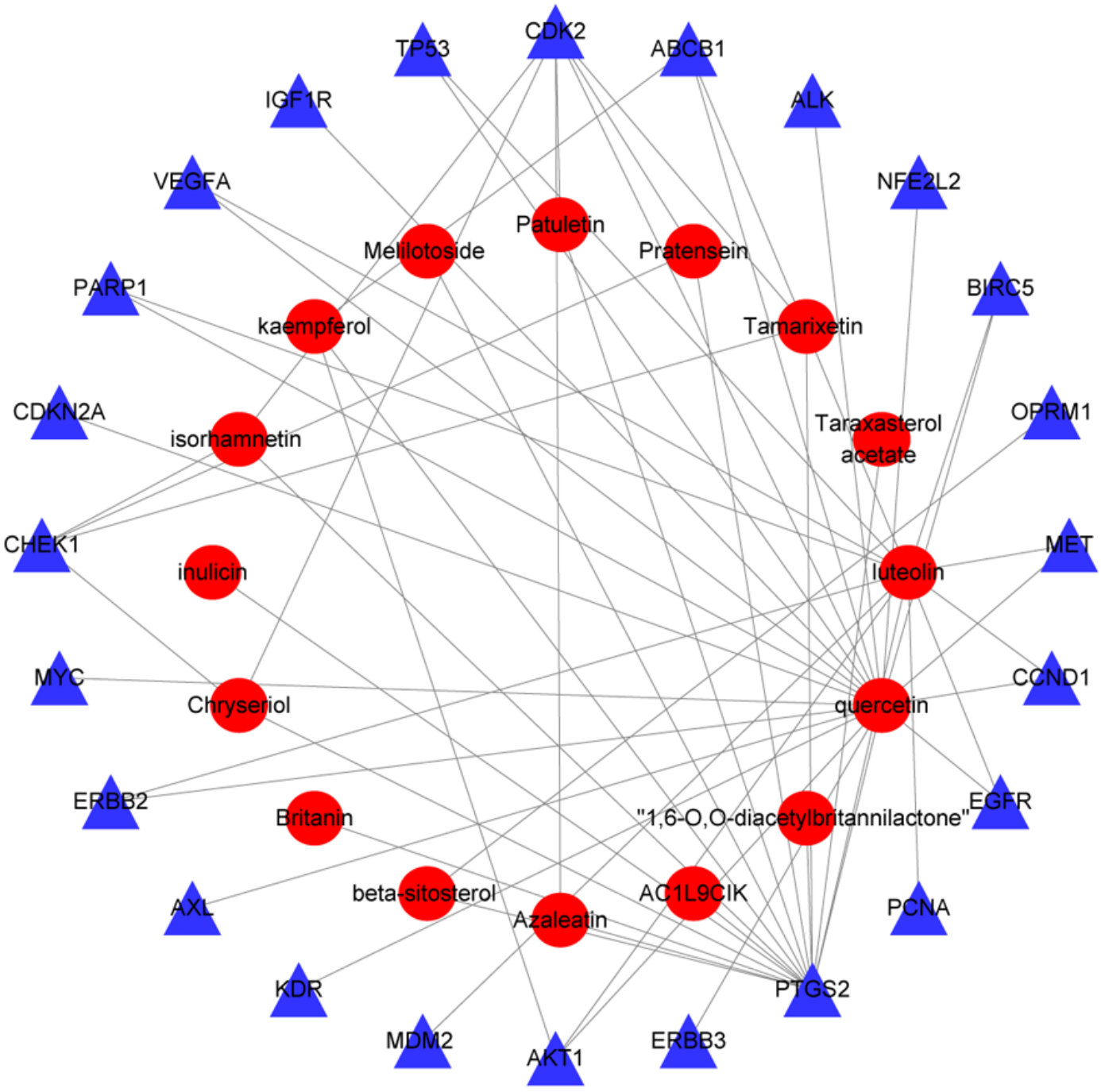

Figure 3

C–T network (red circulars indicate compounds; blue triangles represent compound targets).

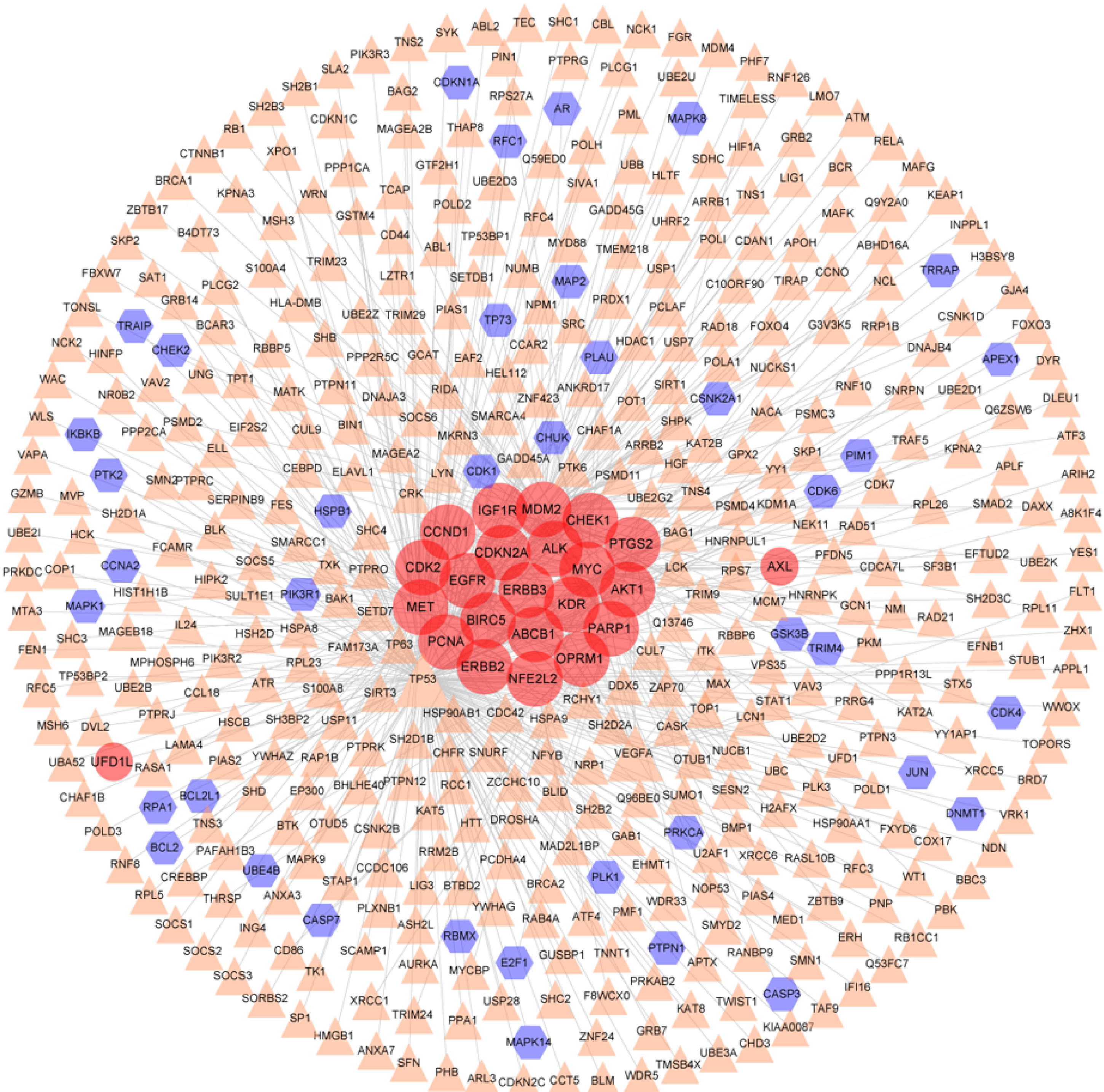

Figure 4

T–T network (red ellipses represent the NSCLC-related targets of IJT, blue hexagon represents compound targets, and orange triangle represents the known therapeutic targets and related human proteins).

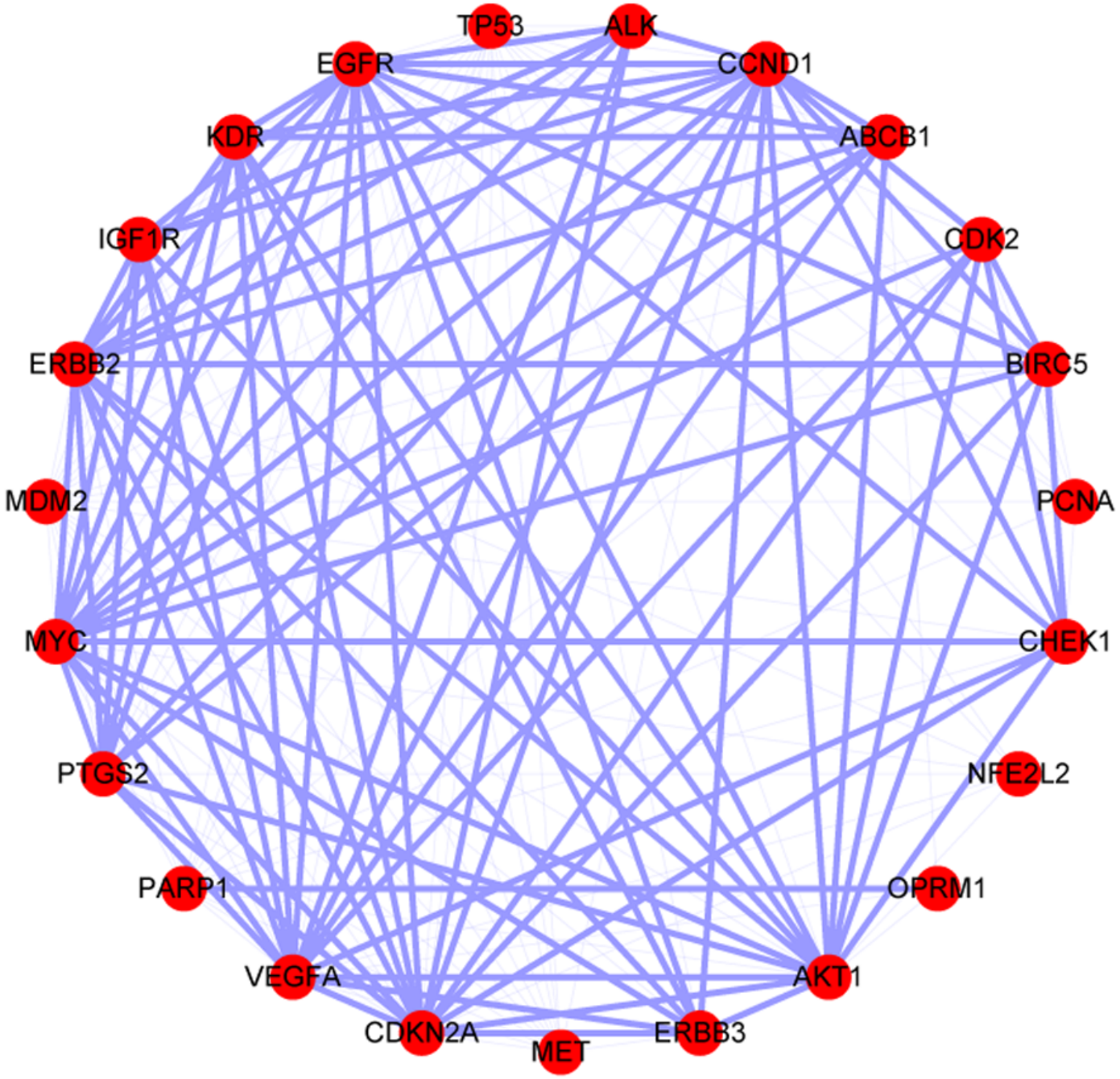

Figure 5

T–T network of hub targets for IJT against NSCLC extracted from Figure 4.

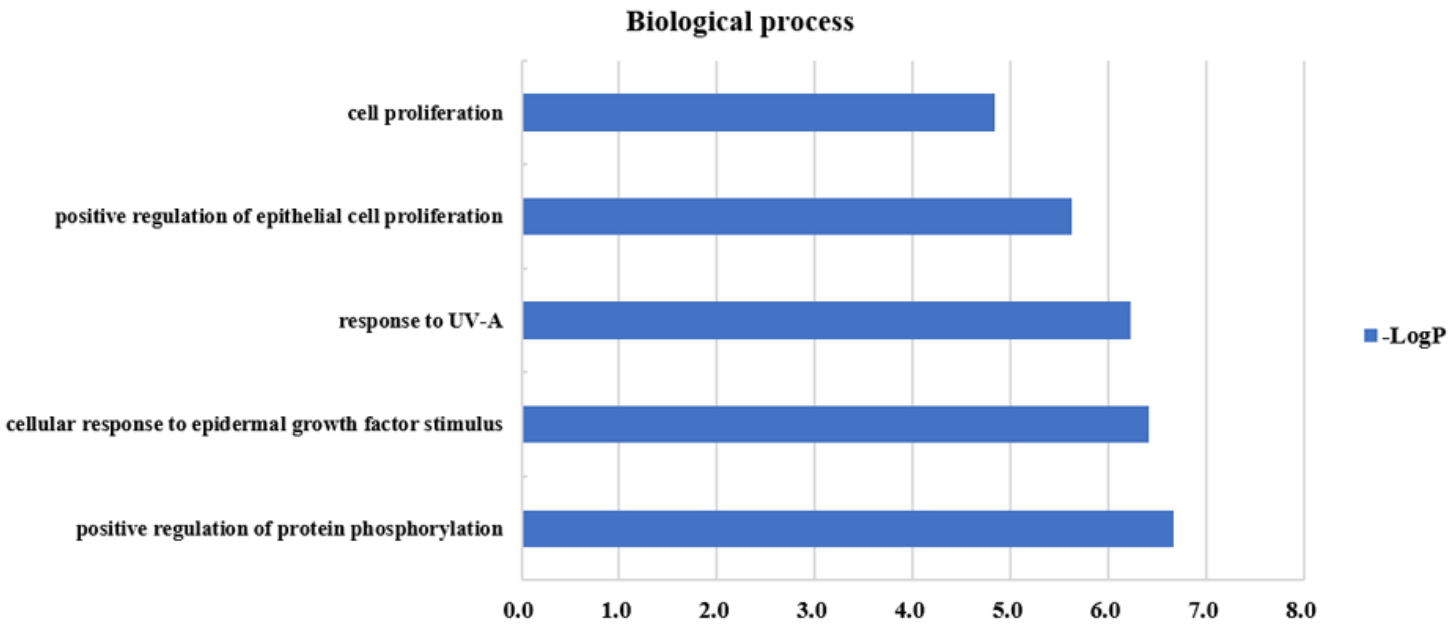

Figure 6

Top five GOBP enriched terms. The x-axis indicates Log10(P), which is the enrichment score of GO terms. The y-axis represents BP categories.

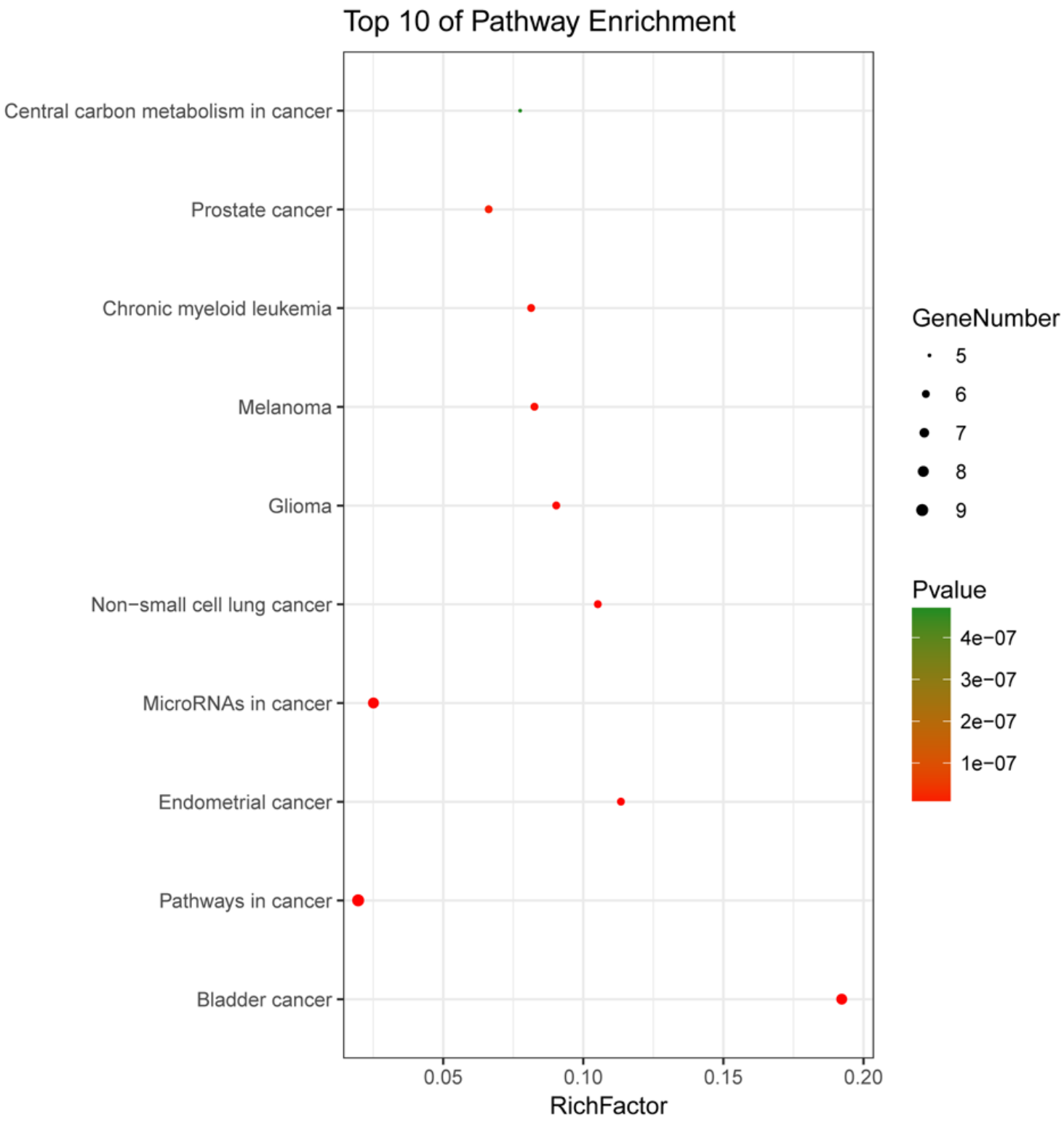

Figure 7

Bubble chart of the top 10 KEGG pathways. The x-axis represents the rich factor, which refers to the 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 indicates expressively enriched KEGG pathways.

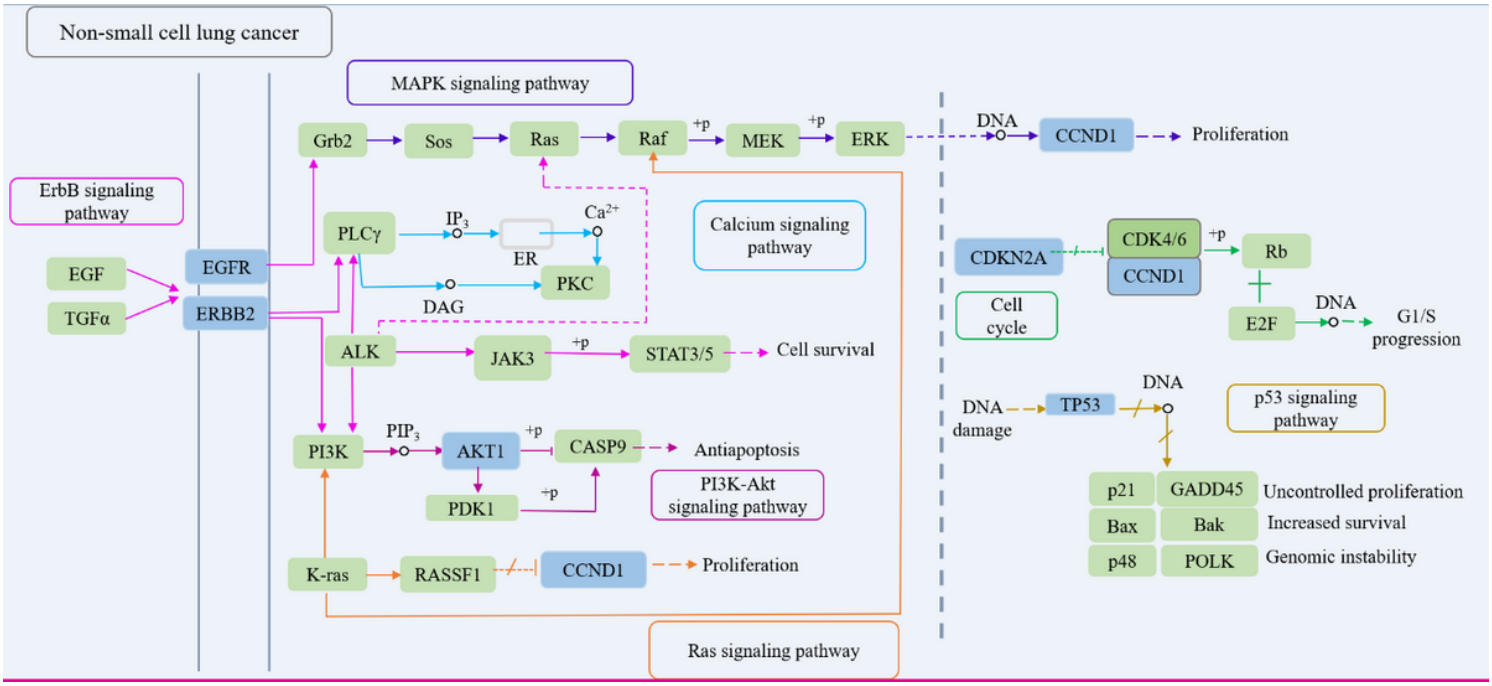

Figure 8

Distribution of targets in IJT on the compressed “NSCLC pathway.” Arrows and T-arrows delineate activation active and inhibition active, respectively, and segments delineate indirectly activation effect or inhibition effect.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- TableS8.xlsx
- TableS1.xlsx
- TableS2.xlsx
- TableS6.xlsx
- TableS5.xlsx
- TableS4.xlsx
- TableS7.xlsx
- TableS3.xlsx
- TableS9.xlsx