

# Exploring the Mechanism of Laminaria for the Treatment of Alzheimer's Disease based on Network Pharmacology

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## Research

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# Abstract

**Background** *Laminaria japonica* has also been reported to have a therapeutic effect on AD, but the mechanism is not entirely clear. To explore the mechanism of *Laminaria* for the treatment of Alzheimer's disease (AD), the “active components-targets” network and the protein-protein interaction (PPI) network were constructed for analyzing targets' functions and pathways.

**Methods** The main active components of *Laminaria* were extracted using the TCMSP database and were predicted and screened by GeneCards. Cytoscape was used to construct the “drug-components-targets-disease” network. STRING and Cytoscape were applied to map the PPI network. The Gene Ontology (GO) terms and KEGG pathways of targets were analyzed by Metascape.

**Results:** Seven active components involving 23 active targets were obtained. The network analysis elucidated that *Laminaria* was mainly involved in cell process, metabolic process, response to stress and other biological processes. CASP3, PPARG, RELA, CCND1 and CASP9 played a key role in treating AD by regulating two small cell lung cancer and Toxoplasmosis.

**Conclusion:** This study demonstrated that *Laminaria* could prevent and treat AD with advantages of multi-components, multi-targets and multi-pathways, which explored a new way for further research on the mechanism of *Laminaria* in the treatment of AD.

## Background

Alzheimer's disease (AD), characterized by impairment of memory, cognitive dysfunction and social disorders, is a chronic neurodegenerative disease[1]. According to *Alzheimer's Disease International*, dementia affects 50 million people worldwide, with a new case of dementia occurring somewhere in the world every 3 seconds. The symptoms of the disease is most common in those aged 60 or older and the neurologic changes caused by AD are irreversible[2]. The aging of the global population is unprecedented. The number of people over 60 in the world is projected to increase by 56% between 2015 and 2030, and by 2050 the global elderly population is projected to more than double. Thus, age remains the greatest risk factor for AD. The pathological hallmarks of AD include amyloid  $\beta$ -protein deposition[3], abnormal phosphorylation of the protein *tau*[4], neuroinflammatory response[5], cholinergic deficit[6], oxidative stress[7], et al. At present, medicine for treatment of AD can only relieve the symptoms and have relatively large side effects, which cannot repair nerve damage and prevent the deterioration of the disease[8–10]. Therefore, it is urgent to systematically elucidate the mechanism of AD and find safe and effective agents against AD.

*Laminaria japonica*, the most common member of the brown algae family, is not only well-known as “longevity food”, but also as traditional Chinese herbal medicine that is used to prevent and treat various diseases for over a thousand years[11]. *Laminaria* is rich in vitamins, minerals, dietary fiber, proteins and polysaccharides[12], which has shown to possess many biological activities such as antibacterial[13], antiviral[14], anti-inflammatory[15], anti-tumor[16], anti-diabetes[17] and antioxidant[18] and protective

effects against liver damage, hypertension, obesity, insomnia[19]. Recently, *Laminaria* has also been reported to have a therapeutic effect on AD, but the mechanism is not entirely clear.

Network pharmacology is an emerging approach to explore the relationship between drugs and diseases that integrates system biology, network analysis, bioinformatics and multi-directional pharmacology[20]. It devotes to understand the drug's pharmacological mechanism and development in the network perspective. Network pharmacology can quickly and efficiently analyzes the mechanism of traditional Chinese medicine in a modern way[21]. In this study, the "drugs-components-targets-disease" network was constructed to analyze the relationship between components of *Laminaria* and AD-related target proteins to investigate the binding affinity and predict the possible binding sites of drugs. This strategy could provide a reasonable basis for further clinical and experimental research in *Laminaria's* action mechanism against AD.

## Materials And Methods

### Collection and selection of main chemical components

The chemical components of *Laminaria* were collected from the Traditional Chinese Medicine Systems Pharmacology (TCMSP) database and analysis platform (<http://tcmsp.com/tcmsp.php>)[22]. Oral bioavailability (OB) is an important pharmacokinetic parameter in drug absorption, distribution, metabolism and excretion, indicating the rate and degree of systemic absorption of active ingredients in oral drugs[23]. The parameters of  $OB \geq 30\%$  are considered to be absorbed and utilized by the body. Drug-likeness (DL) is a necessary condition for preparing compound medicine, the value of which indicates the similarity between the ingredients and known chemical drugs.  $DL \geq 0.18$  is generally considered as an important reference for the activity[24]. According to the recommended criterion in TCMSP database,  $OB \geq 30\%$  and  $DL \geq 0.18$  were used to select the active components.

### Collection of potential targets for Alzheimer's disease

The targets of potentially active components were collected in the TCMSP database. The collected targets were imported into the Uniprot database (<http://www.uniprot.org/>) by name with the "Homo sapiens" setting to obtain human-related protein targets. Different ID types of the proteins were converted to UniProt IDs. The GeneCards (<https://www.genecards.org/>) database was used to obtain the gene name of each target by searching the keyword "Alzheimer's Disease"[25].

### Construction of the "drug-targets-disease" interaction network

The targets of active components of *Laminaria* and the targets of AD were imported into a website called Bioinformatics & Evolutionary Genomics (<http://bioinformatics.psb.ugent.be/webtools/Venn/>), and the

overlapping targets were collected. The target-disease and component-target network was created and merged to construct the component-target-disease network model using Cytoscape 3.7.0 software[26].

## Construction of PPI network

STRING 10.5 (<https://string-db.org/>) is a database of known and predicted protein-protein interactions including direct (physical) and indirect (functional) associations[27]. The AD-related common targets and the potential targets of *Laminaria* were applied to construct the PPI network using STRING 10.5 database with the “Homo sapiens” setting to achieve a comprehensive understanding of the relationships among compounds, targets, and AD. The STRING automatically scores each PPI, and the higher the score, the higher the confidence. In this study, a PPI network consisting of the products of gene expression was constructed based on the 30 data with top scores.

## Analysis of targets' pathways

To further understand the function of targets' application in the signal pathway, the *Laminarin*-AD overlapping targets were introduced into the Metascape database (<https://metascape.org/>), in which these targets were standardized under the “Homo sapiens” setting and the threshold was set as  $P \leq 0.05$ . Gene Ontology (GO) terms[28] and Kyoto Encyclopedia of Genes and Genomes (KEGG)[29] pathways were analyzed. The pathway map of the effect of *Laminaria* on AD was constructed by KEGG Mapper (<https://www.kegg.jp/>). GraphPad Prism 5.0 was used for mapping.

## Results

### Screening of active compounds

A total of 48 compounds in *Laminaria* were obtained from TCMSP. Based on the absorption, distribution, metabolism, excretion calculation, 7 active compounds with  $OB \geq 30\%$  and  $DL \geq 0.18$  were screened. The information of the 7 active compounds in *Laminaria* was shown in Table 1.

Table 1  
Main active compounds in Laminaria

NO.	MOL ID	Name	OB %	DL
1	MOL010615	Saringosterol	43.48	0.62
2	MOL010616	Eckol	87.06	0.63
3	MOL010617	Eicosapnte Macnioc Acid (1553-41-9)	45.66	0.21
4	MOL010625	24-Methylenecholesterol	43.54	0.76
5	MOL001439	Arachidonic acid	45.57	0.2
6	MOL000953	CLR	37.87	0.68
7	MOL009622	Fucosterol	43.78	0.76

## Identification of the targets of Laminaria on AD

A total of 72 protein targets were collected based on the above 7 active compounds. 34 AD-related targets were obtained when the 72 targets were mapped to the UniProt database for normalizing and standardizing naming. There are 8,718 AD-related targets in the GeneCards database. The targets of drug components were compared with that of AD and 23 potential targets related to the treatment of AD with *Laminaria* were selected (Table 2).

Table 2  
Information on potential anti-AD drug targets from *Laminaria*

NO.	Target	Symbol
1	Trypsin-1	PRSS1
2	Coagulation factor VII	F7
3	Estrogen receptor beta	ESR2
4	Apoptosis regulator Bcl-2	BCL2
5	Caspase-9	CASP9
6	Caspase-3	CASP3
7	Prostaglandin G/H synthase 1	PTGS1
8	Progesterone receptor	PGR
9	Mineralocorticoid receptor	NR3C2
10	Retinoic acid receptor RXR-gamma	RXRG
11	Transcription factor p65	RELA
12	G1/S-specific cyclin-D1	CCND1
13	Peroxisome proliferator-activated	PPARG
14	Tumor necrosis factor receptor superfamily member 1A	TNFRSF1A
15	Arachidonate 5-lipoxygenase	ALOX5
16	P-selectin	SELP
17	Beta-galactosidase	GLB1
18	ATP-binding cassette sub-family A member 1	ABCA1
19	Mitochondrial uncoupling protein 2	UCP2
20	Complement C1r subcomponent	C1R
21	Cholesteryl ester transfer protein	CETP
22	ATP-binding cassette sub-family G member 1	ABCG1
23	Multidrug resistance-associated protein 4	ABCC4

## Construction and analysis of the “drug-components-targets-disease” network

Information about *Laminaria's* active components and overlapping targets was imported into Cytoscape 3.7.0 to establish a “drug-components-targets-disease” visualization network. As shown in Figure 1, the purple node represented the drug *thallus Laminariae*, the yellow nodes represented the active components, the green nodes represented overlapping genes between *Laminaria* and AD, and the pink node represented the disease AD. The same target corresponded to different active components, and vice versa, which sufficiently suggested *Laminaria's* characteristics, multi-components and multi-targets.

## Construction and analysis of the PPI network

The PPI network was constructed when the target proteins were introduced into the STRING database and their names were standardized under the “Homo sapiens” setting. As shown in Table 2, one node represented one protein, and the edge between the two nodes indicated the interaction between proteins. It was speculated that CASP3, PPARG, RELA, CCND1, CASP9 were the key targets for the treatment of AD with *Laminarin*.

## Analysis of GO and pathway of targets

GO and KEGG analyses were performed on the targets of active components for the treatment of AD using the Metascape database. The threshold  $P \leq 0.05$  was set to select biological processes and pathways. The GO provides the logical structure of the biological functions, including three aspects: biological process, molecular function and cellular component, and how these functions are related to each other, manifested as a directed acyclic graph. Figure 3 showed the results of GO analysis for the predictive targets of the effect of *Laminarin* on AD, the response to steroid hormone accounted for the largest proportion in the biological process, platelet dense granule was the only one in cellular component, and steroid binding, steroid hormone receptor activity, nuclear receptor activity, transcription factor activity and direct ligand regulated sequence-specific DNA binding were at the top in molecular function.

KEGG was used to analyze the distribution of pathways for predicting the targets of *Laminarin* for AD. As shown in Figure 4, there were 57 enrichment pathways involved in 23 targets, including small cell lung cancer, toxoplasmosis, apoptosis, measles, hepatitis C, influenza A, tuberculosis, kaposi sarcoma-associated herpesvirus infection, Epstein-Barr virus infection, human immunodeficiency virus 1 infection and human cytomegalovirus infection. The key target proteins were enriched in the small cell lung cancer and toxoplasmosis signal pathways, further indicating the characteristics of *Laminarin*, multi-component and multi-pathway. The maps of small cell lung cancer and toxoplasmosis signal pathways depicted with KEGG Mapper were shown in Figure 5 and Figure 6.

## Discussion

Alzheimer's disease is the 5th leading cause of death among people aged 60 years or older and no viable method has been found to prevent and cure AD. *Laminaria* has been reported to have a therapeutic effect on AD, but the mechanism is not entirely clear. Thus, the study of the *Laminaria* mechanism on AD is of great significance. Network pharmacology is an emerging area of pharmacology that utilizes network analysis of drug action. By considering drug actions in the context of the cellular networks, network analysis promises to greatly increase our knowledge of the mechanisms underlying the multiple actions of drugs. In this study, the possible mechanism of the treatment of AD with *Laminaria* was analyzed by network pharmacology.

Through database searching and screening, 7 active components of *Laminaria* were obtained, and 23 overlapping targets of active components and AD were collected. Through the construction and screening of the "drug-components-targets-disease" network, Saringosterol, Eckol, Eicosapnte macnioc acid, 24-Methylenecholesterol, arachidonic acid, CLR, Fucosterol, et al. were predicted to be the active components in the treatment of AD by *Laminaria*.

CASP3, PPARG, RELA, CCND1 and CASP9 were identified as the key genes for the treatment of AD by the PPI network. The target network indicates the characteristics of *Laminarin*, multi-components and multi-pathways. The PPI network shows that there is an interactional and complex relationship among the *Laminarin* target proteins. The results of GO analysis showed that the mechanism of *Laminaria* on AD involved biological processes like cellular processes, metabolic processes and responses to stress, cellular components such as organelles, cell membranes and cytoplasm, molecules like small molecules, cations and metal ions, and signal molecules, transcription factors, receptors, proteins, enzymes and other substances. The analysis on the target pathway showed that the main targets of *Laminaria* for AD were small cell lung cancer and toxoplasmosis.

## Conclusions

The results suggest that *Laminaria* may act on multiple targets and thus play an anti-AD role. At present, there are few reports about these targets. This study may provide a new perspective for further study on the molecular mechanism of the potential targets of *Laminarin* in the treatment of AD. Relevant target verification experiments are in progress.

## Declarations

## Ethics approval and consent to participate

Not applicable

## Consent for publication

Not applicable

# Availability of data and materials

All data are available in the manuscript and they are showed in figures, tables.

## Competing interests

The authors declare that they have no competing interests.

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## Author Contributions

Data curation, writing-original draft, L. Yu; Methodology, S. C. Pei; Software, K. Y. Yuan; Formal analysis, J. Zhang; Validation, J. Y. Zhao; Supervision, review and editing, project administration S. Q. Chai. All authors have read and agreed to the published version of the manuscript.

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Not applicable

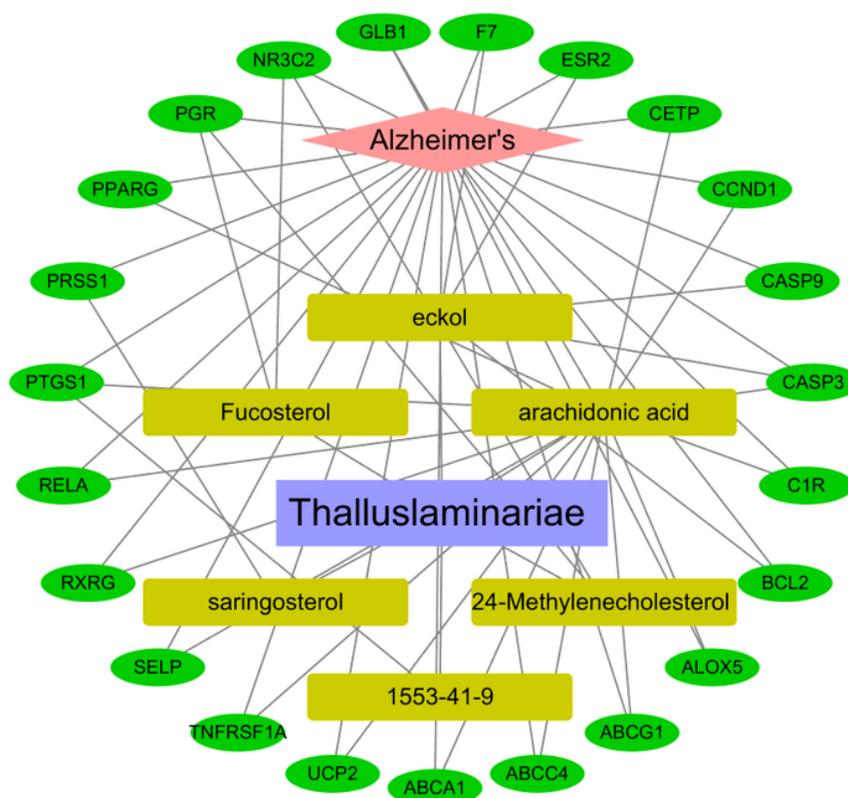
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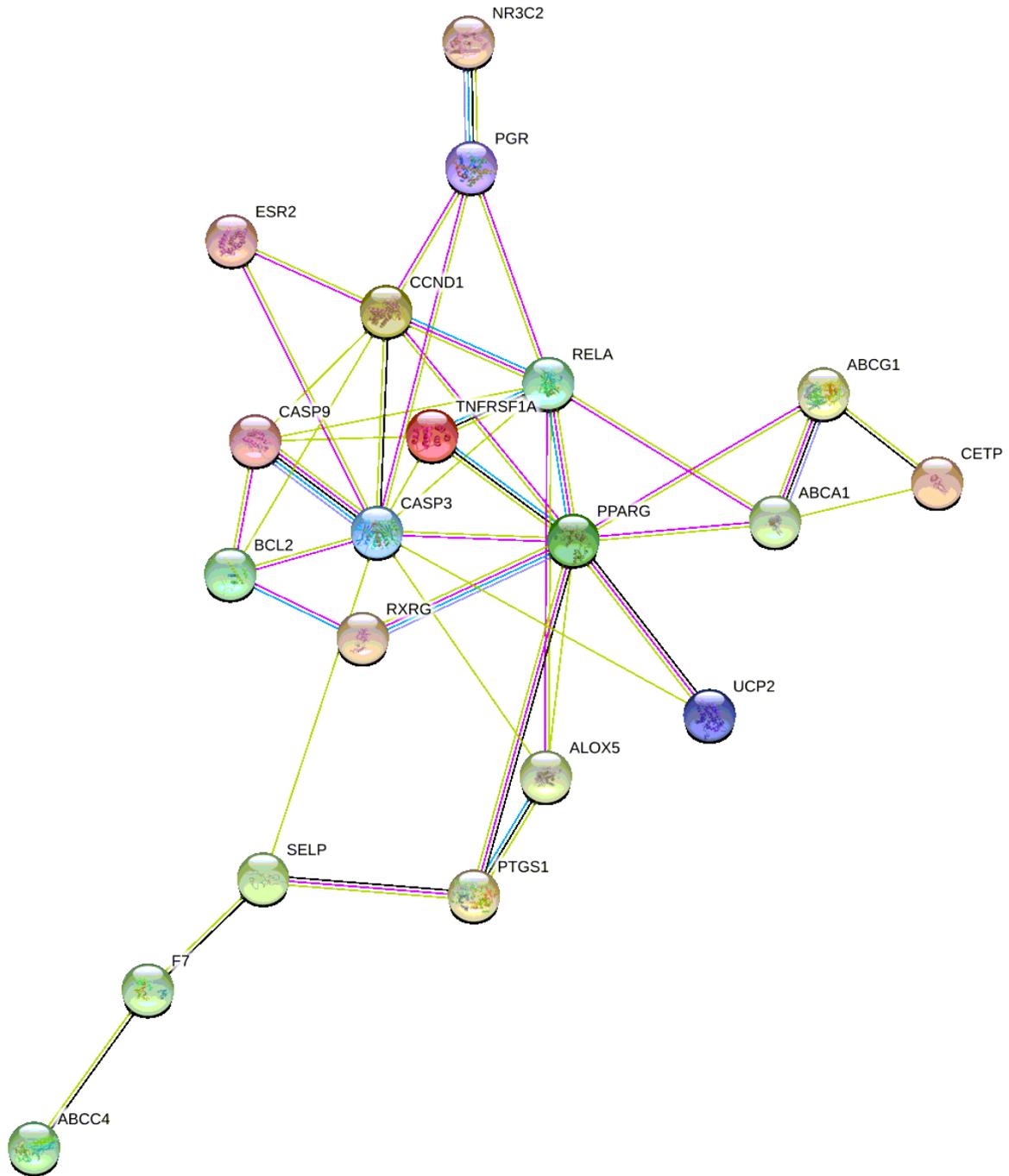
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## Figures



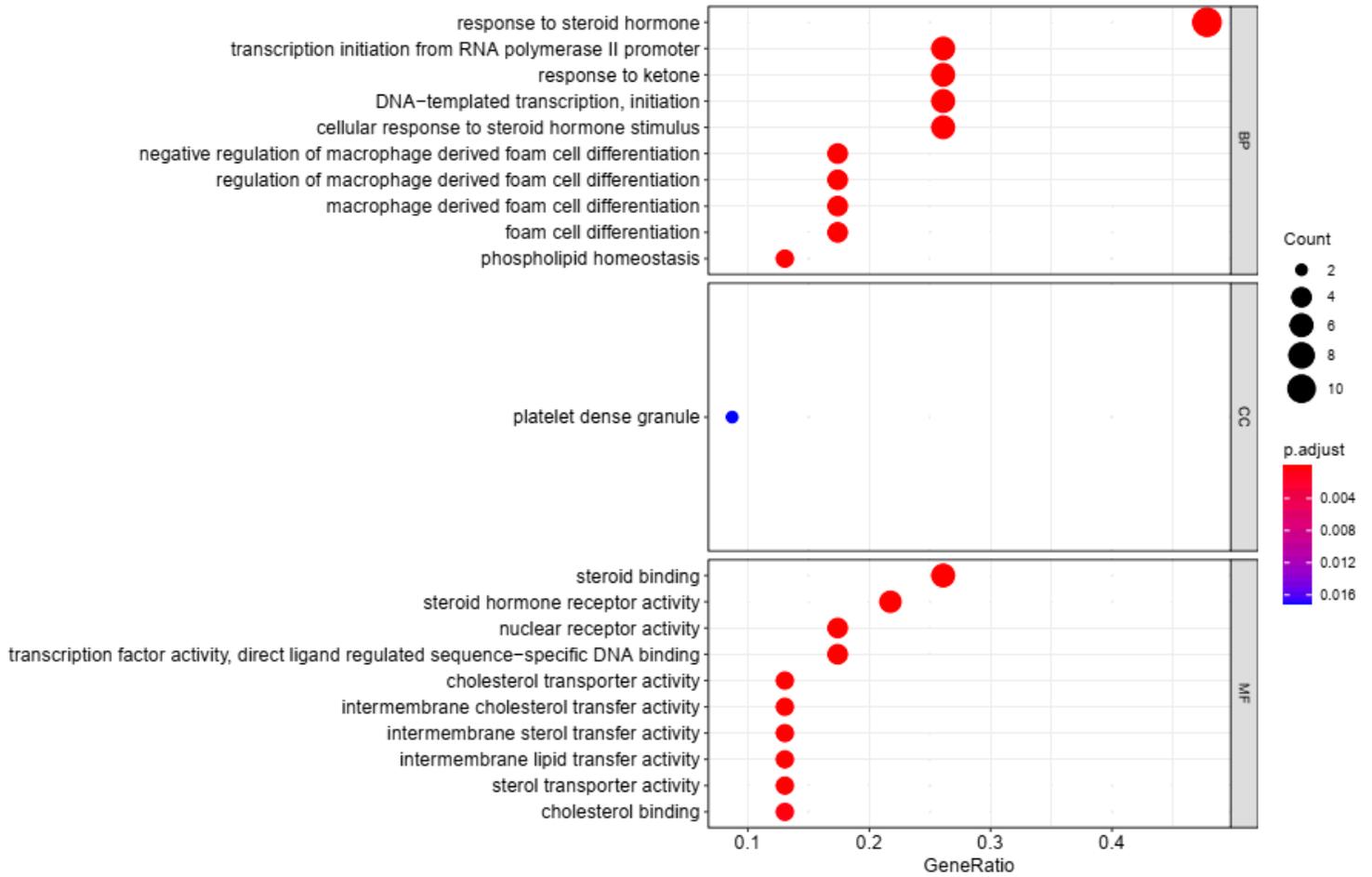
**Figure 1**

The “drug-components-targets-disease” visualization network.



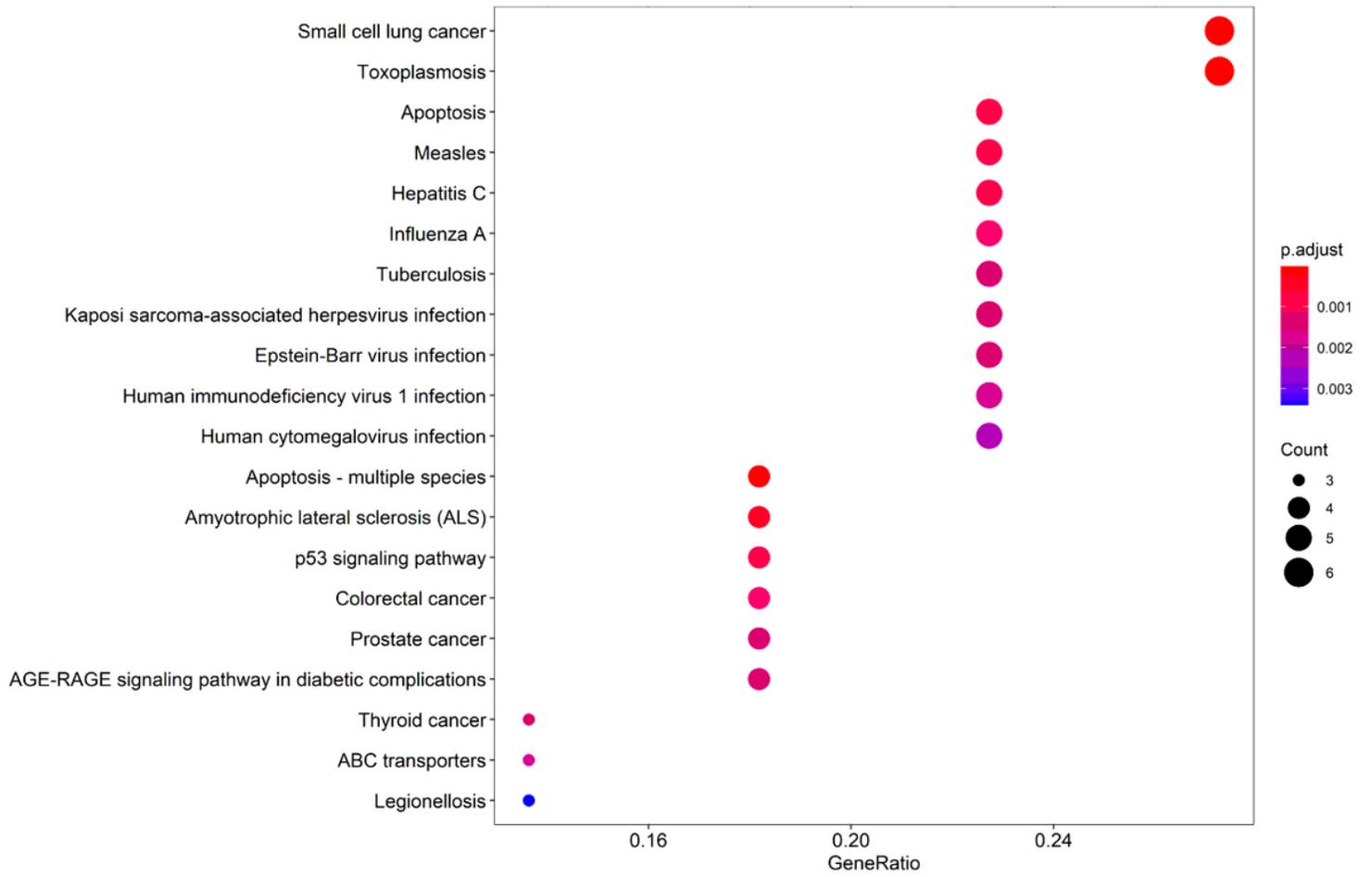
**Figure 2**

The PPI network of Laminarin.



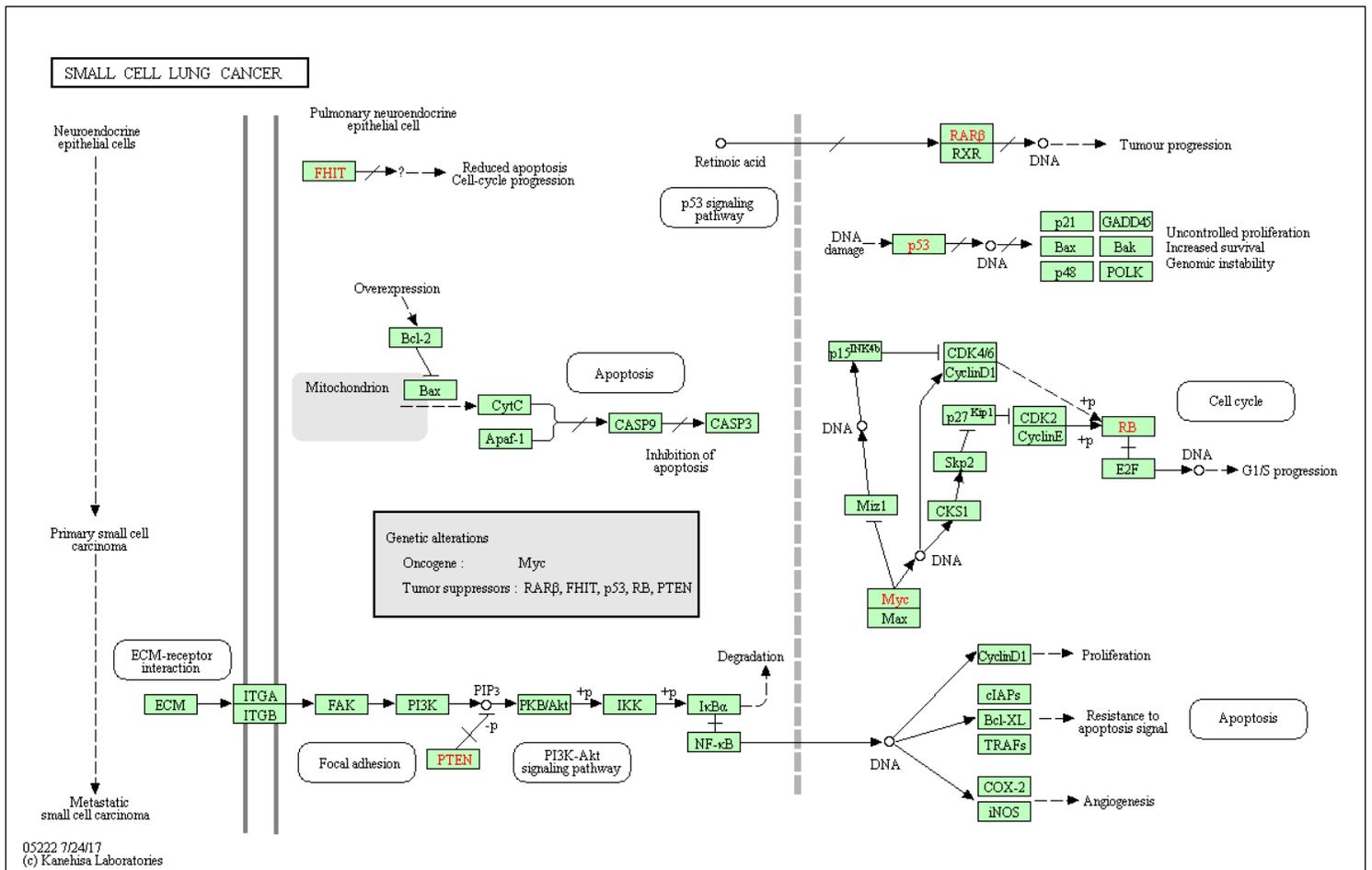
**Figure 3**

Enriched gene ontology terms for biological processes, cellular components and molecular functions of potential targets from the main active components of Laminarin.



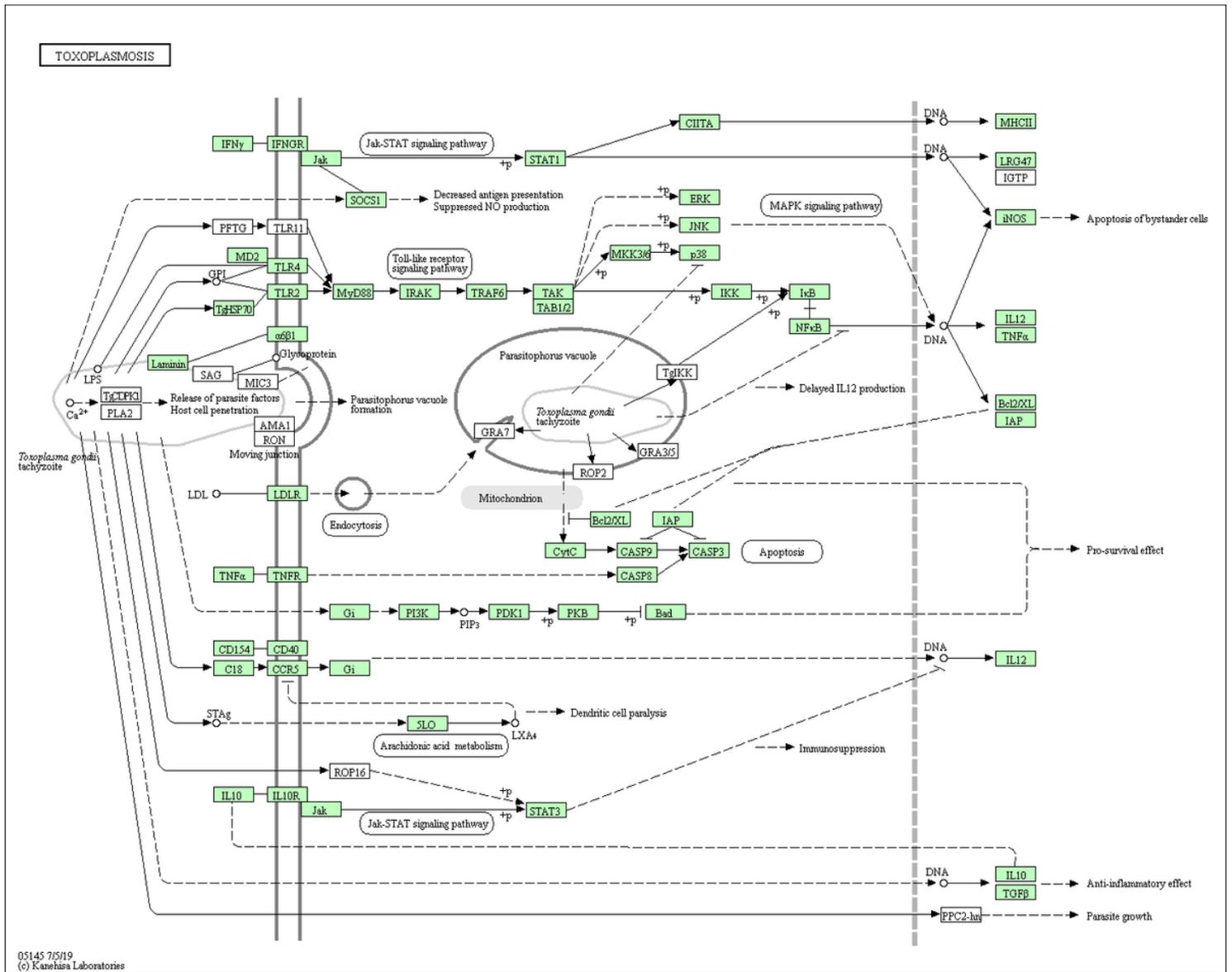
**Figure 4**

Enriched KEGG pathways of potential targets from the main active components of Laminarin.



**Figure 5**

Signal pathway of small cell lung cancer.



**Figure 6**

Signal pathway of toxoplasmosis.