Study on the mechanism of anti-cancer action of *rosa roxburghii tratt* based on network pharmacology

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Abstract. The active chemical components and targets of *rosa roxburghii tratt* were screened by the method of network pharmacology, and the molecular mechanism of its action was discussed. By consulting relevant literature reports, we established drug-disease targets by using databases such as TCMSP, TCMIP, Genecards and OMIM. Using STRING database to construct the protein interaction network between *rosa roxburghii tratt* and cancer target; and Cytoscape software was used to construct the interactive network diagram of "active ingredient-target-disease". The function enrichment analysis of Gene Ontology (GO) and pathway enrichment analysis based on Kyoto Encyclopedia of genes and genomes (KEGG) were carried out by using biological information annotation database (David). We obtained 11 active components and 80 drug-disease targets were obtained, DAVID database was used to carry out GO function enrichment analysis and KEGG pathway enrichment analysis on 80 potential targets, and 303 biological processes and 114 signaling pathways were screened out to participate in the anti-cancer effect of *rosa roxburghii tratt*. The Pathway in cancer has the strongest correlation with cancer, which is related to the regulation of IL6, AR, CASP3 and other key genes. These key genes are related to myricetin, quercetin, kaempferol and other compounds in *rosa roxburghii tratt*. The anti-cancer compounds of *rosa roxburghii tratt* might were myricetin, quercetin, kaempferol and other compounds. The anti-cancer effect of *rosa roxburghii tratt* may regulation of key genes, indirectly affecting the sustained angiogenesis, developing apoptosis and promotion, so as to promote or inhibit the production of cancer.

1. Introduction

*Rosa roxburghii tratt* is a kind of perennial deciduous bush of Rosaceae, which is mainly distributed in southwest mountainous areas such as Yunnan, Guizhou, Sichuan, and also planting in Hubei, Guangdong and other places, now. It has the functions of relieving food, diarrhea and heatstroke. *Rosa roxburghii tratt* has rich nutritional value and broad prospects in the development of food, health products and medicines. Fresh *rosa roxburghii tratt* fruit is rich in vitamins, polyphenols, flavonoids, organic acids, polysaccharides, amino acids and trace elements [1]. Among them, the content of VC is the highest, ranking the first among many fruits, and a certain amount of VK1, VB2, VP and carotene [2]. Other chemical components are mainly flavonoids, and their aglycones are myricetin, quercetin and
kaempferol their triterpenoids are pentacyclic triterpenoid ester glycoside, Euscaphic acid, rosa roxburghii tratt acid, rosa roxburghii tratt glycoside, etc[3]. These chemical components, such as myricetin, quercetin and kaempferol, have been reported to have anti-cancer activity.

Modern medical research showed that rosa roxburghii tratt has a variety of biological effects such as regulating immune function, detoxification, sedation, delaying senility, anti-atherosclerosis, and anti-cancer. In recent years, rosa roxburghii tratt has been shown to inhibit a variety of cancers, such as bladder [4], prostate [5] and liver cancer [6]. At present, CL1 from rosa roxburghii tratt can inhibit the growth of gastric cancer SGC-7901 cells in vitro, and does not significantly inhibit the proliferation and differentiation of hematopoietic stem/progenitor cells into granulocytes [7]. Although, many studies have shown that rosa roxburghii tratt plays an important role in anti-cancer, most of the studies are based on animal and in vitro cell experiments. However, the type and mechanism of cancer treatment are still unclear, which still needs further research and exploration.

Fortunately, the advent of network pharmacology provides a reference for the study of the mechanism of rosa roxburghii tratt in the treatment of cancer [8]. Due to the multi-component, multi-target and multi-level characteristics of traditional Chinese Medicine, Network pharmacology has the characteristics of wholeness, systematicness and focusing on drug interactions, which are consistent with the basic characteristics of traditional Chinese medicine [4]. Therefore, network pharmacology can be used to predict drug targets on the whole, to comprehensively and systematically study the law and mechanism of drug interaction with the body, and to provide theoretical basis for in depth experimental research on rosa roxburghii tratt and guidance of rational clinical drug use.

2. Materials and methods

2.1. Acquisition of active ingredients of rosa roxburghii tratt

By referring to related literatures, the latest reported compounds of rosa roxburghii tratt were searched, and the active chemical components of rosa roxburghii tratt were screened through the screening of oral bioavailability (OB) ≥ 30% and drug-like property (DL) ≥0.18, or the compounds with the activity reported in literatures.

2.2. Prediction of rosa roxburghii tratt target and cancer target

Using the function of TCMSP, TCMIP, BATMAN and other databases to predict the target of the effective components of rosa roxburghii tratt. In the human gene database Genecards and OMIM of Online Mendelian genetic platform, “cancer” is the key word to retrieve the related target genes of cancer, and map and compare with the target genes of active ingredients to screen the common target, which is the target of rosa roxburghii tratt active ingredients for cancer treatment.

2.3. Key target PPI network construction

By searching the STRING database online, importing 80 common targets, defining species as "humans", and PPI network for cancer treatment of rosa roxburghii tratt was constructed. The results are exported in TSV format, and then the topological parameters of each target point in PPI network are obtained through the Network Analyzer plug-in of the software Cytoscape 3.6.1, such as Degree, Betweenness centrality and Closeness centrality, and the median of the three topological parameters was calculated. Selected the targets whose values of the above three topology parameters are greater than all median values, list them in the form of tables, and visualize the targets.

2.4. Go function enrichment analysis and KEGG pathway enrichment analysis

The selected targets were analyzed by using David (https://david.ncifcrf.gov/) database for KEGG pathway analysis and Go (gene ontology) biological process analysis.
2.5. Labeling of KEGG signaling pathway
Using the function of KEGG mapper in KEGG (https://www.genome.jp/kegg/) signal pathway database to mark the target points on the signal pathway closest to cancer, to verify that *rosa roxburghii tratt* plays an anti-cancer role through multiple targets and multiple channels.

3. Results

3.1. Screening of active ingredients
By referencing to relevant literature, ADME parameters (OB≥ 30% and DL≥0.18) were used as criteria for the selection of compounds. Or refer to the existing literature and report that the compound has activity for screening, and a total of 11 active compounds are screened, the results are shown in Table 1.

**Table 1. Active compounds and target numbers of *rosa roxburghii tratt* [9-10]**

| Mol Id    | Chemical compound  | OB%  | DL   | Target number |
|-----------|--------------------|------|------|---------------|
| MOL002008 | myricetin          | 13.75| 0.31 | 16            |
| MOL000098 | quercetin          | 46.43| 0.28 | 76            |
| MOL00422  | kaempferol         | 41.88| 0.24 | 35            |
| MOL001468 | MLT                | 59.62| 0.02 | 8             |
| MOL00131  | EIC                | 41.9 | 0.14 | 6             |
| MOL005500 | linolenate         | 45.01| 0.15 | 2             |
| MOL001308 | oleic acid         | 33.13| 0.14 | 8             |
| MOL001002 | ellagic acid       | 43.06| 0.43 | 10            |
| MOL001641 | METHYL LINOLEATE   | 41.93| 0.17 | 2             |
| MOL002850 | butylated hydroxytoluene | 40.02 | 0.07 | 4             |
| MOL000635 | vanillin           | 52   | 0.03 | 1             |

3.2. Prediction results of potential targets of *rosa roxburghii tratt* in the treatment of cancer
In Genecards and OMIM databases, a total of 4862 cancer-related genes were retrieved with "Cancer" as the keyword, and Cancer genes were matched with *rosa roxburghii tratt* related targets and Venn diagram was drawn, as shown in figure 1.

**Figure 1.** Match of cancer target gene and *rosa roxburghii tratt* target gene
3.3. PPI network analysis

80 intersection targets were imported into the network diagram of the interaction relationship obtained in the STING database, the results were exported in TSV format, and the TSV file was imported into Cytoscape 3.6.1 software. The plug-in Network Analyzer is used to obtain the topological parameters of each target point, such as Degree, Betweenness centrality and Closeness centrality. The median of the three topological parameters is 15, 0.003825445 and 0.541523305, respectively. Degree and Betweenness centrality, Closeness centrality were greater than the median of targets, as shown in Table 2. According to the Degree value of the topological indicator, it is presented in terms of rank 1, 2-11, 12-31, and 32-80, as shown in Figure 2. It is suggested that these targets play an important role in PPI network, indicating that these targets play an important role in the treatment of cancer in *rosa roxburghii tratt*.

![Gene association diagram (PPI network diagram)](image)

Table 2. Analysis of gene topological parameters

| name  | Degree | Betweenness Centrality | Closeness Centrality |
|-------|--------|-------------------------|----------------------|
| IL6   | 52     | 0.1275                  | 0.7500               |
| MAPK8 | 48     | 0.0794                  | 0.7282               |
| CASP3 | 47     | 0.0554                  | 0.7143               |
| VEGFA | 47     | 0.0605                  | 0.7143               |
| EGFR  | 44     | 0.0358                  | 0.7009               |
| ESR1  | 42     | 0.0413                  | 0.6881               |
| MYC   | 42     | 0.0417                  | 0.6881               |
| CCND1 | 38     | 0.0193                  | 0.6579               |
| FOS   | 36     | 0.0556                  | 0.6522               |
| ERBB2 | 34     | 0.0173                  | 0.6410               |
| AR    | 33     | 0.0210                  | 0.6356               |
| PPARG | 32     | 0.0455                  | 0.6250               |
| RELA  | 29     | 0.0100                  | 0.6098               |
| CASP8 | 27     | 0.0123                  | 0.5859               |
| PGR   | 27     | 0.0108                  | 0.6000               |
3.4. Enrichment analysis of Go biological function
80 potential targets were mapped to the DAVID database for GO functional enrichment analysis, and 303 biological processes were obtained. The first 20 biological processes with P<0.01 and FDR<0.05 were screened out, as shown in Table 3. It is shown as a bubble chart, and the result is shown in Figure 3. The results showed that the anti-cancer effect of *rosa roxbughi tratt* was related to the regulation of multiple biological processes, biological processes including positive regulation of transcription from RNA molecular ase II promoter, response to estradiol, negative regulation of apoptotic process, positive regulation of transcription. DNA-templated are involved. Among them, positive regulation of transcription from RNA polymerase II promoter is mainly a process of activating or increasing the frequency, rate or degree of transcription of RNA polymerase II promoter. Response to estradiol is any process by which the stimulation of estradiol (a C18 steroid hormone hydroxylated at C3 and C17 as an effective estrogen) results in a change in the state or activity of a cell or organism. These biological processes reflect the anti-cancer mechanism of *rosa roxbughi tratt*, which involves the abnormality of multiple biological processes in vivo, and also indicate that the active components of *rosa roxbughi tratt* may exert anti-cancer effect by regulating these biological processes.

**Figure 3. GO biological function enrichment analysis bubble chart**
Table 3. GO biological function enrichment analysis results

| ID      | Term                                                                 | Count | Count% | P Value          | FDR            |
|---------|----------------------------------------------------------------------|-------|--------|------------------|----------------|
| GO:00459| positive regulation of transcription from RNA polymerase II promoter | 24    | 30     | 6.99296E-11      | 0.07           |
| GO:00323| response to estradiol                                               | 10    | 12.5   | 4.05895E-10      | 0.07           |
| GO:00430| negative regulation of apoptotic process                            | 16    | 20     | 2.72842E-10      | 0.06           |
| GO:00458| positive regulation of transcription, DNA-templated                | 16    | 20     | 1.44413E-08      | 0.05           |
| GO:00454| response to ethanol                                               | 9     | 11.25  | 3.22447E-08      | 0.05           |
| GO:00075| aging                                                              | 10    | 12.5   | 7.84009E-08      | 0.01           |
| GO:00016| response to hypoxia                                               | 10    | 12.5   | 1.12009E-09      | 0.29           |
| GO:00424| response to drug                                                  | 12    | 15     | 1.72979E-09      | 0.07           |
| GO:00106| positive regulation of gene expression                            | 11    | 13.75  | 4.02959E-07      | 0.06           |
| GO:00713| cellular response to tumor necrosis factor                         | 8     | 10     | 8.39566E-07      | 0.19           |
| GO:00420| wound healing                                                      | 7     | 8.75   | 2.03722E-06      | 0.64           |
| GO:00096| response to toxic substance                                        | 7     | 8.75   | 2.91027E-06      | 0.64           |
| GO:00714| cellular response to hypoxia                                       | 7     | 8.75   | 5.92115E-06      | 0.02           |
| GO:00703| cellular response to hydrogen peroxide                             | 6     | 7.5    | 7.01059E-06      | 0.17           |
| GO:00714| cellular response to organic cyclic compound                       | 6     | 7.5    | 8.32105E-06      | 0.97           |
| GO:00324| response to lipopolysaccharide                                     | 8     | 10     | 1.19777E-05      | 0.42           |
| GO:00069| apoptotic process                                                  | 13    | 16.25  | 1.27132E-05      | 0.87           |
| GO:00103| response to gamma radiation                                       | 5     | 6.25   | 1.2962E-05       | 0.29           |
| GO:00432| response to amino acid                                             | 5     | 6.25   | 1.2962E-05       | 0.29           |
| GO:00466| response to antibiotic                                             | 5     | 6.25   | 1.47609E-05      | 0.19           |

3.5. KEGG pathway analysis

80 potential targets were mapped to the DAVID database and KEGG pathway enrichment analysis was performed. A total of 115 signal pathways were obtained. The first 20 signal pathways with P<0.01 and FDR< 0.05 were screened out and displayed in a bubble chart, as shown in Figure 4. These pathways are closely related to the mechanism of anti-cancer of *rosa roxburghii tratt*, including Pathways in cancer,
Hepatitis B, Proteoglycans in cancer, Pancreatic cancer and other signaling pathways, as shown in Table 4. The corresponding diseases include liver cancer, pancreatic cancer, prostate cancer and bladder cancer. Using the KEGG mapper function in the KEGG signaling pathway database, 80 related target proteins were labeled on the most closely related signaling pathway with cancer. The results showed that 27 target proteins were involved in the regulation of pathways in cancer signaling pathway, as shown in Figure 5. 

**Table 4.** Enrichment analysis results of KEGG pathway

| hsa ID   | Term                              | Count | Count% | PValue  | FDR      |
|----------|-----------------------------------|-------|--------|---------|----------|
| hsa05200 | Pathways in cancer                | 27    | 33.75  | 1.62E-14| 2.01E-11 |
| hsa05161 | Hepatitis B                       | 17    | 21.25  | 1.95E-12| 2.42E-09 |
| hsa05205 | Proteoglycans in cancer           | 15    | 18.75  | 2.30E-08| 2.86E-05 |
| hsa05212 | Pancreatic cancer                 | 10    | 12.5   | 2.61E-08| 3.23E-05 |
| hsa05215 | Prostate cancer                   | 11    | 13.75  | 2.93E-08| 3.63E-05 |
| hsa05223 | Non-small cell lung cancer        | 9     | 11.25  | 1.27E-07| 1.58E-04 |
| hsa04668 | TNF signaling pathway             | 11    | 13.75  | 1.93E-07| 2.40E-04 |
| hsa05219 | Bladder cancer                    | 8     | 10     | 2.30E-07| 2.85E-04 |
| hsa05210 | Colorectal cancer                 | 9     | 11.25  | 2.88E-07| 3.57E-04 |
| hsa04066 | HIF-1 signaling pathway           | 10    | 12.5   | 8.08E-07| 0.001001554 |
| hsa05206 | MicroRNAs in cancer               | 15    | 18.75  | 1.91E-06| 0.00236837 |
| hsa05222 | Small cell lung cancer            | 9     | 11.25  | 3.53E-06| 0.00415496 |
| hsa04917 | Prolactin signaling pathway       | 8     | 10     | 1.05E-05| 0.013010193 |
| hsa05213 | Endometrial cancer                | 7     | 8.75   | 1.88E-05| 0.023262588 |
| hsa05145 | Toxoplasmosis                     | 9     | 11.25  | 2.29E-05| 0.02844756 |
| hsa05134 | Legionellosis                     | 7     | 8.75   | 2.34E-05| 0.02906305 |
| hsa04919 | Thyroid hormone signaling pathway | 9     | 11.25  | 3.17E-05| 0.039312309 |
| hsa04012 | ErbB signaling pathway            | 8     | 10     | 4.01E-05| 0.049741114 |
| hsa04210 | Apoptosis                         | 7     | 8.75   | 5.20E-05| 0.064433604 |
| hsa04510 | Focal adhesion                    | 11    | 13.75  | 7.07E-05| 0.087548194 |

![Figure 4. KEGG pathway enrichment analysis of bubble chart](image)
Figure 5. Labeling of potential targets on Pathways in cancer

By using the software of Cytoscape 3.6.1, the network visualization analysis of 80 common genes, diseases and chemical components were carried out, and the interaction network of anti-cancer of *rosa roxburghii tratt* was constructed. After selecting the corresponding interactive proteins and visualizing them with different colors and shapes, we can directly see the network relationship between the active chemical components and the target. 80 purple targets represent common targets, and the common targets in the purple inner circle are the top 20 with strong correlation. The red represent diseases, there are 11 triangles representing active ingredients of compounds, and the inverted triangles represent drugs. The larger the connectivity, the larger the shape, as shown in Figure 6.

Figure 6. "component-target-disease" interaction network of *rosa roxburghii tratt* anti-cancer effect
4. Discussion

*Rosa roxburghii tratt* has a long history of medicine and food in China, as well as rich resources. At present, *rosa roxburghii tratt* has been developed into Chinese medicine preparation, such as compound Cili agent, in clinical treatment of gastric ulcer, insomnia and other diseases. It has been reported that *rosa roxburghii tratt* can inhibit or kill liver cancer cells, bladder cancer cells and pancreatic cancer cells in cell experiment or pharmacological experiment, but the mechanism of anti-cancer of *rosa roxburghii tratt* is still unclear. Through network pharmacology prediction, the main chemical components of *rosa roxburghii tratt* were myricetin, quercetin, kaempferol, etc., which all had anti-cancer effects. For example, myricetin can inhibit cancer by up-regulating the apoptosis pathway, and can also up-regulate the expression of proapoptotic gene BAX, so as to prompt mitochondria to produce cytochrome C and activate the cytochrome c-mediated apoptosis pathway [11]. Quercetin can significantly inhibit TGF-β1 induced EMT in HCC cells by increasing E-cadherin protein expression and decreasing the expression of N-cadherin and Vimentin proteins [12]. Kaempferol can inhibit SKOV-3 cell proliferation in vitro [13]. These anti-cancer Pathways are mainly related to Pathways in cancer, Hepatitis B, Proteoglycans in cancer and Pancreatic cancer, and their regulation is related to IL6, MAPK8, CASP3, VEGFA and EGFR.

In this study, IL6 gene in the key target screening, Degree, Closeness centrality and Betweenness centrality are all large, ranking the first in comprehensive ranking, which is one of the key targets of *rosa roxburghii tratt*. IL6 protein is produced primarily at the site of acute and chronic inflammation, where it is secreted into the serum and induces transcriptional inflammatory responses through the interleukin-6 receptor A. It plays an important role in the final differentiation of B cells into immunoglobulin secreting cells, and is also involved in the differentiation of lymphocytes and monocytes, acting on T cells, liver cells, hematopoietic progenitor cells and cells of the central nervous system. According to KEGG map, Pathways in cancer are composed of VEGF signaling pathway, PPAR signaling pathway and P53 signaling pathway, etc. By activating or inhibiting IL6, MAPK8, CASP3 and VEGFA gene expression, and indirectly Sustained angiogenesis, Evading apoptosis and effort, promote or inhibit the occurrence of cancer. This article provides relevant evidence for the research of anti-cancer mechanism of *rosa roxburghii tratt*, but the specific mechanism of anti-cancer of *rosa roxburghii tratt* needs further research and verification.

Acknowledgements

This work was financially supported by the Guizhou Domestic First-Class Construction Project [(Chinese Materia Medica) (GNYL [2017] 008)]. The authors thank the government of China for their financial support.

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