Network pharmacology reveals hub bio-active ingredients and possible mechanisms of a Chinese herbal prescription Ru-kang-yin against breast cancer

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Abstract

Background

Ru-kang-yin (RKY), a traditional Chinese herbal prescription, has been clinically used as adjuvant therapy for breast cancer via inhibiting cell invasion, proliferation, and promoting apoptosis. However, its anti-breast cancer bio-active ingredients and possible mechanisms are still unclear. In the present study, the hub bio-active compounds and underlying mechanisms of RKY in treatment of breast cancer were systematically elucidated by the approach of network pharmacology.

Methods

By using network pharmacology approach, a total of 53 nonredundant bio-active components met the drug screening criteria and 155 putative targets of RKY in treatment of breast cancer were identified. Besides, 381 potential breast cancer-related targets were obtained. Among of the targets, 56 shared targets of RKY and breast cancer were acquired.

Results

Based on topological network analysis of PPI network of shared targets, 19 hub therapeutic targets of RKY against breast cancer were obtained. GO and KEGG pathway enrichment analysis of core therapeutic targets showed that the core targets remarkably involved in multiple biological functions and KEGG pathways which mainly participated in inflammatory response, cell proliferation, and apoptosis.

Conclusions

These findings uncover the hub bio-active compounds and underlying mechanisms of RKY against breast cancer and provide crucial information regarding using RKY as a promising candidate for treating breast cancer.

1. Background

In women, breast cancer is among the most commonly occurring cancers and the second largest cause of death worldwide after cardiovascular disease. The statistics from 2019 show approximately 268,600 new cases of invasive breast cancer in US, accounting for about 30% of all new cases among women; the estimation of 41,760 women are expected to die from breast cancer, accounting for approximately 6.8% of all cancers in females.[1] In China, the statistics from 2015 show about 268,600 newly diagnosed invasive breast cancer cases, accounting for about 15% of all new cancers among women, and estimation of 69,500 women died from breast cancer.[2] The estimate of breast cancer mortality rate is 13.4 per million patients in 2020 in Europe.[3] Although important advances in cancer treatment efficacy
and obvious improvements in survival rates, breast cancer remains the most prevalent cancer causes of death in women worldwide, as the limitations of timing, availability, costly medical care, and serious adverse side effects.[4–7] Therefore, it is of utmost importance to exploit a new set of strategies with high efficiency and low toxicity to prevent and treat breast cancer.

In clinical practice, traditional Chinese medicine (TCM) has been widely used in China for the treatment of breast cancer due to its synergistic therapeutic effects and reduced side-effects.[8–14] For instance, a traditional Chinese herbal prescription, Ru-kang-yin (RKY), consisting of 6 herbs: Citri Reticulatae Pericarpium (CRP, Chenpi in Chinese), Coicis Semen (CSE, Yiyiren in Chinese), Radix Bupleuri (RB, Chaihu in Chinese), Astragali Radix (AR, Huangqi in Chinese), Curcumae Rhizoma (CR, Ezhu in Chinese), and Poria cocos (Schw.) Wolf (PC, Fuling in Chinese), has been frequently used in medical clinics for anti-breast cancer therapy,[15] and has exhibited significant effects on inhibiting proliferation and promoting apoptosis of breast cancer cells.[16, 17] However, information regarding core bio-active ingredients and molecular mechanism of RKY against breast cancer remains to be fully explained.

Network pharmacology is one of the newly developed strategies for drug discovery based on the approaches of systems biology, which introduces the theoretical system of "disease-gene-target-drug" to investigate the mechanisms of drug intervention on the disease and drug-body interaction from multi-dimensional perspective.[18–20] For example, the potential mechanism of natural products of radix astragali in treatment of triple-negative breast cancer (TNBC) has been studied using network pharmacology analysis, and the results indicated that key component of astragalus polysaccharides can effectively reduce TNBC cell invasion, proliferation, and promote apoptosis via the PIK3CG/AKT/BCL2 pathway.[21] Also, TCM can improve cancer chemosensitivity. For example, Ai Du Qing formula can improve the chemosensitivity of breast cancer to paclitaxel through inhibiting caveolin-1, which further increase protein expression levels of cell cycle-associated proteins p21/cyclinB1 and apoptosis-related proteins of PARP1, BAX and Bcl-2.[22]

In current work, the hub bio-active compounds and underlying mechanisms of RKY in treatment of breast cancer were systematically elucidated by the approach of network pharmacology. The bio-active compounds in RKY and its linked targets were identified from the public databases. The key compounds of RKY and its connected core targets were also identified by topological network analysis. The molecular function of key bio-active compounds associated breast cancer targets were obtained by GO and KEGG pathway enrichment analysis. The present study uncovered the hub bio-active compounds and underlying mechanism of RKY against breast cancer and provided important information regarding RKY as a promising candidate for treating breast cancer.

2. Methods

2.1. Identification of bio-active compounds and related targets in RKY
The bio-active compounds of RKY were obtained from the Traditional Chinese Medicine System Pharmacology (TCMSP, http://www.tcmspw.com/tcmsp.php) database.[23] Drug screening criteria including oral bio-availability (OB) ≥ 30%, drug-like property (DL) ≥ 0.18, Caco-2 cell permeability (Caco-2) ≥ 0, and drug-like principles were applied to select the bio-active compounds.[24–27] The potential targets of the bio-active compounds of RKY were predicted from TCMSP database, and the official gene names were manually standardized using UniProtKB database (https://www.uniprot.org/).[28]

### 2.2. Identification of breast cancer related targets

The known breast cancer related targets were obtained by searching key word “breast cancer” against Comparative Toxicogenomics Database (CTD, http://ctdbase.org/),[29] Therapeutic Target Database (TTD, http://db.idrblab.net/ttd/),[30] GeneCards database (https://www.genecards.org/),[31] and PharmGKB database (https://www.pharmgkb.org/).[32] All targets identified above were manually standardized as official gene names using UniprotKB database.

### 2.3. Protein-protein interaction and compounds-targets network construction

Potential therapeutic targets of RKY against breast cancer were acquired via intersection of the bio-active compounds-related targets and breast cancer-related targets. These shared potential therapeutic targets were entered into String database (https://string-db.org/)[33] to construct protein-protein interaction network. The PPI network with confidence score > 0.7 was selected and performed to topological network analysis using the NetworkAnalyzer tool of Cytoscape v 3.7.2 software.[34] The final RKY-related key targets were acquired by meeting the value of Degree Centrality (DC), Betweenness Centrality (BC), and Closeness Centrality (CC) with its corresponding threshold value over the median value of each.[35–37] The network of core pharmaceutical bio-active compounds of RKY and key breast cancer-related targets was drawn using Cytoscape v 3.7.2 software.

### 2.4. GO function and KEGG pathway enrichment analyses

Gene ontology (GO) enrichment analysis and KEGG pathway enrichment analysis of the core targets were performed using the Database for Annotation, Visualization and Integrated Discovery (DAVID, https://david.ncifcrf.gov/, v6.8).[38] The false discovery rate value < 0.05 was considered as significantly enriched terms.

### 3. Results

#### 3.1. Bio-active ingredients of RKY

By the TCMSP database screening, total 667 candidate compounds in RKY were retrieved (Table S1). Based on the drug screening criteria of OB ≥ 30%, DL ≥ 0.18, Caco-2 ≥ 0 and drug-like principles, 53
nonredundant bio-active components were ultimately identified including 14 in RB, 5 in CRP, 4 in CR, 13 in PC, 14 in AR, and 9 in CSE (Fig. 1, Table S2).

3.2. Compounds and disease-related targets screening

Total 155 nonredundant compound-related targets were identified by screening out from the TCMSP and UniProtKB databases (Table S3). A total of 381 potential targets related to breast cancer were obtained by searching against the CTD, TTD, GeneCards and PharmGKB databases. Fifty-six potential targets were finally acquired by calculating the intersection of the related targets of bio-active compounds and breast cancer (Fig. 2).

3.3. The hub targets of RKY acting on breast cancer

To identify the hub targets of anti-cancer effect of RKY on breast cancer, a PPI network of 56 targets were constructed by the STRING database. The PPI network composed of 52 targets and 284 link edges followed confidence score ≥ 0.7 (Fig. 3A). According to the criteria of DC value ≥ median DC, BC value ≥ median BC, and CC value ≥ median CC of topological network analysis, the core network composed of 19 targets and 126 link edges were obtained (Fig. 3B). The 19 hub targets were listed in Table 1.
Table 1
Information of the 19 hub targets.

| UniProt ID | Gene Symbol | Protein Name                                      | BC     | CC     | DC  |
|------------|-------------|--------------------------------------------------|--------|--------|-----|
| P05412     | JUN         | Transcription factor AP-1                        | 0.0917 | 0.7805 | 23  |
| P04637     | TP53        | Cellular tumor antigen p53                       | 0.1256 | 0.7805 | 23  |
| P28482     | MAPK1       | Mitogen-activated protein kinase 1 (MAP kinase 1)| 0.0322 | 0.7111 | 21  |
| P31749     | AKT1        | RAC-alpha serine/threonine-protein kinase        | 0.0442 | 0.7111 | 21  |
| P45983     | MAPK8       | Mitogen-activated protein kinase 8 (MAP kinase 8)| 0.0683 | 0.7442 | 21  |
| P01375     | TNF         | Tumor necrosis factor (Cachectin) (TNF-alpha)    | 0.0238 | 0.6531 | 19  |
| P27361     | MAPK3       | Mitogen-activated protein kinase 3 (MAP kinase 3)| 0.0193 | 0.6531 | 17  |
| P35354     | PTGS2       | Prostaglandin G/H synthase 2                     | 0.0165 | 0.64   | 17  |
| P42574     | CASP3       | Caspase-3                                        | 0.0151 | 0.6275 | 16  |
| Q16539     | MAPK14      | Mitogen-activated protein kinase 14 (MAP kinase 14)| 0.0187 | 0.64   | 16  |
| P14780     | MMP9        | Matrix metalloproteinase-9                      | 0.0053 | 0.6154 | 15  |
| P09601     | HMOX1       | Heme oxygenase 1 (HO-1)                         | 0.0111 | 0.6038 | 15  |
| P29474     | NOS3        | Nitric oxide synthase, endothelial              | 0.0154 | 0.6275 | 15  |
| Q04206     | RELA        | Transcription factor p65 (Nuclear factor NF-kappa-B p65)| 0.0108 | 0.6038 | 14  |
| P03372     | ESR1        | Estrogen receptor (ER) (ER-alpha)                | 0.0291 | 0.6275 | 14  |
| P10275     | AR          | Androgen receptor                               | 0.0305 | 0.6038 | 13  |
| P37231     | PPARG       | Peroxisome proliferator-activated receptor gamma| 0.0117 | 0.5714 | 12  |
| P05362     | ICAM1       | Intercellular adhesion molecule 1                | 0.0037 | 0.5714 | 12  |
| Q92731     | ESR2        | Estrogen receptor beta                           | 0.0519 | 0.6038 | 11  |

MW: molecular weight; Hdon: hydrogen-bond donors; Hacc: hydrogen-bond acceptors; OB: oral bioavailability; Caco-2: Caco-2 cell permeability; DL: drug-likeness; DC: degree centrality; BC: betweenness centrality; CC: closeness centrality.

3.4. The bio-active compounds and hub targets network
To investigate the relationship between the bio-active compounds and the hub targets, the interaction network of bio-active compounds and the hub targets was established (Fig. 4). The result showed that 22 bio-active compounds of RKY were critical to the hub targets. Among of the compounds, the top 5 active compounds were kaempferol (MOL000422) which was related to 13 hub targets, nobiletin (MOL005828), related to 10 key targets, formononetin (MOL000392) linked to 9 hub targets, naringenin (MOL004328) with connection to 8 core targets, and isorhamnetin (MOL000378) which was linked to 8 key targets.

3.5. Function enrichment analysis of hub targets

To understand the molecular function of the hub targets, GO analysis and KEGG pathway enrichment were conducted. GO function enrichment analysis (Fig. 5A) showed that the hub targets were primarily distributed in molecular function such as protein binding, enzyme binding, transcription factor binding, transcription regulatory region DNA binding, and MAP kinase activity. Nucleus, nucleoplasm, and cytosol were involved in cellular component. The key targets were also mainly involved in positive regulation of transcription, lipopolysaccharide-mediated signaling pathway, response to drug, and positive regulation of apoptotic process.

KEGG pathway enrichment analysis was performed to further investigate the underlying mechanism of RKY on the treatment of breast cancer. The results showed that the hub targets were highly related to prolactin signaling pathway, TNF signaling pathway, NOD-like receptor signaling pathway, VEGF signaling pathway, estrogen signaling pathway, apoptosis, toll-like receptor signaling pathway, and tumor-related pathways such as colorectal cancer, pancreatic cancer, and prostate cancer (Figure. 5B). These results indicated that RKY have an excellent anti-tumor activity against breast cancer and further verified the reliability of hub targets of RKY.

4. Discussion

At present, RKY, a traditional Chinese herbal prescription, has been clinically used for adjuvant anti-breast cancer therapy. The previous studies demonstrated that RKY has significant effects in inhibiting proliferation and promoting apoptosis breast cancer cells, [16, 17] but its underlying anti-breast cancer bio-active ingredients and mechanism remains abstruse. The present work was aimed to uncover the potential bio-active compounds and underlying mechanism of RKY against breast cancer using network pharmacology.

In this study, total 53 nonredundant bio-active ingredients were obtained according to the drug screening criteria (Fig. 1, Table S3). Based on the bio-active compounds and hub targets network analysis, 22 key bio-active compounds were identified (Fig. 4). Among of them, kaempferol is one of the bioflavonoids which was reported to effectively prevent breast cancer cell growth.[39–41] More studies showed that kaempferol could enhance the expression of pro-apoptotic related proteins, such as caspase-9, caspase-3, caspase-7, PARP, Bax, p21, p53, and pATM.[42, 43] and decrease the expression of anti-apoptotic proteins of pAKT, Bcl2, cyclins A, B, D1, and E, CDK1, PLK-1, IRS-1, MEK1/2, and cathepsin D.[39, 42–47] As a natural methoxylated flavonoid, nobiletin is the second key compound and well-studied to potent the
anti-angiogenic activity in ER⁺ breast cancer cells via regulating Src/FAK/STAT3-mediated signaling pathway through PXN.[48] Another recent report demonstrated that nobiletin could prevent the migration and proliferation and promote apoptosis through regulating the expression of Bax/Bcl-2, caspase-3, and p53 in breast cancer cells.[49] Recently, Surichan et al. found that the bioactivation of nobiletin by the protein family of cytochrome P450 CYP1 could induce G1 arrest in MDA-MB-468 breast cancer cells.[50] Formononetin is one of the bioactive phytoestrogens which was found to suppress the migration and invasion of breast cancer cells of MDA-MB-231 and 4T1.[51] Formononetin also reported to effectively suppress the tumor growth and had the potential function of anti-proliferative and anti-angiogenic activities.[52–55] Naringenin is another flavonoid compound linked to 8 key targets which was found to not only inhibit the pulmonary metastasis of breast cancer cells via suppressing PKC activation, but also suppress the migration of breast cancer cells by inflammatory and apoptosis signaling pathways.[56, 57] Therefore, these bio-active compounds exhibited the effectiveness and synergistic effects of the chemical ingredients in RKY for treating breast cancer.

The results of topological network analysis indicated that 19 hub targets of RKY on breast cancer were obtained. The GO function enrichment analysis showed that these core targets were mainly involved in the function of biological process, such as lipopolysaccharide-mediated signaling pathway, response to drug, positive regulation of apoptotic process, and positive regulation of transcription via protein binding such as enzyme, transcription factor, and transcription regulatory region DNA binding. KEGG pathway enrichment analysis indicated that these core targets were mainly involved in the many signaling pathways, such as prolactin signaling pathway, TNF signaling pathway, NOD-like receptor signaling pathway, VEGF signaling pathway, estrogen signaling pathway, apoptosis, and toll-like receptor signaling pathway. Among these pathways, prolactin signaling pathway mainly participated in regulating the development of mammary gland and the terminal differentiation of mammary epithelial cells.[58] Activation of prolactin signaling pathway in TNBC could suppress tumorigenesis via inducting epithelial phenotypes.[59] TNF was an inflammatory cytokine involved in all processes of tumor development, including cell proliferation, tumorigenesis, tumor progression, angiogenesis, and tumour metastasis.[60, 61] In breast cancer, more studies showed that TNF signaling could promote breast cancer cell migration via increasing the expression levels of membrane-associated proteases in lipid rafts.[62] Recent study indicated that the expression of transmembrane TNF-alpha (tmTNF-α) correlated with breast cancer severity and doxorubicin resistance, and tmTNF-α acted as potential target for treatment of doxorubicin-resistant breast cancer.[63] NOD-like receptor signaling pathway was highly correlated with breast cancer and could promote breast cancer cells proliferation, migration and invasion.[64] VEGF signaling pathway was involved in all process of angiogenesis. Recent study revealed that VEGF signaling could promote homologous recombination in BRCA1 and contribute to cisplatin resistance of TNBC cells.[65] Estrogen signaling elements possessed important roles in development of drug resistance and metabolic adaptation in progression of breast cancer.[66] Toll-like receptor signaling was highly associated with breast cancer chemoresistance via promoting the expression of pro-inflammatory cytokines.[67] Therefore, these results indicated that the bio-active compounds of RKY could change the expression of multiple pathways which were closely associated with breast cancer.
5. Conclusions

In present study, total 53 nonredundant bio-active ingredients were obtained in RKY according to the drug screening criteria. Based on the network pharmacology analysis, 22 key bio-active compounds linked to 19 hub targets were obtained. GO and KEGG pathway enrichment analysis of core therapeutic targets showed that the core targets remarkably involved in multiple biological functions and pathways which mainly responsible to inflammatory response, cell proliferation and apoptosis. Taken together, these findings uncover the hub bio-active compounds and underlying mechanism of RKY against breast cancer and provide more information regarding using RKY as a promising candidate for treating breast cancer.

Abbreviations

RKY: Ru-kang-yin; GO: Gene Ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes; TCM: traditional Chinese medicine; TCMSP: Traditional Chinese Medicine System Pharmacology; CTD: Comparative Toxicogenomics Database; TTD: Therapeutic Target Database.

Declarations

Competing Interest

The authors declare that they have no competing interests

Availability of data and materials

All data and material are public

Ethics approval and consent to participate

No ethics approval and consent are required

Consent for publication

No consent is required

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Authors’ contributions

Shirun Chu, Fang Chen and Delin Xu made substantial contributions to the study design. Shirun Chu, Fang Chen, and Mei Yang were involved in drafting the manuscript. Shirun Chu, and Delin Xu were involved in critically revising the manuscript. All authors read and approved the final manuscript.

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Figures

Figure 1

Herbs and compounds network. The round nodes represent the herbs of RKY, and the square nodes represent the bio-active compounds.
Figure 2

Veen diagram of related targets of bio-active compounds and breast cancer.
Figure 3

Topological network analysis. Blue nodes indicate the primary input targets, and red nodes indicate the hub targets after topological network analysis. (A) primary input targets of PPI network; (B) hub targets of PPI network.
Figure 4

The bio-active compounds and hub targets network. The red square nodes represent hub targets and the blue round nodes represent bio-active compounds. The node size reflects the target degree in network.

Figure 5

GO and pathway enrichment analysis of hub targets of RKY. (A) GO enrichment analysis of hub targets of RKY. (B) KEGG enrichment analysis of hub targets of RKY.

Supplementary Files

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