Exploring the Mechanism of Acetylenic Phenols from Selaginellae Herba in Treating Triple-Negative Breast Cancer Based on Network Pharmacology and Molecular Docking

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Abstract [**Objectives**] To investigate the anti-tumor molecular mechanism of acetylenic phenols against triple-negative breast cancer (TNBC) using network pharmacology and molecular docking approaches. [**Methods**] Based on team's previous *in vitro* activity screening, the most active acetylenic phenols were selected for further analysis. Genes associated with triple-negative breast cancer (TNBC) were retrieved from the GAD and OMIM databases. Using Cytoscape software, a compound-target-pathway interaction network was constructed to visualize the relationships between the acetylenic phenols, their potential targets, and related pathways. Functional enrichment analysis of GO terms and KEGG pathways was performed using the DAVID database to identify key signaling mechanisms. Furthermore, molecular docking was conducted to evaluate the binding interactions between the acetylenic phenols and the potential core targets. [**Results**] Acetylenic phenols exhibit potential anticancer effects by modulating multiple signaling pathways, including the PI3K-Akt pathway, cell cycle pathway, and breast cancer pathway, which are closely associated with the pathophysiological processes of triple-negative breast cancer (TNBC) such as cell proliferation, apoptosis, and cell cycle regulation. Molecular docking results indicated that acetylenic phenols bind effectively to their targets via hydrogen bonding, hydrophobic interactions, and π-stacking, indicating strong binding affinity. [**Conclusions**] Acetylenic phenols exert anti-TNBC effects by modulating key targets, including EGFR, RAF1, ESR1, CHEK1, and CDC25C, and influencing associated signaling pathways. These findings reveal the molecular mechanism underlying their anti-TNBC activity and provide a theoretical foundation for the potential application of acetylenic phenols in TNBC treatment.

Key words Network pharmacology, Molecular docking, Triple-negative breast cancer (TNBC), Acetylenic phenols, Selaginellae Herba

1 Introduction

Triple-negative breast cancer (TNBC) is a subtype of breast cancer characterized by the negative expression of estrogen receptor, progesterone receptor, and human epidermal growth factor receptor-2, accounting for approximately 15% - 20% of all breast cancers. Due to the lack of corresponding targets, there are no effective targeted drugs available clinically. Paclitaxel and platinum compounds remain the commonly used chemotherapeutic agents for the clinical treatment of TNBC[1-2]. However, factors such as chemoresistance, metastasis, and severe toxic side effects lead to unsatisfactory therapeutic outcomes, making TNBC the most lethal subtype of all breast cancers[3].

The extraction of active ingredients from traditional Chinese medicine (TCM) for cancer treatment has become a prominent research focus in recent years, yielding promising results. Selaginellae Herba, which is listed in the Chinese Pharmacopoeia, was documented as early as in *Shen Nong's Herbal Classic* to "mainly treat evils of the five viscera, female cold-heat pain in the yin, and blood stasis". Multiple varieties of Selaginellae Herba are widely used in folk medicine, often for treating conditions such as cancer, hyperglycemia, and thrombocytopenic purpura[4-5].

Modern pharmacological studies have demonstrated that Selaginellae Herba possesses anti-tumor properties, with acetylenic phenols identified as its primary active constituents[6-7]. Our previous research also found that acetylenic phenols significantly inhibited the proliferation of TNBC cell lines MDA-MB-468 and MDA-MB-231; however, the underlying anti-TNBC mechanism remains unclear[8]. Therefore, this study employed network pharmacology and molecular docking to explore the potential molecular mechanisms of acetylenic phenols against TNBC, aiming to provide a theoretical basis for their clinical application in TNBC treatment.

2 Materials and methods

2.1 Screening of acetylenic phenols against TNBC and its potential targets With reference to the relevant literature and activity test data, we determined the potential ingredients in acetylenic phenols for the treatment of anti-TNBC, and used the PubChem (http://pubchem.ncbi.nlm.nih.gov) database to obtain the targets of the active ingredients in acetylenic phenols[9], supplemented with Swiss Target Prediction (http://www.swisstarget-

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prediction. ch/)[10], and Homo sapiens was selected as species for prediction. Target proteins and gene information were corrected by Uniprot (https://www. uniprot. org) database, and the targets with "Homo sapiens" species were screened out, which were acetylenic phenols anti-TNBC targets.

For the screening of pharmacological targets of TNBC, with "triple negative breast cancer, TNBC" as keywords, we collected TNBC related genes from literature, GAD database and OMIM database[11], and mapped the excavated TNBC related genes with drug target genes. The common targets were screened and the potential targets of acetylenic phenols against TNBC were determined.

2.2 Component-target network analysis and protein-protein interaction (PPI) analysis The acetylenic phenols and potential targets were imported into Cytoscape 3.7.2 software to establish a "component-target" network. The potential action targets were imported into the STRING database (https://string-db. org/cgi/input. pl)[12] to construct a PPI network of major targets. The resulting PPI network was analyzed using the Analyze Network tool. Potential core targets were screened based on degree values, and the network was further analyzed.

2.3 GO and KEGG enrichment analysis DAVID 6.7 was used for GO and KEGG enrichment analysis[13], and $P<0.05$ was used as the standard for gene enrichment analysis, and then the enrichment results were visualized.

2.4 Molecular docking simulation The 3D structures of the active compounds were downloaded from the PubChem database and energy-minimized using Chem3D software. The three-dimensional structures of the target proteins were obtained from the PDB database (http://www. rcsb. org), from which the optimal protein crystal structures with complete sequences and high resolution were selected. These structures then underwent preprocessing steps such as adding hydrogen atoms and removing water molecules. Molecular docking simulations were performed using PyRx 0.8 software[14]. The Predicted Binding Affinity scoring system was employed to evaluate the binding strength between the compounds and the targets. Finally, the results were visualized using Pymol 2.1.1 software.

3 Results and analysis

3.1 Screening of acetylenic phenols A total of 17 active compounds from the total acetylenic phenols were screened through literature retrieval and previous activity experiments conducted by our research group. These compounds are Selariscinin H, Selariscinin I, Selariscinin J, (R)-Selaginellin U, (R)-Selaginellin V, Selaginellin U, Selaginellin, Selaginellin A, Selaginellin T, Selaginellin E, Selaginpulvilin A, Selaginellin M, Anemarchalconyn, Selariscinin G, Selariscinin F, Diselaginellins A, and Diselaginellins B, and were identified as potential components for anti-TNBC drugs[8].

3.2 Collection of action targets of acetylenic phenols Potential targets of acetylenic phenols were identified using the PubMed database and Swiss Target Prediction. A total of 285 gene targets were obtained after removing duplicates. Meanwhile, 538 genes associated with triple-negative breast cancer (TNBC) were collected from literature mining, the GAD database, and the OMIM database, also after deduplication. By cross-referencing the drug target genes with TNBC-related genes, 173 common targets were identified as potential mediators of acetylenic phenols against TNBC.

3.3 Component-target network analysis 17 compounds and 173 targets were imported into Cytoscape software to construct the compound-target interaction network. The results show that there are 377 nodes (17 compound nodes, 173 targets, and 356 edges).

3.4 PPI network construction and analysis A PPI analysis was conducted using the STRING database, with the minimum interaction score set at 0.7 and unconnected genes removed. The resulting PPI network contained 170 nodes and 3 398 edges. Nodes with a higher number of edges were considered potentially central to the anti-TNBC activity. This network was further analyzed using the Network Analyzer plugin in Cytoscape to calculate topological parameters. Nodes representing drug-active components with higher degree values were selected for analysis. Targets with a degree value more than twice the median (degree = 16) were identified as key core genes involved in the treatment of TNBC. Finally, 37 core targets were predicted and screened.

3.5 Enrichment analysis and visualization of core targets The biological functions of the core targets were analyzed and categorized into three aspects: biological process (BP), molecular function (MF), and cellular component (CC). A total of 501 GO items with $P<0.05$ were identified, including 397 significant BP terms primarily involved in cellular metabolic processes, response to stimulus, developmental processes, regulation of apoptosis, and G_2/M phase transition of the mitotic cell cycle, among others. In the MF category, 72 out of 84 entries met the significance threshold ($P<0.05$), mainly encompassing small molecule binding, ion binding, metal ion binding, catalytic activity acting on proteins, and enzyme binding. For CC, 32 out of 44 entries were significant, including intracellular anatomical structures, cytoplasm, membrane-bounded organelles, plasma membrane, and organelle lumen. KEGG pathway enrichment analysis was performed using the DAVID database to investigate the roles of the core targets in the prevention and treatment of triple-negative breast cancer (TNBC). The analysis revealed that the 37 core targets participated in 151 signaling pathways, 31 of which were significant ($P<0.05$). These targets are mainly associated with pathways related to TNBC, such as disease-related pathways, metabolic pathways, nitrogen metabolism, estrogen signaling pathway, cell cycle, microRNAs in cancer, and breast cancer pathways.

3.6 Pathway analysis KEGG pathway analysis indicated that most enriched pathways were associated with TNBC. Using the KEGG Mapper Color tool to identify highly reliable pathways, it was suggested that acetylenic phenols may exert anti-TNBC effects through key pharmacological targets including EGFR, RAF1, and ESR1, located upstream in the breast cancer signaling pathway.

Additionally, targets such as CHEK1 and CDC25C, positioned in the midstream of the cell cycle signaling pathway which lies downstream of the breast cancer pathway appear to regulate TNBC cell growth and proliferation.

3.7 Molecular docking simulation Based on the pathway analysis results outlined in Section **3.6**, molecular docking was performed to evaluate the binding affinity between the identified potential pharmacological targets, EGFR, RAF1, ESR1, CHEK1, and CDC25C, and all acetylenic phenol compounds. According to the PyRx docking results, 85 compound-target pairs exhibited a binding free energy of less than −6.0 kJ/mol, suggesting strong binding activity between the compounds and their respective targets. The co-crystallized ligand from each target's original crystal structure was used as the positive control.

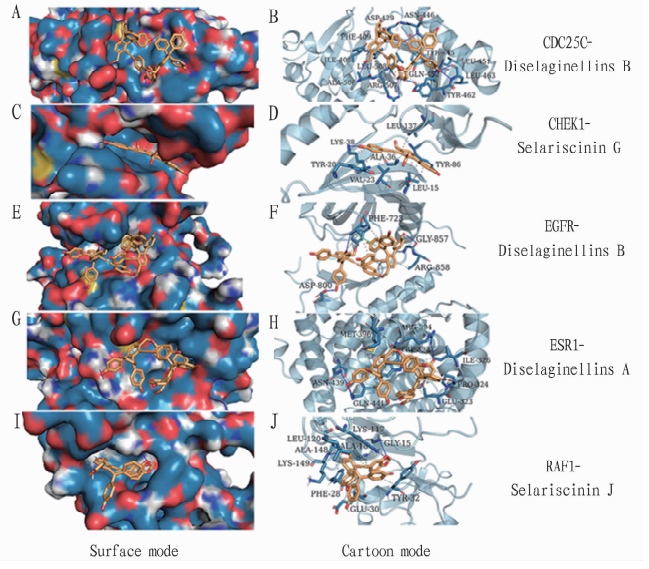
In the analysis of the binding between CHEK1 and Selariscinin G, several key hydrophobic interactions and hydrogen bond interactions were identified. Firstly, the amino acid residues LEU-15A, TYR-20A, VAL-23A, ALA-36A, LYS-38A, TYR-86A, and LEU-137A formed significant hydrophobic interactions with the ligand, with distances ranging from 3.41 Å to 3.82 Å. In particular, LEU-15A participated in hydrophobic interactions with the ligand three times, indicating its importance in ligand binding. Additionally, LYS-38A further strengthened the binding between the ligand and the protein by forming a hydrogen bond of 2.32 Å. These interactions ensure stable binding of the ligand to the target protein, potentially enhancing its bioactivity.

The EGFR binding assay with Diselaginellin B revealed that the ligand forms multiple important interactions with the protein. Key hydrophobic interactions were observed with residues ALA-722A, PHE-723A, and ARG-858A, at distances ranging from 3.38 Å to 3.71 Å. Notably, PHE-723A participated repeatedly in hydrophobic contacts, suggesting its critical role in ligand stabilization. Hydrogen bonds were also formed with ALA-722A, ASP-800A, and GLY-857A, at distances of 2.43, 2.85, and 2.98 Å, respectively, contributing to the precise positioning of the ligand. In addition, π-stacking interactions involving PHE-723A were identified at distances of 4.07 Å and 5.24 Å, further enhancing binding stability.

In the CDC25C binding assay with Diselaginellin B, multiple key interactions were identified. Hydrophobic interactions were observed with ten amino acid residues, including ILE-408A, TYR-445A, and TYR-462A, at distances ranging from 3.02 to 3.89 Å, indicating their important role in stabilizing ligand binding. Furthermore, hydrogen bonds formed by ASP-429A, LEU-451A, and ARG-507A, with distances between 2.02 and 3.53 Å, contributed additional binding affinity. Finally, two π-stacking interactions involving TYR-445A (at 4.86 and 5.17 Å) further enhanced the overall binding stability.

The binding assay of RAF1 with Selariscinin J revealed multiple important interactions. Hydrophobic interactions were formed with amino acid residues including ALA-18A, PHE-28A, GLU-30A, TYR-32A, LYS-117A, and LEU-120A, at distances ranging from 3.17 to 3.96 Å. These interactions contributed to stabilizing the ligand at the protein's binding site. Notably, PHE-28A and LEU-120A participated in multiple contacts, underscoring their importance for binding stability. Additionally, hydrogen bonds were observed between the ligand and residues GLY-15A, ALA-148A, and LYS-149A, with distances of 2.52, 2.80, and 2.70 Å, respectively, further strengthening the ligand's binding to RAF1.

The binding assay of ESR1 with Diselaginellin A identified multiple key interactions. Hydrophobic interactions were observed between the ligand and residues GLU-323A, PRO-324A, ILE-326A, TRP-393A, ARG-394A, MET-396A, and GLU-397A, with distances ranging from 3.01 to 3.98 Å. The participation of TRP-393A and ARG-394A was particularly notable for enhancing binding stability. Additionally, hydrogen bonds were formed by ARG-394A, ASN-439A, and GLN-441A at distances of 2.25 to 2.46 Å, providing further stabilization. A π-stacking interaction involving TRP-393A (distance: 4.73 Å) was also identified, which further consolidated the ligand's binding (Fig. 1).



NOTE Wheat: ligand; blue: bound protein residues; gray dashed line: hydrophobic linkage; dark blue solid line: hydrogen bonding linkage; green dashed line: π-stacking interaction (parallel).

Fig. 1 Analysis of interaction mode between acetylenic phenols and pharmacological target

In conclusion, the binding of acetylenic phenols to TNBC targets is stabilized by a combination of hydrophobic interactions, hydrogen bonds, and π-stacking interactions, which are critical for stable ligand binding and their resulting biological activity. Furthermore, the diacetylenic phenolic derivatives demonstrated superior binding properties compared to their monoacetylenic counterparts.

4 Conclusions

This study systematically elaborated the potential molecular mechanisms by which acetylenic phenols may treat TNBC using an integrated approach of network pharmacology and molecular docking.

Our findings indicate that acetylenic phenols exert anti-TNBC effects through multi-target and multi-pathway synergism, with a potential toxicity advantage over conventional chemotherapeutic agents. Notably, the diacetylenic phenolic structure demonstrated strong binding affinity in molecular docking, suggesting its high potential for drug development. These results provide a theoretical foundation for further research on acetylenic phenols in TNBC treatment and propose a new direction for developing novel anti-cancer agents.

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