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Exploration on the Effects and Molecular Mechanisms of Pterostilbene on Esophageal Cancer Based on Network Pharmacology

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Abstract: ***This study used a network pharmacology approach to analyze the potential role of Pterostilbene (PTS) in esophageal squamous cell carcinoma (ESCC). Compared with traditional ESCC treatments, which often have single targets, limited efficacy, and significant side effects, PTS, as a natural flavonoid compound, offers multiple targets, pathways, strong bioavailability, and blood-brain barrier penetration. These properties may compensate for the shortcomings of conventional drugs. The study constructed the PTS target network and PPI network, systematically analyzing its core targets and related pathways, providing a theoretical foundation for the development of PTS as a new drug for the prevention and treatment of ESCC.***

Keywords: Esophageal squamous cell carcinoma, Pterostilbene, Network pharmacology.

1. Introduction

According to GLOBOCAN data, esophageal cancer ranked as the 11th most common cancer and the 7th leading cause of cancer-related death globally in 2022 [1]. Esophageal squamous cell carcinoma (ESCC) is the predominant type in Asia and Africa [2]. In China, the incidence of esophageal cancer accounts for 43.8% of the global total, and the number of deaths represents 42.1% of the global total, indicating a substantial disease burden [3].

PTS is an important non-flavonoid polyphenolic compound primarily found in blueberries and the wood of the bagged Pterocarpus [4]. PTS has demonstrated advantages in various fields, including neuroprotection, antioxidation, anti-inflammatory, and anticancer properties [5]. making it a promising topic for further research in health prevention. However, there is a lack of relevant studies on its role in esophageal cancer treatment. This study, based on network pharmacology, aims to explore the potential targets and mechanisms of PTS in the treatment of esophageal cancer, providing research insights for its development as a novel drug for the prevention and treatment of esophageal cancer.

2. Materials and Methods

2.1 Collection of Targets for PTS

The SMILES code of PTS was obtained from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/). This SMILES code was then imported into the SuperPred database (http://bioinformatics.charite.de/superpred), BATMAN-TCM database (http://bionet.ncpsb.org.cn/batman-tcm/index.php), and CTD database (https://ctdbase.org/) to identify the potential biological targets of its active components.

2.2 Collection of Disease-Related Targets for ESCC

The keyword “esophageal squamous cell carcinoma” was used to search and filter relevant disease-associated targets from the following databases: GeneCards (https://www.genecards.org/), the Online Mendelian Inheritance in Man (OMIM) database (https://www.omim.org/), and DisGeNET database (https://disgenet.com/). The intersection of the targets of PTS and ESCC was determined using Venny 2.1 software (https://bioinfogp.cnb.csic.es/tools/venny/index.html), by importing the PTS component targets and ESCC-related pathogenic targets to obtain the common targets and generate a Venn diagram.

2.3 Construction of the "PTS-Intersection Target" Network

The intersection targets and PTS components were imported into Cytoscape 3.10.0 to construct the "PTS-Intersection Target" network and analyze the relationship between PTS and the common targets.

2.4 Construction of the Protein-Protein Interaction (PPI) Network

Using the STRING (functional protein association networks) database (https://stringdb.org/), the common targets of PTS and ESCC were imported, with the species set to “Homo sapiens,” to obtain the protein-protein interaction (PPI) network for the common targets. The resulting TSV file was then imported into Cytoscape 3.10.0 to construct the PPI network of the common targets and visualize key genes within the intersected genes.

2.5 GO and KEGG Enrichment Analysis

The common targets were imported into the Metascape platform (http://metascape.org) for Gene Ontology (GO) functional enrichment analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis, with the species set to “Homo sapiens” and a significance threshold of $P<0.01$. The enrichment results were visualized through the Microbial Genomics platform (http://www.bioinformatics.com.cn), focusing on the major

biological processes and metabolic pathways. In the visualization, nodes with different colors represent different types of enrichment results, and the size of the nodes correlates with their significance level.

3. Results Analysis

3.1 Effects of PTS

The targets were predicted, organized, and deduplicated using the BATMAN-TCM, SuperPred, and CTD databases, resulting in a total of 328 targets.

3.2 Identification of ESCC-related Targets

A total of 1795 ESCC-related targets were obtained after screening and retrieval from the OMIM, GeneCards, and DisGeNET databases. A comparative analysis between the targets of PTS components and ESCC-related targets revealed 163 targets for the treatment of ESCC by PTS. The Venn diagram of these targets is shown in Figure 1.

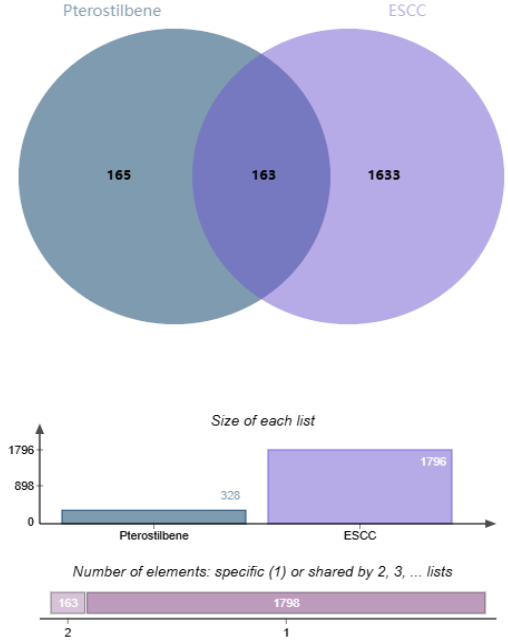

Figure 1: Venn Diagram of PTS Treatment ESCC Intersection Targets

3.3 "PTS-Intersection Targets" Network Analysis

The "PTS-Intersection Targets" network diagram was constructed using Cytoscape 3.10.0 software (Figure 2). The network consists of 162 nodes. In the diagram, the darker the color, the larger the degree value. Circles represent active components of the drug, diamonds represent the intersection targets between the drug and the disease, and the lines indicate the interactions between the active components and the targets.

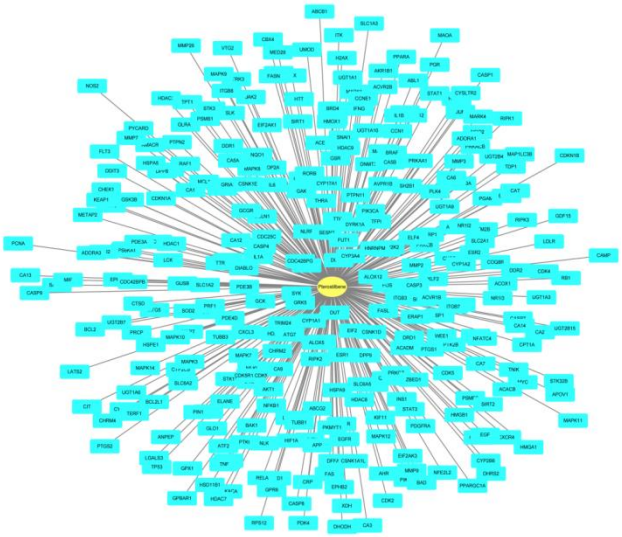

Figure 2: PTS Target Network Diagram

3.4 PPI Network

After importing the PPI network "TSV" file obtained from the String database into Cytoscape 3.10.0 software, key targets were selected using the Centiscape 2.2 plugin. The key target PPI network diagram was then generated. In the diagram, the larger the circle area and the darker the color, the higher the degree value. The PPI network of common targets for PTS treatment of ESCC consists of 162 nodes, 4157 edges, an average node degree of 51.3, an average local clustering coefficient of 0.696, and a PPI enrichment p-value of < 1.0E-16. After filtering with the Centiscape 2.2 plugin, 9 key targets were identified (Figure 3). These key targets include AKT1, MYC, STAT3, BCL2, CASP3, HIF-A, TNF, IL-6, and JUN.

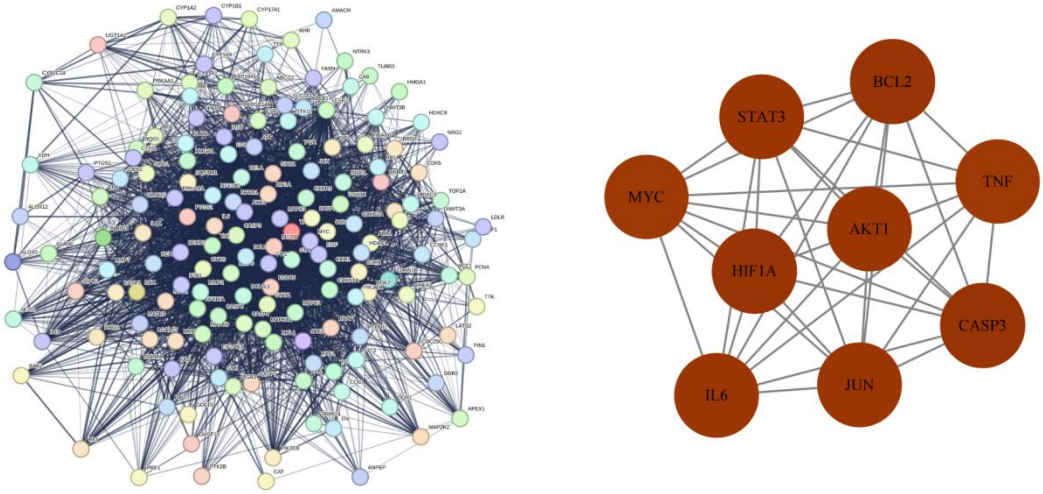

Figure 3: PPI Network PTS Treatment of ESCC Intersection Targets

3.5 GO Functional and KEGG Pathway Enrichment

GO functional enrichment analysis of the intersection targets for PTS treatment of ESCC was performed using the Metascape platform. The top 20 entries, ranked by p-value, were visualized (Figure 4). GO enrichment analysis resulted in 2252 biological processes (BP), 379 cellular components (CC), and 218 molecular functions (MF). The intersection targets showed strong correlations with responses to reactive oxygen species (ROS), cellular response to chemical stress, cellular response to oxidative stress, and responses to hydrogen peroxide, among others.

KEGG pathway enrichment analysis of the intersection targets for PTS treatment of ESCC was also performed using the Metascape platform (Figure 5). The top 20 entries, based on p-value, were visualized. A total of 161 KEGG functional items were obtained, involving pathways such as Tuberculosis, Non-small cell lung cancer, Melanoma, TNF signaling pathway, C-type lectin receptor signaling pathway, Influenza A, PD-L1 expression and PD-1 checkpoint pathway in cancer, Endometrial cancer, Toxoplasmosis, Chemical carcinogenesis, receptor activation, Human T-cell leukemia virus 1 infection, Small cell lung cancer, Prolactin signaling pathway, Chemical carcinogenesis, reactive oxygen species, AGE-RAGE signaling pathway in diabetic complications, Human immunodeficiency virus 1 infection, Epstein-Barr virus infection, Chronic myeloid leukemia, Prostate cancer, Colorectal cancer, Pancreatic cancer, Human cytomegalovirus infection, Measles, Kaposi sarcoma-associated herpesvirus infection, Endocrine resistance, Hepatitis C, and Apoptosis.

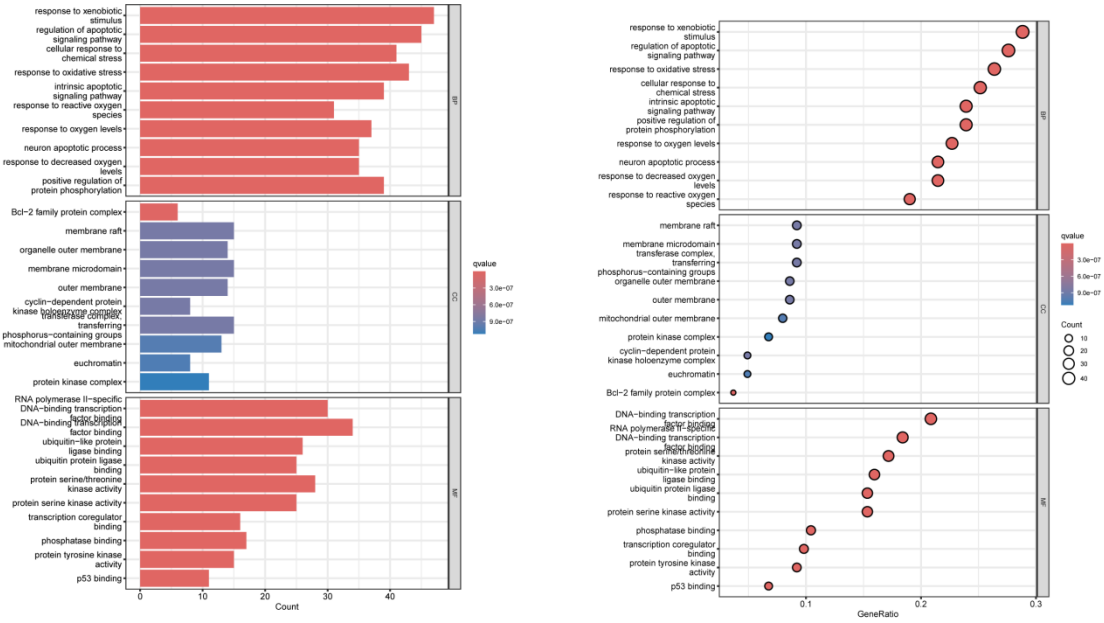

Figure 4: GO Enrichment Analysis

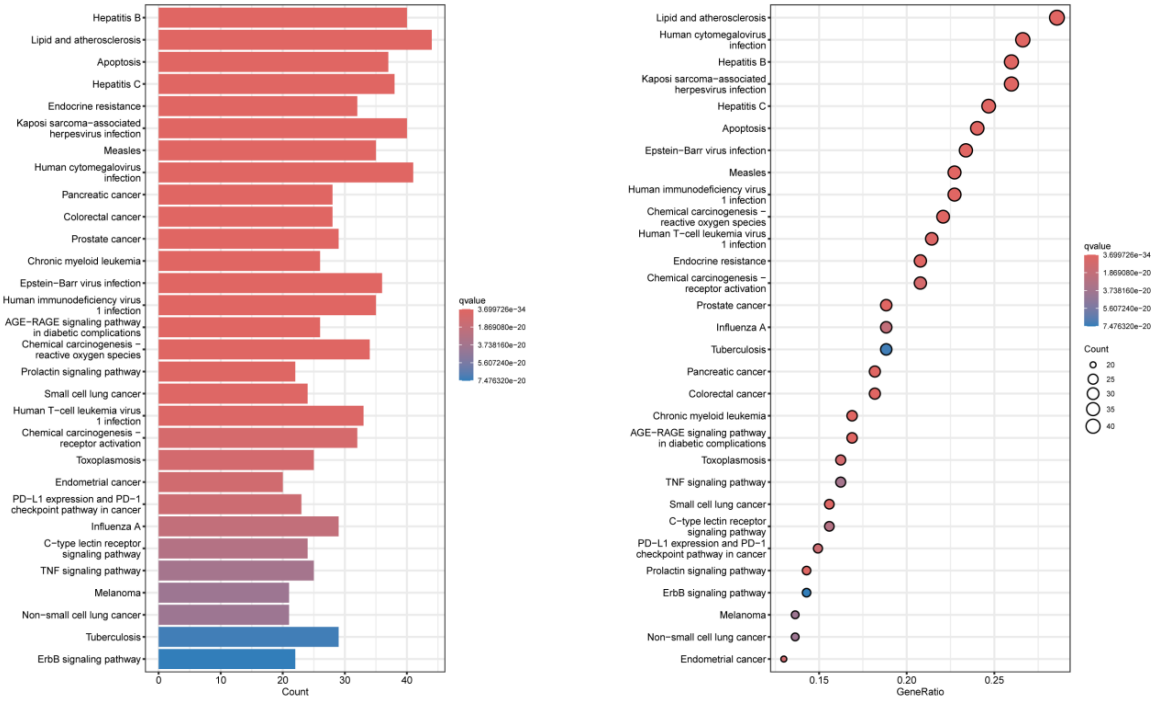

Figure 5: KEGG Enrichment Analysis

4. Discussion

Esophageal cancer is characterized by a high incidence and high mortality rate [6]. The pathological types of esophageal cancer vary by region. Despite some progress in surgery, chemotherapy, radiotherapy, targeted therapy, and immunotherapy, the prognosis for esophageal cancer patients remains poor due to the unrestricted proliferation and metastasis of tumor cells, as well as the prominent issue of drug resistance [7]. Therefore, it is essential to further explore prognostic biomarkers and effective therapeutic targets for esophageal cancer.

This study used a network pharmacology approach to analyze the potential targets and signaling pathways of PTS in the treatment of ESCC. Through screening, key targets such as AKT1, MYC, STAT3, BCL2, CASP3, HIF-A, TNF, IL-6, and JUN were found to be closely related to ESCC. AKT1 is involved in cell survival, proliferation, and anti-apoptotic processes. It is often overactivated in ESCC, promoting tumor growth and drug resistance [8]. MYC expression is frequently upregulated in ESCC, promoting tumor cell proliferation and metabolic changes [9]. STAT3 plays a crucial role in ESCC, particularly in regulating the tumor microenvironment and immune escape [10]. Overexpression of BCL2 is closely associated with drug resistance and invasiveness in ESCC [11]. CASP3, as a key executioner protein of apoptosis, its activity is closely related to programmed cell death In ESCC, the expression of CASP3 may be inhibited, promoting tumor cell survival [12]. HIF-1A is a key regulatory factor under hypoxic conditions, involved in tumor cell metabolic reprogramming, angiogenesis, and enhanced invasiveness [13]. It is associated with tumor metastasis and drug resistance in ESCC. The expression of TNF can affect the tumor immune microenvironment and invasiveness [14]. IL-6, a pro-inflammatory cytokine, plays an important role in tumor immune escape, tumor progression, and chemotherapy resistance [15]. JUN is a component of the AP-1 complex that regulates cell proliferation, differentiation, and stress responses [16]. These targets play key roles in the occurrence and development of ESCC through various molecular mechanisms, making them potential therapeutic targets.

In the GO enrichment analysis, the biological processes affected by PTS in esophageal cancer mainly involve the cell's response mechanisms to external or internal stress, particularly in the areas of tumors, drug responses, oxidative stress, and apoptosis. These stimuli may induce adaptive changes or response reactions in cells, typically processed through mechanisms such as metabolism, detoxification, and transport [17]. This is especially important in drug metabolism in cancer cells and detoxifying organs such as the liver, affecting tumor response to treatment, particularly drug resistance to anticancer agents. PTS may prevent tumor cells from evading death through these signaling pathways, thereby inhibiting tumor growth and metastasis [18].

Additionally, combined with KEGG enrichment analysis, it was revealed that PTS induces the generation of reactive oxygen species (ROS), leading to DNA damage in esophageal cancer cells and inhibiting tumor cell proliferation. Key molecules involved in ROS generation include: metabolic enzymes such as CYP1A1, CYP2E1, NQO1; antioxidant enzymes such as SOD2, GPX; signaling molecules such as MAPK, JNK, AKT, NF-κB [19]; and key cancer regulatory genes such as HIF1A, VEGF. By studying these pathways, a better understanding of the molecular mechanisms by which PTS inhibits esophageal cancer can be achieved, providing theoretical support for the development of new preventive and therapeutic strategies.

Although this study suggests potential mechanisms of PTS in treating ESCC, its efficacy still needs further validation through more experimental research.

As a natural flavonoid compound, PTS has certain advantages in the treatment of ESCC. Compared with the clinical drugs currently used for ESCC, which often have single targets, limited clinical efficacy, and multiple side effects, the multi-pathway, multi-target, strong bioavailability, and blood-brain barrier penetration of the natural compound PTS are advantageous in addressing the limitations of traditional ESCC treatments [21]. This study, using a network pharmacology approach, constructed the PTS target network and PPI network, systematically identifying the core targets and related pathways involved in the anti-ESCC effect of PTS, providing a theoretical foundation for the development of PTS as a new drug for the prevention and treatment of ESCC.

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