Network Pharmacological Examination of Yujin's Efficacy in Stroke Treatment

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Abstract: This study aimed to get to the bottom of the bioactive components in Yujin that pack a punch against stroke and the intricate ways they work their magic. We put the Traditional Chinese Medicine systems pharmacology platform (TCMSP) to work to filter out the key Yujin components and their target proteins. Meanwhile, we rounded up stroke-related targets from a lineup of databases including GeneCards, OMIM, DrugBank, and TTD. By mapping these elements with Venn diagrams, we zeroed in on the common ground. The STRING database helped us weave together a protein-protein interaction (PPI) network. Taking it a step further, we used Cytoscape 3.8.2 to build a comprehensive network linking active components, targets, and pathways. To get the full picture, we dove into Gene Ontology analysis—covering biological processes, molecular functions, and cellular components—through the DAVID database. Finally, to connect the dots on how Yujin gets the job done, we ran a Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis. Yujin boasts three bioactive components that pack a punch against stroke, while simultaneously interacting with twelve crucial target proteins. Our Gene Ontology analysis revealed that Yujin's impact on stroke management touches upon a whopping 1458 biological processes, 38 cellular components, and 143 molecular functions. When we dove deeper with KEGG pathway enrichment analysis, we found that Yujin works its therapeutic magic through several key pathways-think lipid metabolism and atherosclerosis, platinum drug resistance, toxoplasmosis, and more. The beauty of Yujin lies in its multi-target, multi-pathway approach, which makes it a valuable player in both preventing and treating stroke. The mechanisms we've uncovered in this study could pave the way for future experimental research and clinical applications down the road.

Keywords: Network pharmacology; Stroke, Mechanism; Yujin

1. Introduction

Stroke, also known as cerebrovascular accident, is an acute neurological deficit caused by a disturbance in the cerebral blood circulation [1]. It is classified into ischemic stroke and hemorrhagic stroke, with the former being predominant. It is the world's second most common cause of death after heart disease and the leading cause of severe long-term disability [2]. According to statistics from the World Health Organization, the global incidence of stroke is on the rise, and with the intensification of the aging population, the incidence is increasing year by year [3, 4]. Currently, the only approved treatment for ischemic stroke is thrombolytic therapy. However, thrombolytic therapy has time constraints and potential risks of hemorrhage, and the treatment is relatively expensive, making it unsuitable for all stroke patients. Therefore, exploring an effective treatment method with minimal

adverse reactions is key to solving this problem.

Yujin of Curcuma wenyujin, rhizoma of Curcuma longa, rhizoma of Curcuma kwangsiensis, or rhizoma of Curcuma phaeocaulis, all from the Zingiberaceae family, is a dried tuber root. It is cold in nature, pungent and bitter in taste, and has an affinity for the Liver, Heart, and Lung meridians. Its functions include activating blood to alleviate pain, promoting qi to resolve depression, and treating chest and abdominal distension and pain. The main constituents of Tuber are volatile oil and curcuminoids, exhibiting activities such as anti-inflammatory, lipid-lowering, and anti-tumor effects.

Network pharmacology is mainly through the research methods of systems biology, by means of some mathematical methods, at the molecular level, to make a better interpretation of the network interaction relationship between the multiple components of traditional Chinese medicine and the organism [5]. This study investigates the therapeutic mechanism of Yujin for stroke through the analysis of databases such as the TCMSP, STRING, GO, and KEGG.

2. Materials and Methods

2.1 Compilation of Essential Drug Components and Associated Target Receptors

Our research draws on the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP) to meticulously sift through the active components in Yujin [6]. Acknowledging the intricate nature of these components and the specifics of how the human body absorbs them, we implemented stringent criteria. We only included those components that showed significant promise, boasting an oral bioavailability of at least 30% and a drug-likeness index greater than 0.18. Using these parameters, the TCMSP platform subsequently yielded potential therapeutic targets for these potent components.

2.2 Screening Stroke-related Disease Targets and Pharmacological Targets of Juhua in Stroke Treatment

To identify stroke-related therapeutic targets, we mined four comprehensive databases—GeneCards [7], OMIM [8], TTD [9], and DrugBank [10]—using "stroke" as our search term. By constructing a Venn diagram, we pinpointed the overlapping targets between the pharmacologically active components of Yujin and stroke-related genes. These intersecting targets were subsequently extracted as the key therapeutic targets through which Yujin's bioactive components exert their effects in stroke treatment.

2.3 Establishing PPI Connections and Pinpointing Critical Aims

The drug action targets identified through Venn diagram screening were uploaded to the String database platform, where we restricted the species to "Homo sapiens" and set the interaction confidence to its maximum value (0.900), leaving all other parameters at their default settings. Following the elimination of isolated nodes, we constructed a target protein interaction network. Subsequently, using the CytoNCA plugin within Cytoscape 3.8.2[11], we performed network topology analysis to pinpoint the key targets involved in Yujin's stroke intervention.

2.4 GO Functional Annotation and KEGG Pathway Mapping

This research delved into the ways Yujin impacts the biological processes associated with cerebral stroke through an examination of its projected pharmacological targets. Leveraging the ClusterProfiler package in R, we conducted thorough Gene Ontology (GO) annotations and KEGG

pathway enrichment evaluations. The resulting data was then graphically represented to shed light on the fundamental mechanisms and functional interconnections at play.

3. Result

3.1 Yujin Bioactive components Isolation and Target Sites

This research carried out a comprehensive search of Yujin's chemical components using the TCMSP database as our primary resource. By applying strict selection parameters-specifically requiring Oral Bioavailability (OB) of at least 30% and Drug-likeness (DL) scores of 18% or higher-we managed to pinpoint three principal constituents with potential therapeutic benefits, which collectively interacted with 42 different biological targets. To analyze these findings, we utilized Cytoscape software to map out a network topology model illustrating the "Yujin-Target" relationships (depicted in Figure 1). Within this visual representation, the crimson-colored nodes symbolize Yujin's active components, while the light cyan nodes indicate their associated protein targets. The network analysis revealed that components like beta-sitosterol, sitosterol, and naringenin demonstrate impressive connectivity within this framework.

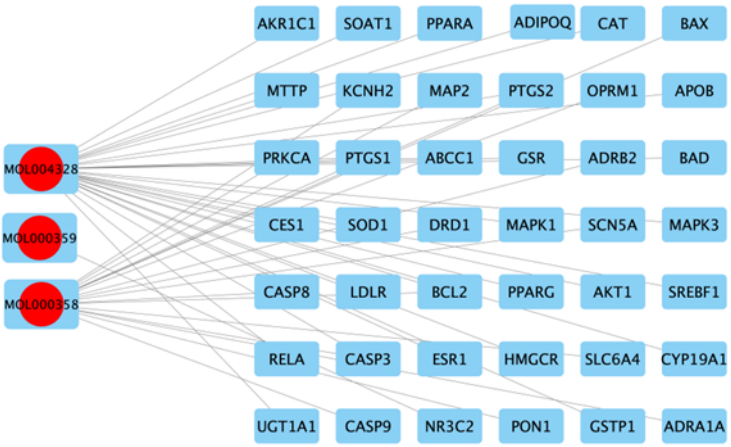

Figure 1: Components-Gene Interaction Network Visualization.

3.2 Identification of Yujin's Therapeutic Targets for Stroke and Associated Pathologies

By leveraging the comprehensive data from four prominent databases-GeneCards, OMIM, TTD, and DrugBank-this investigation successfully compiled a substantial collection of 2915 genes implicated in stroke pathogenesis (for specifics, refer to Figure 2A). Additionally, through the strategic application of Venn diagram analysis, we pinpointed 42 shared targets by examining the overlap between the mechanisms of action of three key Chrysanthemum constituents and the previously mentioned stroke-related genes. These intersecting targets represent the probable molecular foundation through which Chrysanthemum's active components may deliver their therapeutic benefits against stroke (as illustrated in Figure 2B).

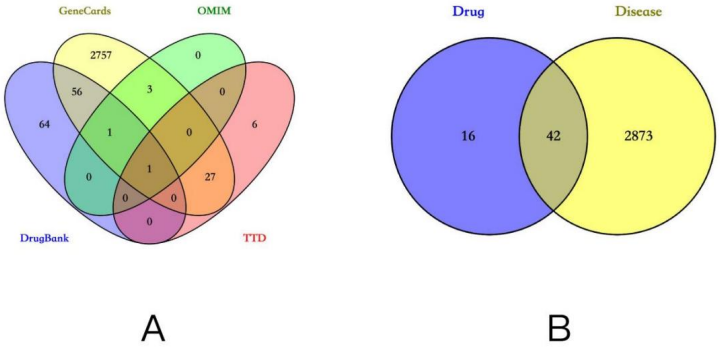

Figure 2: Yujin-Stroke Destination Mapping.

3.3 Develop PPI Network Architecture and Identify Key Targets

To get to the bottom of how Yujin works its magic against stroke, this study took 42 potential targets identified earlier and fed them into the String database to map out a protein-protein interaction network (Figure 3). The CytoNCA plugin was the real workhorse here, putting the network through its paces with a thorough topological analysis. By applying some pretty strict filters-think BC cut-off above 15.1, CC over 0.51, DC topping 20, EC clearing 0.12, LAC exceeding 13.35, and NC surpassing 14.62-the researchers zeroed in on 12 key core players like CASP3, BCL2, and AKT1 (check out Figure 4). These targets likely hold the key to the molecular mechanisms that make Yujin effective in stroke treatment.

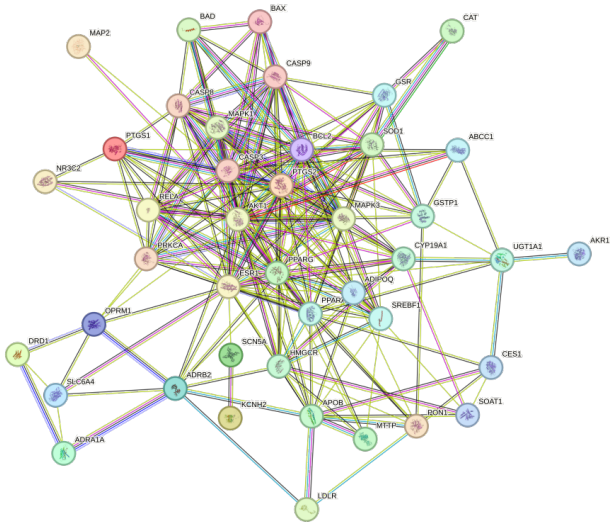

Figure 3: PPI Network.

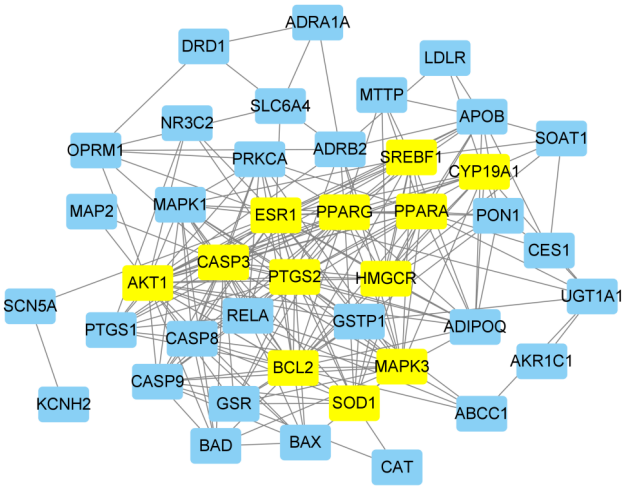

Figure 4: Central Objective Development.

3.4 GO Functional Annotation and KEGG Pathway Mapping

To delve into the inner workings of chrysanthemum's impact on strokes, we imported 42 pharmacological targets from its bioactive components into the DAVID online tool. We subjected these data to both a Gene Ontology (GO) functional analysis and a KEGG pathway enrichment test. We stuck to a stringent P-value threshold of less than 0.05 to guarantee the robustness and dependability of our conclusions. The results of the GO functional enrichment analysis showed 1639 entries, of which 1458 were biological processes (BP), mainly involving response to xenobiotic stimulus, response to nutrient levels, rresponse to oxidative stress, steroid metabolic process, and so on. 38 were cellular components (CC), mainly involving caveola, Bcl-2 family protein complex, plasma membrane raft, membrane raft, membrane microdomain, endoplasmic reticulum lumen, and so on. And 143 were molecular functions (MF), involving antioxidant activity, nuclear receptor activity, ligand-activated transcription factor activity, cysteine-type endopeptidase activity involved in apoptotic process, phosphatase binding, and so on. In this study, the top 10 entries for each category were selected to draw bubble plots, as shown in Figure 5. KEGG pathway enrichment analysis identified 166 significant pathways, mainly involving Lipid and atherosclerosis, Platinum drug resistance, Toxoplasmosis, Hepatitis C, Hepatitis B, etc. In this study, the top 30 signaling pathways were selected to draw bubble plots, as shown in Figure 6.

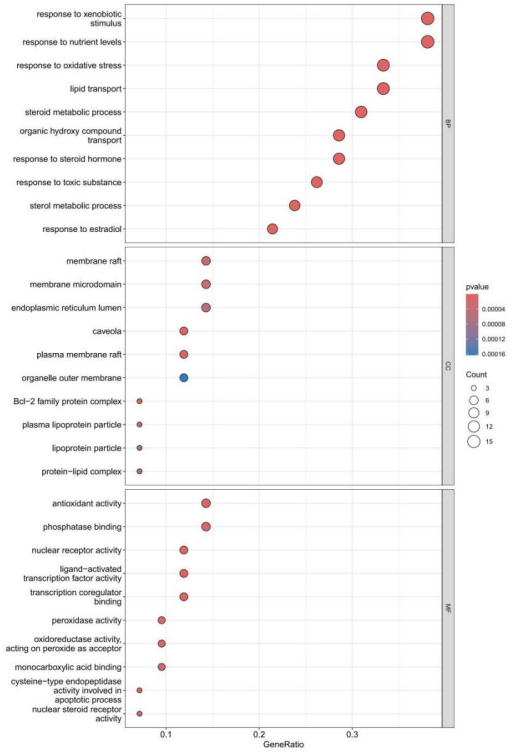

Figure 5: Evaluation of Gene Ontology for Targets in Stroke Interventions.

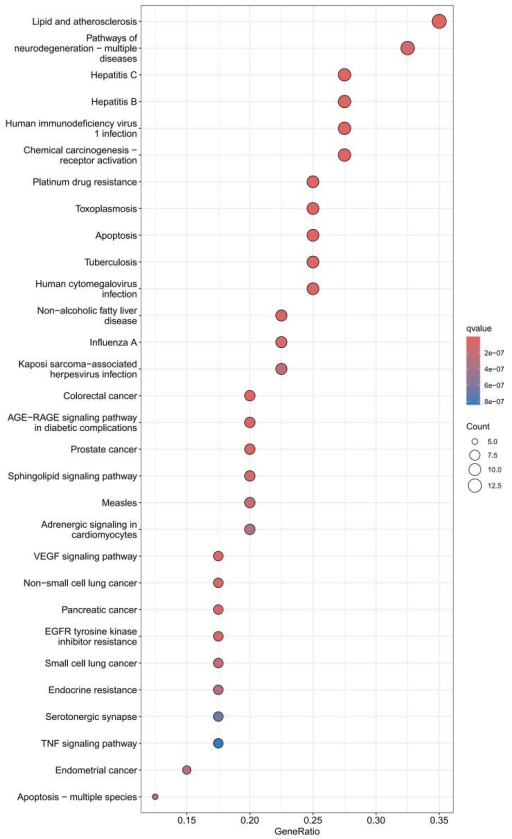

Figure 6: Top 30 KEGG Pathways Associated with Therapeutic Targets for Stroke.

4. Discussion

In the perspective of Traditional Chinese Medicine, the sequelae of stroke fall under the category of "stroke" sequelae [12]. The spasmodic stiffness caused by excessive Yang is often due to pathological factors such as wind, fire, phlegm, and stasis, which disturb the clear orifices from above. When wind and fire mutually incite each other, it is summarized as "excessive Yang". When phlegm and stasis bind together and allow Yang qi to stir and rampage through the meridians, the pattern belongs to excessive Yang. The treatment should be "to treat the rigid with the gentle" [13].

The components of Traditional Chinese Medicine are complex, and they treat diseases by acting through multiple components on multiple targets and pathways. Using the method of network pharmacology to establish a network relationship map of "Traditional Chinese Medicine-active ingredient-action target" can better illustrate the holistic relationship between Traditional Chinese Medicine and targets.

This study employs network pharmacology to investigate the therapeutic mechanisms of chrysanthemum in the treatment of stroke. The screening identified eleven core targets: CASP3, BCL2, and AKT1 and others, which are the key targets through which Yujin exerts its therapeutic effects in stroke treatment.

CASP3 is a member of the cysteine aspartic acid protease (caspase) family, serving as a convergence point for the three classical pathways of cell apoptosis (the cell membrane death receptor pathway, the mitochondrial pathway, and the endoplasmic reticulum pathway), playing a core role in the downstream process of cell apoptosis. Its activation marks the entry of cell apoptosis into an irreversible stage, and thus it is known as the "death execution protease" [14]. The irreversible loss of neurons is a significant feature of many nervous system diseases, and apoptosis, as a form of cell death program, is closely related to stroke. Following CIRI (Cerebral Ischemia-Reperfusion Injury), the release of excitatory amino acids, endoplasmic reticulum stress, inflammation, and calcium overload can all promote the expression of Casp3, a key downstream effector protein in apoptosis, by activating the mitochondrial pathway, the death receptor pathway, and the endoplasmic reticulum pathway, thereby leading to neuronal apoptosis [15].

The BCL2 gene is a significant oncogene in the process of cell apoptosis; the BCL-2 protein it encodes belongs to the anti-apoptotic protein family, and by participating in the endogenous apoptotic pathway, it prevents the release of cytochrome c from the mitochondria into the cytoplasm, thereby inhibiting cell apoptosis [16]. BCL2 belongs to the anti-apoptotic protein family, and its increased expression can reduce neuronal apoptosis. In animal models, BCL2 can effectively inhibit the expression and activation of apoptosis-related proteins such as Caspase-9, exerting a neuroprotective effect [17].

Protein kinase B (AKT1) plays an important role in regulating metabolism, promoting angiogenesis, and regulating vascular endothelial cell function, and can be activated by a variety of upstream cytokines, thereby exerting a protective effect on nerve cells after ischemic injury [18].

This research comprehensively uncovers the multifaceted therapeutic mechanisms of Yujin in stroke treatment. The herb's healing potential appears to stem from a symphony of bioactive components—like beta-sitosterol, sitosterol, and naringenin—hitting multiple bull's-eye targets such as the CASP3, BCL2, and AKT1 genes. Moreover, these components appear to work in concert across various biological highways, including those related to lipid metabolism and atherosclerosis, platinum drug resistance, and toxoplasmosis infection. These findings pave the way for diving deeper into Yujin's molecular underpinnings in stroke therapy and bridging the gap between bench research

and bedside application.

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