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Network pharmacology-based prediction and verification of the molecular targets and pathways for schisandrin against cerebrovascular disease

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[ABSTRACT]

AIM: To illuminate the molecular targets for schisandrin against cerebrovascular disease based on the combined methods of network pharmacology prediction and experimental verification.

METHOD: A protein database was established through constructing the drug-protein network from literature mining data. The protein-protein network was built through an in-depth exploration of the relationships between the proteins. The computational platform was implemented to predict and extract the sensitive sub-network with significant P-values from the protein-protein network. Then the key targets and pathways were identified from the sensitive sub-network. The most related targets and pathways were also confirmed in hydrogen peroxide (H_2O_2)-induced PC12 cells by Western blotting.

RESULTS: Twelve differentially expressed proteins (gene names: NFKB1, RELA, TNFSF10, MAPK1, CHUK, CASP8, PIGS2, MAPK14, CREB1, IFNG, APP, and BCL2) were confirmed as the central nodes of the interaction network (45 nodes, 93 edges). The NF-κB signaling pathway was suggested as the most related pathway of schisandrin for cerebrovascular disease. Furthermore, schisandrin was found to suppress the expression and phosphorylation of IKKα, as well as p50 and p65 induced by H_2O_2 in PC12 cells by Western blotting.

CONCLUSION: The computational platform that integrates literature mining data, protein-protein interactions, sensitive sub-network, and pathway results in identification of the NF-*κ*B signaling pathway as the key targets and pathways for schisandrin.

[KEY WORDS] Schisandrin; Network pharmacology; Cerebrovascular disease; Molecular target; NF-*κ*B signaling pathway

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Introduction

The active ingredients of traditional Chinese medicine (TCM) achieved their therapeutic effectiveness through collectively modulating the molecular network of multi-target agents [1-2]. The unclear molecular mechanisms are an obstacle for the determination of their therapeutic efficacy. The rapid development of network pharmacology has facilitated system level understanding of the interactions of genes, proteins, and has allowed for new methods for uncovering the molecular mechanisms related to the therapeutic efficacy of the active ingredients of TCM [3]. Network pharmacology can make an impact at several areas in target and pathway identification and illumination of drug-target interactions. Many studies have successfully reported interesting biological findings from these networks. A drug-target association network has been used to elucidate the action mechanism of drugs [4]. The drug-drug network and protein-disease network are also employed to discover the association between diseases and target proteins. Theoretical algorithms, such as CIPHER, have been proposed to identify the pathways and key targets [5-6]. Pathway interaction network based on gene expression, protein-protein

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interactions, and cellular pathways have predicted the regulated pathways and extracted information on the sensitive sub-networks for cancer [7]. This network analysis has become a cornerstone for elucidating the mechanisms of TCM. Meanwhile, identifying the potential key targets and pathways for the drugs against the diseases by computational methods is an efficient and time-saving approach for target discovery and experimental verification.

Schisandrin is an active ingredient isolated from the fruit of *Schisandra chinensis* (Turcz.) Baill. (Schisandraceae). It is also one of major effective compounds in the famous Chinese formula ShengMai San [8], which acts as a remedy to treat coronal heart diseases and prevents oxidative injury in the brain [9]. Recent studies have demonstrated that schisandrin exhibits anti-inflammatory and neuroprotective activities *in vivo* and *in vitro*. Schizandrin could attenuate the phosphorylation of JNK, p38, and ERK mitogen-activated protein kinases (MAPK) in primary cultured rat cortical cells induced by glutamate [10]. Meanwhile, it significantly improved amyloid-beta (1-42)-induced short-term and spatial reference memory impairments [11]. In addition, it exerted anti-inflammatory effects on lipopolysaccharide-stimulated RAW 264.7 cells due to a reduction of NF-κB activity and induction of the expression of heme oxygenase-1 [12]. However, the key targets and pathways for schisandrin against cerebrovascular diseases are still unclear. Network pharmacology might facilitate understanding and predicting the key targets and pathways for schisandrin. The predictive results might suggest some clues for further experimental verification. Experimental verification on the predictive model might yield the true reality from the virtual model. The combined method of network pharmacology prediction and experimental verification are preferred to be determined simultaneously to verify the drug target and related signal pathways.

The rat pheochromocytoma PC12 cell line is a commonly used model for studies of neuronal function and stroke-related diseases [13]. The nerve cell with exposure to H_2O_2 has often been used to simulate cerebrovascular or neurodegenerative diseases related to oxidative stress, including stroke and Alzheimer's disease [14-15]. In the present study, the network which built a link between drugs, proteins and pathways was first used to interpret the relationship of the targets and pathways for schisandrin. The sensitive sub-network was extracted from the target database. Then the most related targets and pathways were verified in H_2O_2-induced PC12 cells. These findings are anticipated to shed some new light on the action mechanism of schisandrin against cerebrovascular diseases, and illustrate an approach for the exploration of the mechanisms of TCM formulas which comprise multiple active components.

Materials and Methods

Materials

Schisandrin was purchased from Zelang Medical Technology Co., Ltd.. Nanjing, China (ZL20120309YY, with purity 98%). The pure compound was dissolved and diluted with DMEM medium (Gibco, Grand Island, NY, USA) without serum before each experiment. Polyvinylidene fluoride (PVDF) membranes were purchased from Millipore (Bedford, MA, USA). The primary antibodies were purchased from Bioworld Technology, Inc., Minneapolis, MN, USA. Glyceraldehyde phosphate dehydrogenase (GAPDH) antibody was obtained from Kangchen Bio-Tech, Shanghai, China.

Construction of the regulatory network

Interaction information was retrieved from the literature database Pubmed central of NCBI. The search Mesh terms were specified as 'schisandrin'. The proteins/genes which conformed to the Mesh terms are text-mined from the literature. The proteins/genes contained in the results were filtered with the cerebrovascular disease from the OMIM database (the search field before February 25th, 2013). The proteins/gene/GEO Profiles were standardized in the NCBI database entailed on *Homo sapiens*. Protein-protein/gene- gene information included links to databases, such as BIND, BioGRID, and GeneCards, which represented the major interactome for further bioinformatics analysis. Cytoscape 2.8.3 was developed for reconstruction and visualization of networks [16]. Nodes in the interactome corresponding to the genes and edges represented documented interactions in the visualization. Cytoscape was used as a desktop Java application and downloaded from http://cytoscape.org.

Prediction for drug targets

Network analysis was subjected to GeneMANIA [17] and Clusterone [18] plugin analysis in Cytoscape for network reconstruction and pathway analysis. Clusterone plugin simplified and reconstructed the complicated network by calculating the properties of the sub-networks. It extracted sensitive sub-networks to narrow the range of the network, which represented the central role of the network by containing most of the high degree nodes. Its algorithm had the advantage over other clustering methods by having the higher precision of prediction and showing the proteins at different levels at the same visualization interface. The limitations were that it made more false assumptions than when weighting schemes are used to assess the quality of an interaction. The GeneMANIA plugin was a stand-alone tool for making fast and efficient gene function predictions. GeneMANIA algorithms have been shown to be as good as, or better than, in speed and accuracy compared with other gene function prediction algorithms. The plugin conducted queries using any number of genes and networks, and the resulting predicted networks and genes of functional relationships were available as an annotated Cytoscape network visualization. The size of the GeneMANIA network data was limited by the amount of available memory and disk space. The basic parameters were as default. The P-value was considered to be the index for selecting the in-weights sub-network. A P-value of less than 0.05 showed the