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Mechanism of action of Salvianolic Acid B by module-based network analysis

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Abstract. Salvianolic Acid B (Sal B) is one of the main medicinal ingredients of Radix Salvia miltiorrhiza (Danshen) and possesses a variety of pharmacological effects. The purpose of this study was to discover the new mechanism of action of Sal B based on the protein interaction network (PIN) analysis. A PIN of Sal B was constructed with 852 nodes and 8,626 interactions. By fast agglomerate algorithm based on the edge clustering coefficients (FAG-EC), 11 modules were detected from the network. Gene ontology (GO) enrichment analysis of the modules demonstrated that the roles of Sal B played in cardiovascular disease were related to multiple biological processes, which could represent the characteristics of Chinese Material Medica (CMM) as a whole to regulate the disease. The most interesting finding of this work was that the anti-inflammatory effect of Sal B was due to the immune response of T lymphocytes by regulating IL-2 family, CD3E, CD79A, MAP3K7 and PRKCQ. Therefore, the module-based network analysis will be an effective method for better understanding CMM.

Keywords: Protein interaction network, module, mechanism of action, Salvianolic Acid B, GO enrichment analysis

1. Introduction

Radix Salvia miltiorrhiza (Danshen) has been widely used to treat cardio-cerebral vascular diseases for hundreds of years in China. Salvianolic acids are the most abundant water-soluble compounds in Danshen, among which the most abundant and bioactive is Sal B [1,2]. Studies have shown that Sal B possesses many biological activities, to name a few, Sal B shows protective effect on myocardial ischemia-reperfusion injury [3,4] by increasing vascular endothelial growth factor (VEGF) activation [5]; it can also relieve cerebral ischemia injury by reducing neural damages [6,7]; Sal B also has inhibitive effects on platelet adhesion and aggregation, which are the important basis for its cardiovascular effect [8,9]. Furthermore, Sal B has many other activities, such as anti-inflammatory and anti-oxidative, improving coronary microcirculation and so on [10−14].

Network pharmacology is a novel subject based on the construction of multi-layer networks of "disease-phenotype-gene-drug" to predict the drug targets in a holistic view[15]. Protein-protein interactions (PPIs) are major bearers of the biological process. Therefore, PPIs become a hot topic in recent studies for understanding cellular functions and for therapeutic reasons. PIN of diverse organisms and disease models have been constructed to reveal the mechanism of diseases [16,17], or

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to discover novel drug in an integrated system [18]. The GO[19] project is a collaborative effort to construct ontologies which facilitate biologically meaningful annotation of gene products. It provides a collection of well-defined biological terms, spanning biological processes, molecular functions and cellular components. The GO annotation is considered to be a very helpful vehicle for investigating PPIs.

In this work, a network pharmacology approach has been applied to analyze the molecular mechanism of action of Sal B. PPIs were adopted in constructing a biological network, since proteins executed nearly all cell functions associated with enzymes, channels and transporters. The PIN of Sal B is provided with scale-free property and modularity, proven by the topology analysis. Through the module-based network analysis, discoveries were made in the mechanism of action of Sal B.

2. Materials and methods

2.1. Network construction

The targets information of Sal B was extracted from ChEMBL (https://www.ebi.ac.uk/chembl/#) and STITCH3.1 (http://stitch.embl.de/). ChEMBL [20] is a database of bioactive drug-like small molecules, and it contains binding, functional and ADMET information for the compounds. STITCH [21] is a search tool for interactions of chemicals, integrating information about interactions from metabolic pathways, crystal structures, binding experiments and drug–target relationships.

The PPIs information was obtained from the databases of DIP, BIND, HPRD, BioGRID, MINT, and IntAct, which are integrated in BisoGenet1.41.00 [22]. BisoGenet is a multi-tier application for visualization and analysis of biomolecular relationships, and the client tier is a Cytoscape [23] plugin, which manages the user input, communication with the Web Service, visualization and analysis of the resulting network. The targets retrieved from the database were used to construct a PIN as seed nodes, and their neighbor nodes with a distance of 1.

2.2. Network analysis

A plugin for Cytoscape Network Analyzer[24] was employed to compute and display a comprehensive set of topological parameters, including the network diameter, density, centralization, heterogeneity and so on. Compared with the random network, properties of scale-free and modularity of the PIN were investigated.

The algorithm FAG-EC[25] was applied to mine functional modules in the PIN, which identified functional modules based on the edge clustering coefficients. Compared with other algorithms, FAG-EC is time saving and could produce overlapped complexes. The complex size threshold was set to 2, and the choice of the clique size threshold was 3. Based on the detected modules, GO enrichment analysis was utilized to predict possible biological roles of the modules by evaluating the involved biological processes, using the BinGO[26] plugin for Cytoscape.

3. Results and Discussion

3.1. Construction of the network

Using “Salvianolic Acid B” as the key word, 6 and 5 human proteins were respectively extracted

from STITCH 3.1 and ChEMBL (gained the data on December of 2012), the targets and their binding affinities are listed in Table 1. The plasma concentrations of Sal B range 10.8–259.4 μg/mL[27], and Sal B can prevent the elevation or decrease of the proteins with dose from 0.1 μ M to 10 μ M[28-31]. The 11 targets were taking as an initial list of identifiers of genes of BisoGenet. The generated network contained 852 nodes and 8626 edges. The nodes present genes and the edges indicate their relations.

Table1

The list of targets and their binding affinities of Sal B.

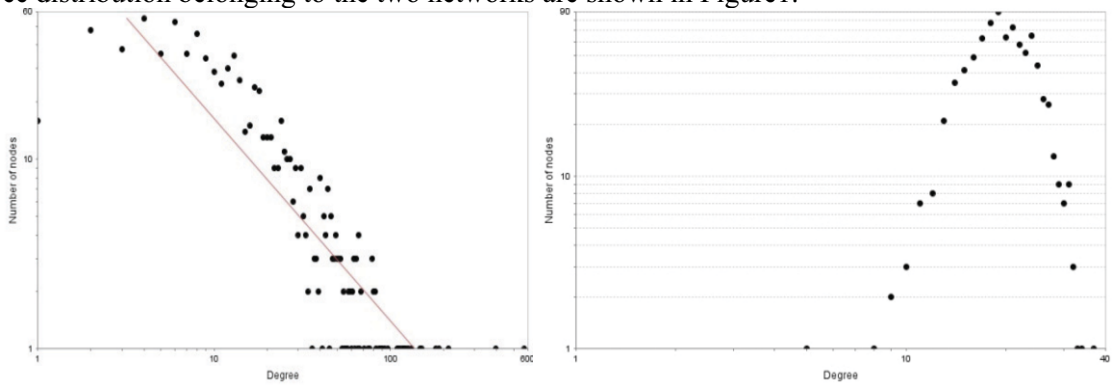
Targets	UniProt ID	IC50(μ M)	Source
MMP9	P14780	-	STITCH
PTGS2	P35354	-	STITCH
BCL2L1	Q07817	-	STITCH
BAX	Q07812	-	STITCH
SH3BP5	O60239	-	STITCH
MMP2	P08253	-	STITCH
SRC	P12931	90	ChEMBL
LCK	P06239	41	ChEMBL
STAT1	P42224	-	ChEMBL
STAT3	P40763	-	ChEMBL
STAT5B	P51692	-	ChEMBL

Notes: Sal B can inhibit the elevation or decrease of other proteins [28-31], so the IC50 are not available.

3.2. Network analysis

3.2.1. Topological analysis

It has been proposed that biological networks possess scale-free topology, if the degree distribution follows a power law distribution $P(k) \sim k^{-\gamma}$[32]. The node degree distribution was calculated, and the result indicated that the PIN of Sal B followed the power law with a degree exponent of 1.068(R^2=0.757). And the random network complied with the Poisson distribution. The graphs of degree distribution belonging to the two networks are shown in Figure1.



(A) (B)

Fig.1. The degree distributions of the networks. (A) It is the degree distribution of the PIN of Sal B, the network followed the power law distribution, the equation is $y=191.51x^{-1.068}$, so the PIN of Sal B is scale-free. (B) It is the degree distribution of the random network, which belongs to the possion distribution.

Table 2

The simple Parameters of protein interaction network of Sal B and random network

networks / Parameters	PIN of Sal B	Random Network
Clustering coefficient	0.397	0.024
Connected components	1	1
Network diameter	5	4
Network radius	3	3
Network centralization	0.653	0.020
Shortest path	725052(100%)	725052(100%)
Characteristic path length	2.324	2.581
Network density	0.024	0.024
Network heterogeneity	1.612	0.218

Notes: The connected component is 1 that indicates the network has no other subnetworks. The network diameter is the greatest distance between any pair of vertices and the radius of a graph is the minimum eccentricity of any vertex. Network centralization is a network index that measures the degree of dispersion of all node centrality scores in a network. And network heterogeneity can characterize the degree of uneven distribution of the network.

The clustering coefficient is a measure of the "all my friends know each other" property, which could reflect the modularity of the network. The clustering coefficient of the PIN of Sal B was 0.397, while that of random network was 0.024. Evidently, the PIN of Sal B was more modularity. These results suggested that the network exhibited scale-free property and modular architecture. All the topological parameters are completed shown in Table 2.

3.2.2. Clustering and GO enrichment analysis

With the FAG-EC algorithm, 11 modules were identified. All 11 modules included 841 of the total 852 proteins. The two largest modules and the rest small ones connected the seed nodes were extracted, and all of them are presented in Figure2.

It has been shown that functional enrichment analysis is conducive to gain insights into the shared underlying biological function of the proteins associated with a module [33]. Functional enrichment was carried out using BinGO. For each module the most significant GO biological processes were assigned. The results are shown in Table 3.

Module 1 and module 2 contain almost 93% proteins of the PIN, and the proteins participate in many biological processes. From the biological processes the proteins occupying, it could predict that Sal B plays a pharmacodynamics with the biological processes, such as positive regulation of biological or cell process, signal transduction, cell communication, cell adhesion, regulation of cell death and proliferation, positive regulation of metabolic process, response to oxidative stress and so on.

The anti-inflammatory mechanism of Sal B is to be discussed as an example. The results show that module 3, 5, 6 and 8 are related to regulation of immune system and inflammatory process.

Module 3 is closely related to the Interleukin (IL)-2 family, which plays a major role in promoting and maintaining T lymphocyte populations by mediating JAK/STAT, MAPK and PI3K pathways[34]. Previous study had demonstrated that Sal B could suppress IFN-γ-induced JAK-STAT1 activation in endothelial cell [35]. This indicates that the module-based network analysis method is reliable to some extent.

Module 5 and module 8 contain proteins such as CD3E, CD79A and CD79B. Immunohistochemical studies demonstrated expression of both T-cell and B-cell–associated antigens, including CD3 and CD79A, which could make T-cell lymphoma aberrantly express [36]. Module 6 possesses proteins like MAP3K7, FYB and PRKCQ. Studies had shown that the deletion of the gene encoding TAK1 (MAP3K7) might result in reduced development of thymocytes and regulating T cells expressing the

transcription factor Foxp3[37]. Protein kinase Cθ (PRKCQ) is an established component of the immunological synapse, and PKCθ-deficient T cells could strongly reduce the interleukin 2 production and T cell proliferative response [38]. However, studies had shown that the anti-inflammatory effect of Sal B were related to other biological processes, such as blockingCOX-2 expression, neuroprotective effect and attenuating vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1) expression in TNF-α stimulated human aortic endothelial cells [28,39,40]. Through the results, another mechanism of action had been forecasted for Sal B to resist inflammation, it is the ability of promoting and maintaining T lymphocyte populations by regulating IL-2 family, CD3E, CD79A, MAP3K7 and PRKCQ. And further confirmation is needed. Flow cytometry can be employed to assess the percentages and absolute counts of human lymphocyte subsets in whole blood and plasma, concentration of proteins can be measured by enzyme-linked immunosorbent assay (ELISA).

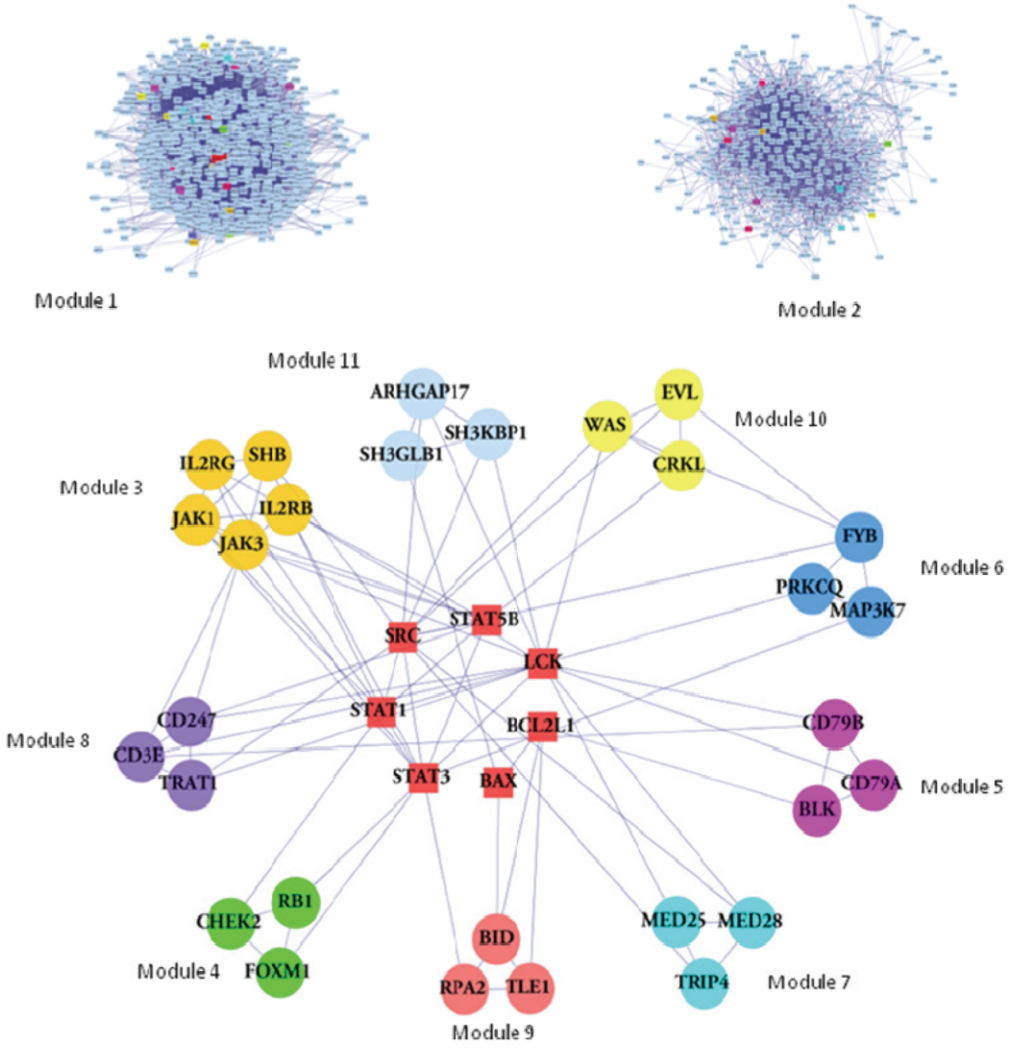


Fig.2. Modules in the PIN of Sal B. With the FAG_EC algorithm, 11 modules are extracted from the network. The two largest ones are module 1and module 2, and modules from 3 to 11 are arranged around a few seed nodes.

Table 3

GO biological process terms of the modules display partially.

Modules	GO terms	P-value
Module 1	signal transduction,	1.3388E-152
	positive regulation of biological process,	2.9138E-150
	cell communication	4.0365E-150
	positive regulation of cellular process	5.6601E-144
	regulation of cell death	5.3641E-99
	blood coagulation	3.4415E-53
	platelet activation	2.7873E-42
Module 2	positive regulation of cellular process	2.233E-97
	positive regulation of metabolic process	4.8447E-87
	transmembrane receptor protein tyrosine kinase signaling pathway	8.6397E-52
	cell adhesion	1.2198E-21
	response to oxidative stress	4.13E-09
	positive regulation of lipase activity	1.35E-09
Module 3	response to interleukin-15	1.19E-09
	interleukin-2-mediated signaling pathway	2.16E-08
	interleukin-4-mediated signaling pathway	6.48E-08
Module 4	cell cycle phase transition	1.09E-06
	mitotic cell cycle phase transition	1.09E-06
Module 5	B cell receptor signaling pathway	1.94E-09
	antigen receptor-mediated signaling pathway	1.35E-07
	regulation of immune system process	9.77E-05
Module6	T cell receptor signaling pathway	6.13E-08
	antigen receptor-mediated signaling pathway	1.35E-07
Module 7	positive regulation of chromatin binding	9.86E-05
	positive regulation of mediator complex assembly	9.86E-05
Module8	T cell receptor signaling pathway	6.13E-08
	antigen receptor-mediated signaling pathway	1.35E-07
Module 9	regulation of response to stimulus	1.94E-03
	macromolecular complex assembly	6.50E-03
Module 10	actin polymerization or depolymerization	1.62E-04
	actin filament organization	1.08E-03
Module 11	regulation of protein insertion into mitochondrial membrane involved in apoptotic signaling	2.96E-04

Notes: P-value is the probability of obtaining the observed effect, a very small P-value indicates that the observed effect is very unlikely to have arisen purely by chance, and therefore provides evidence against the null hypothesis.

4. Conclusions

In this paper, a module-based network analysis approach was proposed to expound the mechanism of Sal B. The biological process of Sal B mainly contains positive regulation of biological or cell process, signal transduction, cell communication, cell adhesion, regulation of cell death and proliferation, positive regulation of metabolic process, response to oxidative stress and so on. The anti-inflammatory effect is the focus of discussion. Another mechanism of action is forecasted for Sal B to resist inflammation, that is, the ability of promoting and maintaining T lymphocyte populations by regulating IL-2 family, CD3E, CD79A, MAP3K7 and PRKCQ. However, further experiments are needed to confirm the conclusions.

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