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Review

Network Pharmacology-based Approaches Capture Essence of Chinese Herbal Medicines

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ABSTRACT

Traditional Chinese Medicine (TCM), a crucial component of the current medical system, has been extensively used in clinical practice due to its valuable therapeutic efficacy, and its potentials as an important source of new pharmacophores. TCM is characterized by holistic theory, which emphasizes maintaining the balance of the patient's whole body using herbal formulae (fangji in Chinese) composed of mixtures of herbs with multiple bioactive ingredients. Because of the complex nature of these formulae, it is necessary to develop systematic methods to identify their bioactive ingredients and to clarify their mechanisms of action. With the rapid progress in bioinformatics, systems biology, and polypharmacology, "network pharmacology", which shifts the "one target, one drug" paradigm to the "network target, multi-component" strategy, has attracted the attention because it can not only reveal the underlying complex interactions between a herbal formula and cellular proteins but detect the influence of their interactions on the function and behavior of the system. Growing evidence shows that the network pharmacology strategy can be a powerful approach to modern research on TCM. The present paper focuses on the basis of network pharmacology and the recent progress in its methodology, illustrates its utility in screening bioactive ingredients and elucidating the mechanisms of action of TCM herbal formulae, analyzes its limitations and problems, and discusses its development direction and application prospects.

Key words

bioactive ingredient; Chinese herbal medicine; molecular mechanism; network pharmacology; network target

1. Introduction

Traditional Chinese Medicine (TCM), which is based on empirical applications and experience distillation over thousands of years, has become a crucial component of the current medical system and has been extensively used as complementary and alternative health care in clinical practice. TCM is characterized by holistic theory and emphasizes maintaining the balance of the patient's whole body using herbal formulae (Fangji in Chinese), which are complex mixtures of herbs consisting of multiple bioactive ingredients. There are a number of synergistic and antagonistic interactions among the various bioactive ingredients of TCM. Moreover, these compounds bind to the corresponding target proteins transiently, simultaneously, or weakly, which in combination enables to treat the complex diseases in a

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systematic manner. Due to their complex nature, the ingredient profiling and molecular mechanisms of TCM herbal formulae remain elusive due to the limitations of reductionism approach, which has hindered the application of Chinese herbal formulae in mainstream medicine and the modernization of TCM.

With the rapid progress in bioinformatics, systems biology and polypharmacology, "network pharmacology" (Hopkins, 2007; 2008), shifting the "one target, one drug" paradigm to the "network target, multi-component" strategy (Li, 2011), have attracted the attention because they can not only reveal the underlying complex interactions between a herbal formula and cellular proteins, but detect the influence of their interactions on the function and behavior of the human system, of which the key idea is in line with the holistic theory of TCM. Actually, network-based TCM studies (Li et al, 2007; Li, 2007) were earlier than the term "network pharmacology". Li's lab has established a series of network-based TCM research strategies since 2007 (Li and Zhang, 2013), and proposed the new concept of "network target" (Li, 2011). TCM network pharmacology integrates TCM theory with molecular networks and utilizes "network target" as a key concept that focuses on the systematic effects of drug targets on the biological network (Li, 2011). This framework aims to decipher the mechanisms of the therapeutic effects of drugs, or TCM herbal formulae, and to understand their possible toxicity and unknown pharmacological activities. Various network topological and dynamic features, such as degree, closeness, betweenness, modularity, feedback, connectivity, and propagation, have been used to investigate the combinatorial rules and holistic regulation effects of herbal formulae (Yang et al, 2015). Thus, the network pharmacology strategy can provide a powerful means for modern research on TCM.

The present paper focuses on the basis of network pharmacology and the recent progress on its methodology, illustrates its utility in screening bioactive ingredients and elucidating the mechanisms of action of TCM herbal formulae, analyzes its limitations and problems, and discusses its development direction and application prospects.

2. Methods and tools of TCM network pharmacology

2.1 "Network target" theory

Network target, a key concept in network pharmacology that firstly proposed by Li (Li, 2011; Li et al, 2011), is an attempt to define a disease-specific molecular network as a therapeutic target for the designation of appropriate treatments. Because many complex human diseases stem from the disruption of molecular networks functions, it is not surprising that the underlying mechanism of a herbal formula acting on a disease process is to reverse the imbalance of a disease-specific network rather than to regulate single molecules. When creating a TCM herbal formula which is a complicated chemical system involving a mixture of many types of chemical compounds, TCM clinicians combine herbs under the guidance of a unique and vital "Emperor-Minister-Assistant-Messenger" (Jun-Chen-Zuo-Shi in Chinese) rule. The "Jun" (emperor) herb is the principal herb in the formula and is responsible for treating the main disease or principal syndrome; The "Chen" (minister) herb assists the "Jun" herb in promoting a curative effect; The "Zuo" (assistant) herb is applied to modulating the effects of the "Jun" and "Chen" herbs, including alleviating toxicity and generally improving drug efficacy; Finally, the "Shi" (messenger) herb plays an indispensable supporting role in harmonizing the actions and enhancing the functions of the other herbs. This combinatorial principle has been demonstrated to act on the "network target" of a disease-specific network, which can be constructed using the interactions of disease-related genes or gene products, signaling pathways and the co-functions of biological processes. Network targets can be defined as the key components with topological importance in a disease-specific molecular network according to the calculation of various topological features. Network target theory provides the means to comprehensively identify all possibly affected targets and their interactions for deciphering the mechanisms of the therapeutic effects of drug treatments, including TCM herbal formulae, and clarifying their possible toxicity and unknown pharmacological activities (Li et al, 2007).

2.2 Features of disease/drug specific molecular network

In TCM network pharmacology, a "network" is often constructed to illustrate the associations between herbal formulae and specific diseases using the links between various herbs/herb ingredients containing a herbal formula and the corresponding targets or the links between drug targets and disease-related genes. The major features of biological network include topological, functional, and dynamic features. Following the network construction, the evaluation of these features can provide a quantifiable description of the complex biological system and its response to various drug/herbal treatments. Here, we briefly describe these features that pertain to biological networks.

Topological features: For each node i in an interaction network, the following features can be calculated to assess its topological property, for example: (1) "Degree" is defined as the number of links to node i. (2) "Node betweenness" is viewed as a measure of how many shortest paths between pairs of nodes that run through node i. (3) "Closeness" is defined as the inverse of the farness, which is the sum of node is distance to all other nodes. The closeness centrality can be used to evaluate the distance from node i to all other nodes in the network. As a node's degree/node betweenness/closeness centrality becomes larger, the importance of the node in the interaction network increases (Wang et al, 2012). (4) K-core analysis is an iterative process in which the nodes are removed from the networks in the order of least connected to most connected (Wuchty and Almaas, 2005). The core of maximum order is viewed as the main core or the highest k-core of the network. A k-core sub-network of the original network can be generated by recursively deleting vertices

from the network, the degree of which is less than k, leading to the construction of a series of sub-networks which gradually screen the globally central region of the original network. On this basis, a "K value" is used to assess the centrality of node i. For each edge e in an interaction network, the "edge betweenness" is often used to assess the importance of a specific interaction in the network and is defined as the frequency of an edge placed on the shortest paths between all pairs of vertices in the network (Narayanan et al, 2011). The edges with the highest betweenness values are most likely to lie between functional modules.

Functional features: Based on the degree distribution, a biological network can be divided into some subgraphs, which represent groups of nodes that link to each other forming small subnetworks and are also called motifs (Milo et al, 2002; Barabási et al, 2011). They are often associated with several optimized biological functions. Moreover, most biological networks can be also divided into several modules using a high degree of clustering. Since they represent highly interlinked local regions in the network, the nodes in the same module are often believed to exert specific biological functions which introduce the concept of a functional module, an aggregation of nodes of similar or related function in the same network neighborhood (Vidal et al, 2011).

Dynamic features: Network complexity may be caused by robustness, feedback, fine tuning, spatial and temporal compartmentalization and dynamic modular structure between and within functional modules under different conditions (Han, 2008). The dynamical features of biological networks can be assessed by molecular phenotypes, modular structures, and conditional connectivity (Vidal et al, 2011; Dhal et al, 2014).

2.3 Algorithms, software, and databases related to TCM network pharmacology

Network pharmacology, an emerging discipline based on the disease-gene/target-drug multilevel network, has been used to identify drug targets and to improve the efficiency of drug discovery. The research underpinning of this framework has increasingly motivated medical researchers to identify the place of intersection between TCM and network pharmacology. Table 1 summarizes a series of TCM network pharmacology-related algorithms, including the network-based prediction of disease genes (Wu et al, 2008; Yao et al, 2011), drug targets, and drug functions (Zhao and Li, 2010; Li and Zhao, 2010; Gu et al, 2010), the construction of disease-specific networks and herb networks (Wu et al, 2008; Li et al, 2010a; 2006; 2010b; 2012; 2008), drug-gene-disease co-module quantitative analyses (Zhao and Li, 2012; Zu et al, 2015; Wang et al, 2014; 2013), software for network analysis and visualization (Leydesdorff et al, 2013; Shannon et al, 2003; Brown et al, 2009), and Table 2 lists public resources with drug information (Wishart et al, 2008; Kuhn et al, 2010; Papadatos and Overington, 2014; Cheng et al, 2014), compounds and herbs used in TCM (Chen, 2011; Xue et al, 2013; Hao et al, 2011; Ru et al, 2014; Li et al, 2011), disease-related molecules (Hamosh et al, 2005; Zhang et al, 2008), molecular interactions (Szklarczyk et al, 2015; Chen et al, 2009; Matthews et al, 2009; Brown and Jurisica, 2005; Aranda et al, 2010; Prasad et al, 2009; Ceol et al, 2010; Lehne and Schlitt, 2009; Beuming et al, 2005), and biological pathways (Wixon and Kell, 2000).

The development of the existing algorithms, software and databases related to TCM network pharmacology, as noted above, has provided novel methodologies and opportunities for identifying bioactive ingredients and biomarkers, potentially revealing mechanisms of action and exploring the scientific evidence of herbal formulae on the basis of complex biological systems. From 2006 to 2011, Li's laboratory created a total of 14 network-based algorithms [CIPHER (Wu et al, 2008), drug CIPHER (Zhao and Li, 2010; Li and Zhao, 2010), comCIPHER (Zhao and Li, 2012), CIPHER-HIT (Yao et al, 2011), DMIM (Li et al, 2010), NADA (Li et al, 2010), NIMS (Li et al, 2008; 2009; 2011), SAF (Yan et al, 2010), LMMA (Li et al, 2006), CSPN (Huang and Li, 2010), sGSCA (Wang et al, 2014), ClustEx (Wang et al, 2013), GIFT (Gu et al, 2010) and DGPsubNet (Zu et al, 2015)], and two databases [HerbBioMap (Li et al, 2011) and dbNEI (Zhang et al, 2008)], which embody "network target" as a key concept of TCM network pharmacology and provide the methodological support for screening bioactive ingredients, clarifying the synergistic effects among herbs, and investigating "disease-syndrome-formulae"-associated mechanisms and biomarkers of various herbal formulae, such as Liuwei Dihuang Pill (Liang et al, 2014), Qinluo Yin (Zhang et al, 2013), Wutou Decoction (Zhang et al, 2015), and the Gegen Qinlian Decoction (Li et al, 2014).

3. Application of network pharmacology-based approaches to identify bioactive ingredients of Chinese herbal medicine

Chinese herbs are valuable sources of potential new pharmacophores. About thirty percent of the top-selling drugs are originated from ingredients of Chinese herbs (Strohl, 2000). Bioactive ingredients are defined as ingredients that can be separated and have pharmacological activities. Accumulating studies have made great efforts to extract and analyze the bioactive herbal ingredients. Tang et al (Tang et al, 2004) isolated potent antioxidant ingredients from 33 Chinese herb extracts by high-performance liquid chromatography (HPLC) and mass spectrometry (MS) *in vitro* and assessed their potential to treat diseases involving oxidative stress. Li et al (2012) performed a high-throughput in silico screen and obtained a group of bioactive ingredients with desirable pharmacodynamics and pharmacological characteristics from the Compound Danshen Formula. Ludwiczuk et al (2011) performed a bioactivity-guided approach based on a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay for growth inhibition and quantitative real-time polymerase chain reaction (PCR) for tumor necrosis factor-α (TNF-α) inhibitory activity to identify the anticancer constituents from the leaves of *Alnus sieboldiana* Matsum. (Betulaceae). However, it may not be an appropriate approach

Table 1 Algorithms and software related to TCM network pharmacology

Types	Names	Description	Application	References
Algorithms	CIPHER	Network-based prediction for disease genes	Predicting disease genes	(Wu et al, 2008)
	CIPHER-HIT	Modularity-based disease gene prediction	Predicting disease genes	(Yao et al, 2011)
	drug CIPHER	Network-based prediction for drug (herbal ingredient) targets and functions	Predicting drug targets and functions	(Zhao and Li, 2010; Li and Zhao, 2010)
	GIFT	Global optimization-based inference of chemogenomic features from drug-target interactions	Determining drug-target interactions	(Gu et al, 2010)
	DMIM	Herb network construction and co-module analysis for herbal formulae	Investigating the molecular mechanisms of drugs	(Li et al, 2010)
	LMMA	Disease-specific molecular network construction	Investigating the underlying mechanisms of diseases	(Li et al, 2006)
	NADA	Network-based assessment for drug (herbal ingredient) action	Investigating the molecular mechanisms of drugs	(Li et al, 2010)
	NIMS	Network-based identification of multi-component synergy and drug (herbal ingredient) combinations	Drug combination design	(Li et al, 2008; 2009; 2011)
	SAF	Synergism assessment factor	Drug combination design	(Yan et al, 2010)
	comCIPHER	Drug-gene-disease co-module analysis	Investigating the molecular mechanisms of drugs	(Zhao and Li, 2012)
	DGPsubNet	Systematic analysis of new drug indications by drug-gene-disease coherent subnetworks	Identification of novel drug indications and their molecular basis	(Zu et al, 2015)
	sGSCA	Inferring pathway crosstalk networks using gene set co-expression signatures	Systematically investigating the molecular mechanisms of complex diseases on both pathway and gene levels at the same time	(Wang et al, 2014)
	ClustEx	Disease-specific responsive gene module identification	Identification of the responsive gene modules during disease progression	(Wang et al, 2013)
	CSPN	Disease-specific pathway network construction	Investigating the underlying mechanisms of diseases	(Huang and Li, 2010)
Software	Pajek	A program for Windows, for analysis and visualization of large networks having some tens of thousands of vertices based on experiences gained in development of graph data structure and algorithms libraries	Network analysis and visualization	(Leydesdorff et al, 2013)
	CytoScape	An open source software platform for visualizing complex networks and integrating these with any type of attribute data	Network analysis and visualization	(Shannon et al, 2003)
	NAViGaTOR	A powerful graphing application for the 2D and 3D visualization of biological networks	Network analysis and visualization	(Brown et al, 2009)

Table 2 Public resources related to TCM network pharmacology

Names	Descriptions	Applications	References
DrugBank	A unique bioinformatics and cheminformatics resource that combines detailed drug (i.e. chemical, pharmacological, and pharmaceutical) data with comprehensive drug target (i.e. sequence, structure, and pathway) information	Collecting information on drugs and the corresponding targets	(Wishart et al, 2008)
STITCH	A resource to explore known and predicted interactions of chemicals and proteins. Chemicals are linked to other chemicals and proteins by evidence derived from experiments, databases and the literature	Search tool for interactions of chemicals	(Kuhn et al, 2010)
ChEMBL	A manually curated chemical database of bioactive molecules with drug-like properties	Search tool for interactions of chemicals	(Papadatos and Overington, 2014)

To be continued

Continued Table 2

Names	Descriptions	Applications	References
PubChem	A database of the biological activities of small molecules	Collecting information on the biological activities of small molecules	(Cheng et al, 2014)
OMIM	An online catalog of human genes and genetic disorders	Collecting information on disease related genes	(Hamosh et al ,2005)
KEGG	A database resource for understanding high-level functions and utilities of the biological system, such as the cell, the organism and the ecosystem, from molecular-level information, especially large-scale molecular datasets generated by genome sequencing and other high-throughput experimental technologies	Collecting information on pathways involved by certain molecules	(Wixon and Kell, 2000)
String	A database of known and predicted protein interactions which include direct (physical) and indirect (functional) associations	Search tool for the retrieval of interacting genes/proteins	(Szklarczyk et al, 2015)
HAPPI	An online database of comprehensive human annotated and predicted protein interactions, which was created by extracting and integrating publicly available protein interaction databases, including HPRD, BIND, MINT, STRING, and OPHID, using database integration techniques	Collecting information on protein-protein interactions	(Chen et al, 2009)
Reactome	A free, open-source, curated and peer reviewed pathway database	Intuitive bioinformatics tools for the visualization, interpretation and analysis of pathway knowledge to support basic research, genome analysis, modeling, systems biology and education	(Matthews et al, 2009)
OPHID	A web-based database of predicted interactions between human proteins, which combines the literature-derived human PPI from BIND, HPRD and MINT, with predictions made from Saccharomyces cerevisiae, Caenorhabditis elegans, Drosophila melanogaster, and Mus musculus	Collecting information on protein-protein interactions	(Brown and Jurisica, 2005)
InAct	A freely available, open source database system and analysis tools for molecular interaction data	Collecting information on molecular interactions	(Aranda et al, 2010)
HPRD	A centralized platform to visually depict and integrate information pertaining to domain architecture, post-translational modifications, interaction networks and disease association for each protein in the human proteome	Collecting information on protein-protein interactions, post-translational modifications, protein expression and subcellular localization	(Keshava Prasad et al, 2009)
MINT	A database of functional interactions between proteins	Collecting information on protein-protein interactions	(Ceol et al, 2010)
DIP	A database that documents experimentally determined protein-protein interactions	A comprehensive and integrated tool for browsing and efficiently extracting information about protein interactions and interaction networks in biological processes	(Lehne and Schlitt, 2009)
PDZBase	A manually curated protein-protein interaction database developed specifically for interactions involving PDZ domains	Collecting information on protein-protein interactions involving PDZ domains	(Beuming et al, 200)
TCM Database @Taiwan	The world largest and most comprehensive free down small molecular database on TCM for virtual screening currently	Collection of compounds and herbs of TCM	(Chen, 2011)
TCMID	Recording TCM-related information collected from different resources and through text-mining method	Collection of compounds and herbs of TCM	(Xue et al, 2013)

To be continued

Continued Table 2

Names	Descriptions	Applications	References
HIT	Recording TCM-related information collected from different resources and through text-mining method	Collection of compounds and herbs of TCM	(Hao et al, 2011)
TCMSP	A unique systems pharmacology platform of Chinese herbal medicines that captures the relationships among drugs, targets, and diseases, and includes chemicals, targets, and drug-target networks, and associated drug-target-disease networks, as well as pharmacokinetic properties for natural compounds involving oral bioavailability, drug-likeness, intestinal epithelial permeability, blood-brain-barrier, aqueous solubility, etc.	Collection of compounds and herbs of TCM	(Ru et al, 2014)
CHEM-TCM	Digital database of individual molecules, constituents of plants used in the traditional Chinese herbal medicine, consisting of four major parts: chemical identification, botanical information, predicted activity against common Western therapeutic targets, and estimated molecular activity according to traditional Chinese herbal medicine categories	Collection of compounds and herbs of TCM	http://www.chemtcm.com/database.htm
HerbBioMap	A molecular data source for herbs and TCM phenotypes	Collection of compounds and herbs of TCM	(Li et al, 2011)
dbNEI	A database for neuro-endocrine-immune interactions and drug-NEI-disease network	Collection of NEI molecules and interactions	(Zhang et al, 2008)

to use separated ingredients with good pharmacological activities *in vitro* to infer the efficacy of Chinese medicines *in vivo* because the concentration of bioactive ingredients in herbs is often very low and the testing concentration of *in vitro* experiments are not realistic under *in vivo* conditions. Moreover, the ingredient contents often vary widely because of their distribution of different parts of the herbs, the growth environment, and other factors. In this context, network pharmacology-based approaches have been proposed to integrate the ingredient contents and their interactions with the corresponding targets that may be responsible for their pharmacological activities *in vivo*.

"Qing-Luo-Yin" (QLY), an anti-rheumatoid arthritis (RA) herbal formula in the Xin-An medical family, consists of four herbs: Ku Shen (*Sophorae Flavescentis Radix*), Qing Feng Teng (*Sinomenii Caulis*), Huang Bai (*Phellodendri Chinensis Cortex*), and Bi Xie (*Dioscoreae Spongiosae Rhizoma*). Zhang et al (2013) identified several anti-angiogenic and anti-inflammatory active ingredients, including kurarinone, matrine, sinomenine, berberine, and diosgenin, from the 235 ingredients of QLY by predicting the target profile of each ingredient using drug CIPHER and performing a network target analysis. The synergistic effects of major bioactive ingredients, such as matrine and sinomenine, were demonstrated on the regulation of the TNF-α- and VEGF-induced signaling pathways involved in RA. Several ingredient groups, such as saponins and alkaloids, were also identified as active components in QLY.

Ge-Gen-Qin-Lian Decoction (GGQLD), an ancient and effective treatment for "dampness heat" syndrome, which causes diarrhea and dysentery, originated from Treating on Cold Damage Disease (Shanghan Lun), compiled by Zhong-jing Zhang. GGQLD consists of four herbs: *Puerariae Lobatae Radix* (Gegen), which is the principle herb, and *Scutellariae Radix* (Huangqin), *Coptidis Rhizoma* (Huanglian), and *Glycyrrhizae Radix* et *Rhizoma Praeparata cum Melle* (Gancao), which are used as adjuvant herbs to assist the effects of Gegen. GGQLD has been extensively used in the treatment of Type 2 diabetes (T2D). Li et al (2014) performed a network target analysis to reveal that 4-hydroxymephenytoin, a core component of Ge-Gen, might be involved in the antidiabetic ingredients of GGQLD, which can stimulate endogenous insulin secretion and ameliorate insulin resistance in 3T3-L1- based insulin resistance models.

Xuesaitong (XST) Injection, a Chinese medicinal preparation consisting of the total saponins from *Panax notoginseng* (Burk.) F. H. Chen (Sanqi), has been extensively used in China for the treatment of cardiocerebrovascular diseases (CVDs) such as thrombosis, myocardial infarction, cerebral infarction, and coronary heart disease, and its beneficial effect has been demonstrated through long-term clinical practice. Wang et al (2014) constructed a CVD network according to the interactions among CVD-related proteins and performed an analytical quantification of the total saponins to screen the chemical ingredients, followed by the target exploration for these ingredients using gene expression analysis, text mining, and computational approaches. Then, the topological features of the targets of these ingredients in the CVD network, along with the contents of ingredients, were combined to calculate a content-weighted index for the integrative evaluation of ingredient efficacy. As a result, the authors identified notoginsenoside R_1 and ginsenosides Rg_1, Rb_1, Rd, and Re as the main bioactive ingredients of XST against myocardial infarction, which was also validated by *in vivo* analysis.

Sini Decoction (SND), a classical formula consisting of *Aconiti Lateralis Radix Praeparaia*, *Zingiberis Rhizoma*, and *Glycyrrhizae Radix et Rhizoma Praeparata cum Melle*, has been fully shown to be clinically effective in treating

doxorubicin (DOX)-induced cardiomyopathy. Chen et al (2014) integrated GC/LC-MS-based metabolomics and network pharmacology approaches to indicate total alkaloids, the major active ingredients of *Aconitum carmichaelii*, as the principal ingredient in the SND formula, whereas total gingerols, total flavones, and total saponins, the major active ingredients of *Zingiberis Rhizoma* and *Glycyrrhizae Radix et Rhizoma Praeparata cum Melle*, respectively, served as adjuvant ingredients. Chen et al (2014) also showed that phosphoinositide 3-kinase gamma, insulin receptor and glucokinase are targets of bioactive ingredients in this formula.

Modified Simiaowan (MSW) is widely used in the clinical to treat gouty diseases. Zhao et al (2015) constructed three drug-target networks, including the "candidate ingredient-target network" which links candidate ingredients and targets; the "core ingredient-target-pathway network" which connects core potential ingredients and targets through related pathways; and the "rationality of herb combinations of the MSW network" to collect 30 core ingredients in MSW and 25 inflammatory cytokines and uric acid synthetases or transporters, which are effective for gout treatment through related pathways.

Zhi-Zi-Da-Huang Decoction (ZZDHD), which consists of four crude herbs−*Gardeniae Fructus*, *Rhei Radix et Rhizoma*, *Aurantii Immaturus Fructus*, and *Sojae Semen Praeparatum*− has been used for centuries to treat alcoholic liver disease. An and Feng (2015) performed molecular docking and network analysis to screen multiple bioactive compounds of ZZDHD based on four key original enzymes (cytochrome P450 2E1, xanthine oxidase, inducible nitric oxide synthase, and cyclo-oxygenase-2) involved in ethanol-induced oxidative stress damage. The authors predicted the relationships of bioactive ingredients to the targets, which were further verified by experimental pharmacological studies.

Taken together, these efforts suggest the efficiency of network-based approaches in the identification of bioactive ingredients of Chinese herbs in a systematic level.

4. Application of network pharmacology-based approaches to clarify mechanisms of action of Chinese herbal medcines (CHM)

Chinese herbal formulae contain a large number of ingredients that are too complex to be assessed by traditional experimental methods based on the "one gene, one drug, one disease" paradigm. It is therefore reasonable to utilize network pharmacology-based approaches to elucidate their actions and the underlying mechanisms according to the "Jun-Chen-Zuo-Shi" combinatorial rule. By combining the prediction of the target profiles of all available ingredients in an herbal formula with systematic network target analysis, network pharmacology can define TCM from a systems perspective and at a molecular level, providing a new method for studying TCM.

Following the identification of bioactive ingredients of QLY, Zhang et al (2013) further performed network target analysis to find that Qing Feng Teng (*Sinomenii Caulis*; Chen herb), Huang Bai (*Phellodendri Chinensis Cortex*), and Bi Xie (*Dioscoreae Spongiosae Rhizoma*; Zuo-Shi herbs) could augment or modulate the therapeutic effects of Ku-Shen (*Sophorae Flavescentis Radix*, the Jun herb in QLY) by targeting the compensatory pathway and feedback loop in the TNF/IL1B/VEGF-induced NFκB pathway.

Liu-Wei-Di-Huang (LWDH), formulated to tonify the "*Yin* deficiency pattern" in TCM, has been applied in clinical settings to treat various complex diseases, such as hypertension and esophagus carcinoma. Li et al (Li et al, 2010; Liang et al, 2014) constructed a multilayer herb-biomolecule-disease network by manually curating the genes and diseases related to LWDH and revealed a metabolism-immune network imbalance underlying TCM Cold Syndrome and Hot Syndrome, a pair of typical syndromes reflecting the Yin-Yang imbalance of the human body. Then, the authors predicted the biological targets of LWDH ingredients by analyzing the chemical group composition of the ingredients, exploring their chemical characteristics and distribution in chemical space, and measuring their drug-likeness properties. They also found the most well-represented target molecules of LWDH compared to the null model with Poisson binomial statistics and ranked the ingredients in LWDH by defining an efficacy score. Following the prediction of the diseases potentially treated by LWDH, the authors constructed a network that integrated compound-target and target-disease relationships and a network that reflected the common biological processes related to compounds and diseases. Moreover, they found that the predicted targets of LWDH were highly connected in a protein-protein interaction (PPI) network, and LWDH treated diseases through both shared and separated biological molecules and processes via different groups of compounds. Finally, the effects of seven compounds belonging to different chemical groups were verified by the subsequent experiments.

With the success of network-based active ingredient identification, Li et al (2014) also revealed that GGQLD could regulate key biological processes in T2D development, such as glucose homeostasis and response to an insulin stimulus, through network target analysis.

Wu-tou Decoction (WTD), which is prepared from a basic formula of five Chinese herbs−*Aconiti Radix* (Wu Tou), *Ephedrae Herba* (Ma Huang), *Astragali Radix* (Huang Qi), *Paeoniae Radix Alba* (Bai Shao), and *Glycyrrhizae Radix* (Gan Cao)−was originally recorded in *Synopsis of Golden Chamber* (Chinese name: Jin Gui Yao Lue) written by the Chinese medical sage Zhong-jing Zhang. WTD has been extensively used for the treatment of RA, constitutional hypotension, and hemicrania. Zhang et al (2015) constructed the interaction network of the putative targets of WTD and known RA-related targets. The authors selected hub nodes based on the values of four topological features, including degree, node betweenness, closeness, and k-coreness. Following functional analyses and experimental validations *in vitro* and *in vivo*, the authors indicated that WTD might attenuate RA partially by restoring the balance of the

nervous, endocrine, and immune (NEI) systems and subsequently reversing the pathological events during RA progression.

Qishen Yiqi (QSYQ), composed of *Astragalus Membranaceus Radix*, *Salvia Miltiorrhiza Radix*, *Notoginseng Radix et Rhizoma* and *Dalbergiae Odoriferae Lignum*, is a Chinese medicine prescription for treating ischemic heart disease. The QSYQ Dropping Pill was approved by the China Food and Drug Administration in 2003. Wu et al (2014) constructed an acute myocardial ischemia (AMI) specific organism disturbed network by integrating data of disease-associated genes, protein-protein interactions, and microarray experiments. Then, the "Network Recovery Index" for the Organism Disturbed Network (NRI-ODN) was developed to assess the therapeutic efficacy of QSYQ and its ingredients. As a result, the authors quantified the network recovery ability of QSYQ and its component herbs by NRI-ODN and clarified the synergistic effects of QSYQ on treating AMI. Among four component herbs, *Astragalus Membranaceus Radix* and *Salvia Miltiorrhiza Radix* showed a stronger recovery ability than *Notoginseng Radix et Rhizoma* and *Dalbergiae Odoriferae Lignum*, which also conformed to the "Jun-Chen-Zuo-Shi" rule of QSYQ.

Gansui Banxia Tang (GSBXT), which consists of five ingredients−*Kansui Radix* (Gansui), *Pinelliae Rhizoma* (Banxia), *Glycyrrhizae Radix* (Gancao), *Paeoniae Alba Radix* (Baishao) and *Mel* (Fengmi)−is a popular complementary and alternative medicine modality for treating hepatocellular carcinoma (HCC). To investigate the therapeutic effects and the pharmacological mechanisms of GSBXT on reversing an HCC imbalanced network, Zhang et al (Zhang et al, 2014) developed a comprehensive systems approach to integrate disease-specific and drug-specific networks. The authors revealed the relationships of the ingredients in GSBXT with their putative targets and with HCC-relevant molecules and HCC-related pathway systems.

The Fuzheng Huayu Tablet (FZHYT), which contains herbs such as *Salvia Miltiorrhiza Radix* (Danshen Injection), *Cordyceps*, and *Persicae Semen*, has been used to treat hepatitis B-caused liver cirrhosis (HBC). Chen et al (2015) integrated microRNA expression profiling and network target analysis to infer that FZHYT might play a critical function in the HBC treatment process and directly regulate many important pathways, including but not limited to the cell cycle, the p53 signaling pathway, and the TGF-β signaling pathway.

These network-based investigations provide more insight into a better understanding of the pharmacological mechanisms of Chinese herbal formulae and may also offer a powerful means for the further exploration of the chemical and pharmacological bases of TCMs.

5. Challenges and perspectives

Growing evidence shows that TCM network pharmacology approaches offer a novel research paradigm for translating TCM from an experience-based medicine into an evidence-based medicine system and provide new insights into the therapeutic basis of CHM, which will accelerate TCM drug discovery as well. However, there are considerable challenges ahead in achieving the development of TCM network pharmacology.

First, determining how to use a "network" as a mathematical and computable means to illustrate various connections between herbal formulae and diseases, particularly in complex biological systems, is a principal problem that researchers should address with as soon as possible.

Second, with the dawn of the big-data era and the rapid progress of multiple "omics" technologies (including genomics, transcriptomics, proteomics, and metabolomics), determining how to establish an efficient network-based platform to screen new drug targets and disease-related genes and to optimize Chinese herbal formulae by integrating "omics" data, disease/drug-specific networks and experimental validations is a challenge.

Third, Chinese herbal formulae, usually as oral administration, may go through a series of drug metabolism including absorption, distribution, metabolism, and excretion (ADME) process, and then exert the corresponding pharmacological effects following reaching target tissues (Yu et al, 2012). Since pharmacokinetics is characterized as "what the body does to the drug" and pharmacodynamics refers to "what the drug does to body", it is necessary to integrate TCM network pharmacology with pharmaco-kinetics and pharmacodynamics.

Forth, TCM network pharmacology highlights a "network target, multicomponent therapeutics" approach (Li et al, 2011). According to this approach, a network may be identified as the therapeutic target of Chinese herbal formulae. And a network-targeted effect switch could explain the new mechanism associated with herb medicine (Li, 2015). Further, it is more important to develop novel and efficient algorithms to evaluate the integrative effects within a comprehensive network target.

Finally, network pharmacology-based approaches must integrate a large amount of database information, as well as clinical and experimental data. However, there exist limitations in the insufficient accumulation and poor quality of TCM-related data, the heterogeneity, and the non-reproducible nature of the existing data. Researchers should strengthen the construction of TCM bioinformatics and chemical information databases and improve their applications. Moreover, it is necessary to conduct interdisciplinary research by combining efforts from biology, biomedicine, clinics, bioinformatics, and pharmacology for the sustainable development of TCM network pharmacology.

In summary, although TCM network pharmacology is still in its infancy, such great advancements in this field will undoubtedly bring about a conceptual change in drug discovery and make an important contribution to the modernization and globalization of TCM.

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