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Review

Network Pharmacology for Traditional Chinese Medicine Research: Methodologies and Applications

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ABSTRACT

The inception of network pharmacology comes from the advance in "multi-target, multi-drug" paradigm and opens up a new field for pharmaceutical science. Traditional Chinese medicine (TCM) is well-known for its use of medicinal herb combinations to treat the functional disorders induced by diseases through a holistic view, which naturally followed the principal of network pharmacology. In this review, the methodologies of network pharmacology in TCM studies were summarized. Specifically, the methodologies for network construction and network analysis were detailed by following three TCM study cases. The perspectives for TCM network pharmacology were also provided.

Key words

network pharmacology; network toxicology; traditional Chinese medicine

1. Introduction

Traditional Chinese medicine (TCM) was derived from the accumulation of empirical knowledge from thousands of years of clinical practice. Unlike clinical practice in Western medicine nowadays, TCM emphasized to balance out the functional disorders induced by diseases and treat them with a synergetic combination of herbs, and therefore provided a more systematic effect on diseases (Wang et al, 2011). Bridging TCM theory with molecular biology and explaining the combination principles of TCM formula might provide us a unique view for disease progress and drug development (Bai et al, 2014). From the perspective of modern medical science, TCM formula militates against diseases through the strength of integrated pharmacological effects from multiple components that target complex disease networks, which leads to the recovery from a distorted equilibrium of biological systems. In this sense, complexity of TCM in chemical composition and molecular mechanisms offers the unique advantages in complex therapeutics, which targets multiple cellular processes simultaneously, being suggested as a valid approach for treating complex diseases (Iyengar, 2013; Liu, 2014).

Western medicine flourished with many clinically effective drugs discovered by following the paradigm of "one target, one drug". However, reports suggested that new drug approvals decreased in recent years while the investment in drug development significantly increased (Pammolli et al, 2011), indicating the increased difficulty associated with this approach. With the growing understanding of complex diseases, drug discovery gradually shifted to a new "multi-target

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multi-drug" paradigm, with the purpose at systemically modulating multiple targets in complex diseases.

The inception of network pharmacology (Hopkins, 2007) comes from the advance in the new paradigm and opens up a new field for designing combination drugs. TCM formula, the combination system of herbs, naturally followed the principal of network pharmacology. With the advances in systems biology and bioinformatics, network pharmacology of TCM has attracted the attention of researchers (Fan et al, 2011; Wang et al, 2012).

In this review, we summarized the methodologies of network pharmacology in TCM studies, referred later as TCM network pharmacology, which can be generally divided into network construction and network analysis. We detailed the methodologies for network construction and network analysis by following three TCM network pharmacology case studies and the perspectives.

2. Methodologies of TCM network pharmacology

Figure 1 illustrates the basic flowchart of the methodologies of TCM network pharmacology, including network construction and network analysis. Network construction focuses on the approaches to discover the relationships among network components, while network analysis highlights the search for making the relationships/rules hidden from the established network.

The biological entities of network studies can be gene, protein, or even TCM syndromes. With available nodes in the network, there are several approaches to define the edges for constructing a network as discussed below. Meanwhile, the network analysis approaches have demonstrated their usefulness in understanding molecular mechanisms of TCM for their therapeutic or toxicological effects. For example, topology-based methods and module analysis have been applied in the network analysis of TCM to help understand cellular targets, pathways, and biological processes affected by TCM. Furthermore, network pharmacology helps to explain the relationship between TCM formulae and diseases or syndromes, which can lead to rational design of TCM combinations and the possible co-medication with synthetic pharmaceuticals.

2.1 Network construction

Networks are composed of vertex and edge. Normally, the vertex, or so called node, is the investigated subject or entity. Network construction refers to the process of applying algorithms to establish the linkages (edges) between vertices in line with specific research purposes. A network can be homogenous or heterogeneous depending on the components that make up the vertices of the network. The homogeneous network is more popular, such as protein-protein interaction (PPI) or drug-drug likeness network. The heterogeneous networks have different subsets of vertices which have no connection within the vertices subset. A network with k types

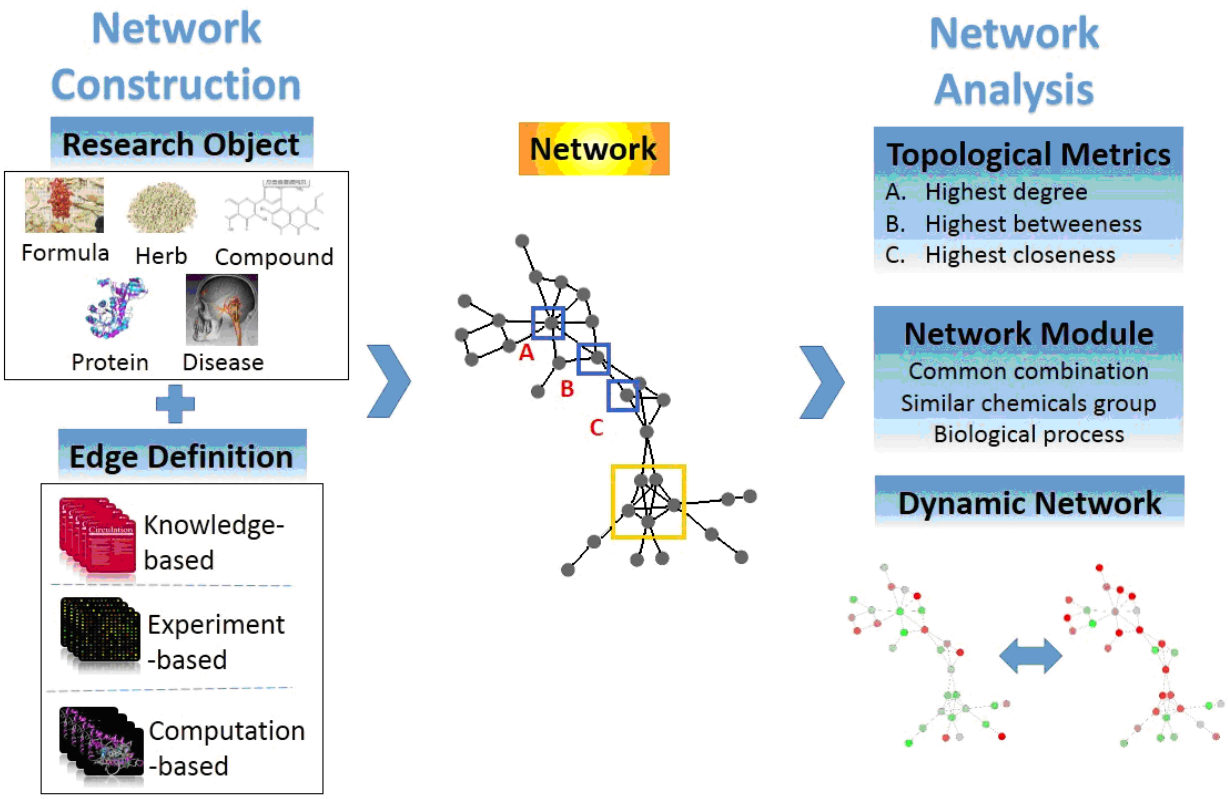

Figure 1 Framework of TCM network pharmacology

Nodes in blue boxes corresponding to the properties in topological metrics; Nodes in yellow box represent a possible module in the network based on the high connectivity; Red, green, and grey nodes within networks represent the up-regulated, down-regulated, and unchanged nodes, respectively

of vertices is named k-partite network, of which the most common is bipartite (e.g. drug-target network). Furthermore, these two types of networks can be combined to generate complex networks that have edges between and within the subsets of vertices.

Defining edge (vertices connection) is important in network construction. Network construction strategies can be grouped based on their data sources: knowledge-based, experiment-based, and computation-based.

2.1.1 Knowledge-based strategy

In the knowledge-based strategy, vertices are connected based on pre-existing knowledge. Literature mining and database integration, such as relation learning or nature language process, have been well employed to knowledge curation (Yandell and Majoros, 2002; Bunescu et al, 2005).

TCM syndrome is a disease classification system used to describe the disorders in a patient based on the TCM theoretical framework. It is therefore important to understand the pathogenesis of the disease. However, these syndromes were recorded in the documents written in Chinese, which differed from English in language structure and therefore became a great challenge for data mining. Zhou et al (2007) developed a character-based literature mining method (bubble-bootstrapping) in order to detect the disease characters of syndromes from TCM related literatures. This method and a co-occurrence frequency weight formula were applied together to inferring the syndrome-disease relations. Using the disease-gene relations retrieved from MEDLINE, a weighted gene network was constructed to study the relationship between TCM syndromes and corresponding genes. A TCM syndrome example, kidney-*yang*-deficiency, was used to demonstrate this network-based approach to identify a list of genes that were possibly involved in this syndrome (Zhou et al, 2007).

Therapeutic efficacy of TCM formulae is rooted in their complexity of chemical composition and molecular mechanisms. Integration of multiple databases to construct a disease-centric network for a TCM formula is an effective way to gain comprehensive understanding of their biological effects. A PPI network for rheumatoid arthritis was derived from OMIM (Hamosh et al, 2005), GAD (Becker et al, 2004), KEGG (Ogata et al, 1999), DrugBank (Wishart et al, 2006), HIT (Ye et al, 2011), and STRING (Jensen et al, 2009), and was built to study a well-known formula for this disease. This network included genes, drugs, and their targets, as well as compounds from the formula and their corresponding targets. This network was helpful in discovering proteins and active ingredients responsible for anti-rheumatic activity of this formula, and their findings were verified by published studies (Fang et al, 2013).

The knowledge-based strategy can be used for pragmatic network modeling, ranging from homogenous and heterogeneous networks to complex network modeling (Li et al, 2014). Types of heterogeneous networks include chemical-target network (Fang et al, 2013; Pei et al, 2013; Wang et al, 2013a; Xue et al, 2013; Zhang et al, 2013b) and target-disease network (Li et al, 2014) while homogeneous network modeling includes proteins (Chen et al, 2012; Liang et al, 2013; Sun et al, 2013) and Chinese drugs network (Long et al, 2013). The network is constructed by relatively reliable data from published studies. On the other hand, its applications were usually limited by the information available.

2.1.2 Experiment-based strategy

In the experiment-based strategy, the connection between network entities was established based on the correlation discovered from experimental data. This strategy needs high-throughput data, and therefore omics technologies such as microarray, proteomics, and metabonomics were widely used for this propose (Califano et al, 2012; Lu et al, 2012; Li et al, 2013).

Our group recently proposed a case study of Qishen Yiqi formula (Li et al, 2014), a widely used Chinese medicine formula in China for treating cardiovascular diseases. We identified the differentially expressed genes of microarray assays on Qishen Yiqi formula used for treating the myocardial infarction of rats by a cardiovascular disease related database (Wu et al, 2013) and constructed a compound-target-pathway network. Under the guide of network analysis, we successfully validated the effects of several components of Qishen Yiqi formula on targets. Another example carried out by Yue et al (2008) was a proteomic approach-based study on ganoderic acid D, a main compound from *Ganoderma lucidum*. They used MALDI-TOF MS/MS to identify 21 differentially expressed proteins in ganoderic acid D-treated human cervical carcinoma cell line, by which a minimal network extracted from a PPI network with proteins by one step expansion was constructed. Further analysis suggested that the 14-3-3 protein might play an important role in the cytotoxicity mechanism of ganoderic acid D, which partially explained the anticancer effect of *G. lucidum*.

Experiment-based strategies can be used to discover new biomarkers or previously unknown regulatory pathways for TCM. However, it sometimes failed to provide reliable and repeatable conclusion partly due to the noise in the data generated from these high-throughput or omics technologies.

2.1.3 Computation-based strategy

Computation-based strategy involves predictive algorithms to generate the links between vertices. Many methodologies have been applied, including molecular docking (Li et al, 2012; Su et al, 2013; Zheng et al, 2013; Shi et al, 2014), machine learning methods (Li et al, 2012; Tao et al, 2013; Wang et al, 2013b; Xu et al, 2013; Yao et al, 2013), and similarity-based comparison (Xu et al, 2013).

Qi-enriching and blood-tonifying herbs are two groups of widely used Chinese herb medicine. Zhou et al (2013) used these two groups of herbs as a case study for the elucidation of how TCM restores balance and harmony in patients. They built a compound-target network in which interactions were predicted by a combination method of docking, support vector machine, and random forest (Zhou et al, 2013). Functional

analysis on the network showed that *qi*-enriching herbs had a potential effect on enhancing physical strength and immune system, while blood-tonifying herbs might improve hematopoiesis function (Liu et al, 2013).

Many approaches have been applied for the similarity comparison. For instance, Keiser et al (2007) utilized a ligand set similarity algorithm to predict couples of unexpected drug-protein interactions. These predictions were experimentally confirmed in subsequent studies (Keiser et al, 2007). Wu et al (2013) developed an ensemble approach which calculated drug similarity based on the anatomical therapeutic chemical classes.

The results from several computation-based studies have been validated (Wu et al, 2008; Yue et al, 2008; Sirota et al, 2011; Wu et al, 2011). Considering the uncertainty of the data generated, the computation-based approach is normally regarded as a complementary option to the experimental or knowledge-based strategies for its advantages of little cost and high throughput.

2.2 Network analysis

Network analysis can lead to the identification of novel biomarkers and discrimination of new functional network modules. Three types of methods are summarized in Table 1, including topological metrics analysis, module analysis, and dynamic network analysis.

Table 1 Methodologies of network pharmacology for TCM research

Network modeling	Methodologies	Summary	Recommended references
network construction	knowledge-based	more reliable information by utilizing the existed knowledge	Zhou et al, 2007; Fang et al, 2013
	experiment-based	an explorative method for discovering new information	Yue et al, 2008; Wang et al, 2013
	computation-based	low cost and high throughput method as a complementary method	Liu et al, 2013; Xu et al, 2013
network analysis	topological metrics analysis	quantitative description for the property of a network	Zhang et al, 2014
	module analysis	identification and classification of resemblance vertices	Li et al, 2010; Song et al, 2013
	dynamic network analysis	display difference between diverse states of network	Taylor et al, 2009; Wu et al, 2013

2.2.1 Topological metrics analysis

Network topological metrics analysis focuses on the topological characteristics of a network or its components. In topological metrics analysis, node centrality is a widely used measurement with three main metrics: degree, closeness, and betweeness. The degree of a node is defined by the number of connected edges. In a directed network, in-degree and out-degree represent the number of adjacent head and tail endpoints, respectively. Generally, a hub is a high degree node in a network. Closeness is related to the position of nodes in a network with the boundary nodes having small closeness. The closeness of a node equals the inverse of the total sum of all the shortest paths to every other node. The betweenness of a node represents the frequency of the given node's participation in all shortest paths. A node with high betweenness might suggest a primary role in information diffusion.

Gansui Banxia Decoction (GBD) is a classic TCM formula to treat hepatocellular carcinoma. Zhang et al (2014) developed a systematic approach to investigate molecular mechanisms of GBD. Protein-protein interactions and predictive relationships between ingredients in GBD and their targets were used to construct the network. Through network topological metrics analysis, Zhang et al (2014) discovered that GBD targeted genes showed higher node centrality in the hepatocellular carcinoma related gene network compared with the median centrality among all the nodes. Further experiments validated the predicted compound-target relations, and the possible mechanism of GBD on hepatocellular carcinoma was suggested based on the results derived from the network-based approach.

Network topological metrics analysis can lead to identifying previously unknown information by calculating the properties of a network, yet the realistic meaning of these properties remains unclear (Wu et al, 2011). For instance, studies showed that only a small fraction of disease related genes had large degree or connection to hubs (Goh et al, 2007).

2.2.2 Module analysis

The sub-networks, also regarded as a network module or community, are groups of vertices that may have resemblance roles in network (Fortunato, 2010). Unlike high targeting synthetic pharmaceutical drugs, a TCM formula contains dozens even hundreds of chemicals that impact a set of targets and biological processes. It is a challenging task to reveal potential mechanisms of TCM. Song et al (2013) predicted chemical-target interactions and information from multiple database to construct a complex network of TCM compounds and proteins for Shufeng Jiedu Formula, a TCM formula against influenza infections. Then they applied a well-known module detection method, Girvan-Newman algorithm (Girvan and Newman, 2002), to identify four distinct modules enriched with functional pathways. Results suggested that the anti-influenza effect of Shufeng Jiedu Formula might relate to the combinational effects of ingredients on EGFR/HER2 signaling pathway, PTEN/AKT pathway, and others.

Synergistic effect is a unique strength of TCM formulae which are built up on the Chinese *yin-yang* and five-element theories but are difficult to be understood by the clinicians in Western medicine practice. In a case study of Liuwei Dihuang Formula, Li et al (2010) performed a "co-module" analysis based on the modules in disease PPI network. They

found that the average shortest path was significantly smaller between Liuwei Dihuang targeting genes and indication genes than the former and random disease gene sets. Based on this finding, they predicted possible drug pairs with synergistic effects on targets and consequently demonstrated it in an experimental study.

2.2.3 Dynamic network analysis

Dynamic network analysis is committed to investigating the similarities and differences between dynamic states of bio-systems. It was limited to the applications in quantitative data and ordinarily omics data, for the purpose of mapping a network with immobile structure.

Taylor et al (2009) constructed a network of breast cancer related genes by literature mining and then mapped the microarray data of patients with different prognosis. The results from dynamic network analysis showed that some genes were not significantly differentiated in expression between patient groups, while their correlation coefficient of expression with other genes changed instead. These findings partly explained the reason why "signature gene" set failed in disease prediction (Ein-Dor et al, 2005). The correlation coefficients offered possible indicators for cancer prognosis. Currently, there are still very few TCM studies applied to dynamic network analysis, although it has been widely used in other areas (Elo et al, 2013; Anglani et al, 2014).

3. Application cases

Utilizing a systemic concept, network pharmacology is considered as a potential approach to bridging TCM and modern sciences. To demonstrate this concept, we selected three case studies as below.

3.1 Rational TCM formula design

Li et al (2013) developed an integrated platform for rationally designing combination drugs. First, they proposed a concept of "network target" (Li et al, 2013) instead of "multiple target" (Normile, 2003), which represented not a list of targets but a network that provided quantitative measure to the effects of drug combinations. A case study of anti-rheumatoid arthritis formula Qingluo Yin was carried out, and a multilayer drug-gene-disease network was constructed by combining the three types of aforementioned strategies. Next, the co-module analysis was performed on the network to detect the combination rules of the formula and the synergistic ingredients. Finally, they found that toxicity was reduced, and the effects between ingredients and their targeting biological processes were enhanced. These findings were independently confirmed by literature and experiments.

3.2 Efficacy evaluation of TCM formula

The efficacy evaluation of TCM formula is difficult because it contains a large number of ingredients with complex effects in biological pathways and mechanisms. A network pharmacology approach was proposed to assess the efficacy of Shenmai Injection composed of *Ginseng Radix* et *Rhizoma Rubra* (red ginseng) and *Ophiopogonis Radix* (Wu et al, 2013). Five sets of genome-wide transcriptomic data (i.e. control, disease, red ginseng treatment, *Ophiopogonis Radix* treatment, and Shenmai Injection treatment) were used to construct a biological network using the pathways enriched by the differentially expressed genes. A network recovery index was proposed to quantitatively evaluate the recovery effect derived from the treatment of Shenmai Injection and herbs. The results indicated that the network recovery indexes in red ginseng and *Ophiopogonis Radix* treatment groups were significantly lower than those in the Shenmai Injection group, suggesting that the combination of red ginseng and *Ophiopogonis Radix* (i.e. Shenmai Injection) had a synergic treatment effect. The findings were subsequently validated by echocardiography experiments.

3.3 Dissection of active ingredients

It is well-known that the efficacy of TCM formula is not necessarily represented by the activity of a single ingredient, but instead, by the interaction among a set of ingredients. Wang et al (2014) presented a case study to demonstrate the dissection of active ingredients of TCM utilizing the network approach. Using Xuesaitong (a TCM formula used for treating cardiovascular diseases) as an example, they constructed a knowledge-based network of cardiovascular disease. They determined the saponins in Xuesaitong and carried out the microarray experiments on Xuesaitong for treating the myocardial infarction of rats (Wang et al, 2013c) to identify differentially expressed genes within the cardiovascular disease network and acquired compound-target interactions from literature mining. Taking into consideration, the importance of genes within the cardiovascular disease network and ingredient content of Xuesaitong, Wang et al (2014) designed a degree-based network algorithm for assessing efficacy and identified a combination of five ingredients by a set point of 95% whole formula efficacy. The efficacy of this combination of five ingredients was proven to share the similar effects in myocardial infarction treatment with Xuesaitong Formula *in vivo* as demonstrated in the subsequent experiments.

3.4 Therapeutic mechanism of TCM

The relationship between TCM and disease/TCM syndrome can be transferred into a network context. Understanding disease-specific molecular network is the key to understand the therapeutic mechanism of TCM (Zhang et al, 2013b). Zhang et al (2013b) analyzed the published papers and presented that the network target construction, target prediction, and drug-gene-disease co-module analysis were very important for the study on therapeutic mechanism of TCM: (1) construction of disease-specific network as therapeutic target and construction of disease-specific networks in the molecular level or pathway-pathway

interaction level by combined knowledge and high throughput omics data; (2) target prediction and extraction of herbal formulae treating specific diseases; (3) co-module analysis based on the network target such as drug-gene-disease co-module analysis between ingredient targets from herbal formulae and disease-specific network target and network target-based computational screening of active ingredients and synergistic therapeutic combinations.

3.5 Network toxicology for TCM safety studies

Network toxicology is another intriguing field for TCM research (Fan et al, 2011). The relationship between toxins and toxic targets is complicated (Yabuuchi et al, 2011). In network toxicology, adverse outcome in human and toxicological mechanisms of medications can be investigated through modeling the complex relationships among adverse reactions, targets, and chemical entities (Brouwers et al, 2011; Zhou et al, 2013). Specifically, the knowledge about the chemical entities, genes, proteins, toxicological endpoints, and adverse reactions needed to be collected from literature, public database, and experiments. A network can be constructed based on comprehensive relationships among the nodes (e.g. genes, proteins, toxicological endpoints, and adverse reactions), and network analysis will help to infer the unknown relationships among the interesting nodes (e.g. active ingredients and targets). Notably, the advance of systems toxicology offered unprecedented opportunities to provide high throughout omics data to enhance the construction of network among adverse reactions, genes, targets, and chemical entities. For the TCM studies, the network modeling can be utilized to identify the active toxic ingredients in herbs, to understand the toxicological mechanisms, and to predict the potential adverse outcomes in human and contradiction of herb combinations (e.g. TCM pairs “18 antagonisms and 19 mutual inhibitors”) (Long et al, 2013). Overall, network toxicology can be a promising tool that can provide the scientific evidences to support the safety evaluation of TCM.

4. Limitations and challenges of TCM network pharmacology

The inception of TCM network pharmacology provides us a new opportunity to get a systematic insight of TCM. The current studies have proven their potential to add value for TCM researches, and it will lead to a new avenue for the studies of therapeutic mechanism and safety evaluation of TCM.

However, due to the complexity of TCM in chemical composition and molecular mechanisms, very few study cases directly demonstrated the relationship between multi-targets and multi-ingredients in a herbal formula by utilizing the network pharmacology methodologies. Meanwhile, the network pharmacology can help to generate the hypothesis which needs to do further experimental validation. Unfortunately, no reported study case has experimentally demonstrated the causality relationship between the efficacy or toxicity and active ingredients in a herbal formula that was generated from network modeling. Although the target-target and target-disease relationships are accumulated in literature, the relationships between targets and active ingredients of herbs are almost blank, which largely impedes the application of network pharmacology in TCM.

As shown in Table 2, the researches in TCM network pharmacology are growing fast and have covered almost every related topic, even though some challenges still need attention. First, network pharmacology required massive data availability; However, the current TCM related data are obviously insufficient

Table 2 TCM related databases

Annotation	Database names	Description	Address
S	TCM database@taiwan (Chen, 2011)	The largest small molecular database on TCM which provides more than 60 000 TCM compounds with 2D and 3D structures.	http://tcm.cmu.edu.tw/
C, B	TCMGeneDIT (Fang et al, 2008)	The data mining from biomedical literature, this database provides associative information about TCM formulae, genes, diseases, formula effects, and TCM ingredients.	http://tcm.lifescience.ntu.edu.tw/
S, C, B	TCM-ID (Ji et al, 2006)	The data obtained from relevant books and journals, which consist of 1197 TCM formulae covering 4111 disease, 1104 TCM herbs, and 9862 ingredients. Molecular 3D structures are partially provided.	http://tcm.cz3.nus.edu.sg/group/tcm-id/tcmid.asp
C, B	TCMID (Xue et al, 2013)	The results from combinations of database integrations and text mining, which consist of 8159 TCM herbs, 25 210 ingredients, 6826 drugs, 17 521 targets, 46 914 TCM formulae, and 3791 diseases.	http://www.megabionet.org/tcmid/
S, C, B	TCMSP (Ru et al, 2014)	TCM systems pharmacology database contains data extracted from databases and a few computation results. It consists of 29 384 ingredients, 3311 targets, and 837 associated diseases based on 499 Chinese herbs in *Chinese Pharmacopoeia*. It also provides pharmacokinetics properties of compounds along with structures and constructed networks made up of compounds, targets, and diseases. Part of the compound-target interactions are prediction.	http://sm.nwsuaf.edu.cn/lsp/tcmsp.php
C, B	TCM-PTD	The predictive relation between TCM ingredients and targets with 490 TCM herbs, 12 629 ingredients, 1354 targets, and 1241 FDA approved chemical drugs.	http://tcm.zju.edu.cn/ptd

S: structure data; C: TCM formulae, herbs, and compounds; B: biological information including genes, proteins, targets, and diseases

in scale and scope, especially in the area of high quality omics data. Therefore, the accumulation of TCM data still has a long way to go in the near future. Second, due to the redundancy and uncertainty of bionetworks, algorithms specifically designed for biological problems, especially for TCM, are still significantly lacking (Ding et al, 2012; Yang et al, 2014). Finally, most of the current studies focus on explaining the mechanism of mode-of-action or the synergic effects of ingredients and toxicity of TCM was largely an ignored field in which the network pharmacology has potential to play an important role.

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