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Traditional Chinese medicine network pharmacology: theory, methodology and application

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[ABSTRACT] Traditional Chinese medicine (TCM) has a long history of viewing an individual or patient as a system with different statuses, and has accumulated numerous herbal formulae. The holistic philosophy of TCM shares much with the key ideas of emerging network pharmacology and network biology, and meets the requirements of overcoming complex diseases, such as cancer, in a systematic manner. To discover TCM from a systems perspective and at the molecular level, a novel TCM network pharmacology approach was established by updating the research paradigm from the current "one target, one drug" mode to a new "network target, multi-components" mode. Subsequently, a set of TCM network pharmacology methods were created to prioritize disease-associated genes, to predict the target profiles and pharmacological actions of herbal compounds, to reveal drug-gene-disease co-module associations, to screen synergistic multi-compounds from herbal formulae in a high-throughput manner, and to interpret the combinatorial rules and network regulation effects of herbal formulae. The effectiveness of the network-based methods was demonstrated for the discovery of bioactive compounds and for the elucidation of the mechanisms of action of herbal formulae, such as Qing-Luo-Yin and the Liu-Wei-Di-Huang pill. The studies suggest that the TCM network pharmacology approach provides a new research paradigm for translating TCM from an experience-based medicine to an evidence-based medicine system, which will accelerate TCM drug discovery, and also improve current drug discovery strategies.

[KEY WORDS] Traditional Chinese medicine; Network pharmacology; Network target; Theory; Methodology; Application

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1 TCM Network Pharmacology: An Emerging Subject

Traditional Chinese medicine (TCM) has developed over thousands of years and has accumulated abundant clinical experience, forming a comprehensive and unique medical system. The administration of TCM herbal formulae is a remarkable feature of treatment based on Syndrome (*ZHENG* in Chinese) differentiation, as well as holistic thinking in TCM theory. Recently, TCM has excited worldwide interest [1-4]. However, understanding the scientific basis of TCM herbal formulae at the molecular level and from a systems perspective is still one of great challenges for evidence-based TCM [3-4]. The recent application of cutting-edge technologies in analytical chemistry and chemical biology to characterize commonly used herbs or herbal formulae has provided the means to identify the active ingredients in TCMs and their biological targets [5-11]. Indeed, it is likely that the investigation of the molecular basis of herbal formulae will increase the acceptance of TCM worldwide [12]. Such efforts have facilitated the identification of the main active ingredients and synergistic ingredient pairs and, in some cases, have led to drug discoveries based on TCM. However, as a large number of ingredients are included in TCM herbs or herbal formulae, and many molecular changes are involved in diseases and TCM Syndromes, the combinatorial rules and roles of most herbal formulae in complex diseases remain to be elucidated. Although some studies have successfully reported the extraction of a single active ingredient from an herb or herbal formula, and the identification of its biological activities and targets, a better understanding of how the multiple ingredients in an herbal formula act in synergy, and what effect they can have on

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multiple targets of a disease, is both biologically and clinically important for modern studies of TCM [12].

With the rapid progress of bioinformatics, systems biology and polypharmacology, network-based drug discovery is considered a promising approach toward more cost-effective drug development [13-16]. In TCM, the perspective of holism has long been central to herbal treatments for various diseases. Characterized by holistic theory, and a rich experience in multicomponent therapeutics, TCM herbal formulae offer bright perspectives for treating complex diseases in a systematic manner. Thus, bridging the emerging network science and ancient TCM will provide novel methodologies and opportunities for discovering bioactive ingredients and biomarkers, potentially revealing mechanisms of action, and exploring the scientific evidence of herbal formulae on the basis of complex biological systems. As a beginning of the "TCM network pharmacology", Li proposed that there was a possible relationship between TCM Syndrome and molecular networks in 1999 [17], and then in 2007 he established a network-based TCM research strategy [18] and conducted a network study for Cold/Hot Syndromes and Hot/Cold herbal formulae [19]. Subsequently, Li updated the TCM research framework as an "Herb network-Biological network-Phenotype network" [20] and proposed a new concept of "Network target" [21]. A series of methods were also created by Li's laboratory to provide methodological support for TCM network pharmacology [22-44] (Table 1). At nearly the same time, the subject of "Network Pharmacology" was proposed in 2007 and 2008 [45-46], and it is rapidly becoming a cutting-edge research field in current drug studies and the next-generation mode of drug research.

Here, recent progress in this laboratory on the theory, methodology and application of TCM network pharmacology is reviewed to provide a reference for the modernization of TCM by combining computational and experimental efforts.

Table 1 Concepts, methods and databases created by LI Shao's laboratory in TCM network pharmacology

Category	Term	Description	Year	Ref.
Concepts	TCM network pharmacology	Hypothesis of the relationship between TCM Syndrome and molecular networks	1999	[17]
		Proposed a network-based TCM research framework related to TCM network pharmacology	2007	[18]
		A network-based case study on Cold/Hot herbal formulae and Hot/Cold Syndromes	2007	[19]
		Proposed the "Herb network-Biological network-Phenotype network"	2009	[20]
		Proposed the new concept of "Network target"	2011	[21, 22]
Methods	CIPHER	Network-based prediction for disease genes	2008	[24]
	drugCIPHER	Network-based prediction for drug (herbal ingredient) targets and functions	2010	[23, 35]
	comCIPHER	Drug–gene–disease co-module analysis	2012	[34]
	CIPHER-HIT	Modularity-based disease gene prediction	2011	[25]
	DMIM	Herb network construction and co-module analysis for herbal formulae	2010	[31]
	NADA	Network-based assessment for drug (herbal ingredient) action	2010	[32]
	NIMS	Network-based identification of multi- component synergy and drug (herbal ingredient) combinations	2011	[22, 36, 37]
	Drug combination model	A formal model for analyzing drug combination effects	2010	[33]
	LMMA	Disease-specific biomolecular network construction	2006	[26]
	CSPN	Disease-specific pathway network construction	2010	[27]
	ClustEx	Disease-specific responsive gene module identification	2010	[28]
Databases	HerbBioMap	A molecular data source for herbs and TCM phenotypes	2010	[40]
	dbNEI	A database for neuro-endocrine-immune interactions and drug-NEI-disease network	2006, 2008	[38, 39]

2 Network Target: A Key Concept of TCM Network Pharmacology

2.1 *Network: a computable representation of complex biological systems*

In TCM network pharmacology, a "network" is a mathematical and computable representation of various connections between herbal formulae and diseases, particularly in complex biological systems. Fig. 1 shows the basic network topological measures that allow for the characterization of different drug treatments from a network perspective. For instance, in the "Herb network-Biological network-Phenotype network" [20], a "node" denotes the following: (i) a gene, gene product or any biological entity in the biomolecular network including the protein-protein interaction network, gene regu-

latory network, genetic interaction network, metabolic network, and signaling network; (ii) an herb, herb ingredient or drug in the herb network; (iii) a clinical phenotype of a disease in the phenotype network. An "edge" is an association, interaction, or any other well-defined relationship. The "degree" of a node is the number of edges connected to it. The "betweenness" of a node is the number of shortest paths that go through a given node. The nodes with high centrality (e.g., network degree, modular structure, and betweenness) can be viewed as key nodes in a network. Network parameters such as degree, betweenness, shortest path and modules can be used to measure directly the targeted key druggable proteins or protein interactions, and indirectly the targeted key undruggable proteins by network propagation. The introduction of a "network" in drug discovery incorporates the assessment of network topology, as well as dynamics, and thus offers a quantifiable description of the complex biological system and its response to various drug/herbal treatments.

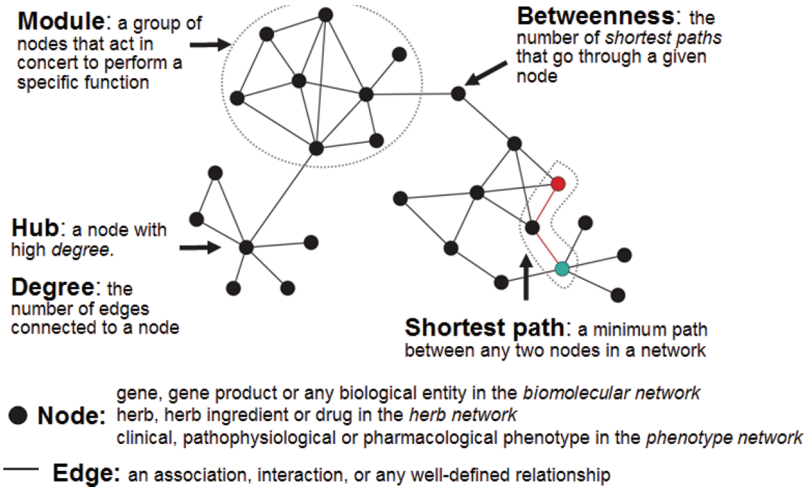

Fig. 1 Some basic measurements of network topological properties

2.2 *Paradigm shift from the "one target, one drug" to the "network target, multi-components"*

TCM network pharmacology highlights a paradigm shift from the current "one target, one drug" strategy to a novel version of the "network target, multi-components" strategy. As shown in Fig. 2, the new concept of "network target" was proposed by this laboratory as a core in the "Herb network-Biological network-Phenotype network". The "network target" is an attempt to treat a disease-specific biomolecular network as a therapeutic target to help design appropriate treatments [18-22]. Researchers have realized that the disruption of biomolecular networks can act as sensors and drivers of common human diseases [47-48]. Therefore, it was considered that the mechanism of action of a herbal formula is to adjust, not for single molecules, but for imbalances in the status of disease-specific networks, which refers to the network interaction and node activity or expression in a given disease context. Moreover, an herbal formula is a complicated chemical system involving a mixture of many types of chemical compounds, and the "Multiple targets" model is not sufficient to account for the combinatorial principle of Sovereign-Minister-Assistant-Envoy (*Jun-Chen-Zuo-Shi* in Chinese) of herbal formulae. Based on these concerns and related studies [18-44], it was proposed that the principles of herbal formulae are considered to act on the "Network target" of specific diseases. This concept attempts to comprehensively describe all of the possible vulnerable targets for clarifying the efficiency and toxicity of drug treatments, such as herbal formulae. It represents an evolutionary approach to the problems of the design and optimization of network-based multi-component therapeutics [21-22].

In the "Network target" theory, the establishment of molecular connections between drug/herbal formulae and diseases/TCM Syndromes is crucial. These molecular connections are derived from a disease-specific network, which can be formed from the interactions of genes or gene products, signaling pathways and the co-functions of biological processes. The network targets are those key components with important topological, dynamic and functional properties in a disease-specific molecular network. Moreover, as the efficiency and toxicity of drug treatments can be predicted by network-based approaches [21-23, 49], the network targets provide the means to decipher the mechanisms of the therapeutic effects of drugs, or TCM herbal formulae, as well as the means to understand their possible toxicity and unknown pharmacological activities. As such, network targets have the potential to determine drug or TCM herbal formulae on-target and off-target effects that may serve as the basis for the rational design of drug combinations.

2.3 *Network target vs Multiple targets*

In comparison with the former concept of "multiple targets", several unique characteristics of network targets are important to bear in mind. First, a network target is defined in relation to a particular disease and, accordingly, each disease has its own unique network target. Second, the mechanisms of drugs/herbal formulae and diseases/TCM Syndromes

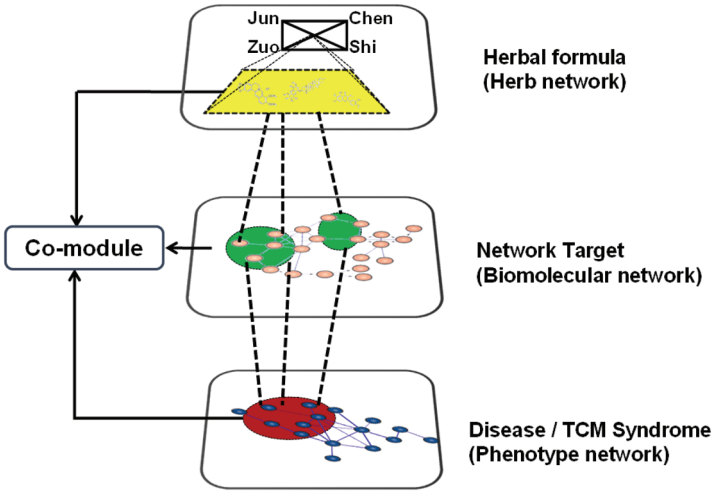

Fig. 2 The framework of TCM network pharmacology and the network target concept

should be simultaneously considered in the network target theory. A disease-specific network target may not be identical to other network targets, but it is likely to overlap with these other network targets, which means that a particular drug or herbal formula can be used to treat different diseases. Several drug-specific network targets can be implicated in a disease network, indicating that these drugs or herbal formulae can treat the same disease. Third, from a network perspective, the systematic modulation of diseases or TCM Syndromes is achieved by targeting specific network targets due to network propagation, although some components of such network targets are not druggable. Lastly, a network target can provide predictive and quantitative measures to the mechanistic role of drugs or herbal formulae in the treatment of diseases. These characteristics aid in the network target identification process, an important step in TCM network pharmacology.

3 Methodologies of TCM Network Pharmacology

Methodology is the mainstay of any new subject. Network pharmacology is a multidisciplinary research field that integrates a large amount of information to make new discoveries by combining both computational and experimental approaches. The computational approaches mainly include graph theory, statistical methods, data mining, modeling, and information visualization methods. The experimental approaches include various high-throughput omics technologies and biological and pharmacological experiments. As listed in Table 1 and illustrated in Fig. 3, a series of TCM network pharmacology methods were created, including the network-based prediction of disease genes [24-25], drug targets [23] and drug functions [22,23], the construction of disease-specific networks [26-29], the construction of herb networks [31], and a drug-gene-disease co-module quantitative analysis [31-34]. These methods and patented key procedures [35-37] and databases [38-40] have provided a solid platform for performing network target studies. Indeed, the promise of the network target approaches in drug discovery is best illustrated in the area of TCM. These approaches have continuously led to (i) the identification of active ingredients and synergistic ingredient pairs in TCM herbal formulae [22, 31-32, 41-42] and (ii) exploration of the network characteristics of the classic theory of TCM herbal formulae, such as Cold or Hot herb properties [19], and the combinatorial rules of '*Jun-Chen-Zuo-Shi*' [31]. Furthermore, these network characteristics can be exploited to predict clinical biomarkers of TCM herbal formulae and rationally design multi-component therapeutics.

3.1 *Network-based global prediction of disease genes and drug targets*

The realization of the full potential of network target approaches is dependent on identifying the genes and proteins related to diseases and TCM Syndromes, and the target profiles of the drugs and herbal ingredients. This will require the use of new technologies, including systems or network biology with associated computational approaches, because of the high cost of experimental biology methods, such as functional genomics and chemoproteomics, and their inability to perform whole-genome screening for diseases or drugs. Based on these concerns, the TCM holistic analogism has been employed to develop the comparative analysis methods of complex network systems. This strategy has allowed the successful prediction of disease-related genes and drug target profiles. Two methods were developed in our laboratories, CIPHER (Correlating protein Interaction network and PHEnotype network to pRedict disease genes) [24] and drugCIPHER (Correlating protein Interaction network and chemical/PHEnotype network to pRedict drug targets) [23]. They have demonstrated highly accurate and genome-wide inference capabilities for disease-related genes and drug targets, and were used for building disease-biomolecular networks and drug-biomolecular networks, respectively. To predict drug target profiles, drugCIPHER can be used to infer drug-target interactions on a genome-wide scale. Three linear regression models were proposed that relate drug therapeutic

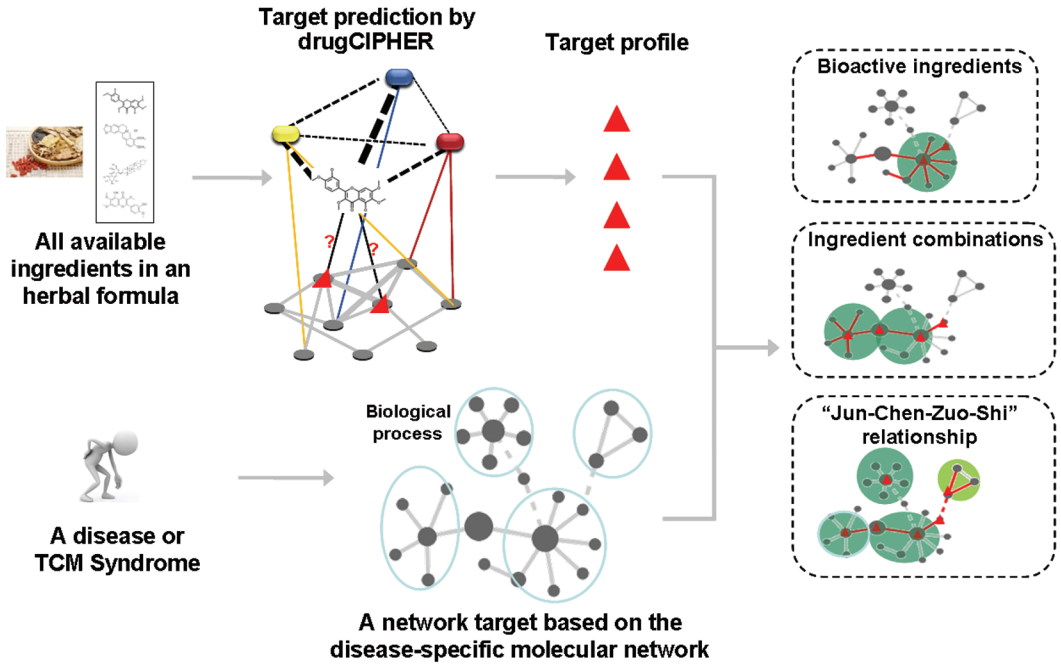

Fig. 3 A general schematic diagram of TCM network pharmacology in the discovery of an herbal formula

similarity, chemical similarity and their combination, respectively, to the relevance of the targets on the basis of a protein-protein interaction network. Based on drugCIPHER, a genome-wide map of drug biological fingerprints can be constructed with high accuracy. This work demonstrated that the integration of phenotypic and chemical indexes in pharmacological space and protein-protein interactions in genomic space can speed the genome-wide identification of drug targets, and also find new applications or side effects for existing drugs or herbal ingredients [23]. Another scoring system of CIPHER for disease-related gene prediction is based on a similar integrative model. The CIPHER method calculates the correlation between the closeness profile of the candidate genes in the PPI network and the similarity profile of the query phenotypes, and assigns a score to the candidate gene [24]. Recently, a modularity measurement was introduced into the algorithm framework of CIPHER to improve the prediction accuracy [25].

CIPHER and drugCIPHER are in accordance with the rationale of "like attracts like". The interactome and functional relationship networks can be integrated to reveal genes or proteins potentially involved in disease pathogenesis or drug mechanisms of action. Several characteristics are implicated in these methods. First, the relationships among numerous diseases or drugs are included instead of the investigation of only a single disease or drug. Second, the information of the interactome, and the knowledge of all the available known disease-gene or drug-target relationships can be integrated to determine the molecular basis of a given disease or drug (herbal compound). Lastly, such systematic approaches can be used for the large-scale prediction of diseases or drugs on a whole-genome level to obtain a comprehensive molecular understanding of diseases or drugs. These two methods provide the possible means to map diseases, TCM Syndromes, the drugs and the herbal formulae, into the biomolecular network.

3.2 *Construction of a disease- or TCM syndrome-specific biomolecular network*

As the functional interdependencies between the molecular components in a human cell are ubiquitous, a disease or TCM Syndrome is rarely a consequence of an abnormality in a single gene. Rather, it may reflect an imbalance of complex intracellular and intercellular network states that link tissue and organ systems [18, 20]. The inherent mechanisms of a disease or a TCM Syndrome can be characterized with the biomolecular network model. Based on the network resulting from CIPHER and drugCIPHER, the biomolecular network specific for a disease or TCM Syndrome can be further constructed by combining various types of datasets, including multi-level high-throughput omics data, biomedical literature, and the human interactome maps and databases. For example, through integrating these datasets, a construction strategy for a network of a particular disease or TCM Syndrome was proposed [26]. Various datasets have also been integrated to analyze and evaluate the network balance at the molecular and signaling pathway levels [27-30]. Integrative network construction and analysis strategies are applicable to cancer-related inflammation and angiogenesis [26-30], as well as Cold Syndrome and Hot Syndrome [19, 43-44].

3.3 *Drug-gene-disease co-module analysis based on a network target*

The drug-gene-disease co-module analysis based on a network target [31, 34, 39] is primarily focused on mapping disease phenotypes and herbal compounds into biomolecular

networks and then conducting qualitative and quantitative analyses of their molecular interactions. Such an analysis can help discover active ingredients and their synergistic combinations, elucidate the mechanisms of action of herbal formulae, and develop modern rational drug design strategies for TCM. The basic principle of the drug-gene-disease co-module analysis is that the interactions between diseases and drugs can be viewed as the interaction between disease-specific molecules and drug-targeting proteins in the network target. By calculating the node importance and the distance from the network target, one can gain a comprehensive insight into drug efficiency and toxicity. Meanwhile, this computational model also can identify the synergistic drug combinations of numerous therapeutic agents and allow the quantitative evaluation of synergistic effects and the better understanding of the molecular mechanisms of the observed combinatorial effects based on the network target. This novel method (network target-based identification of multicomponent synergy, or "NIMS") is a first-step computational approach toward the identification of synergistic drug combinations at the molecular level, and it has been used to select synergistic agent pairs from TCM herbal formulae for a pathological process demonstrated by angiogenesis [22]. Clearly, such an approach would reduce the search range and experimental cost for multicomponent therapeutics.

To understand the synergistic effects of drugs in a dose-response manner, a formal model for quantitatively analyzing drug combination effects was also proposed by simulating the kinetics of the key elements (e.g., a biochemical pathway) in the network target [33]. As an example in that study, a model of the TNF-NFκB pathway, using dose-response data for therapeutic agents targeting proteins in the pathway, identified synergistic combinations between certain agents, such as the IKK inhibitor PS-1145 and the HSP90 inhibitor geldanamycin [33]. This suggests that this approach can help identify reasonable targets for creating effective ingredient combinations from herbal formulae.

4 Applications of TCM Network Pharmacology

Powered by the above methodologies of network-target-based TCM network pharmacology [18-44], a novel strategy can be established to elaborate the combinatorial rules of TCM herbal formulae and discover active ingredients and synergistic ingredient pairs for TCM drug development, as illustrated in Fig. 3.

4.1 *Identification of active herbal ingredients and synergistic combinations*

One of the great challenges in the modernization of TCM is to identify the active herbal ingredients and ingredient pairs that produce the therapeutic effects or the adverse effects. The "prediction and discovery" of active ingredients or synergistic ingredient pairs in herbal formulae is recognized as a major goal of TCM network pharmacology. We selected a Xin-An medical family's anti-rheumatoid arthritis (RA) herbal formula "Qing-Luo-Yin" (QLY) [50] as an example. This formula consists of four herbs, Ku-Shen (*Sophora flavescens*), Qing-Feng-Teng (*Sinomenium acutum*), Huang-Bai (*Phellodendron chinensis*) and Bi-Xie (*Dioscorea collettii*). Following the steps shown in Fig. 3, some anti-angiogenic and anti-inflammatory active ingredients, such as kurarinone, matrine, sinomenine, berberine, and diosgenin, can be identified among the 235 ingredients of QLY by predicting each ingredient's target profile using drugCIPHER and performing a network target analysis [42]. Moreover, the synergistic effects of major ingredients, such as matrine and sinomenine, in QLY can be identified, and may be derived from the feedback loop and compensatory mechanisms by targeting the TNF- and VEGF-induced signaling pathways involved in rheumatoid arthritis [22, 32, 42]. Several ingredient groups such as saponins and alkaloids that act as active components in QLY were also identified [42].

To identify more herbs and their ingredients that are active in angiogenesis, the herbs in QLY were treated as seeds, and a method was established called the Distance-based Mutual Information Model (DMIM) to extend the herb pairs from 3685 collateral-related herbal formulae [31]. The DMIM approach, combining mutual information entropy and the '*Jun-Chen-Zuo-Shi*' relationship, offers a new approach for identifying herb networks, which in turn, can recommend candidate effective herb pairs with synergistic and antagonistic relationships [31]. From such an herb network, some novel angiogenesis inhibitors were discovered, such as vitexicarpin from Man-Jing-Zi (*Fructus viticis*) in an herbal co-module with Huang-bai in QLY [31]. Using target prediction and experimental validation, vitexicarpin was found to target key molecules (AKT and SRC) in the VEGF pathway to exert anti-angiogenic effects [41]. These efforts demonstrate the effectiveness of the network target approach in identifying active ingredients (as well as ingredients that caused side effects) and synergistic ingredient pairs from numerous compounds in a given formula.

4.2 *Understanding the combinatorial rules of TCM herbal formulae*

Because many pathological processes are involved in a complex disease, and because they can be organized into different functional modules in a disease-specific biomolecular network, it is reasonable to assume that the "*Jun-Chen-Zuo-Shi*" principle of herbal formulae can be explained by the actions of herbs on network-based functional modules. Given the ability to predict the target profiles of all available ingredients in an herbal formulae, it is possible to reveal the combinatorial rule of "*Jun-Chen-Zuo-Shi*"" based on the target interactions on the disease-specific molecular network. Moreover, the interactions of the target proteins of herbal ingredients may contribute to the "Emergence" of the comprehensive effects of TCM herbal formulae [21-22]. Therefore, it is promising to interpret the scientific basis and combinatorial rules of herbal formulae by the net-

work target analysis of herbal ingredients, as indicated by the example of QLY. According to the procedures in Fig. 3, the network target analysis for all available ingredients in QLY further suggests that Ku-Shen (*Sophora flavescens*; *Jun* herbs) acts on the targets enriched in RA-related pathological processes, such as inflammation, the immune response and angiogenesis. Qing-Feng-Teng (*Sinomenium acutum*; *Chen* herb), and Huang-Bai (*Phellodendron chinensis*) and Bi-Xie (*Dioscoreae collettii*; *Zuo-Shi* herbs) can augment or modulate the therapeutic effects of the *Jun* herb by targeting RA-related pathological processes, particularly, by synergistically acting on the compensatory pathway and feedback loop in the TNF/IL1B/VEGF-induced NFκB pathway [42]. These results address the possible molecular basis underlying the combinatorial rules and pharmacological activities of QLY.

4.3 *Elucidation of the Formula-Syndrome relationship*

A characteristic signature that distinguishes herbal formulae and multicomponent therapeutics is the clinical guidance of TCM Syndrome theory. The Formula-Syndrome relationship can be reflected by the rules of "the same treatment for different diseases" and "the same disease with different treatments" in TCM. Network target approaches have been applied to explore the mechanisms as well as biomarkers of the Formula-Syndrome relationship, and they greatly facilitate the mechanistic interpretation of herbal formulae and TCM Syndrome. For instance, the co-module analysis presented by the Liu-Wei-Di-Huang (LWDH) formula nicely illustrates "the same treatment for different diseases" [31]. This is demonstrated by a study that showed that multiple LWDH formula-treated diseases share a common network target associated with the neuro-endocrine-immune (NEI) pathways, as well as the imbalance of the human body [31]. Further investigation suggested that the key genes regulated by the LWDH formula are enriched in NEI pathways, and are also significantly close to the genes associated with cancer, diabetes and hypertension in the network target ($P < 0.000\ 1$). These LWDH-treated diseases share an overlapping molecular basis and show high phenotypic similarity ($P = 0.025$) [31]. To understand the interplay between networks and TCM Syndromes, and to determine the mechanism of "the same disease with different treatments", molecular networks associated with Cold Syndrome or Hot Syndrome were identified, providing useful indicators for predicting Cold or Hot Syndrome diseases [19]. Indeed, evidence from experiments using an collagen induced arthritis rat model indicates that the Hot formula (*Wen-Luo-Yin*) acts on the hub nodes of the Cold Syndrome network, whereas the Cold formula (*Qing-Luo-Yin*) tends to target the hub nodes of the Hot Syndrome network, which is in agreement with the TCM therapeutic principles of "Warming the Cold and Cooling the Hot". The fact that the network can lead to the differentiation of the effects of Cold and Hot formulae suggests that the network target will become an essential component of TCM Syndrome research strategies. In addition, the networks of Cold and Hot Syndromes have been applied to clinical research for prediction of network biomarkers, suggesting that the Cold and Hot networks can give rise to different clinical phenotypes [43-44]. Therefore, increasing efforts are being made in TCM Syndrome studies based on the theory of network targets, which may be promising for TCM modernization and, in the future of personalized medicine [51].

4.4 *Rational design and optimization of drug discovery from herbal formulae*

TCM is a rich source of therapeutic leads for the pharmaceutical industry, and also has a well-developed theory of prescriptions. TCM network pharmacology, as an integrative approach based on the network target theory, can provide some feasible strategies and recommendations to increase the success rate of modern drug discovery. For example, vitexicarpin and several ingredient pairs in QLY (discussed above) have been demonstrated as possible anti-inflammatory and anti-angiogenesis treatments using network target approaches [22, 41-42]. Additionally, TCM network pharmacology can also be used to refine the experience by identifying and optimizing the synergistic and antagonistic herb combinations in an herbal formula, which in turn benefits combinatorial drug development. Therefore, the goal of rational design and optimization based on TCM network pharmacology is determining the best method of designing an optimal prescription of multiple components with a clear understanding of its pharmacology and potential drug-related adverse effects. In the future, this question will be further explored as follows: (1) recognizing the optimal ways that natural products work against a disease-specific network target; (2) quantifying the biological impact of network perturbations caused by natural products and their combinations with high efficacy and low side effects; and (3) detecting and making use of the characteristics of the combinatorial rules of TCM herbal formulae from a network perspective.

5 TCM Network Pharmacology: a New Avenue for Discovering Traditional Chinese Medicines

More than 10 000 herbs used in more than 100 000 herbal formulae have been recorded in traditional Chinese medicine [3], posing a huge challenge to the pursuit of a better understanding of the molecular mechanisms of TCM herbal formulae. Our recent studies have demonstrated that the TCM network pharmacology strategy has a wide variety of practical applications for understanding TCM herbal formulae (Fig. 4). Meanwhile, with the availability of various databases and data resources related to TCM and biological systems [52-64] (Table 2), many researchers have continued to modernize TCM in the context of network pharmacology. As listed in Table **3**, researchers have conducted many network-based computational and experimental studies to detect effective substances and determine the mechanisms of action of herbal

formulae against many diseases [65-73]. In 2012, a special issue on TCM network pharmacology was launched in *Evidence-Based Complementary and Alternative Medicine*, and more studies are expected. Through these valuable analyses and explanations, the mysteries of TCM can be more thoroughly revealed.

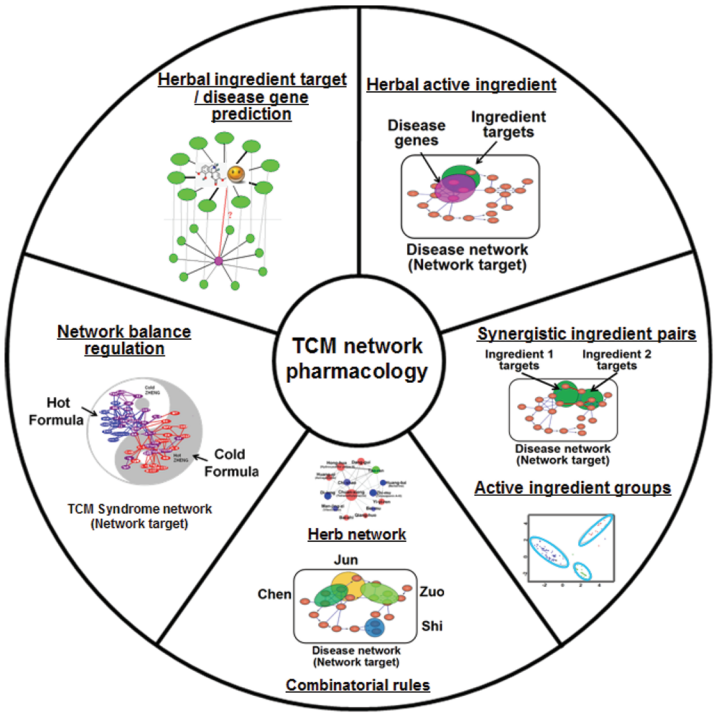

Fig. 4 Summary of TCM network pharmacology applications

Table 2 Some public databases and resources related to TCM network pharmacology

Category	Name	Description	Web	Ref.
Herb related databases	TCM-ID (Traditional Chinese Medicine Information Database)	Contains 1 197 formulae, 1 098 medicinal herbs and 9 852 herbal ingredients.	http://tcm.cz3.nus.edu.sg/group/tcm-id/	[52]
	TCM Database@Taiwan	Contains more than 20 000 pure compounds isolated from 453 TCM ingredients.	http://tcm.cmu.edu.tw	[53]
	TCMGeneDIT	Provides association among TCM and genes, diseases, TCM effects and TCM ingredients through text-mining	http://tcm.lifescience.ntu.edu.tw	[54]
	CHMIS-C (Comprehensive Herbal Medicine Information System for Cancer)	Contains 203 cancer-related molecular targets, 527 anticancer herbal formulations, 937 individual ingredients and 9 366 phytochemicals	http://sw16.im.med.umich.edu/chmis-c	[55]
	TCMID (Traditional Chinese Medicines Integrated Database)	Contains 47 000 prescriptions, 8 159 herbs, 25 210 compounds, 6 828 drugs, 3 791 diseases and 17 521 related targets collected from different resources and through text-mining	http://www.megabionet.org/tcmid/	[56]
Biomolecular network resources (only shows protein-protein interaction databases)	HPRD (Human Protein Reference Database)	Human protein-protein interaction data manually extracted from the literature	http://hprd.org/	[57]
	MINT (Molecular Interaction database)	Focuses on experimentally verified protein-protein interactions in scientific literature	http://mint.bio.uniroma2.it/mint/	[59]
	STRING (Search Tool for the Retrieval of Interacting Genes/Proteins)	A database of known and predicted protein interactions derived from four sources: Genomic Context, High-throughput Experiments, Coexpression and Previous Knowledge	http://string-db.org	[59]
	DIP (Database of Interacting Proteins)	Experimental protein-protein interaction data	http://dip.doe-mbi.ucla.edu	[60]
	BioGRID	Integrates protein-protein interaction data through comprehensive curation efforts	http://thebiogrid.org/	[61]

(continued)

Category	Name	Description	Web	Ref.
Phenotype network resources	OMIM (Online Mendelian Inheritance in Man)	A comprehensive, authoritative compendium of human genes and genetic phenotypes	http://www.omim.org	[62]
	UMLS (Unified Medical Language System)	Contains more than 2 million names for 900 000 concepts from biomedical vocabularies, and 12 million relations among these concepts	http://www.nlm.nih.gov/research/umls/	[63]
	HPO (Human Phenotype Ontology)	Uses information from OMIM and the medical literature and contains approximately 10 000 terms	http://www.human-phenotype-ontology.org	[64]

Table 3 Selected TCM network pharmacology studies in international journals

Herb / Herb ingredients / Herbal formula	Related disease / TCM Syndrome / Target	Year	Ref.
Qing-Luo-Yin (Hot-Cooling herbal formula) Wen-Luo-Yin (Cold-Warming herbal formula)	Cold / Hot Syndromes in rheumatoid arthritis	2007	[19]
Ganoderic acid D from Ling-Zhi (*Ganoderma lucidum*)	Cancer	2008	[65]
Realgar-*Indigo naturalis* formula	Acute promyelocytic leukemia	2010	[66]
Liu-Wei-Di-Huang Pill	Diabetes mellitus, Rheumatoid arthritis, Atherosclerosis, Lung cancer etc.	2010	[31]
Chuan-Xiong (*Rhizoma Chuanxiong*) herb network from 3865 Collaterals-related herbal formulae	Angiogenesis disorders	2010	[31]
61 herb / herb ingredients including Qing-Feng-Teng (*Sinomenium acutum*), etc.	Anti-angiogenesis synergistic combination screening	2011	[22]
Qishenkeli	Coronary heart disease	2012	[67]
Yishen Juanbi Tablet	Rheumatoid arthritis	2012	[68]
Rhein from Da-Huang (*rhubarb*)	Target prediction	2012	[69]
10 anti-AD herbal ingredients	Alzheimer	2012	[70]
Compound Dan-Shen Formula	Cardiovascular disease	2012	[71]
VIT from Man-Jing-Zi (*Vitex rotundifolia*)	Anti-tumor angiogenesis	2013	[41]
Qing-Luo-Yin	Rheumatoid arthritis	2013	[42]
Maxingshigan-Yinqiaosan Formula	H1N1 Influenza	2013	[72]
Radix Curcumae formula	Cardiovascular disease	2013	[73]

In general, the TCM network pharmacology approach has two distinguishing features and potentials: it is predictable and systematic. This approach is different from the traditional method of "trial and error" and could make the drug discovery process predictable owing to the computational powers of this approach and its capacity to manage large amounts of data. Moreover, this approach is also different from the reductionist method, and can make the systematic study of herbal formulae achievable. Therefore, although TCM network pharmacology is still in its infancy, such a novel approach will initiate new directions and lead a probable revolution in the modernization of TCM, and also contribute new insights into the current drug discovery field.

References

[1] Corson TW, Crews CM. Molecular understanding and modern application of traditional medicines: triumphs and trials [J]. *Cell*, 2007, **130**(5):769-774.

[2] Chan K. Progress in traditional Chinese medicine [J]. *Trends Pharmacol Sci*, 1995, **16**(6):182-187.

[3] Qiu J. Traditional medicine: a culture in the balance [J]. *Nature*, 2007, **448**(7150):126-128.

[4] Stone R. Lifting the veil on traditional Chinese medicine [J]. Science, 2008, **319**(5864):709-710.

[5] Wang L, Zhou GB, Liu P, *et al*. Dissection of mechanisms of Chinese medicinal formula Realgar-Indigo naturalis as an effective treatment for promyelocytic leukemia [J]. *Proc Natl Acad Sci USA*, 2008, **105**(12): 4826-4831.

[6] Lam W, Bussom S, Guan F, *et al*. The four-herb Chinese medicine PHY906 reduces chemotherapy-induced gastrointestinal toxicity [J]. *Sci Transl Med*, 2010, **2**(45):45ra59.

[7] Hoessel R, Leclerc S, Endicott JA, *et al*. Indirubin, the active constituent of a Chinese antileukaemia medicine, inhibits cyclin-dependent kinases [J]. *Nat Cell Biol*, 1999, **1**(1): 60-67.

[8] Yue QX, Cao ZW, Guan SH, *et al*. Proteomics characterization of the cytotoxicity mechanism of ganoderic acid D and computer-automated estimation of the possible drug target network [J]. *Mol Cell Proteomics*, 2008, **7**(5): 949-961.

[9] Park SJ, Ahmad F, Philp A, *et al*. Resveratrol ameliorates aging-related metabolic phenotypes by inhibiting cAMP phosphodiesterases [J]. *Cell*, 2012, **148**(3):421-433.

[10] Zhou H, Sun L, Yang XL, *et al*. ATP-directed capture of bioactive herbal-based medicine on human tRNA synthetase [J]. *Nature*, 2013, **494**(7435):121-124.

[11] Titov DV, Gilman B, He QL, *et al*. A subunit of TFIIH, is a

target of the natural product triptolide [J]. *Nat Chem Biol*, 2011, **7**(3):182-188.

[12] Xu Z. Modernization: One step at a time [J]. *Nature*, 2011, **480**(7378):S90-S92.

[13] Keith CT, Borisy AA, Stockwell BR. Multicomponent therapeutics for networked systems [J]. *Nat Rev Drug Discov*, 2005, **4**(1):71-78.

[14] Jia J, Zhu F, Ma X, *et al*. Mechanisms of drug combinations: interaction and network perspectives [J]. *Nat Rev Drug Discov*, 2009, **8**(2):111-128.

[15] Schadt EE, Friend SH, Shaywitz DA. A network view of disease and compound screening [J]. *Nat Rev Drug Discov*, 2009, **8**(4):286-295.

[16] Levinson AD. Cancer therapy reform [J]. *Science*, 2010, **328**(5975):137.

[17] Li S. Possible relationship between traditional Chinese medicine ZHENG and molecular networks. The First Academic Annual Meeting of the China Association for Science and Technology. 1999.

[18] Li S. Framework and practice of network-based studies for Chinese herbal formula [J]. *J Chin Integrat Med*, 2007, **5**(5): 489-493.

[19] Li S, Zhang Z, Wu L, *et al*. Understanding ZHENG in traditional Chinese medicine in the context of neuro-endocrine-immune network [J]. *IET Syst Biol*, 2007, **1**(1): 51-60.

[20] Li S. Network systems underlying traditional Chinese medicine syndrome and herb formula [J]. *Curr Bioinformat*, 2009, **4**(3):188-196.

[21] Li S. Network target: a starting point for traditional Chinese medicine network pharmacology [J]. *China J Chin Mater Med*, 2011, **36**(15):2017-2020.

[22] Li S, Zhang B, Zhang NB. Network target for screening synergistic drug combinations with application to traditional Chinese medicine [J]. *BMC Syst Biol*, 2011, **5**(S1): S10.

[23] Zhao S, Li S. Network-based relating pharmacological and genomic spaces for drug target identification [J]. *PLoS ONE*, 2010, **5**(7):e11764.

[24] Wu X, Jiang R, Zhang MQ, *et al*. Network-based global inference of human disease genes [J]. *Mol Syst Biol*, 2008, **4**: 189.

[25] Yao X, Hao H, Li Y, *et al*. Modularity-based credible prediction of disease genes and detection of disease subtypes on the phenotype-gene heterogeneous network [J]. *BMC Syst Biol*, 2011, **5**:79.

[26] Li S, Wu LJ, Zhang ZQ. Constructing biological networks through combined literature mining and microarray analysis: a LMMA approach [J]. *Bioinformatics*, 2006, **22**(17): 2143-2150

[27] Huang Y, Li S. Detection of characteristic sub pathway network for angiogenesis based on the comprehensive pathway network [J]. *BMC Bioinformatics*, 2010, **11**(S1): S32.

[28] Gu J, Chen Y, Li S, *et al*. Identification of responsive gene modules by network-based gene clustering and extending: application to inflammation and angiogenesis [J]. *BMC Syst Biol*, 2010, **4**: 47.

[29] Gu J, Li S. Towards integrative annotating of the cell-type specific gene functional and signaling map in vascular endothelial cells [J]. *Mol Biosyst*, 2012, **8**(8):2041-2049.

[30] Chen Y, Gu J, Li D, *et al*. Time-course network analysis reveals TNF-alpha can promote G1/S transition of cell cycle in vascular endothelial cells [J]. *Bioinformatics*, 2012, **28**(1): 1-4.

[31] Li S, Zhang B, Jiang D, *et al*. Herb network construction and co-module analysis for uncovering the combination rule of traditional Chinese herbal formulae [J]. *BMC Bioinformatics*, 2010, **11**(S11): S6.

[32] Li LS, Zhang NB, Li S. Ranking effects of candidate drugs on biological process by integrating network analysis and Gene Ontology [J]. *Chin Sci Bull*, 2010, **55**(26): 2974-2980.

[33] Yan H, Zhang B, Li S, *et al*. A formal model for analyzing drug combination effects and its application in TNF-alpha-induced NFkappaB pathway [J]. *BMC Syst Biol*, 2010, **4**: 50.

[34] Zhao S, Li S. A co-module approach for elucidating drug-disease associations and revealing their molecular basis [J]. *Bioinformatics*, 2012, **28**: 955-961.

[35] Li S, Zhao S. Network-based identification of drug target and drug action: China, 201010218468.X [P]. 2010-10-21.

[36] Li S, Zhang NB, Zhang B. Method of network-based identification of multicomponent synergy and compositions for use as effective component of anti-angiogenesis medicines: US, 8112230 B2[P].

[37] Li S, Zhang N, Zhang B. Network-based identification of synergistic drug combination: China, ZL200810239284.4 [P]. 2008-10-23.

[38] Zhuang YL, Li S, Li YD. dbNEI: a specific database for neuro-endocrine-immune interactions [J]. *Neuro Endocrinol Lett*, 2006, **27**(1-2):53-59.

[39] Zhang J, Ma T, Li Y, *et al*. dbNEI2.0: building multilayer network for drug-NEI-disease [J]. *Bioinformatics*, 2008, **24**(20): 2409-2411.

[40] Li S, Zhang B, Yuan J. A HerbBioMap database. China Software Copyright: 2011SR076502.

[41] Zhang B, Liu L, Zhao S, *et al*. Vitexicarpin acts as a novel angiogenesis inhibitor and its target network [J]. *Evid Based Complement Alternat Med*, 2013: 278405.

[42] Zhang B, Wang X, Li S. An integrative platform of TCM network pharmacology and its application on an herbal formula, Qing-Luo-Yin [J]. *Evid Based Complement Alternat Med*, 2013: 456747.

[43] Ma T, Tan C, Zhang H, *et al*. Bridging the gap between traditional Chinese medicine and systems biology: the connection of Cold Syndrome and NEI network [J]. *Mol Bio Syst*, 2010, **6**(4): 613-619.

[44] Jiang B, Liang X, Chen Y, *et al*. Integrating next-generation sequencing and traditional tongue diagnosis to determine tongue coating microbiome [J]. *Sci Rep*, 2012, **2**: 936.

[45] Hopkins AL. Network pharmacology [J]. *Nature Biotechnol*, 2007, **25**(10): 1110-1111.

[46] Hopkins AL. Network pharmacology: the next paradigm in drug discovery [J]. *Nature Chem Biol*, 2008, **4**(11): 682-690.

[47] Schadt EE. Molecular networks as sensors and drivers of common human diseases [J]. *Nature*, 2009, **461**(7261): 218- 223.

[48] Barabási AL, Gulbahce N, Loscalzo J. Network medicine: a network-based approach to human disease [J]. *Nat Rev Genet*, 2011, **12**(1): 56-68.

[49] Hoeng J, Deehan R, Pratt D, *et al*. A network-based approach to quantifying the impact of biologically active substances [J]. *Drug Discov Today*, 2012, **17**(9-10): 413-418.

[50] Li S, Lu A, Wang Y, *et al*. Suppressive effects of a Chinese herbal medicine Qing-Luo-Yin extract on the angiogenesis of collagen induced arthritis in rats [J]. *Am J Chinese Med*, 2003, **31**(5): 713-720.

[51] Jan van der Greef. Perspective: All systems go [J]. *Nature*, 2011, **480**: S87

[52] Ji ZL, Zhou H, Wang JF, *et al*. Traditional Chinese medicine information database [J]. *J Ethnopharmacol*, 2006, **103**(3): 501.

[53] Chen CY. TCM Database@Taiwan: the world's largest traditional Chinese medicine database for drug screening in

silico [J]. *PLoS One*, 2011, **6**(1): e15939.
[54] Fang YC, Huang HC, Chen HH, *et al*. TCMGeneDIT: a database for associated traditional Chinese medicine, gene and disease information using text mining [J]. *BMC Complement Altern Med*, 2008, **8**:58.
[55] Fang X, Shao L, Zhang H, *et al*. CHMIS-C: a comprehensive herbal medicine information system for cancer [J]. *J Med Chem*, 2005, 48(5):1481-1488.
[56] Xue R, Fang Z, Zhang M, *et al*. TCMID: Traditional Chinese Medicine integrative database for herb molecular mechanism analysis [J]. *Nucleic Acids Res*, 2013, **41**: D1089-D1095.
[57] Goel R, Harsha HC, Pandey A, *et al*. Human Protein Reference Database and Human Proteinpedia as resources for phosphoproteome analysis [J]. *Mol Biosyst*, 2012, **8**(2): 453- 463.
[58] Licata L, Briganti L, Peluso D, *et al*. MINT, the molecular interaction database: 2012 update [J]. *Nucleic Acids Res*, 2012, **40**: D857-D861.
[59] Franceschini A, Szklarczyk D, Frankild S, *et al*. STRING v9.1: protein-protein interaction networks, with increased coverage and integration [J]. *Nucleic Acids Res*, 2013, **41**: D808-D815.
[60] Xenarios I, Salwínski L, Duan XJ, *et al*. DIP, the Database of Interacting Proteins: a research tool for studying cellular networks of protein interactions [J]. *Nucleic Acids Res*, 2002, **30**(1): 303-305.
[61] Chatr-Aryamontri A, Breitkreutz BJ, Heinicke S, *et al*. The BioGRID interaction database [J]. *Nucleic Acids Res*, 2013, **41**: D816-D823.
[62] Hamosh A, Scott AF, Amberger JS, *et al*. Online Mendelian Inheritance in Man (OMIM), a knowledgebase of human genes and genetic disorders [J]. *Nucleic Acids Res*, 2005, **33**: D514-D517.
[63] Bodenreider O. The Unified Medical Language System (UMLS): integrating biomedical terminology [J]. *Nucleic Acids Res*, 2004, 32: D267-D270.
[64] Robinson PN, Mundlos S. The Human Phenotype Ontology [J]. *Clin Genet*, 2010, 77: 525-534.
[65] Yue QX, Cao ZW, Guan SH, *et al*. Proteomics characterization of the cytotoxicity mechanism of ganoderic acid D and computer-automated estimation of the possible drug target network [J]. *Mol Cell Proteomics*, 2008, **7**(5): 949-961.
[66] Zhao J, Jiang P, Zhang W. Molecular networks for the study of TCM pharmacology [J]. *Brief Bioinform*, 2010, **11**(4): 417-430.
[67] Wang Y, Liu Z, Li C, *et al*. Drug target prediction based on the herbs components: the study on the multitargets pharmacological mechanism of qishenkeli acting on the coronary heart disease [J]. *Evid Based Complement Alternat Med*, 2012: 698531.
[68] Jiang M, Lu C, Chen G, *et al*. Understanding the molecular mechanism of interventions in treating rheumatoid arthritis patients with corresponding traditional Chinese medicine patterns based on bioinformatics approach [J]. *Evid Based Complement Alternat Med*, 2012: 129452.
[69] Zhang A, Sun H, Yang B, *et al*. Predicting new molecular targets for rhein using network pharmacology [J]. *BMC Syst Biol*, 2012, **6**: 20.
[70] Sun Y, Zhu R, Ye H, *et al*. Towards a bioinformatics analysis of anti-Alzheimer's herbal medicines from a target network perspective [J]. *Brief Bioinform*, 2012 Aug 11.
[71] Li X, Xu X, Wang J, *et al*. A system-level investigation into the mechanisms of Chinese Traditional Medicine: Compound Danshen Formula for cardiovascular disease treatment [J]. *PLoS One*, 2012, **7**(9): e43918.
[72] W Dai, JX Chen, P Lu, *et al*. Pathway pattern-based prediction of active drug components and gene targets from H1N1 influenza's treatment with Maxingshigan-Yinqiaosan Formula [J]. *Mol Biosyst*, 2013, **9**(3): 375-385.
[73] Tao W, Xu X, Wang X, *et al*. Network pharmacology-based prediction of the active ingredients and potential targets of Chinese herbal Radix Curcumae formula for application to cardiovascular disease [J]. *J Ethnopharmacol*, 2013, **145**(1): 1-10.

中药网络药理学：理论、方法与应用

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【摘　要】 中医学将病人当作一个具有不同状态的系统来进行治疗，具有悠久的历史，积累了大量的方剂。中医学的整体观念与网络药理学、网络生物学等前沿科技的核心思想具有相通之处，也符合了对肿瘤等复杂疾病进行系统性治疗的需求。“中药网络药理学”这一新领域的研究旨在从系统层次和分子水平揭示中药方剂的奥秘，促进中药研究从当前的“单一靶标，单一药物”模式转向“网络靶标，多成分药物”的新模式。近年来，中药网络药理学的一系列方法得以创建，包括基于网络的疾病基因预测、中药成分的靶标谱和药理活性预测、药物-基因-疾病的共模块分析、中药方剂多成分协同作用的大规模筛选、中药方剂的配伍规律和网络调节机理分析等。这些方法在清络饮、六味地黄丸等方剂的药效物质与作用机理等方面得到了有效应用。研究结果表明中药网络药理学能够为中医学从基于经验的医学迈向基于证据的医学提供新的途径，并能加速中药药物发现的进程，同时改进当前的药物研究策略。

【关键词】 中医药；网络药理学；网络靶标；理论；方法；应用

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