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•Review•

TCM network pharmacology: A new trend towards combining computational, experimental and clinical approaches

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[ABSTRACT] Traditional Chinese medicine (TCM) is a precious treasure of the Chinese nation and has unique advantages in the prevention and treatment of diseases. The holistic view of TCM coincides with the new generation of medical research paradigm characterized by network and system. TCM gave birth to a new method featuring holistic and systematic "network target", a core theory and method of network pharmacology. TCM is also an important research object of network pharmacology. TCM network pharmacology, which aims to understand the network-based biological basis of complex diseases, TCM syndromes and herb treatments, plays a critical role in the origin and development process of network pharmacology. This review introduces new progresses of TCM network pharmacology in recent years, including predicting herb targets, understanding biological foundation of diseases and syndromes, network regulation mechanisms of herbal formulae, and identifying disease and syndrome biomarkers based on biological network. These studies show a trend of combining computational, experimental and clinical approaches, which is a promising direction of TCM network pharmacology research in the future. Considering that TCM network pharmacology is still a young research field, it is necessary to further standardize the research process and evaluation indicators to promote its healthy development.

[KEY WORDS] Network pharmacology; Traditional Chinese medicine; Network target; Computation; Experiment; Clinical approach
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The Origin and Development of TCM Network Pharmacology

With the rise of interdisciplinary fields such as bioinformatics and systems biology, research strategies for exploring the interactions between drugs and diseases have gradually shifted from isolated studies to systematic and holistic analysis. Since then, scholars in China and abroad have started performing biomedical research based on networks. In 1999, Li proposed a hypothesis regarding the relationship between traditional Chinese medicine (TCM) syndromes and biomolecular networks [1]. In 2002, Li suggested that TCM formulae may regulate the complex disease-related gene networks by exerting "tiny and multiple effects", and ultimately have an "emerging" effect [2]. In 2007, Li published an article on the biological basis of TCM syndromes from the network perspective [3]. Later, in the same year, he proposed a network-based TCM formula research framework [4]. After the publication of the abovementioned works, the term "network pharmacology" was proposed internationally for the first time in October 2007 [5]. In an article published in 2008, network pharmacology was hailed as the next paradigm in drug discovery [6]. In 2009, Pan published "New paradigm for drug discovery based on network pharmacology " in Chinese Journal of New Drugs & Clinical Remedies [7]. The origin of network pharmacology is listed in Table 1.

Since network pharmacology was proposed, some concepts similar to network pharmacology have also been proposed to promote the development of network pharmacology, such as systems pharmacology [10], network toxicology [11], integrative pharmacology [12] and modular pharmacology [13]. They all utilize the idea of network, and carry out systematic research on the mechanism of herbal formulae and biological basis of syndromes. These fields are booming and have the potential to combine with network pharmacology, which is expected to bring sustainable development and new breakthroughs in TCM research [14].

Network pharmacology explains the foundation of complex biological systems from a network perspective. Re-

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Table 1 The origin of network pharmacology

Timeline	Research Content	Ref.
1999	TCM syndromes are related to molecular networks	[1]
2002	Network regulating effect of TCM prescriptions	[2]
2007	Biological network of Hot syndrome and Cold syndrome	[3]
2007	Network pharmacology	[5]
2008	Network pharmacology: the next generation medicine research model	[6]
2009	Herb network-biological network-phenotype network	[8]
2009	New paradigm for drug discovery based on network pharmacology	[7]
2011	Network target: a starting point of network pharmacology research on TCM prescriptions	[9]

searchers can understand the health and disease state of the human body by establishing and analyzing the biological network, and can use the biological network as a target to design effective drug intervention methods [15]. Unlike the traditional pharmacology research strategy, network pharmacology does not study the interaction between a single disease, a single target, and a single drug in isolation but measures the regulatory effect of drugs on the biomolecular network from a systematic and holistic perspective, which is characterized by its systematic nature, relevance and predictability.

Traditional Chinese medicine (TCM) has played a key role in the origin and development of network pharmacology. TCM is a valuable treasure with thousands of years of clinical experience in China. It is characterized by the holistic view and syndrome differentiation and has its own characteristics and advantages in the treatment of complex diseases. The holistic characteristic and rich experience of TCM, on the one hand, highlights the limitations of the reductionist medical research mode. On the other hand, it brings forth a new generation of studies featuring network. TCM network pharmacology is a methodology based on biological network that reveals the biological foundation among complex diseases, syndromes and herbal formulae. To systematically reveal the biological basis of holistic diagnosis and treatment in TCM, Chinese scholars have taken the lead in putting forward the new concept of the "network target", which refers to the key in the biomolecular network that can associate disease, TCM syndrome and Chinese herbal medicine, and can quantitatively represent the overall regulatory mechanisms of herbal formulae, including key molecules, key pathways, and key modules [16]. Therefore, the word "network" in TCM network pharmacology includes networks composed of many elements such as TCM herbs, targets, diseases, and syndromes. The research scope of TCM network pharmacology includes both Chinese herbal medicine and TCM syndrome. Since 2007, the concept of network target has been constantly exploring new frontiers and applications, to illustrate the advantages of TCM. A series of methods and applications have been proposed, adding new impetus and vitality to the coordinated development of network pharmacology in TCM.

In recent years, the rapid development of computing methods represented by big data and artificial intelligence and high-throughput, multi-omics technologies has effectively promoted the development and wide application of network pharmacology methodology. Furthermore, network pharmacology provides new ideas and methods for analyzing massive biomedical data and building a bridge from data to knowledge. Under such mutual promotion, network pharmacology has developed rapidly, and its influence has gradually expanded. As shown in Fig. 1, based on the statistics of Web of Science (WOS) and China National Knowledge Infrastructure (CNKI), the number of literature published in the field of network pharmacology both in China and overseas has been steadily and rapidly increasing (Figs. 1A, B). The development trend of network pharmacology in recent years shows that traditional medicine is an important part of network pharmacology research, which is reflected by the fact that traditional medicine studies account for more than half of the literature in the field of network pharmacology (Fig. 1C). Traditional Chinese medicine studies account for a large proportion of these studies related to traditional medicine, and the research field of TCM network pharmacology is developing more rapidly than that of TCM pharmacology (Fig. 1D). Traditional medicine network pharmacology studies are also being conducted in India, South Korea, Africa, and other countries and regions [17–20].

In terms of research content, the application fields of network pharmacology have continuously expanded, and achievements have been made in pharmacodynamic materials, the discovery of disease and syndrome markers, drug repositioning, and other fields. Network pharmacology research methods are also rapidly improving, from single-layered to multi-layered, dynamic networks, and from relying on public data to combining computational, clinical, and experimental data (Fig. 2). The research on network pharmacology shows the development trends of the in-depth interaction among computation, clinical investigation and experiment, and the cross among mathematics, biology, and medicine. In network pharmacology research, it is often necessary to design experiments to verify the computational results. In particular, TCM has the characteristic of syndrome differentiation, and syndrome is difficult to be simulated by cell or an-

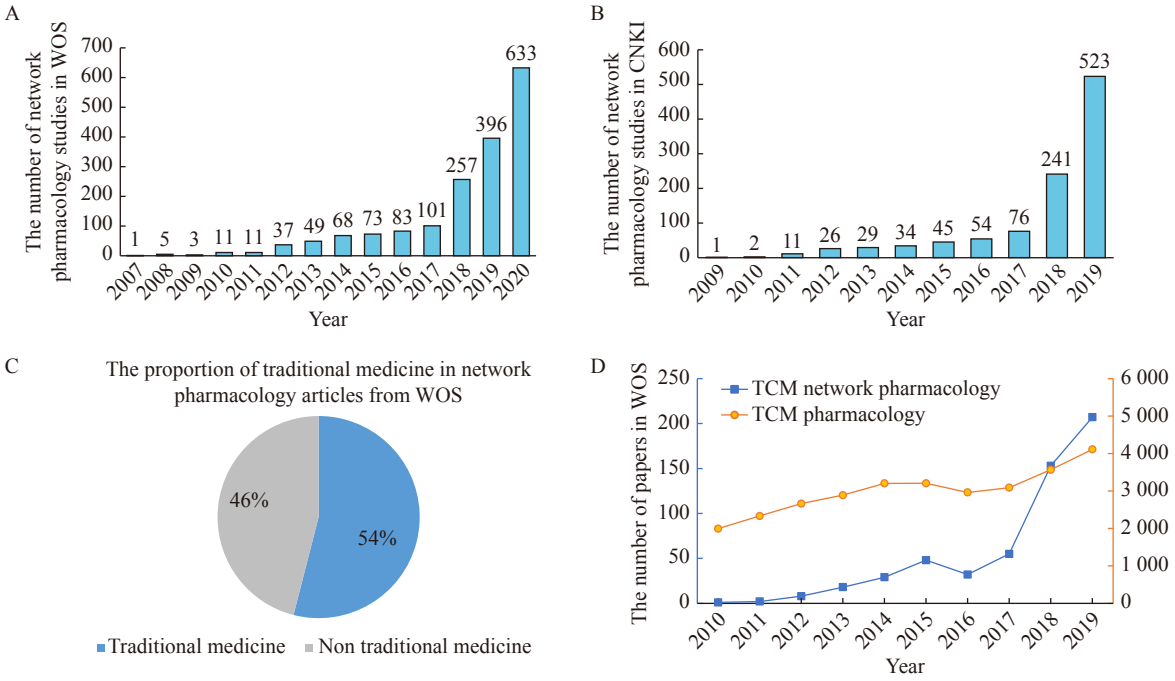

Fig. 1 The number of articles in the network pharmacology field and related fields according to the year of publication. A, B, The numbers of papers about network pharmacology in WOS (A) and CNKI (B). C, Proportion of traditional medical network pharmacology in network pharmacology articles in WOS. D, The number of papers about TCM network pharmacology and TCM pharmacology in WOS

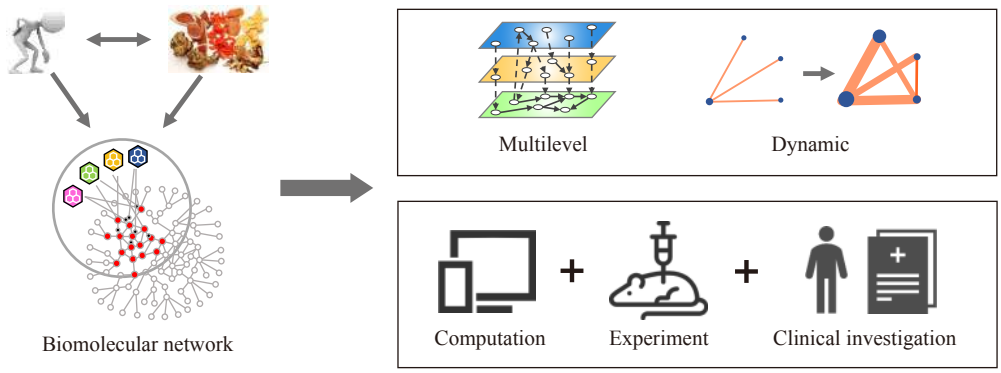

Fig. 2 The development trend of TCM network pharmacology

imal model, so clinical data is more needed. On the other hand, with the progress of high-throughput technologies, the biomedical data has accumulated to an astonishing amount and continues to grow rapidly. Network pharmacology provides a feasible way to obtain an overall understanding of TCM syndromes and herbs from these massive clinical and experimental data. Therefore, the combination of computational, experimental and clinical approaches is a promising direction of network pharmacology.

Network Pharmacology Takes Artificial Intelligence Algorithms and Big Data as the Core

Network pharmacology emerged and developed together with bioinformatics, systems biology, network medicine, artificial intelligence, big data science, and other related research fields. The core of network pharmacology is the network target theory. Based on the network target theory, big data, and artificial intelligence, researchers have developed various network-based drug or disease research models and algorithms. These methods have developed from single-layered to multi-layered networks, static to dynamic networks, and have been combined with frontier technologies such as neural network, deep learning, and single-cell sequencing, bringing new insights to network pharmacology. Li *et al*. revealed the overall associated modular rules of the relationship between phenotypes-biomolecules-compounds and thus took the lead in modeling and identifying genome-wide disease-causing genes and drug targets [21-22]. They also performed, for the first time, the whole-genome prediction of TCM syndrome-related gene profiles and TCM compound

target profiles. Since network pharmacology was proposed, a series of high-precision intelligent algorithms have been established, such as large-scale prediction of the synergistic effects of drugs and TCM compounds based on biological networks [23]. Some related studies are shown in Table 2.

Prediction of drug targets and binding modes

The identification of drug targets is an important step in drug discovery. In recent years, various drug-target interaction (DTI) prediction algorithms have been developed. Predbinding applies support vector machine (SVM) and random forest (RF) to large-scale protein-ligand binding affinity prediction, and identifies some of the important characteristics in the RF model [24]. The Herb-Target Interaction Network (HTINet) model based on representation learning integrates data related to Chinese and Western medicine into a multi-source heterogeneous network according to symptoms. Through network embedding, low-dimensional representations of herbs and proteins were obtained respectively. Then a supervised classification model was constructed based on the representation obtained to predict herb-target interactions [25]. DTINet also uses low-dimensional vectors for feature representation and then makes predictions through a vector space projection scheme [26]. MONN, based on a multi-objective neural network, can predict the non-covalent interactions and binding affinities between compounds and proteins through structure-free information [27]. DeepDTA uses sequence information of drugs and targets to predict their binding affinity values *via* a convolutional neural network (CNN) [28].

With the development of research, researchers are not satisfied with the ability to predict the affinities of drugs and proteins, and further attempts have been made to predict their binding patterns and interaction mechanisms. Based on the interaction data between drugs and proteins, a statistical model called GIFT was constructed to infer the interactions between substructures of drugs and protein domains [29]. Global optimization was introduced to this model for the first time, which helped to reveal the potential mechanism of drug-protein interactions. VIsualized Structure–Activity Relationship (VISAR) is an algorithm and visualization tool for analyzing drug-protein binding patterns based on deep neural networks [30]. This method can convert the information learned by the neural network into a form that is easy for people to understand and can help reveal the contribution of compound substructures to the overall activity. A semi-supervised deep learning model called DeepAffinity [31], which unifies recurrent and convolutional neural networks, uses labeled and unlabeled data jointly to predict affinity. Furthermore, attention mechanisms are embedded to improve its interpretability.

New indications and drug repositioning

Drug-target interactions combined with disease-gene relationships can be used to identify indications for drugs and to provide guidance for drug repositioning. One of the network-based methods quantifies the relationship between disease-related proteins and drug targets in the human protein-protein interactome for drug repositioning [32]. Researchers used routine healthcare data containing more than 220 million patients to test the predictive effect, and used in vitro pharmacological experiments to test the potential mechanism of repositioned drugs. Ranking-system of Anti-Cancer Synergy (RACS) is a semi-supervised learning model that utilizes drug pharmacological properties, drug targeting networks and transcriptomic profiles to predict drug combinations for cancer [33]. Mechanism and Drug Miner (MD-Miner) builds patient-specific signal transduction networks by integrating known disease genes with patients' gene expression profiles and carries out personalized drug repositioning by combining the drug targeting network [23].

Understanding the occurrence and progression of disease and syndrome

In addition to drug target prediction, network-based algorithms have also been developed to explain the occurrence and progression of diseases from a holistic perspective. A quantitative mathematical model was proposed to explore the universal evolution process of complex diseases by integrating clinical omics data with evolutionary dynamics [34]. The researchers systematically reveal the internal relationship between the metabolism-immune imbalance associated with Cold and Hot syndrome and inflammation-induced tumorigenesis, providing an example for the integration of Chinese and Western medicine. The DIAMOnD algorithm identifies disease or TCM syndrome modules in the interaction network starting from known disease or TCM syndrome-related biomolecules. For example, the function of the asthma disease module was verified through computational and experimental methods [35]. CIPHER-SC [36] applies graph convolution on the context-aware network and achieves a complete end-to-end learning architecture for disease-gene association inference. It is the first algorithm to incorporate single-cell transcriptome data to the biological network and enables cell-type-specific prediction. A novel predictive method of disease-gene association based on graph convolutional network and matrix factorization, with the ability to deal with nonlinear associations, was proposed [37]. In addition, TCM network pharmacology is used to study the differences and associations between different TCM syndromes. Researchers constructed RNA networks of hepatitis B patients with different syndromes and revealed the molecular mechanisms of different syndromes through dynamic network analysis [38]. In another study, the dynamic network biomarker (DNB) method was used to explain the dynamic changes of TCM syndromes [39].

Databases used in network pharmacology

Network pharmacology research also integrates many authoritative databases in the field of medicine. Most of these databases start from drugs and compounds from TCM herbs or herbal formulae and use network pharmacology to establish the relationship between drugs and diseases or syndromes. For example, the drug and chemical database ChEMBL [41] provides data on the physical and chemical

Table 2 Cases of network pharmacologic algorithms in the last 5 years

Scope	Name	Description	Year	Ref.
Prediction of DTIs and binding modes	GIFT	Global optimization-based inference of chemogenomic features from drug–target interactions	2015	[29]
	VISAR	Interactive tool for dissecting chemical features learned by deep neural network QSAR models	2020	[30]
	HTINet	Target prediction algorithm of TCM based on representation learning	2019	[25]
	DeepDTA	Drug-target affinity prediction model based on CNN	2018	[28]
	DTINet	DTIs prediction based on heterogeneous networks	2017	[26]
	Pred-binding	Large-scale protein-ligand affinity prediction algorithm	2016	[24]
Prediction of new indications and drug repositioning	Drug effects *via* network proximity	Drug repositioning method based on network pharmacology	2018	[32]
	RACS	Combination of network and transcriptome to find synergistic chemotherapy drugs	2015	[33]
	MD-Miner	Identify patient-specific potential drugs	2017	[40]
Understanding the occurrence and progression of disease and syndrome	CIPHER-SC	Disease–gene relationship inference based on graph convolution and single-cell transcriptome	2020	[36]
	DIAMOnD	Identifying disease model from interactome	2015	[35]
	GCN-MF	Identify disease-gene association through graph convolution network and matrix factorization	2019	[37]
	Dynamical network analysis	Dynamic change and biomarkers of different syndromes in Chronic Hepatitis B	2019	[38]
	Multiscale model	Quantitative analysis model of molecular–cell– system multiscale network dynamics	2017	[34]

properties, biological activities, targets, and druggability of natural products, including TCM compounds. The information on FDA-approved drugs in Drugbank [42] provides the gold standard for pharmacoinformatics research. OMIM [43], HPO [44], UMLS [45], and other disease databases provide abundant and reliable annotation information for exploring disease-related phenotypes and pathogenic genes. Information in protein-protein interaction databases such as STRING [46] and HPRD [47] serves as a bridge between drugs and diseases for the construction of drug intervention networks for diseases.

For TCM, there are some specialized databases containing prescriptions, syndromes and other massive resources accumulated from long-term clinical practice. For example, TCMID [48] contains information on TCM prescriptions, herbs, compounds, and their targets collected through text mining, promoting the mechanistic analysis of TCM by establishing the relationship between TCM compounds, diseases, and disease genes/proteins. TCMGeneDIT [49] is a database containing information on TCM herbs, compounds, TCM functions, genes, and diseases and their relationship, and helps people understand the possible mechanisms of TCM through gene regulation relationships. ETCM [50] provides information on TCM herbs, formulae, and their chemical components, and predicts targets of the compounds according to their chemical fingerprint similarity with known drugs. Users can explore the relationship between herbs, herbal formulae, compounds, gene targets, pathways, and diseases on the website. SymMap [51] is a TCM database focusing on the association of syndromes. The database contains TCM syndromes, herbs, symptoms, syndrome-related diseases, TCM compounds, and drug targets. The associations between these six types of entities form a heterogeneous network. HIT [52] records direct or indirect targets of TCM active compounds reported in literature, and also provides various reference materials for users' reference. TCM Database@Taiwan [53] contains 37 170 compounds from 352 herbs for download. BATMAN-TCM [54] is an online analysis platform for the mechanisms of TCM, which is used to reveal the interaction between the active compounds of TCM and physiological processes. BATMAN-TCM is committed to revealing the mechanism of TCM using the strategy of "multicomponent-multitarget-multipathway". Many network pharmacology algorithms also make use of the information in the database to find the hidden rules from the massive amount of information. These algorithms and databases together promote the vigorous development of network pharmacology.

TCM Network Pharmacology with Clinical Investigation

Network pharmacology is closely related to clinical research. On the one hand, clinical investigation is an important data source for network pharmacology research. On the other hand, the key targets, modules, compounds, biological pathways and other predictions obtained through network pharmacology analysis need to be verified, and clinical trial is the most rigorous and convincing verification method (Fig. 3). Network pharmacology research combined with

clinical data has led to a large number of progress in fields such as the biological basis of diseases and syndromes, identification of biomarkers, and analysis of the mechanism of TCM prescriptions. Some related cases are shown in Table 3.

Discovery of disease and syndrome markers

Clinical data play an important role in network pharmacology-based research on disease and syndrome biomarkers. By exploring the differences between different syndromes or patients and healthy controls at the molecular level, the expression profiles of characteristic genes or the regulatory network of functional genes can be established to identify key genes and functional proteins. The mechanism underlying the occurrence and progression of diseases and syndromes can then be clarified, and specific genes and protein markers can be found. Network pharmacology was applied to predict the prognosis related biological network of pancreatic cancer [55]. A precise prognosis biomarker panel consisting of five key nodes of the network was discovered and verified in a multicenter clinical study, and its prognostic effect was significantly better than that of major clinicopathological factors. For the first time, a single-cell network related to the transformation of gastritis to early gastric cancer was constructed in patients with Hot Syndrome-related manifestations. The breakthrough discovery of gastric early-malignant cells and their biomarkers has provided new insights for the early prevention and control of gastric cancer [56]. A series of studies have been carried out to identify the molecular characteristics of rheumatoid arthritis (RA) patients with TCM Cold and Hot Syndromes. Authors combined genome-wide expression analysis and network pharmacology to identify the relationships between gene expression networks and TCM syndromes [61–63]. Li *et al*. constructed a network balance model and found markers of Cold Syndrome and Hot Syndrome in patients with chronic gastritis, indicating that patients with

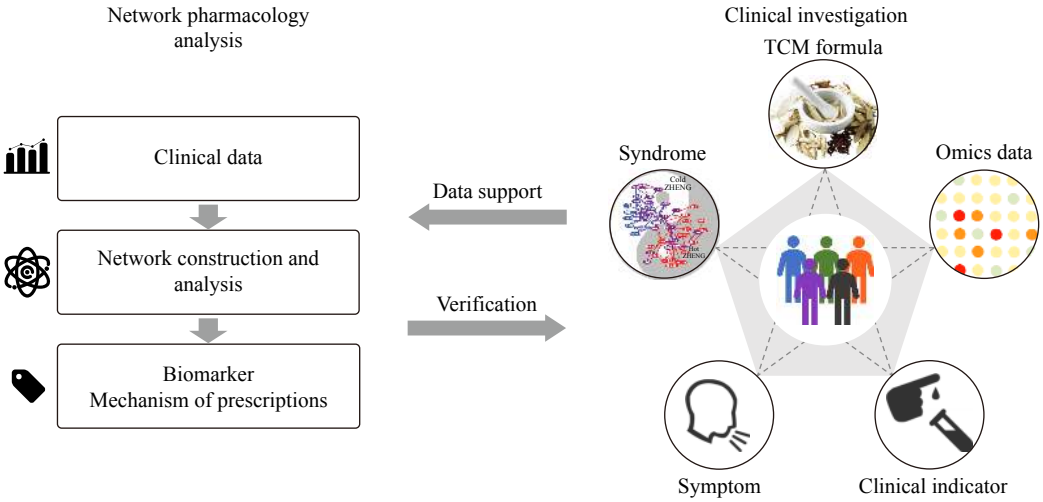

Fig. 3 Combination of network pharmacology and clinical investigation

Table 3 Cases of TCM network pharmacology combining clinical investigation

Term	Description	Year	Ref.
Biomarkers of diseases and syndromes	Prognostic biomarker of pancreatic ductal adenocarcinoma	2020	[55]
	Gastric early-malignant cell and its biomarkers	2019	[56]
	Biomarkers of obesity with metabolic syndrome	2019	[57]
	Blood markers of ischemic stroke	2019	[58]
	Markers for Hot and Cold syndrome in chronic gastritis	2013	[59]
	Tongue coating microflora biomarker for syndromes	2012	[60]
	Genes and biological processes related to Hot and Cold syndrome in RA	2012	[63]
Mechanisms of herbal formulae and syndromes	Yinxieling for psoriasis	2019	[64]
	Dengzhan Shengmai capsule for vascular cognitive impairment	2019	[65]
	Prescription optimization and individualized improvement	2018	[66]
	Disease-syndrome relationship and TCM syndrome evolution in chronic hepatitis B	2019	[39]
	The biological basis of spleen qi deficiency syndrome	2020	[69]

Cold Syndrome have a lower level of metabolism and that patients with Hot Syndrome have increased immune regulation [59]. Jiang *et al*. used next-generation sequencing technology to study the relationship between tongue diagnosis in TCM and tongue coating microflora and constructed a tongue flora imbalance network related to Cold and Heat Syndromes. The results showed the potential of tongue coating microflora as a biomarker for syndromes [60]. Besides, network pharmacology has been used to identify biomarkers of obesity with metabolic syndrome [57] and ischemic stroke [58] from clinical data.

Mechanisms of TCM formulae and syndromes

Using network pharmacology methods combined with clinical trials, the mechanism and efficacy of Dengzhan Shengmai capsule for the treatment of vascular cognitive impairment were verified . To verify the mechanism and material basis of a TCM prescription, YinXieLing, serums from patients with psoriasis were collected before and after YinXieLing treatment for proteomic testing and further identification of psoriasis biomarkers [65]. Yang *et al*. proposed a multistage method combined with complex network analysis to identify effective TCM prescriptions for specific diseases. Furthermore, the effective drug-symptom relationship was identified to provide help for personalized prescription [66]. Su *et al*. focused on the disease-syndrome relationship and accumulated a series of results on the same TCM syndrome for different diseases and different TCM syndromes for the same disease [38-39, 67-68]. These results revealed the differences, dynamic transformation and biomarkers of different TCM syndromes in chronic hepatitis B and cirrhosis. Clinical transcriptomic data were used to explore the biological basis of spleen qi deficiency syndrome, and abnormal modules in the biomolecular network in patients were found [69]. Compared with experiments on cell and animal models, clinical trials can reflect the real conditions of patients and provide more accurate data on disease mechanisms and drug effects, leading to more convincing results. Network pharmacology combined with clinical research is attracting increasing attention to help people further understand the mechanisms of diseases and drugs.

Network Pharmacology with Experimental Approach

Earlier network pharmacology studies often used public databases. In recent years, researchers have increasingly combined computation with experiments (Table 4). For example, when studying the mechanism of a drug, herb, or herbal formula, computational findings often need to be experimentally verified in a cell or animal model. Emerging experimental technologies such as high-throughput screening, single-cell sequencing, and gene editing have promoted the development of network pharmacology. The introduction of new technologies not only provides richer data but also provides additional information for the framework of network pharmacology research (Fig. 4).

Identifying biological functions of TCM herbs and active compounds

Traditional Chinese medicine often exerts therapeutic effects by affecting multiple targets. A distinctive characteristic of network pharmacology is to analyze the targets of herbs and their compounds by combining computational and experimental methods and to understand the mechanism based on the biological molecular networks of diseases and syndromes. Furthermore, the network regulation mechanisms and biolo-

Table 4 Cases of the combination of network pharmacology and experimental technology

Category	Object	Technology	Year	Ref.
TCM herbs and active compounds	Tetramethylpyrazine	Enzyme activity and adenosine receptor assay	2015	[70]
	Tanshinol borneol ester	Co-culture tube formation assay	2019	[71]
	Berberine and Coptidis Rhizoma (*Huanglian*, HL)	NMR metabolomics	2019	[72]
	Artemisinin	Chemical proteomics	2015	[73]
	Health-Strengthening Herbal Medicine	High throughput transcriptomics	2018	[74]
TCM herbal formulae	Liu-wei-di-huang	CRISPR-Cas9	2019	[75]
	Qing-luo-yin	Metabonomics	2018	[76]
	Cyclocarya paliurus Formula	High throughput transcriptomics	2018	[77]
	Qijian mixture	Metabonomics, gut microbiota	2018	[78]
Disease and TCM syndrome biomarkers	Biomarkers of gastric early-malignant cells	Single-cell RNA sequencing	2019	[56]
	Tongue coating microbiome biomarker for gastritis	Metagenomic	2019	[79]
	Dynamic network biomarkers for changes in the TCM syndrome	RNA microarray	2019	[39]
	Urinary metabolite markers of blood stasis syndrome	Metabonomics	2020	[80]
	Network of immune cells and new cell type markers	Proteome and secretome	2017	[81]

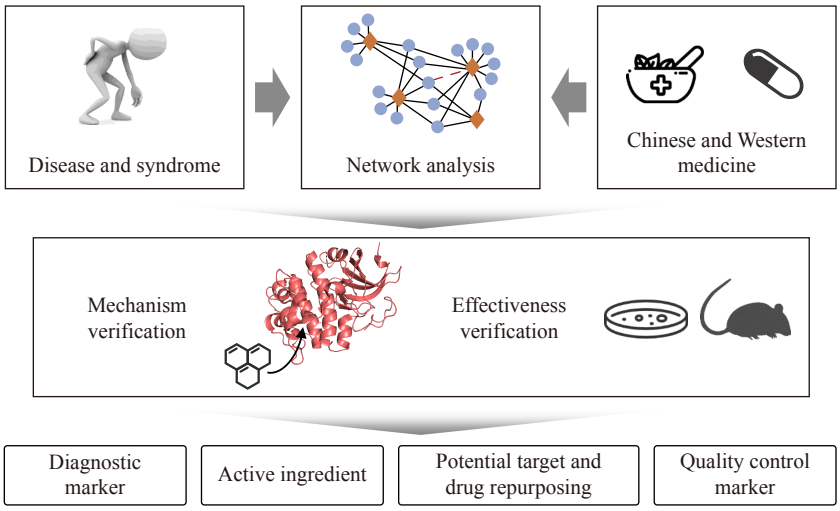

Fig. 4 Combination of network pharmacology and experimental technology

gical functions of TCM can be determined, and new active compounds can be found. The screening strategy based on network pharmacology revealed that tetramethylpyrazine may alleviate methotrexate-induced oxidative damage by acting on phosphodiesterase or the adenosine pathway, and further evaluated the effect and mechanism of tetramethylpyrazine in rats [70]. Tanshinol borneol ester (DBZ) is a derivative of Dantonic, a botanical drug for angina pectoris. Through target prediction and enrichment analysis, network pharmacology analysis found that DBZ may regulate multiple angiogenesis-related pathways. Furthermore, the effect of DBZ in promoting angiogenic activity and its mechanism were tested through experiments [71]. Li *et al*. used NMR metabolomics to study therapeutic effects of berberine and Coptidis Rhizoma (Huanglian, HL) on sepsis, and found that both berberine and HL could reverse the changes in energy metabolism and amino acids metabolisms caused by sepsis [72]. A study on the mechanism of artemisinin used experimental and network methods to discover that the 124 proteins covalently bound to artemisinin proteins are related to various biological processes of Plasmodium falciparum, providing a more complete picture of the mechanism of artemisinin and its derivatives [73]. Zheng *et al*. proposed an innovative high-throughput research strategy that combines computational and experimental methods of network pharmacology. A systematic analysis of the network regulation mechanism of 47 health-strengthening (Fu-Zheng) TCM herbs commonly used in cancer treatment was carried out, suggesting the potential value of health-strengthening herbs in immunity and tumor prevention [74].

Exploring the biological basis of TCM herbal formulae

Research on the mechanism of TCM prescriptions mainly analyzes the targets of compounds in the formula and evaluates the relationship between these targets and key modules of disease and syndrome biomolecule networks by combining computation and experiment. By analyzing the distribution of these targets in the biomolecular network, researchers are able to explore the scientific connotation of combination rules of TCM prescriptions, explain the traditional efficacy mechanism of prescriptions, and find new indications. Guo *et al*. proposed a set of methods integrating network computing and experiments for analyzing molecular networks of complex diseases. With the help of omics information, network prediction algorithms and CRISPR-Cas9, a biomolecular network of inflammation-induced tumorigenesis was constructed, and several functional modules were identified. This work also revealed the multiple effective compounds from Liu-wei-di-huang on synergistic modules [75]. Qing-Luo-Yin (QLY) is a traditional Chinese medicine formula for treating rheumatoid arthritis with Hot Syndrome. Network pharmacological analysis and metabolomics techniques together revealed the anti-rheumatic mechanism of QLY and that the combined use of QLY and methotrexate can reduce side effects [76]. Combining regulatory network analysis and transcriptome experiments, the mechanism of *Cyclocarya paliurus* formula extractum (CPE) for preventing diabetes was explored. The results indicated that CPE treatment inhibited gene expression levels related to inflammation and apoptosis pathways and reduced liver injury in diabetic rats [77]. Gao *et al*. chose four TCM herbs commonly used in the treatment of type 2 diabetes to form a new prescription, Qijian mixture, and preliminarily confirmed its hypoglycemic effect. The potential mechanism of Qijian mixture was speculated based on network pharmacology analysis and the effect of the prescription on the metabolism and gut microbiota [78].

Identification of disease and syndrome biomarkers

Advances in detection technology, especially in combination with network pharmacological methods have provided new opportunities for the identification of disease and syndrome biomarkers. Single-cell RNA sequencing was used to identify gastric early-malignant cells and their biomarkers from patients with Hot syndrome-related symptoms [55]. After the establishment of tongue coating microflora networks for Cold and Hot syndrome [59], metagenomic sequencing further

indicated that the tongue-coating microbiome may be a potential non-invasive biomarker for gastritis with TCM syndromes, including the precancerous cascade [78]. Lu *et al*. performed RNA microarray analysis on blood samples from chronic hepatitis B patients and healthy controls, then used the dynamic network biomarker (DNB) algorithm to obtain the DNBs for TCM syndrome evolution [39]. Based on urinary metabolomics analysis, 21 metabolites were identified as biomarkers during the development of blood-stasis syndrome [80]. A social network of human immune cells was constructed through a proteomic approach, which aided in the discovery of new cell type markers and intercellular connections [81]. Network pharmacology utilizes existing experimental technology while also exploring new experimental methods to meet its own needs. Experiments have become an important part of the network pharmacology research framework, providing data support and verification for research in various fields.

Challenges and Development Directions of Network Pharmacology

Network pharmacology-based studies on TCM and even western medicine are systematic, relevant, and predictive features. These studies have expected to provide support for clinical drug optimization and the interpretation of mechanisms of traditional medicines and have broad application prospects. However, the further development of network pharmacology faces many challenges. A key challenge is how to integrate large amounts of clinical and experimental data to promote precision-oriented diagnosis and treatment, as well as to promote the innovation and development of TCM. There are also some limitations. The data quality of public databases is uncontrollable and heterogeneous, so it is urgent to establish uniform and rigorous standards. Network pharmacology describes the interactions of complex biological systems as networks. How to better understand the internal network regulation mechanism of diseases and syndromes, and how to better reveal the biological basis of TCM, still need more exploration from the aspects of algorithm development, experimental and clinical application. Network pharmacology can be used to understands complex biological systems from a network perspective. At present, the quality of network pharmacology research is rather imbalanced. In response to the above challenges and limitations the World Federation of Chinese Medicine Societies has developed the Network Pharmacology Evaluation Methodology Guidance. This guidance standardizes the principles, procedures and evaluation indexes of data collection, network analysis and experimental verification in the research process to promote the healthy development of this discipline.

It has been noted that the research in network pharmacology is showing some trends, such as the in-depth integration of computational, experimental and clinical approaches, and the intersection of multidisciplinary. Through the in-depth cross-integration of various kinds of information, research in network pharmacology is expected to be useful for studying TCM formulae and complex diseases and TCM syndromes. With the accumulation of experimental data related to TCM, the development of network computing methods and experimental techniques, network pharmacology will be able to be integrated more deeply with the relevant disciplines and its research quality will be improved. On this basis, TCM network pharmacology is expected to promote the development and modernization of TCM, provide evidence and guidance for the accurate use of TCM, and promote the great rejuvenation of TCM.

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