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Review

Integration strategy of network pharmacology in Traditional Chinese Medicine: a narrative review

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Abstract

Traditional Chinese Medicine (TCM) has been extensively used as a mainstay for treating various pathologies. Combing the pharmacology and systems biology approaches, the network pharmacology (NP) approach was developed to predict the probable mechanism underlying the therapeutic effect of TCM. However, approaches solely based on NP cannot effectively elucidate the curative mechanism in a holistic and reliable manner due to limitations in NP-based methods and complexity of TCM components. Thus, integration strategies combining NP with other approaches are increasingly being used. Since the interdisciplinary research in TCM has received much attention in the advent of the big data era of which the NP-based integration strategy is broadly used, the strategy is clearly elaborated in the present review. We summarized several NP-based integration strategies and their applications in TCM studies, including multi-omics approach, gut microbiota study, chemical information analysis, data-mining, and network toxicology study.

Keywords: network pharmacology; toxicology; medicine, Chinese traditional; integration strategy; review

1. INTRODUCTION

Traditional Chinese Medicine (TCM) has been used to treat diseases in China for thousands of years.[1,2] However, considering the paucity of a comprehensive understanding of the mechanisms underlying the therapeutic effects of TCM and its herbal formulas, TCM is considered only as an alternative treatment strategy.[3] Unlike synthetic drugs, TCM drugs possess “multi-component”, “multi-target”, and “multi-pathway” characteristics. Therefore, a complex network of herb-component-target-pathway-disease, metabolites, and gut microbiota must be comprehensively explored to elucidate mechanisms of TCM.

In the advent of the big data era, comprehensive studies of TCM can be achieved in a new modality. The concept of NP was conceived by Hopkins.[4] NP integrates systematic data to analyze the holistic process of interactions between compounds and the human body. The application of NP in TCM was developed subsequently by Li.[5] The term "TCM Systems Bioinformatics (TCMSB)" was proposed and the experimental paradigms of TCM network pharmacology were established gradually.[6,7] The systems pharmacology-based framework of TCM was introduced systematically by Wang *et al*.[8] The construction of "component-target-disease (CTD)" network was put forward and the application of CTD in TCM was attempted.[9,10] The conception of "Integrative pharmacology" was put forward by Xu *et al*.[11] The association between the parameters of absorption, distribution, metabolism, excretion (ADME) and pharmacologic actions was constructed. The utility of NP in studying various TCM formulae was analyzed.[12]

The narrow conception of NP refers processes including screening of active ingredients, fishing of targets, founding of disease targets database and enrichment analysis. These processes constitute a bottom-up approach from ingredients in TCM to their probable curative mechanisms. We regard NP as a pilot research to propose hypotheses of mechanisms to be validated. Therefore, studies reporting evaluation of putative mechanisms using NP-based approaches usually involved evaluation using NP-based methods.

However, NP has several limitations if it is used alone: (a) NP is a pilot prediction with massive hypotheses to confirm, and thus the probable false-positive results cannot be avoided. For example, the similarity of the structures between ligand and protein were matched to output probable targets, of which the result varies

dramatically across different platforms. It is clear that many false-positive results exist, and experimental validation plays a role.[7,13] (b) The NP prediction methods are too simple to holistically evaluate the effects and toxicity profile of TCM. TCM has massive ingredients, and some of them undergo changes in decoction procedures;[14,15] thus, the combined therapeutic effect of TCM is not equivalent to the combined therapeutic effects of constituent compounds. (c) The dose of the TCM drug decides if it is therapeutically beneficial or poisonous.[16] The relative abundance of compounds in TCM varies. Some compounds can hardly reach the effective concentration in the human body, and they cannot be precisely excluded from NP-based analysis. (d) The reliability of ADME-based screening is still debatable. For instance, we screened the confirmed active ingredients (Atractylenolide Ⅰ, Ⅱ, and Ⅲ) of *Atractylodes macrocephala* Koidz. by setting oral bioavailability (OB) and drug-likeness (DL) thresholds as OB ⩾ 30% and DL ⩾ 0.18 in TCMSP,[17] but all the three active ingredients were excluded.

Thus, relying merely on the NP approach will not aid in a reliable way. The concept of "Integration Strategy" is put forward, which refers to a systematic comprehensive strategy that assembles methods for multi-domain investigation. Thus, the broad conception of NP is put forward.[18] Considering the limitations of NP, integration strategies based on NP are frequently being applied in TCM research, and NP-related tools and platforms are increasingly being used.

In the present review, systematic searching of recent publications of NP-based integration strategy in the deciphering of mechanisms of TCM was conducted in databases, which will help in the subsequent exploration of TCM (Figure 1).

2. MULTI-OMICS APPROACH

2.1. Proteomics

Protein is the fundamental constituent of all cells of the body. Millions of proteins play vital roles in life activities. The proteomic approaches aim to analyze cells holistically by detecting the inner dynamic processes of proteins. Besides, the potential disease targets can also be predicted by screening differential protein expression levels using techniques such as iTRAQ labeling quantitative proteomics.[19,20]

Generally, the protein-protein interaction (PPI) analysis is conducted to select the hub proteins for biological enrichment analysis-Kyoto Encyclopedia of Genes and Genomes (KEGG) and Gene Ontology (GO) analysis-to

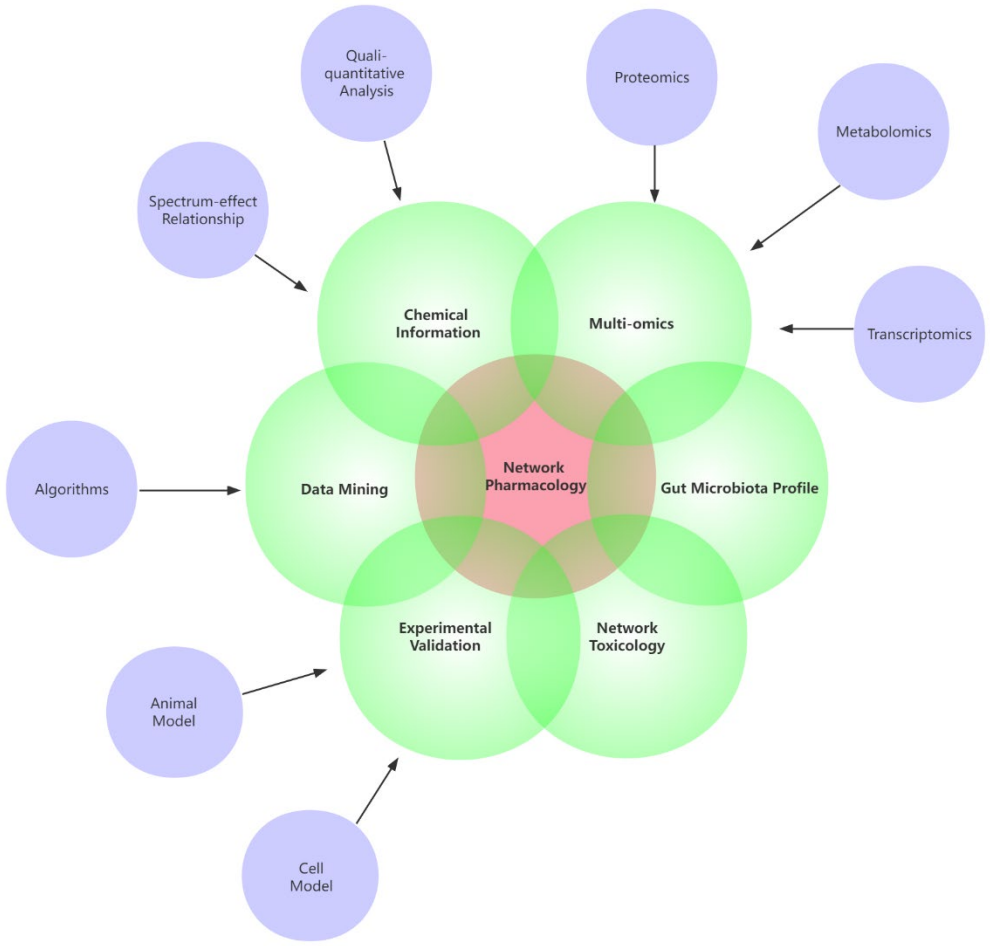

Figure 1 Integration strategy of network pharmacologyThe Venn diagram reflects the interdisciplinary approach based on network pharmacology.

predict related pathways, molecular functions, biological processes, and cellular components of specific species. PPI analysis is a systems biology method to analyze the topological structure of a protein-protein network, widely used in both proteomics and NP. [21,22]

Liu *et al* [23] used a novel integration strategy based on NP and proteomics approaches. After detection of differential protein expression using proteomic approaches, molecular docking-a NP technology- was conducted to evaluate the binding affinity between proteins and ligands. Docking evaluation gave probable key ligands and hub proteins for validation in the case of sheer number of differential proteins and probable ligands in TCM. Liu while screening ligands from the literature, could also have utilized NP databases to find more active ingredients for docking studies. The further pharmacological validation of monomers was conducted including but not limited to the screened ligands (some reported active ingredients might have been ignored by purely manual screening). This line of thinking was used when the NP databases were utilized to find potentially active ingredients.[24] However, since the reliability of ADME filtering results of compounds using NP-related platforms is debatable, NP prediction ought to be combined with the reported data.

Moreover, the Cytoscape software was used to develop the network of the differential proteins[25] or ligands in TCM[26] and calculate the topological parameters like degree centrality using the network analyzer. Cytoscape network analysis is similar to the docking approach, with the difference that Cytoscape network analysis is based on topological configuration between objects and the docking analysis is based on the molecular structure of pharmacophores.

2.2. Metabolomics

As a major approach of systems biology, metabolomics is aimed at analyzing the metabolites with a relative molecular mass of less than 1000.[27] Metabolomics has been widely used to study TCM.[28,29]

With the aim to develop an integration strategy to make the metabolomics approach more targeted, Wang identified 595 differential metabolites in *Astragalus membranaceus* (Fisch.) Bunge extract while evaluating its anti- liver fibrosis properties. Six of these metabolites were analyzed by NP-based methods, and the potential metabolite–target–component interaction network was developed by MBROLE 2.0 platform. The topological analysis that followed indicated the probable hub targets and key metabolites.[30]

The multi-omics approach has a problem of too much data, which poses difficulty in collecting effective data. Besides conducting PPI analysis between differential proteins and selecting the hub proteins for biological enrichment analysis, the key differential metabolites can also be identified by carting out network analysis before metabolism pathway analysis. Therefore, the NP-based tools provide a promising approach to narrow down the selected scope for more efficient validation.

Besides, NP-based methods can build bridges between the extract and monomers to some degree. Hua *et al* [31] utilized the NP-based method after the analysis of differential metabolites of a TCM formula and predicted the active components and pathways. Wang *et al* [32] developed a workflow combining molecular docking, affinity MS, and metabolomics to determine potential ligands from TCM.

An important aim of metabolomics is to identify the diagnostic bio-marks. Integration strategy can also be used combining network toxicology (NT) and metabolomics to relate the toxicity bio-marks with TCM components and explore its toxicity mechanisms.[24,33] Zhang *et al* [35] developed an integrated strategy for accurately screening the bio-marks using NP and metabolomics.

2.3. Transcriptomics

The transcriptomics approach is targeted at all transcripts of genes and used to investigate the transcribed RNA. The main technologies based on transcriptomics are high-throughput sequencing and gene chip analysis.[36] The differential gene expression profile obtained by transcriptomics can be utilized to uncover the deep-layered mechanisms underlying the treatment of diseases.[37,38] Currently, the transcriptomics approach has wide-ranging applications in TCM research.[39,40] Xing *et al* [41] collected the known transcriptomics data in Gene Expression Omnibus (GEO) database to analyze the differential gene expression profile and obtain the differentially expressed genes (DEGs). The DEGs can be compared with the target-fishing results of active components in TCM. A similar report is recently published.[42]

If DEGs cannot be screened in public databases, RNA sequencing (RNA-seq) or gene chip analysis should be adopted. He *et al* [43] utilized the RNA-seq approach to analyze the DEGs for enrichment analysis, followed by PPI analysis. Zhou *et al* [44] utilized mRNA microarray and NP-based analysis to find DEGs. Besides, the RNA-seq approach can also be used to validate the results obtained by NP-based analysis.[45] Screening of differential genes by transcriptomics approach and identification of probable hub targets by NP-based analysis are aimed to obtain the targets playing a role for further validation; these methods are complementary.

Furthermore, multi-omics[24,46] and NP combined with transcriptomics, proteomics, and metabolomics approaches were used to uncover the mechanism of TCM in a stereoscopic and holistic manner.

3. GUT MICROBIOTA STUDY

The gut microbiota in the human body constitutes a microecological system and the balance of which can affect the normal physiological functions and susceptibility to several diseases since the gut microbiota acts on biochemical processes like catabolism and anabolism of proteins.[47,48] The gut microbiota study is

becoming a research hotspot nowadays, and emerging theories including the gut-liver axis[49] and gut-lung axis[50] are proposed. This technique is broadly used in mechanism studies of TCM.[51]

Zhang *et al* [52] reviewed the role of NP and suggested that the database recording relation between TCM and gut microbiota should be further refined. Herein, a novel database of gut microbiota, the gutMEGA database,[53] will be introduced. This database has a collection of metagenomics data of 59 132 quantification events for 6 457 taxa at seven different levels. A similar database is GMrepo[54] that holds a great quantity of meta-data of gut samples and the associated human phenotypes. The gut microbiota database was used in various individual studies,[55] but no TCM studies utilized them to this day. These databases were believed to provide a promising prediction to decipher the probable mechanism of TCM related to microecology.

Gao *et al* [56] explored the systematic relationship between proteins, metabolites, and gut microbiota using NP, metabolomics, and gut microbiota sequencing to investigate the potential mechanism underlying the curative effect of Qi-Jian Mixture in type-2 diabetes. Ding *et al* [57] confirmed the activity of the TCM formula Ge-Gen-Qin-Lian decoction against LPS-induced acute injury and predicted the potential targets using NP-based methods. The mechanism of the lung-gut axis was further elucidated through transcriptomics and metabolomics approaches.

4. CHEMICAL INFORMATION ANALYSIS

4.1. Quali-quantitative analysis

For the NP-based methods have limited capacity in excluding the ingredients that are present in extremely low concentrations, the quali-quantitative analysis of TCM with the NP method has been utilized. Thus, the credibility of NP prediction increases remarkably. Banerjee *et al* [58] identified thirteen components by LC-MS based metabolite screening, and the components were further conducted NP-based analysis. The Mahuang Fuzi Xixin decoction was conducted qualitative analysis to identify the ingredients. Twenty-four ingredients were identified to match the ingredients recorded in databases. These ingredients were further analyzed by NP-based methods and the toll-like receptor and T-cell receptor pathways were highlighted.[59]

4.2. Pharmacokinetic study and ADME screening

The high content of ingredients in the TCM does not always mean a high therapeutic concentration in the body due to the complex nonlinear process of drug metabolism.[60] Therefore, the pharmacokinetic study is considered. An investigation was performed over the type II diabetes mellitus based on the pharmacokinetic target components of Sanye Tangzhi formula.[61] Five components were absorbed and eliminated efficiently. These components were further conducted NP-based analysis. A qualitative and pharmacokinetic based integration NP strategy was performed to investigate the curative mechanisms of *Phlomis brevidentata H.W.Li Radix* for the treating pharyngitis and pneumonia.[62] As a result, twenty-three chemical compounds were identified, three of which were found exhibiting similar ADME characteristics and further anlyzed through NP-based methods and five targets were highlighted.

Besides pharmacokinetic study, the ADME properties of TCM compounds can also be evaluated by NP tools.[63,64] For example, the simplified molecular input line entry specification (SMILES) sequence of compounds can be entered into the SwissADME platform[65] to predict the ADME parameters. However, as described above, the reliability of ADME-based screening is debatable. Thus, the details of several platforms that can provide ADME properties are summarized to motivate further related researches (Table 1).

4.3. Spectrum-effect relationship approach

The spectrum-effect relationship approach is viable in TCM research.[72,73] The spectrum-effect relationships were utilized to investigate the correlation between the curative effect of TCM and their fingerprints. The fingerprints of the TCM exact and drug-containing serum are collected and profiles, including a spectrum of high performance liquid chromatography (HPLC), gas chromatography (GC), were built. By comparing the spectra using analytical methods such as gray relational, cluster, and principal component analysis, the differential chromatographic peaks are screened and identified to predict the potential active compounds.[74] However, reports on the integration strategy of NP and the spectrum-effect relationship approach are scarce, which can be due to the high workload and time consumption. As an effective approach to screen the probable bioactive ingredients in TCM, spectrum-effect relationship approach may play a role in further research. Conclusively, the chemical information analysis of TCM is believed to provide stronger support for the follow-up NP-based analysis. The methods of NP and chemical information analysis are mutually potentiating and the integration strategy can help to increase the reliability of NP.

5. DATA-MINING AND THE INNOVATIVE APPLICATIONS OF NP

It is known that selecting TCM from tens of thousands of studies is difficult.[75] To avoid the omission of valuable information, data-mining of TCM is utilized recently.[76,77] Data mining approaches can find TCM combinations of significant therapeutic value using methods[78,79] such as frequency analysis, correlation rule mining, and recurrent neural network.

The modular characteristics of TCM that have curative effect in endometriosis were explored.[80] The search of modern and ancient literatures was conducted to prepare

Table 1 Platforms that can provide ADME properties

Name	Description	Website	Reference
TCMSP	A unique systems pharmacology platform of Chinese herbal medicines that captures the relationships between drugs, targets, and diseases.	https://tcmsp-e.com/	17
Swiss ADME	A tool allows you to compute physicochemical descriptors as well as to predict ADME parameters, pharmacokinetic properties, druglike nature and medicinal chemistry friendliness of one or multiple small molecules to support drug discovery.	http://www.swissadme.ch/	65
DrugBank Online	A comprehensive database containing information on drugs and drug targets. As both a bioinformatics and a cheminformatics resource, detailed drug data with comprehensive drug target information were combined.	https://go.drugbank.com/	66
CancerHSP	A database contains 2439 anticancer herbal medicines with 3575 anticancer ingredients. For each ingredient, the molecular structure and nine key ADME parameters are provided.	https://lsp.nwu.edu.cn/rj1/CancerHSP2.htm	67
ChEMBL	A database of bioactive drug-like small molecules, it contains 2-D structures, calculated properties and abstracted bioactivities	https://www.ebi.ac.uk/chembl/	68
ChemSpider	A free chemical structure database providing fast text and structure search access to over 100 million structures from hundreds of data sources.	http://www.chemspider.com/	69
TCMIP	An integrative pharmacology-based research platform of TCM, including comprehensive and standardized information for the commonly used herbs and formulas of TCM.	http://www.tcmip.cn/TCMIP/index.php	70
ADMETlab 2.0	A platform for systematical evaluation of ADMET properties, as well as some physicochemical properties and medicinal chemistry friendliness.	https://admetmesh.scbdd.com/	71

Notes: TCMSP: Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform; ADME: Absorption, distribution, metabolism, and excretion; CancerHSP: Anticancer herbs database of systems pharmacology; ChEMBL: A database of bioactive drug-like small molecules; TCMIP: Integrative Pharmacology-based Research Platform of Traditional Chinese Medicine.

for data mining. After screening, 551 literatures were performed analysis including descriptive analysis, frequent itemset mining and recurrent neural network analysis. The herbs with therapeutic benefits were further analyzed by NP. A natural product library was developed by data mining and screened it for ADME properties using NP.[81] Other studies combining NP and data mining are also published.[82-84]

Taking the NP and data mining as a whole, an optimized strategy is formed. Furthermore, this kind of optimization is not merely limited to the NP-based analysis, but exists throughout the investigation, namely the innovative application of NP.

Generally, the NP method links TCM ingredients with the disease targets, but the probable curative effects of TCM cannot be explored for lack of pharmacology-related data. A new model of "targets-(pathway)-targets"(TPT) was proposed to overcome these concerns by utilizing the concept of "module".[85] Exemplified by the study of Zuo *et al* [86]. After the processes of target-fishing and enrichment analysis, the miscellaneous targets were divided into eight modules by the Louvain algorithm[87,88] according to the molecular function and biological process; different modules share different functional propensities and correspond to different pathways. The contribution scores of modules toward diseases were calculated by the contribution-scoring algorithm to evaluate the relationship between modules and diseases. Therefore, diseases related to the module with the highest score were further analyzed, and the hub targets were screened from the key module.

An *in silico* drug repurposing model for coronary disease was developed.[89] Specifically, the drug, target and the PPI interaction networks of the coronary disease genes were developed. The permutation testing was then performed to evaluate the natural products and drugs to be prioritized for association with the disease. The calculated values were corrected by the Benjamini-Hochberg approach[90] and the level of association was characterized by Z-score. After analysis, the drugs and natural products that showed a novel action mechanism against coronary disease were selected. Similarly, Hu *et al* [91] used the Prince algorithm[92] to infer new associations between *Hedyotis diffusa Willd*. and various genes using an iterative network propagation method. After the analysis of gene families, the relevant genes and pathways were identified.

6. NT

The efficacy and safety profiles of synthetic drugs, as well as TCM, are of utmost importance.[93] For instance, aristolochic acid causes nephrotoxicity.[94,95] *Aristolochia manshuriensis Kom*. of the Aristolochiaceae family was previously used in a TCM formula. Despite the processing of TCM, the potential safety issues cannot be neglected. Therefore, the toxicological studies of TCM and its formulas are crucial. With the modernization of TCM development, more experimental toxicology studies were conducted recently.[96,97]

Similar to network pharmacology, toxicology also has a prediction method NT. NT was conceptualized in 2011.[98] NT entails the prediction and pilot analysis of TCM toxicity using the network model. Further, approaches such as NP are used to conduct the enrichment analysis. Similar to NP, the NT method can also be integrated with several research approaches. Exemplified by the study of Liu *et al*.[99] It was reported that an active ingredient in

Tripterygium wilfordii Hook. f. named celastrol triggered cardiotoxicity in zebrafish embryo;[100] however, the underlying mechanisms were unclear. NT-based method combining the metabolomics study was taken. The palmitic acid and TNF pathway were selected for experimental toxicology validation through analysis of differential metabolites, potential hub targets and pathways. Hou *et al* [101] combined the spectrum-toxicity relationship with the NT-based method to determine the probable hepatotoxic ingredients in TCM.

TCM is the coordination of efficacy and toxicity,[102] these targets that did not associate with the therapeutic effect were regarded to be harmful, and thus the NT method is like that in NP. Numerous integration strategies were reported recently.[33,34,103]

7. CONCLUSIONS

The rapid development of systems biology has derivatized NP as well as several algorithms, tools, platforms, and software. These NP-based approaches have been broadly utilized in TCM-related research. There are many difficulties in the comprehensively investigation of bioactive ingredients and systematically explanation of curative mechanisms of TCM due to the characteristics of "multi-component", "multi-target", and "multi-pathway". The NP-based methods can provide ideas for addressing the issues involved in TCM-related researches. Essentially, NP is a pilot research that provides valuable references for the follow-up pharmacodynamics and mechanism study, but the analysis solely based on NP can cause the absence of experimental evidence and probable false-positive results. With the aim of overcoming these limitations, integration strategies have emerged.

In perspective, the integration strategy of NP should focus on its irreplaceable advantages. NP is convenient and timesaving with large amount of information. Moreover, many methods of NP-based analysis are indispensable, such as PPI, GO and KEGG analysis, which is closely linked with most multi-omics approaches to analyze the data.

Conclusively, TCM studies are still in infancy, and more TCM-related basic research is essential. NP-based methods and the integration strategies can preliminarily predict the potential material basis of TCM and its probable mechanism, which can build a bridge between Chinese and Western medicine. Using the integration strategies, we explored many possibilities in this review that are expected to be helpful for subsequent studies. We believe that more integration studies will be conducted in the future to advance the TCM research.

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