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Review

Prediction of quality markers of traditional Chinese medicines based on network pharmacology

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ABSTRACT

Network pharmacology is a powerful tool to reflect the pharmacologically active effects, mechanism of action and toxic activity of traditional Chinese medicines (TCMs). The ingredients of TCMs, associated with quality control of TCM products, are those fundamental chemicals that exhibit biological activities. A great amount of effort has been made by scientists in that field in order to improve the quality of TCMs, though the approaches to determine their quality and the TCM theory and compatibility rules remain ambiguous. Now some methods and technologies must be applied to predict and explore the quality marker (Q-marker) for quality control, as well as to clarify the factors affecting the quality of TCM, which may give new insight into rational ground of establishment of appropriate quality control and assessment system. In this review paper, authors focus on the prediction of quality markers of TCMs by network pharmacology based on three aspects: (1) from network medicine to network pharmacology, (2) complex network system of traditional Chinese medicine, and (3) predicting TCM quality markers based on network pharmacology. Authors proposed the research pattern on network pharmacology based on biological and medical networks, and further TCM network pharmacology based on substantial basis of TCM formulae, and the idea of "effect-ingredient-target-fingerprint" to predict and recognize the TCM Q-marker was the ultimate goal. In addition, authors yet noted how to make full use of the advantages of network toxicology to provide new ideas for the toxicity study of complex TCM systems and the prediction of TCM toxicity markers.

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1. Introduction

Traditional Chinese medicines (TCMs) is a traditional Chinese medicine system developed over thousands of years of application by Chinese pharmacologists. Due to its complexity, how to recognize and apply modern science and/or technology for research and innovative development of TCMs remains as a huge challenge.

According to the characteristics of TCMs, for instance multi-herbs, multi-ingredients, multi-targets, multi-functions, and multi-pathways, the conventional approaches used in study of TCMs have great difficulties in recognition of regulation of synergy effect and diseases as an entire group. Current development of network pharmacology is starting with multi-target approach to explore active ingredients and their potential targets and functions. Therefore, this method shows an irreplaceable advantage and widespread application in the studies of active ingredients, targets of action and therapeutic functions in the TCM study (Anastasio, 2015; Nüesch, Häuser, Bernardy, Barth & Jüni, 2013; Pang et al., 2018; Ru, Li & Wang, 2014; Xue, Shi, Li & Song, 2018; Zhang et al., 2017).

Although many researchers have dedicated to improve the quality of TCMs, the approaches to determine the quality, on the basis of TCM theory and compatibility rules remains unclear. It is considered that due to the complexity of TCMs, new methods and technologies must be applied to explore the potential quality markers for quality control, also clarify the factors affecting the quality of TCMs, which may give insight into rational ground of establishment of appropriate quality control and assessment system (Liu, Guo, Liu, 2018, Liu, Liu, Guo, 2018).

In view of the difficulty in understanding the origin of TCMs and the complexity of TCM theory, scientists at home and abroad have applied a variety of modern research methods to investigate the chemical fingerprints, chemical markers, and bioassay methods, and others. Prof. Chang-xiao Liu has proposed the fundamental principles and basic research approaches and patterns for TCM quality research and a new concept of TCM quality marker (Q-marker) with five basic rules (Liu et al., 2016; Liu et al., 2017, Liu Guo et al., 2018, Liu, Liu et al., 2018). According to the new concept and theory of Q-marker of TCMs, the development of TCM scientific research, modern technologies and methods have attracted the scientists to pay attention to the prediction of Q-markers (Li et al., 2018; Liu, Li & Liu, 2018; Zhang et al., 2018a; Zhang et al., 2018b). The published research articles focused on the methods and theories of discovery, identification of quality markers by applying biology, pharmacology, toxicology, pharmacokinetics and data mining to evaluate and validate the Q-markers.

In terms of data mining and network analysis, the published research paper (Bai et al., 2018; Hou et al., 2018; Jiang, Zhang, Chen, Li &, Liu 2017; Li et al., 2018; Xiong & Peng, 2016; Xu, Xu, Zhang, Zhang & Liu, 2018; Zhang et al., 2018c) is also supportive of the feasibility in predicting TCM Q-markers based on network pharmacology approach. From the perspective of network medicine and network pharmacology, this paper discussed several basic techniques and methods for predicting TCM Q-markers based on network analysis, and provided experimental design basis for the implementation of TCM Q-markers through the application of these technologies and methods.

2. From network medicine to network pharmacology

Based on advances in medicine, chemistry and biology and network science, network medicine is the application of network science towards identifying, preventing, and treating diseases. The field on network medicine focuses on using network topology and network dynamics towards identifying diseases and developing medical drugs in disease therapeutics. When using interatomic networks, someone can discover and classify diseases, as well as develop treatments through knowledge of its associations and their role in the networks. Biological networks, such as protein-protein interactions and metabolic pathways, are utilized by network medicine. Disease networks, which map relationships between diseases and biological factors, also play an important role in the field. Epidemiology is extensively studied using network science as well; Social network and transportation network are used to model the spreading of disease across populations. Network medicine is therefore a medically focused area of systems biology.

The term “network medicine” was coined and popularized in a scientific article by Albert-László Barabási, published in *The New England Journal of Medicine*, in 2007. Barabási states that biological systems, similarly to social and technological systems, contain many components that are connected in complicated relationships but are organized by simple principles. Using the recent development of network theory, the organizing principles can be comprehensively analyzed by representing systems as complex networks, which are collections of nodes linked together by a particular relationship. For networks pertaining to medicine, nodes represent biological factors (biomolecules, diseases, phenotypes, etc.) and links (edges) represent their relationships (physical interactions, shared metabolic pathway, shared gene, shared trait, etc.).

Three key networks for understanding human disease are the metabolic network, the disease network, and the social network. The network medicine is based on the idea that understanding complexity of gene regulation, metabolic reactions, and protein-protein interactions and that representing these as complex networks, which will shed light on the causes and mechanisms of diseases. It is possible, for example, to infer a bipartite graph representing the connections of diseases to their associated genes using OMIM database.

Network pharmacology is a rapidly developing field based on systems pharmacology that emphasizes the effect of drugs on both the interactome and the diseasome (Hopkins, 2008). The drug-target network (DTN) can play an important role in understanding the mechanisms of action of approved and experimental drugs (Yıldırım, Goh, Cusick & Barabás, 2007). The network theory view of pharmaceuticals is based on the effect of the drug in the interactome, especially the region that the drug target occupies. For a complex disease, combination therapy is recommended in this field, since one active pharmaceutical ingredient (API) aimed poly-pharmacology to study target-effect in the entire disease module (Hopkins, 2008). The concept of disease modules can be used to aid in drug discovery, drug design, and the development of biomarkers for disease detection. There can be a variety of ways to identify drugs using network pharmacology; A simple example of this is the “guilt by association” method. This states if two diseases are treated by the same drug, a drug that treats one disease may treat the other. Drug repurposing, drug-drug interactions and drug side-effects have also been studied in this field. Network pharmacology is a distinctive new approach for drug discovery. It involves the application of network analysis to determine the set of proteins most critical in any disease, and then chemical biology to identify molecules capable of targeting that set of proteins. By addressing the true complexity of disease and seeking to harness the ability

of drugs to influence many different proteins, network pharmacology differs from conventional drug discovery approaches, which is generally based on highly specific targeting of a single protein. Network pharmacology has the potential to provide new treatments for complex diseases where conventional approaches have failed to deliver satisfactory therapies.

Network pharmacology is a new approach and strategy for drug design based on the rapid development of systems biology and multi-directional pharmacology. It involves system biology, network biology analysis, gene connectivity and redundancy, and genes. Network pharmacology transcends the shackles of single-target thinking and provides a new strategy for new drug discovery based on multi-target research strategies. Network pharmacology is based on network biology and multi-directional pharmacology (Fig. 1). The new idea of drug design, discovery and development will help to expand the available drug target space, which is one of the new strategies for new drug research and development.

Current network analysis and relevant methods and techniques, such as computer graphics, high-power radiation sources, computational processing power, virtual screening, and any other approaches available construct a powerful network. The development of tools has laid a solid foundation. Through the integration of network searching algorithms and biological activity prediction methods and other related software tools, the establishment of a reliable network library, protein network library, disease network library and drug network library and sub-structure-target database continuous improvement, enrichment and improvement, the network pharmacology has become a research hotspot in Chinese medicine research and development of new drugs and complex TCM systems.

3. Traditional Chinese medicine is a complex network system

Traditional Chinese medicine (TCM) generally plays a therapeutic or regulating role in accordance with the compatibility principle of “King, minister, assistant and guider” under the guidance of TCM theories (Lu & Huang, 2014; Liu, 2017). The therapeutic or recuperative effects of traditional Chinese medicine are recognized on the basis of its medicinal materials. The bio-response substantial basis of TCM refers to the generic name of chemical components contained in TCM that can reflect the clinical efficacy of drugs (Wang, 2015).

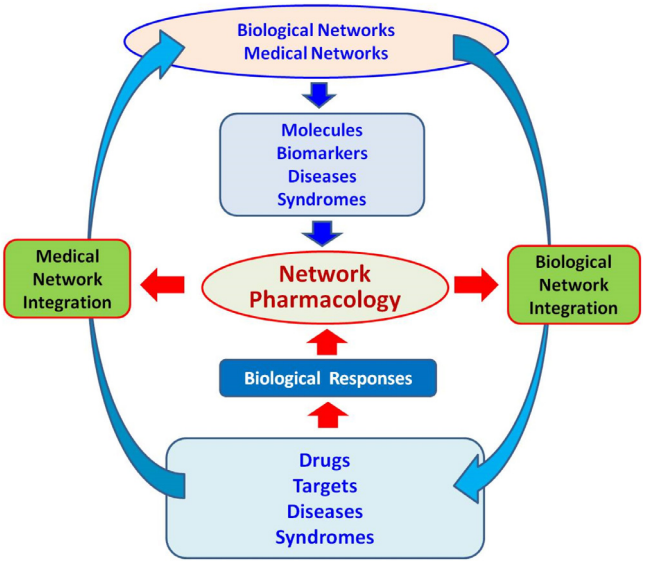

Fig. 1. Network pharmacology based on biological networks and medical networks.

To understand the substantial basis of traditional Chinese medicine, the composition of the chemical components of traditional Chinese medicine must be clarified first. The chemical composition of a single TCM is very complex, and the complexity of the chemical composition of the compound of several or even more than ten herbs will be far beyond our imagination. In recent years, mass spectrometry has been widely used in the study of chemical components of TCM and the study of small molecule compounds in metabolomics, which has made great progress in clarifying the basis of medicinal materials and the mechanism of action of TCM (Feng, Christopher & Alan, 2012; Xia, Bao, He, Deng & Zhou, 2017; Yang, Tong, Li, Li & Liu, 2016). These are only from the source of matter for the elucidation of its functional characteristics. The differences existing in efficacy between drug compositions and interactions between complex substances make it really difficult to understand the complex TCM system.

Chinese TCM scientists have applied network pharmacology approaches into the basic and application studies of TCM. Network pharmacology is an approach based on systems biology, polypharmacology and molecular networks, which has been extensively applied to analyze relationships between drugs and diseases in recent decade (Hong et al., 2017a, 2017b). In particular, it has attracted considerable attention among Chinese medicine researchers for its ability to predict and illustrate interactive relationships between numerous components and targets of TCMs (Li, Yuan, Pan, Wang & Chen, 2017; Mao et al., 2017).

Network-based pharmacological analysis is an ideal approach for investigating the mechanisms of action for herbs and formulae and their potential bioactive components are at molecular and systematic levels. In addition, in silico study is a good tool in predicting the potential active components and mechanisms of action of TCMs, which renders more effective subsequent exploration with experimental approaches (Mao et al., 2017).

In network pharmacological analysis, it is fundamental to understand the properties and biological activity of TCM compounds, and relevant data information can be obtained from those public network databases. The constituent compounds and related targeted genes of the herbs were identified by BATMAN-TCM (Bioinformatics analysis tool for molecular mechanism of TCMs) (Liu et al., 2016). Liver disease targets were recognized by VENNY 2.1 (Oliveros et al., 2007). The network of compounds-targets was constructed by Cytoscape 3.7.0 which is a comprehensive bioinformatics software platform designed for visualizing biological pathways and molecular interaction networks (Cytoscape 3.7.0, 2018). The application can also integrate these networks with annotations, gene expression profiles and other data. Although Cytoscape was originally designed for biological research, now it is a general platform for complex network analysis and visualization. It supports a lot of standard network.

Lipinski’s rule (LR) of five also known as the Pfizer’s rule of five or simply the rule of five is a rule of thumb to evaluate druglikeness or determine if a chemical compound with a certain pharmacological or biological activity has chemical properties and physical properties that would make it a likely orally active drug in humans. The rule was formulated by Christopher A. Lipinski in 1997, based on the observation that most orally administered drugs are relatively small and moderately lipophilic molecules (Lipinski, Lombardo, Dominy & Feeney, 2001, 2004). The pharmaceutical properties’ analysis was performed according to LR and the absorption, distribution, metabolism, and excretion system.

4. Predicting TCM quality markers based on network pharmacology

4.1. Development of concept of TCM network pharmacology

TCMs are complex system with multi-herb combination, multi-ingredients, multi-targets, multi-mechanisms of action and applied to complex biological systems. According to Chinese researcher's description (Li, 1999a; Li, 1999b, 2007; Li et al., 2007; Li, 2009; Li, Fan, Jia, Lu & Zhang, 2014), the concepts on TCM network pharmacology should cover the following aspects, TCM network pharmacology is basic application science to study hypothesis of the relationship between TCM syndrome and molecular networks, TCM formulae regulation for syndrome and disease balance using network-biology, network-phenotype and network- targets. A lot of studies have demonstrated that the TCM network pharmacology strategy has a wide variety of practical applications for understanding TCM herbal formulae. Therefore, with the development of systematic biology science and technology, with the deep understanding of complex traditional Chinese medicine system, the TCM network pharmacology will be continuously optimized.

4.2. Advance on TCM network pharmacology

In the past decade, a number of studies on TCM network pharmacology have shown that the application of network pharmacology is valuable for the study of the relationship between effective materials and efficacy-safety of complex TCMs, and the network study has a very promising prospective in study of quality and efficacy-safety of TCMs. A remarkable feature of TCMs is the use of TCM formulae. Understanding the mechanisms of action and combinatorial rules of herbal formulae is of great significance in TCM modernization and is also one of the important steps in modern drug research and development of TCM products with various factors to affect the intrinsic quality and material basis of TCM products. Characterized by holistic theory and rich experience in multi-component therapeutics, TCM formulae offer bright prospects for the control of complex diseases in a systematic manner (Chen et al., 2016).

Based on the materialistic epistemology, material existence is essential for all biological effects, and the drug effect on "symptoms" and "diseases" in traditional Chinese medicine should be recognized and studied. As shown in Fig. 2, the basic principle of the drug-gene-disease interaction 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.

In the complex network system of TCMs, a large number of chemical ingredients, which presented in medicinal materials, decoctions and prescription formulae, is the basis of constructing its network-pharmacology system. In summary, the biological-network, target-network, symptom-network and disease-network of TCMs are impartible from these chemical ingredients. The material changes in the preparation process of drugs are also impartible either from these chemical ingredients, as well as those regulation genes and relevant metabolism process.

Domestic practitioners have made remarkable achievements in applying network pharmacology to solve the relationship between the efficacy and multi-ingredients of TCMs, and have published several highly valuable research reports. For example, pharmacodynamic effects of main chemical components of *Sophora flavescens* Ait. (Hong et al., 2017c), *Polygoni Multiflori Radix* (Hong et al., 2017a), *Schisandrae Chinese Fructus* (Deng et al., 2018), *Lindera aggregate* (Sims) Kosterm (Yang, Duan, Yan & Zhang, 2018), *Scutellararia barbata* D. Don (Bai et al., 2018b), Dengataiye Tablets (Wang & Liu, 2018), Guanxinning Injection (Zuo et al.,), Yupingfeng

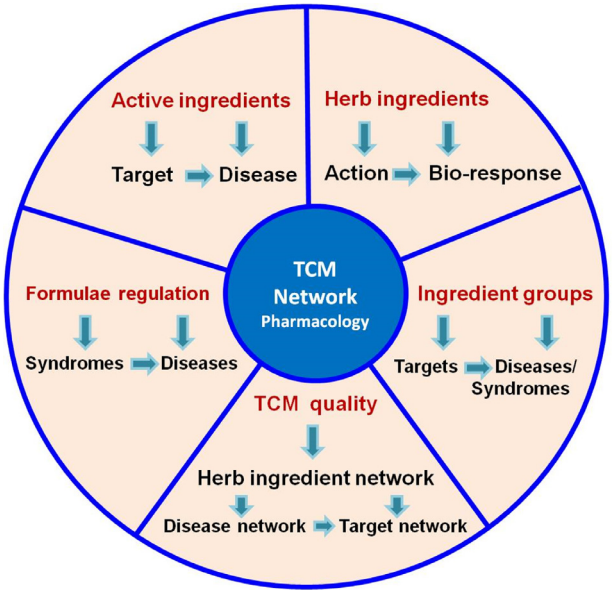

Fig. 2. TCM network pharmacology based on material basis of TCM formulae.

Decoction (Pang et al., 2018), and Naodesheng Formula (Wang et al., 2017) based on network pharmacology. These studies provide references for systematic exploration on the pharmacological actions and mechanism, multi-targets and multi-pathways.

4.3. Predicting TCM quality markers based on network pharmacology

In 2016, Liu et al. proposed a new concept of the quality marker (Q-marker) of TCMs, which aims to identify the quality indicator reflecting safety and therapeutic effects and establish a scientific rational method for quality control of TCM (Liu et al., 2016a; Liu et al., 2017). According to active ingredients with the relationship of quality and biological response (effect and safety under TCM theory and function), The proposed five basic principles of Q-marker are as follows: "(1) Q-marker exists in herbs, pieces, extracts, unilateral or compound formulations; (2) Q-marker should be analyzed through either qualitative and quantitative approach; (3) Q-marker is a biological effect associated with efficacy and safety; (4) Q-markers are substances with related biological effects based on TCM theory and compatibility theory; and (5) Q-marker is a substance with transmissibility and traceability in the process of TCM production and preparation in the new concept on TCM Q-marker" (Liu Guo et al., 2018, Liu, Liu et al., 2018). Therefore, we developed a pattern on network of "effect-ingredient-target-fingerprint" to predict and recognize the TCM Q-marker based on network pharmacology (Fig. 3).

On the basis of theory of TCM and clinical efficacy, the scientific quality evaluation and control system in combination with modern technologies are of particular importance for ensuring the effectiveness and safety of TCM products (Liu et al., 2016a; Liu et al., 2017; Li, Fan, Jia, Lu & Zhang, 2014; Yang et al., 2017; Liu et al., 2016). We carried out an integrated approach "effect-compound-target-fingerprint" to determine or predict Q-markers based on network pharmacology, and used this model to illustrate the confirmation process of Q-marker selection and application (Liao et al., 2018).

According to their internal relation, predicting and screening the potential Q-markers, a holistic integrative-strategy for Q-marker prediction and application is presented (Fig. 4).

Using LC-MS or UPLC-MS and network pharmacology method to investigate the active ingredients of TCMs (Liao et al., 2018),

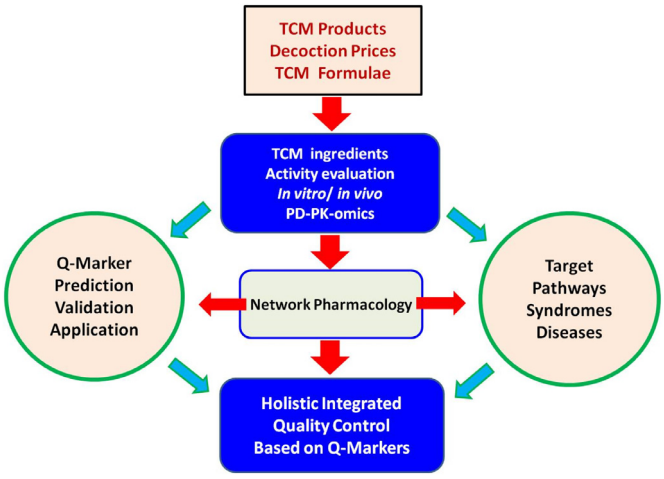

Fig. 3. A pattern on network of "effect-ingredient-target-fingerprint" to predict and recognize TCM Q-marker based on network pharmacology.

the ingredients-targets-pathways network constructed for the Q-markers was validated by activity evaluation system *in vitro* and *in vivo* based on network pharmacology. The "components-targets-pathways" regulated by *Alisma orientale* analysis was used to screen or predict the target and Q-marker of *A. orientale*. On the basis of these Q-markers, establishing the metabolite profile of *A. orientale* will greatly ensure the clinical efficacy. Based on the chromatographic fingerprints, researchers suggested that the integrated Q-markers were used for the holistic quality control system of TCM products.

4.4. Network toxicology of TCMs

A specific drug has sidle pharmacological effect and toxicity associated with treatment. Therefore, during the study of a drug, toxicity must be carefully considered, apart from its effectiveness and safety issues. The concept of network toxicology, initially proposed by Chinese scientists in the world back to 2011, is an important method for biomedicine research developed from network pharmacology (Fan, Zhao, Jin, Shen & Liu, 2011). Network toxicology used network to analyze and predict the toxicity of a specific drug, which plays an important role in the toxicity component prediction of TCMs (Fan et al., 2011; Liu et al., 2015, 2018).

The research process of network toxicology includes: (1) extracting genes, proteins, toxicity, side effects and other factors from literatures and empirical database; (2) taking genes, proteins and toxicity as nodes in the network, the network model is built by calculating the mutual relations between nodes; (3) on this basis of the relationship among genes, proteins, toxicity and side effects can be speculated, so as to study the toxicological properties of drugs and drug-related mechanism of toxicity (Fan et al., 2011). The development of network toxicology is closely related to network pharmacology, gene histology, proteomics and other systems biological technologies. Network toxicology is used to predict the toxic components of drugs, evaluate the safety of drugs, and promote the research of new drugs (Fan et al., 2011; Xing, Hui & Ya, 2015).

The network TOXNET databases of chemicals has been established to provide toxicological information related to a large number of known compounds. And some of them are closely related to traditional medicines and natural products with a large number of known compounds. The development and application of related database of network toxicology and new bioinformatics tools is one of the most important aspects of toxicology research, which will help to explain and study the large amount of biological information generated in the field of TCM toxicology (Youns, Hoheisel & Efferth, 2010). Table 1 shows the contents from the toxicology database of the national library of the United States (TOXEN, 2015).

Based on the predicted results of network toxicology, Li et al. established a method to preliminarily identify toxic compounds and comprehensively interpret the mechanism of toxicity of those toxic compounds. In the context of definite biological properties and chemical properties, the toxicity Q-marker was finally confirmed. Authors provide a study method for the toxicity Q-marker of TCM, which helps to systemically and comprehensively reveal

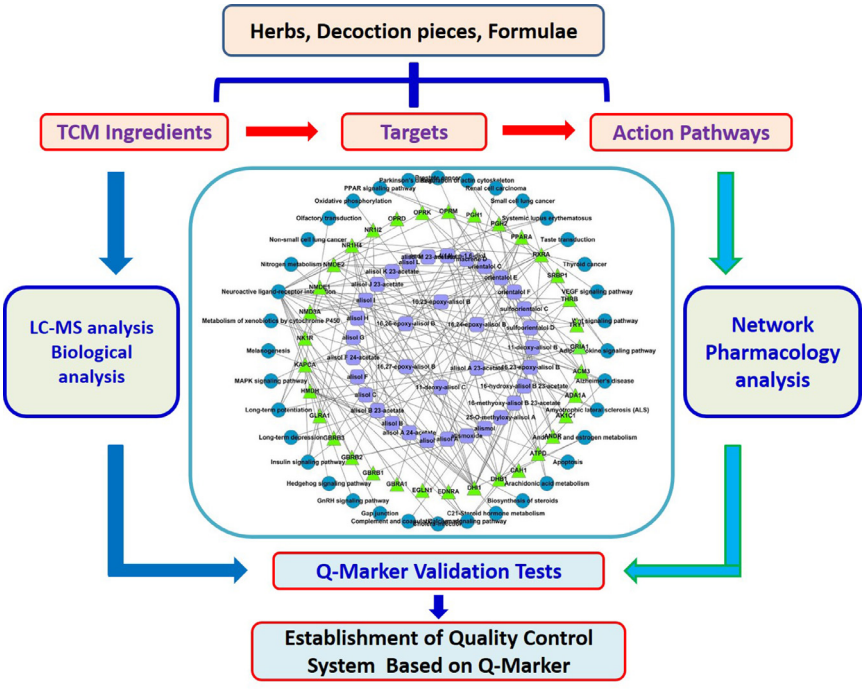

Fig. 4. Integrated strategy for Q-marker prediction and application.

Table 1
Contents from TOXNET database.

No.	Databases	Contents
1	Most visited by toxnet users	(1) HSDB: Hazardous Substances Data Bank. Peer-reviewed toxicology data for over 5000 hazardous chemicals (2) TOXLINE: 4 million references to literature on biochemical, pharmacological, physiological, and toxicological effects of drugs and other chemicals (3) ChemIDplus: Dictionary of over 40 0000 chemicals (names, synonyms, and structures)
2	Breastfeeding & drugs	LactMed: Drugs and Lactation Database. Drugs and other chemicals to which breastfeeding mothers may be exposed
3	Developmental toxicology literature	DART: Developmental and Reproductive Toxicology Database. References to developmental and reproductive toxicology literature
4	Chemical releases & mapping	(1) TOXMAP: TOXMAP: Environmental Health Maps provides searchable, interactive maps of EPA TRI and Superfund data, plus US Census and NCI health data (2) TRI: Toxics Release Inventory. Annual environmental releases of over 600 toxic chemicals by U.S. facilities
5	Genomics	CTD: Comparative Toxicogenomics Database. Access to scientific data describing relationships between chemicals, genes and human diseases
6	Household product safety	Household Products Database: Potential health effects of chemicals in more than 1 0000 common household products
7	Occupational exposure to chemicals	Haz-Map: Links jobs and hazardous tasks with occupational diseases and their symptoms
8	Risk assessment	(1) IRIS: Integrated Risk Information System. Hazard identification and dose-response assessment for over 500 chemicals (2) ITER: International Toxicity Estimates for Risk. Risk information for over 600 chemicals from authoritative groups worldwide
9	Animal testing alternatives	ALTBIB: Resources on Alternatives to the Use of Live Vertebrates in Biomedical Research and Testing
10	Archived, no longer updated	(1) CCRIS: Chemical Carcinogenesis Research Information System. Carcinogenicity and mutagenicity test results for over 8000 chemicals (1985–2011) (2) CPDB: Carcinogenic Potency Database. Standardized analyses of the results of 6540 chronic, long-term animal cancer tests (1980–2011) (3) GENE-TOX: Genetic Toxicology Data Bank. Peer-reviewed genetic toxicology test data for over 3000 chemicals (1991–1998)

the internal toxicity mechanism of TCM. The in-depth study of the toxicity Q-marker provides the material basis and technical support for the safety evaluation of TCM (Li et al., 2018).

The known Public databases based on prediction research of *Ganoderma lucidum* (Leyss. ex Fr.) Karst. and related compounds, build the network forecasting model, combined with fluorescence detection technology identified including ganoderic acid B, ganoderic acid D, kaempferia galanga phenolic active ingredients such as, through the network of pharmacology, six core targets and the center of the four target genes, may act as a potential tumor markers (Zhao & He, 2010). Tang, Huang, Lee & Chen (2017) studied a new Chinese medicine from TCM database matrix metaloproteinases inhibitor, using high-throughput virtual screening technology can help find natural compounds combined with zinc, and quantitative structure-activity relationship (QSAR) model was constructed to predict the qualitative metalloproteinases bioactive natural compounds. The results showed that the south rattan, lorbon and unilobin can interact with zinc binding sites, indicating good prediction effect.

The research methods of network toxicology are widely used in the field of TCM. The toxicity of TCMs is an important part of the modernization of TCMs. Network toxicology provides a simple, accurate and reliable tool for the preliminary screening of toxic substances and plays an important role in the prediction of hepatotoxicity and nephrotoxicity in TCMs (Liu et al., 2018). However, due to the complexity of chemical composition and molecular mechanism of TCMs, the scientific processing of TCMs (such as the process with drug toxicity reduction) and the complexity of the compatibility of TCM (such as drug taste compatibility of drug reduction and effect enhancement) also bring new challenges to the quality control of TCMs.

4.5. Prediction and validation process of quality markers for complex Chinese medicine formulae

The prediction and validation process of quality markers of Chinese medicine complex formulae was shown in Fig. 5. First, it needs to analyze the composition of compound compatibility principle, consider according to the taste natures of King, minister, assistant and guide drugs, in accordance with the standard preparations of single drug and their combination and the whole party composition of the complexity of the characteristics of the standard preparation fingerprints to possible predicted quality markers of primary substance. Secondly, on this basis, integrated pharmacological and network pharmacological methods should be applied to carry out pharmacodynamic (PD), pharmacokinetic (PK) and toxicological (Tox) studies, and to carry out studies on the drug-drug interactions and the interactions between of target and disease in marker validation test. Thirdly, the quality markers related to quality were obtained in combination with the validation results, and using the quality markers to establish a quality evaluation system for complex Chinese medicine formulae.

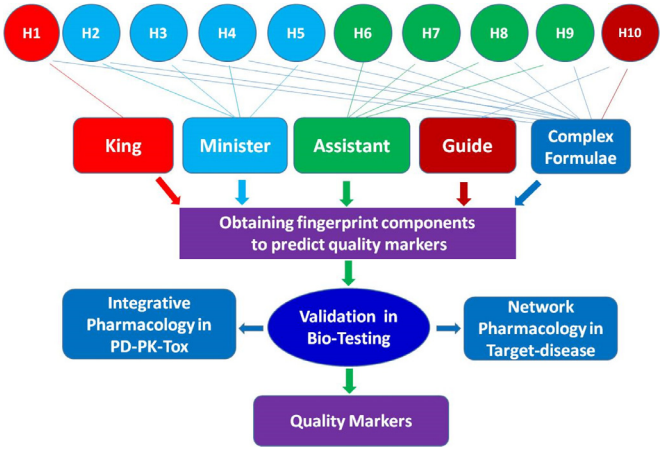

Fig. 5. Prediction and validation process of quality markers of complex Chinese medicine formulae.

For example, the modern prescription of Biqi Capsule comes from the famous doctor Tuo Hua in the Han Dynasty who passed on the experience of “one pill of elixir”, which is the represen-

tative prescription for the treatment of chronic diseases such as rheumatism and arthritis. The compound is the monarch, minister, adjuvant and each ingredient is clearly reflected in the side of a square, the modulation processing powder of *Strychnos nux-vomica* Linn. as "king" drug (H1), *Codonopsis pilosula* (Franch.) Nannf. (Dangshen in Chinese), *Atractylodes lancea* (Thunb.) DC, *Poria cocos* (Schw.) Wolf and *Salvia miltiorrhiza* Bge. as "minister" drugs (H2, 3, 4, 5), *Panax notoginseng* (Burk.) F. H. Chen, *Ligusticum chuanxiong Hort., Achyranthis Bidentatae Radix* and earthworm as "assistant" drug (H6, 7, 8, 9), and make the medicine licorice (H10) as a "guide" drug, the combination formulae of TCM is fitted the "King, minister, assistant and guide" compatibility principle.

The Pk study revealed the mechanism of enhancing efficacy and reducing toxicity of the combination of Biqi Capsules, which provided reliable scientific basis for rational clinical application. To dismantle the compatibility of Biqi Capsule, PK studies have shown that official medicine group and adjuvant could significantly prolong the Tmax of strychnine and brucine, delay the absorption, avoid excessive absorption to produce side effects, would continue to minister, assistant, sealing side of single drug group and drug compatibility, with no apparent PK differences (Liu et al., 2017). This study revealed the influence of the interactions of Minister, Assistant and Guide on the PK of "King" drug, reflecting the scientific compatibility of Biqi Capsules.

In Biqi Capsule, strychnine can control the amount of strychnine to the required level after being processed and mixed. Therefore, when establishing the quality standard of the product, the quantity of the two alkaloids of "King" drug was determined as the quantitative measurement index. In this study, the quantity of these two alkaloids in Biqi Capsule, "King" drug is a quantitative measurement index, which is not only Q-marker but also PK-marker.

5. Discussion

In 2016, Liu et al. proposed a new concept of the Q-marker of TCMs, which is applied to identify the quality indicator reflecting safety and therapeutic effects and establish a scientific rational method for quality control of TCMs (Liu et al., 2016; Liu et al., 2017, Liu Guo et al., 2018, Liu, Liu et al., 2018). The traditional Chinese medicine (TCM) or herbal medicine (HM) monographs are science-based quality standards that provide detailed specifications for identity, content of bioactive constituents or quality markers, and limit contaminants, adulterants, and potentially effective and toxic substances. The multiple tests included in each monograph complement provide an appropriate pharmaceutical quality characterization for the used TCMs or HMs. The chromatographic analytical procedures are validated to provide characteristic profiles for the identity and/or accurate determination of the content of Q-markers. Therefore, it is critical on how to determine those corresponding ingredients of TCMs in prediction and determination of TCM Q-markers.

The network pharmacology analysis is able to show the relationships of the pathways/targets with chemical ingredients of TCMs, and pathways/targets with herbal functions for therapy of diseases in TCM clinical therapeutics. We know that the network analysis clearly explains the interaction between "ingredient-target-pathway". The network relation of the ingredients, targets and pathways of TCMs provided the theoretical basis for mining and analyzing TCM Q-marker.

It is noted how to make use of the advantages of network toxicology to provide new ideas for the toxicity study of complex TCM systems and the prediction of TCM toxicity markers remains a huge challenge. The applicability of network toxicology method is also limited to some extent. Therefore, in the future research, how to combine the network toxicology prediction model with the chemical structure characteristics of TCM components to establish a suitable traditional Chinese medicine toxicity prediction system is the focus of further research.

Declaration of Competing Interest

The authors have declared that there is no conflict of interest.

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