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**Review**

# Network Pharmacology Bridges Traditional Application and Modern Development of Traditional Chinese Medicine

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ABSTRACT

Traditional Chinese medicine (TCM) has developed over thousands of years and has accumulated abundant clinical experience, forming a comprehensive and unique medical system. Emerging evidence has begun to illustrate TCM as an area of important medical rediscoveries. This paper briefly introduced the concept, significance, and technology of network pharmacology based on network biology and systems biology. It focused on the theoretical system and potential prospect of TCM network applied in TCM research and development including predicting new drug targets, action mechanism, new drug discovery; evaluating pharmacodynamics, pharmacokinetics, safety, toxicology, quality control, and bioinformatics of drugs. We also discussed the opportunities and challenges in the development and application of network pharmacology in the modernization of TCM research.

*Key words*

network biology; network pharmacology; systems biology; traditional Chinese medicine



## 1. Introduction

Traditional Chinese medicine (TCM) has been developed over thousands of years and has accumulated abundant clinical experience, forming a comprehensive and unique medical system (Liu and Xiao, 1992; Liu et al, 2000). TCM, derived from ancient China, is the mainstream medicine in China and also an important part in the world healthcare systems (Normile, 2003; Xue and Roy, 2003; Qiu, 2007). In Chinese history, physicians and pharmacists have summarized their experiences in the prevention and treatment of various diseases, and formed a systematical medical theory. They comprehensively understand the beneficial and side effects of Chinese herbal medicines (CHM) and write their prescriptions

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as effective and safe herbal formula. Generally, TCM treats diseases by the synergistic effects of multiple components of Chinese materia medica (CMM) or Chinese herbal medicines (CHM) in TCM formula. Obviously, this principle coincides with the characteristics of syndrome differentiation by TCM and holistic view of TCM treatment.

Although the scientific bases of TCM await further consolidation due to historic reasons, emerging evidence has begun to illustrate TCM as an important area for medical rediscoveries. This review briefly introduced the concept, significance, and technology of network pharmacology based on network biology and systems biology. This paper focused on the theoretical system and the potential prospect of TCM network pharmacology, summarizing its application in modernization of TCM. In this review, we also discussed the opportunities and challenges on the development and application of network pharmacology in the modernization of CMM/CHM research.

## 2. Development of network pharmacology based on network biology and systems biology

In the field of biological sciences, the term "network" has appeared in published literatures as early as 1936, firstly used by Abramson and Margolin (1936). We did not find "network research" papers published from 1936 to 1960.

### *2.1 Network biology*

Network theory is a branch of mathematics, which is related to networks of interacting objects. With the development of biological sciences, McCarthy firstly proposed "network biology" as a branch of the biological sciences in 2003 (McCarthy, 2003). In 2004, Barabási and Oltvai published an important review paper to elucidate the topic about network biology: understanding the functional organization of cells. They pointed out that "a key aim of postgenomic biomedical research is to systematically catalogue all molecules and their interactions within a living cell". There is a need to understand how these molecules and the interactions between the determined function of this enormously complex machinery in isolation and the surrounded other cells. The new concept of network biology will be a revolution for the view of biology and disease in the 21st century (Barabási and Oltvai, 2004).

Network biology can help researchers to understand the functional organization of cells. The theory predicts that modulating multiple nodes simultaneously is often required for modifying phenotypes. Taken together, the observation of phenotypic robustness after gene deletion and network biology theory indicates that in several instances exquisitely selective compounds may exhibit a lower-than-desired efficacy for the treatment of disease (Barabási and Oltvai, 2004). Barabási et al considered that the rapid advances in network biology indicated that cellular networks were governed by universal laws and offered a new conceptual framework that could potentially revolutionize our view of biology and disease pathologies. Thus, the compounds that selectively acted on two or more targets of interest should be more efficacious than single-target agents. Network biology may also play a role in drug target identification.

### *2.2 Systems biology*

Notably in the 1960s systems theory and biology attracted considerable interest among eminent scientists, mathematicians, and engineers. With the availability of quantitative data on the transcriptome and proteome levels, there is an increasing interest in formal mathematical models of gene expression and regulation. Some international conferences, research institutes, and research groups interested by systems biology have appeared in recent years and developed systems theory (Wolkenhauer, 2001). The concept of systems biology is to study biological systems by systematically perturbing them biologically, genetically, or chemically; monitoring the gene, protein, and informational pathway responses; integrating these data; and ultimately, formulating mathematical models to describe the structure of the system and its response to individual perturbations (Ideker et al, 2001). Systems biology deals with complex and comprehensive living systems involving a finite number of hierarchically ordered components, which form interacting networks affected by, and responding to, various perturbations within the system itself and from the environment (Boerries et al, 2012). Systems biology is an analytical approach to investigate the relationships among system's components in order to understand its emergent, i.e. network-level properties (Arrell and Terzic, 2010).

### *2.3 Network pharmacology*

Network pharmacology is based on the principles of network theory and systems biology. Systems biology aims at the integration of biological complexity at all levels of biological organization, which can be cell, organ, organism, or population. In that time, therefore, the concept of network pharmacology is built on the belief that targeting multiple nodes in interconnected molecular systems, rather than individual molecules, could lead to better efficacy and fewer adverse effects (Hopkins, 2007; 2008).

Yıldırım and his co-researchers applied the network analysis to drugs and drug targets. They integrated publicly available drug data with genetic-disease associations, gene-expression information and protein-protein interaction data, and investigated the relationships among approved drugs (Yildirim et al, 2007). When the researchers mapped drug targets onto human protein interaction data, they found that drug targets tended to have more interactions than average proteins but fewer interactions than essential proteins to a statistically significant degree. These results suggest that drug targets tend be nodes positioned in a "goldilocks" region of biological networks lying between the essential hubs and redundant peripheral nodes. (Yildirim et al, 2007). If drug targets are positioned at nodes that are too highly connected, they would likely be essential proteins, whose perturbation

may lead to toxicity. On the contrary, if drug targets were positioned at nodes that are periphery, they would likely be redundant with little effect on disease phenotype if perturbed. Therefore, the statistical network analysis could be a useful tool for prioritization of potential drug targets.

DrugBank is a unique bioinformatics/chem-informatics resource that combines the detailed drug data with comprehensive drug target information. Wishart et al (2006) used DrugBank as a comprehensive resource for *in silico* drug discovery and obtained some useful information. Although their analysis was limited by the relatively scarce amount of public drug-screening data compared with proprietary screening and literature databases, and by the incomplete mapping of the human protein interactions, the authors nevertheless observed a rich network of pharmacological interactions between drugs and their targets. Indeed, the drugs acting on single target appear to be the exception. Using network analysis of integrated data sets and network distance metric, they were able to distinguish between palliative drugs which relieve symptoms, and drugs that act directly on disease genes. The network distance metric highlights the increase in the drugs target and the genes associated with disease. Their findings add to our growing understanding of the role of pharmacological action of drugs (Hopkins, 2007).

In the study of Gohn et al, authors studied human disease network. The network analysis on OMIM database of genetic associations showed that the genetic origins of most diseases were shared with other diseases: among 1284 disorders catalogued in OMIM, 867 share at least one gene with another disorder. They found that essential human genes were likely to encode hub proteins and were expressed widely in most tissues. In contrast, they also found that the vast majority of disease genes were nonessential and showed no tendency to encode hub proteins, and their expression pattern indicated that they localized in the periphery of the network (Goh et al, 2000). This study suggested that disease genes would also play a central role in the human interactions. Hopkins (2007) considered that this study provided motivation to the growing interest in recent years in drug repurposing or indication-discovery strategies.

Developing methods to aid polypharmacology design can help to improve efficacy and predict unwanted off-target effects. Kesier et al (2007) began with 65 000 ligands annotated into sets for hundreds of drug targets. The recognition, informed by system biology, that the drugs for many disease states may require multiple activities to be efficacious may indeed holds the clues to designing a new generation of drugs that perturb biological networks rather than individual targets.

Combining systems biology with network biology may enable a new network pharmacology approach to be applied to drug research. The network of drug action is builted by drug-target networks and biological networks (Figure 1). Thus, network pharmacology could be used to investigate the complex dynamics of interconnected organic and molecular systems in drug discovery and development.

According to statistics by "Pubmed" net and the retrieved relevant network pharmacology literatures, the number of published papers (Figure 2) related to the network pharmacology increased year by year.

It was also found that the English and Chinese literatures on TCM network pharmacology were increased year by year from "Pubmed" and "CNKI" nets, which indicated increasing interest on network pharmacology applied in TCM (Table 1).

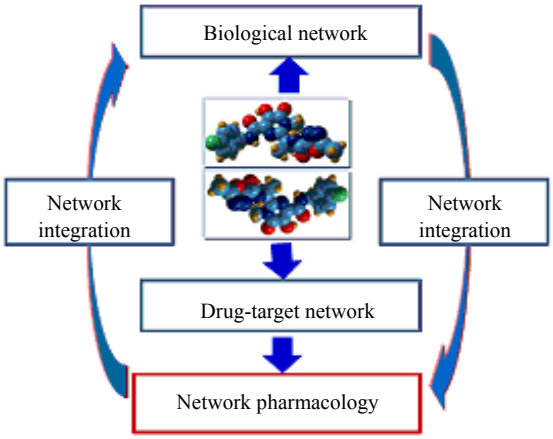


**Figure 1 Illustration of network pharmacology**

Network pharmacology is a network of drug action built by drug-target networks and biological networks.

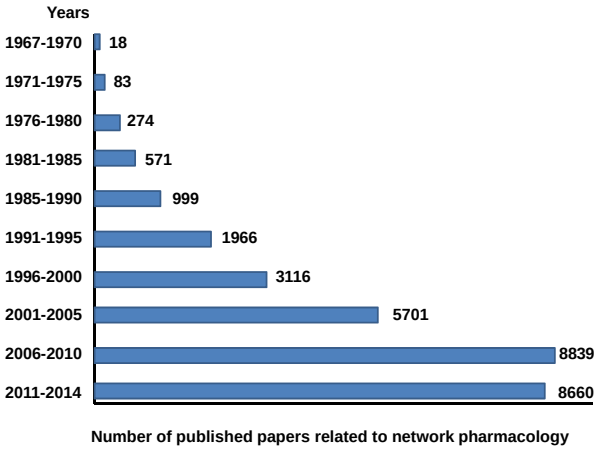


**Figure 2 Published papers from 1967 to June 2014 (total count 30 227) searched in Pubmed database with key words**

**Table 1 Paper numbers on TCM network pharmacology searched from Pubmed and CNKI database (2007−June, 2014)**

| Language | 2007 | 2008 | 2009 | 2010 | 2011 | 2012 | 2013 | 2014 |
|---|---|---|---|---|---|---|---|---|
| English | 1 | 4 | 4 | 9 | 13 | 27 | 26 | 24 |
| Chinese | 0 | 0 | 2 | 6 | 15 | 47 | 34 | 32 |
| Total | 1 | 4 | 6 | 15 | 28 | 74 | 60 | 56 |

### *2.4 Signification of network pharmacology in TCM research*

The overall concept and dialectical thought of TCM lay down the theoretical base for the dialectical treatment and prescription of CHM. The theory reflects multi-component,

multi-target point, and system regulation similar to the study thought of network pharmacology. Due to the features of the multi-component, multi-target, and synergistic effect existed in TCM, the effective substances and mechanisms of action are not always clear for each CHM prescription. Thus, the qualities of CHM are out of control, and a scientific and correct evaluating system is waiting to be established. In fact, the variability of complex chemical substances in CHM is caused by intrinsic and extrinsic factors such as species, difference, organ specificity, seasonal variation, age, cultivation, harvest, storage, processing methods and manufacturing practices. Therefore, establishing TCM network pharmacology for CHM prescription will be far more difficult than that for western drugs composed by single compound with only one or two targets in actions.

The network pharmacology is a novel subject based on the construction of multi-layer networks of disease-phenotype-gene-drug to predict the drug targets in a holistic view, and promotes the efficiency of drug discovery. Network pharmacology produces a new drug development idea from multi-target points beyond single target point so as to exert profound influence. Methodologically, network pharmacology integrated the notions of comprehensive research and systematic assessment, which agreed with the characteristics of holistic view and treatment based on syndrome differentiation in TCM. Establishing TCM network pharmacology, network toxicology, and network CHM decoction pieces could provide the technical support for the safety and rational administration of CHM and provide a new way in the research of CHM. The network pharmacology becomes a new strategy for modern TCM research. The strategies of the network pharmacology could promote the modernization and internationalization process of TCM, conduce to the research and development of TCM, and have the important significance for the integration of Chinese and Western medicines.

## 3. Application of network pharmacology in TCM research and development

With the rapid progress in bioinformatics, systems biology, and polypharmacology, network pharmacology is considered to be a promising approach toward understanding to multiple ingredients and multiple targets of a disease (drugs-targets-diseases). Characterized by holistic TCM theory and a rich experience in TCM therapeutics, the CMM/CHM formulae offer the bright perspectives for treating complex diseases in a systematic manner. Thus, bridging the emerging network science, including network biology and network pharmacology, ancient TCM will provide novel modern methodologies and technology and face the development opportunities in TCM basic and clinical researches. Here, we summed the recent progress in the theory, methodology, and application of TCM network pharmacology.

The recent progress in applying the network pharmacology in TCM research is shown in the following seven aspects, such as predicting new drug targets, action mechanism, new drug discovery, drug evaluation for PD/PK, safety and toxicology, quality control, and bioinformatics (Table 2).

### *3.1 Predicting new drugs and targets*

In the research and development of small molecular drugs, the database of the FDA-approved drugs and their targets for effects have been employed to create the networks of drug-protein interactions (Paolini et al, 2006; Yildirim et al, 2007) or to model similarities of chemical structure between

**Table 2 Recent progress on network pharmacology of TCM research and application**

| Study aspects | Application | References |
|---|---|---|
| Predicting new drug targets | Systems-pharmacology analysis; computational pharmacological comparison; prediction of the active ingredients and targets; drug-target interaction; multi-level data integration and so on | Liu et al, 2013a; Liu et al, 2013b; Zheng et al, 2013; Chen, Liu, and Yan, 2012; Tao et al, 2013; Fang et al, 2013; Li et al, 2014 |
| Action mechanism | Action mechanism and evaluation; drug discovery and combination with applications; action mechanisms of prescriptions; network pharmacology analysis | He et al, 2013; Cheng et al, 2014; Chen et al, 2014; Sun et al, 2014; Li et al, 1991; Shi et al, 2014; Li et al, 2014; Liang, Li, and Li, 2014 |
| New drug discovery | Drug discovery; network analysis in disease-drug; Quantitative proteomic analysis; polypharmacology for drug discovery. | Wang et al, 2013; Tao et al, 2013; Liu et al, 2013; Gu et al, 2013; Liu and Du, 2010 |
| Drug evaluation for PD and PK | Active compounds screening; efficacy and chemical constituents; pharmacokinetics; high through-put technology | Li et al, 2012; Xiang, Cai, and Zeng, 2012; Liu , Yang, and Chen, 2012; Liu et al, 2006; Jiang et al, 2005; Cao et al, 2011 |
| Safety and toxicology | Safety; toxicology; net work toxicology; clinical toxicology | Cao et al, 2012; Nin et al, 2011; Fan et al, 2012 |
| Quality control | Quality control; standard; identification | Tian et al, 2013; Wu et al, 2013; Liu et al, 2013a; Wang et al, 2012 |
| Bioinformatics | Proteomics; metabolomics; signaling networks; drug-gene-disease analysis | Zhang et al, 2013a; Zhang et al, 2013b; Fan et al, 2012; Zheng et al, 2013; Xu et al, 2013 |

drugs and potential ligands for the prediction of drug-target interactions (Keiser et al, 2007; 2009). In 2012, Simon et al employed 1177 FDA-approved small molecular drugs by investigating the interaction profiles based on in silico docking/scoring methods to a series of virtual non-target protein binding sites and contrasting these profiles with 177 major drug categories of the same series of FDA-approved drugs (Simon et al, 2012). Statistical analyses confirmed a close relationship between the studied effect categories and interaction profiles of small molecule drugs. The prediction power was independent of the composition of the protein set used for interaction profile generation. Perhaps general chemical and physicochemical properties of molecules are of importance for the potential interactions in general, whereas pharmacophores (i.e. specific stereochemical groups), are crucial for specific high-affinity interactions. However, TCM is an ancient system used in disease treatments for several thousand years. Up to now, about 100 000 CHM formulae have been recovered, and each of them normally contains several herbs. TCM treatment is different from single chemical drugs in relation of drug-target-disease.

The complex system CHM formulae are usually prescribed based on TCM syndrome patterns. Therefore, TCM network pharmacology is to establish the links between network molecular targets and TCM syndrome-diseases patterns. CHM formula exerts its therapeutic effects through interactions between the herbal ingredients and dysfunctional proteins related to the diseases. However, the action between the ingredients and targets may be changed to increase the therapeutic efficacy and reduce the adverse effects of CHM in the cell and function. Therefore, it is very important to establish herb-ingredient-target-drug network methods and study on the relationships among herbs, ingredients, targets and drugs.

Complex networks possess the characteristics that are very important for the investigation of drug discovery and drug treatment. However, understanding target is basic for the discovery and drug treatment. Generally, building a network involves two opposite approaches: one is bottom-up approach on the basis of established biological and pharmacological knowledge and the other is to use available data (such as compounds database of TCM) and statistical analysis.

As shown in Figure 3, TCM network pharmacology highlights the network model building from the compounds database to identify the potential and active compounds, build drug-targets network and targets-disease network, and analyze the molecular mechanism of action on CHM for TCM theory study and new drugs research and development.

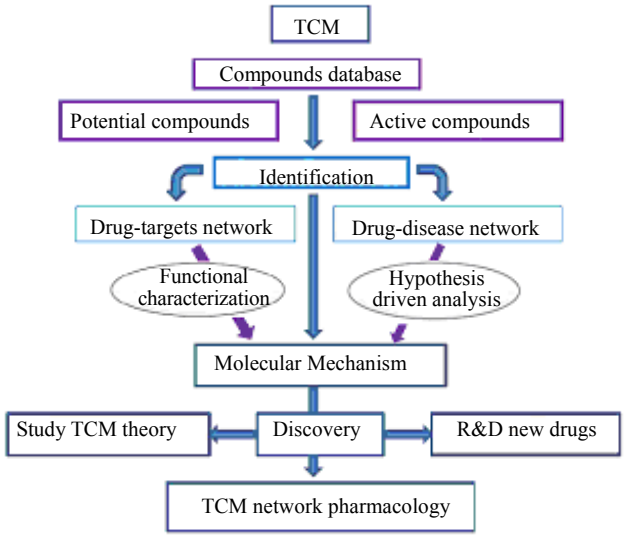


**Figure 3 Network pharmacology model illustration for target-drug-disease analysis of TCM**

In a paper by Li et al (2014), authors considered that the network pharmacology in TCM would provide the novel methodologies and new opportunities for discovering bioactive ingredients and endogenous/exogenous biomarkers, revealing the mechanisms of action and exploring the scientific evidence of numerous herbs and CHM formulae in TCM on the basis of complex biological systems of human body. They also considered that TCM network pharmacology can greatly promote the progress in network pharmacology. They have grouped together 27 papers in a special issue on TCM network pharmacology. The topics ranged from the research paradigm of network pharmacology based on TCM theory and practice, the available databases and computational tools in TCM network pharmacology, to the applications of the network pharmacology in TCM. Those papers highlighted some specific themes and concepts of network target, mechanisms of CHM formulae in TCM, and targets identification of herbal ingredients.

It is critical to predict the potential drug-target interactions from heterogeneous biological data, which helps not only to understand the various interactions and biological processes, but also to develop novel drugs and improve human medicines. Chen et al (2012) developed a network method based on the heterogeneous network (NRWRH) to predict the potential drug-target interactions on a large scale under the hypothesis. They compared with traditional

supervised or semi-supervised methods, and NRWRH makes full use of the tool in the network for data integration to predict drug-target associations. It integrates three different networks (protein-protein similarity network, drug-drug similarity network, and known drug-target interaction network) into a heterogeneous network by known drug-target interactions and implements the random walk on this heterogeneous network. In this study, using four classes of important drug-target interactions including enzymes, ion channels, GPCRs, and nuclear receptors, they found that NRWRH could significantly improve the previous methods in terms of cross-validation and potential drug-target interaction prediction.

In a paper by Liu et al (2013b), researchers predicted the potential new drugs and targets of two sets of tonic herbs, *qi*-enriching herbs (QEH) and blood-tonifying herbs (BTH) in TCM. Using a pattern recognition model based on the artificial neural network and discriminant analysis for assessing the molecular difference between QEH and BTH was developed. It is indicated that QEH compounds have high lipophilicity while BTH compounds possess high chemical reactivity. Additionally, using a systematic investigation integrating absorption, distribution, metabolism, and excretion (ADME) prediction, target fishing and network analysis were performed and validated on these herbs to obtain the compound-target associations for reconstructing the bio- meaningful networks. They proposed a new modeling system, combining oral bioavailability screening, pattern recognition, multiple drug targets prediction and validation, network pharmacology, to investigate the material basis of representative CHM recipe with *qi*-enriching and blood-tonifying function. Their results suggested that the QEH enhanced physical strength, immune system, and normal well-being, acting as adjuvant therapy for chronic disorders while BTH stimulated the hematopoiesis function.

To explore the pharmacological mechanism of Si-Wu-Tang (SWT), a CHM formula widely used for the treatments of gynecological diseases, Fang et al (2013) incorporated microarray data of SWT with the herbal target database TCMID to analyze the potential activity mechanism of herbal ingredients and targets of SWT. They detected 2405 differentially expressed genes in the microarray data, and found that 20 of 102 proteins targeted by SWT were encoded by these DEGs and could be targeted by two FDA-approved drugs and 39 experimental drugs. The pathway enrichment analysis showed that the 20 predicted targets were consistent with those of 2405 differentially expressed genes, elaborating the potentially pharmacological mechanisms of SWT. Further study from a perspective of protein-protein interaction (PPI) network showed that the predicted targets of SWT function cooperatively performed their multi-target effects.

They provided their therapeutic effects through chemical molecules and targeting proteins (e.g. enzymes) related to the pathological processes of diseases. Thus targets of both herbal ingredients and drugs can bridge the gap between TCM and conventional medicine. Based on this assumption, a network integrating herbs, ingredients, targets and drugs was constructed (Figure 4). This network visually shows the relationships between herbs, ingredients, targets and drugs, and is a meaningful attempt in bridging TCM and conventional medicine. They also analyzed 27 other CHM formulae which could also treat the gynecological diseases. The subsequent result provides the additional insights to understand the potential mechanisms of SWT in treating amenorrhea.

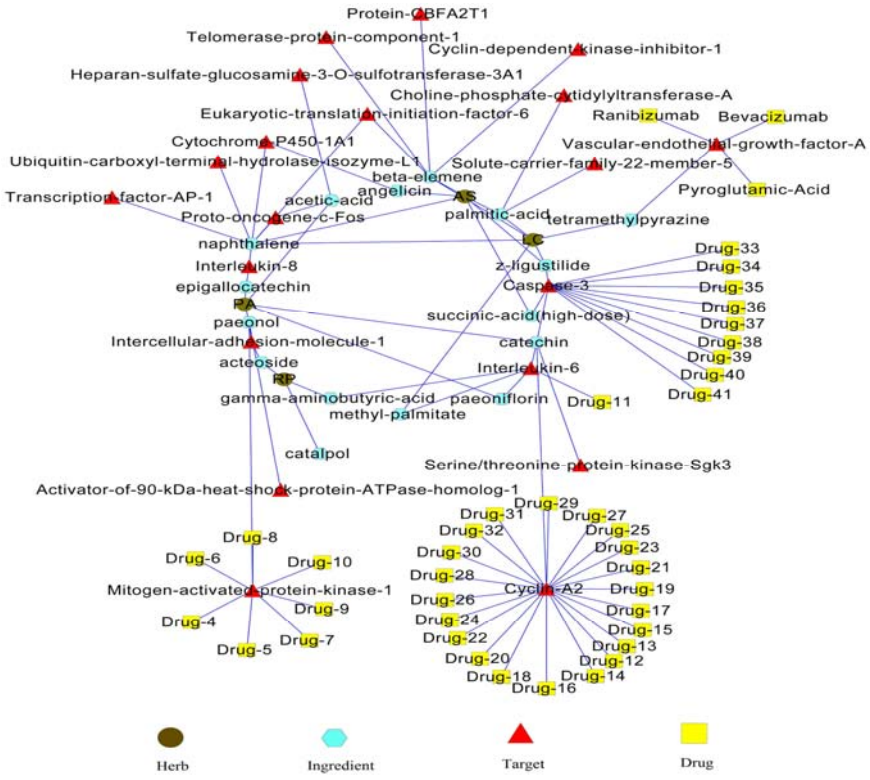


**Figure 4 Herb-ingredient-target-drug network for SWT (Fang et al, 2013)**

Sovereign-Minister-Assistant-Guide (Jun-Chen-Zuo-Shi) is the four basic elements and principles of CHM prescription (Figure 5). The four basic elements must be integrated and interacted with each other to form "point-line-area-cubic" relationship in order to regulate TCM actions in therapy (Liu et al, 2012).

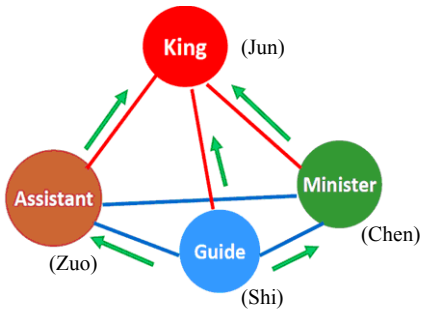


**Figure 5 Four basic elements of Sovereign-Minister-Assistant-Guide (Jun-Chen-Zuo-Shi) in TCMs**

Tao et al (2013a) successfully explained the mechanism of efficiency of *Curcumae Radix* Formula for the prevention of cardiovascular and cerebrovascular diseases (CCVD), and predicted the potential targets of the CHM, which facilitates to elucidate the compatible mechanism of the complex prescription, Sovereign-Minister-Assistant-Guide, and provides a basis for an alternative approach to investigate novel CHM formula on TCM network pharmacology level. Their results indicated that *Curcumae Radix* shared the most common targets with *Gardeniae Fructus*, while less common targets with *Moschus* and *Borneolum*. The integrated network shows that *Curcumae Radix* represents the principal component for the prevention of CCVD, and three other medicines serve as adjuvant ones to assist the effects of the principal components.

The two herbs comprising *Salviae Miltiorrhizae Radux* (SMR) and *Notoginseng Radix* (NR) has been used as a classical formula for cardiovascular diseases (CVD) in TCM treatment. For fully elucidating the pharmacology of SMR and NR in this herb pair, Zheng et al (2013) studied the mechanisms of SMR and NR at the molecular level for the treatment of CVDs, using the systems pharmacology approach, integrating ligand clustering, chemical space, docking simulation, and network analysis, to investigate the two herbal medicines. The distributions of chemical space among the compounds from SMR and NR were discrete, with the existence of small portions of overlap, and the majority of the compounds did not violate "Lipinski's rule of five". Docking indicated that the average numbers of targets correlated with each compound in SMR and NR were 5.0 and 3.6, respectively. Furthermore, the network analyses revealed that SMR and NR exerted the different modes of action between compounds and targets. They suggested that the method of computational pharmacology could be able to intuitively trace out the similarities and differences of the two herbs and their interaction with targets from the molecular level, and that the combination of the two herbs may extend their activities in different potential multidrug combination therapies.

### *3.2 Studies on pharmacological and toxicological mechanism of TCM*

Application of *in silico* compound-protein interactions to pharmacological and toxicological mechanism classification and mechanism make the understanding of the action characterization as well as the prediction probabilities and difference possible.

In early 1991, Wang et al examined the commonly used CHM on their ability to depress the release of λ phage from lysogenic strain in the inductees on SOS network gene expression in *Escherichia coli* GW1107 and GW1060. They found the compound exerted an inhibitory effect on SOS response occurred at 42 degrees C in *E. coli* GW1060 (recA441), but has no effect on SOS network gene expression in *E. coli* GW1107 (lexA51). It is suggested that *Polygonatum Radix* may contain an inhibitor of RecA protease (Wang et al, 1991).

According to the compound-protein interaction network results, He et al's study showed the connectivity layout of phytochemical components with different target proteins within the network in pharmacological action. The principal components analysis (PCA) was carried out based on the predictive probabilities, and pressor targets could be divided into three large classes, neurotransmitter receptors, hormones receptors, and monoamine oxidases. They also found that steroid glycosides seemed to be closed to the region of hormone receptors, and a weak difference existing between them (He et al, 2013). This work explored the possibility of pharmacological or toxicological mechanism classification using compound-protein interactions.

Proteomics has brought the breakthroughs in the study of TCM. Sun et al used a combined method of two-dimensional polyacrylamide gel electrophoresis and matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF/TOF-MS) to study the proteomics of *Yin-Chen-Hao-Tang* (YCHT), a CHM formula with the hepatoprotective effects on alleviating the various types of liver injury. They identified the up-regulation of possible target proteins after YCHT treatment. Fifteen modulated proteins were identified, seven of which were found to be significantly altered by YCHT for metabolism, energy generation, chaperone, anti-oxidation, signal transduction, protein folding, and apoptosis. Functional pathway analysis suggested that these proteins were closely related in the protein-protein interaction network and the modulation of multiple vital physiological pathways. Thus, the data help to understand the molecular mechanisms of hepatoprotective effect of YCHT (Sun et al, 2013).

Cheng et al studied the anti-inflammatory mechanism of *Qingfei Xiaoyan* Pills with network pharmacology (Cheng et al, 2013). Their results showed that the real time-PCR further confirmed the anti-inflammatory effects of *Qingfei Xiaoyan* Pills were due to active ingredients, such as arctigenin, cholic acid, and sinapic acid, intervening focal adhesion, Fc epsilon RI signaling, and ERK/MAPK pathways. The novel approach of drug-target-pathway presents an effective strategy for the

study of TCMs. Li et al (2012) used the systems biology method to study the mechanisms of CHM for the treatment of CVD. Shi et al (2014) used the network pharmacology method to study the mechanisms of traditional prescription medicine, Bushen Huoxue formula treating chronic kidney disease.

The feature of multi-components and multi-targets makes medical herbs serve as valuable resources for network-based multi-target drug discovery. In a study, Li et al (2014b) reported the integration system pharmacology for drug discovery and combination, which was applied to predicting the efficacy, adverse effects, and toxicity of CHM in the treatment of CVD. Their study obtained three results: (1) a disease-specific drug-target network was constructed and examined at systems level to capture the key disease- relevant biology for the discovery of multi-targeted agents; (2) considering the integration of disease complexity and multi-level connectivity, a comprehensive database of literature-reported associations, chemicals, and pharmacology for herbal medicines was designed; (3) a large-scale systematic analysis combining pharmacokinetics, chemogenomics, pharmacology, and systems biology data through computational methods was performed and validated experimentally. These results are useful for a superior output of information about systematic drug design strategies for complex diseases. The strategy of integrating technologies is expected to create new opportunities for TCM research.

Li et al (2014a) applied the network pharmacology approach to determine the active compounds and action mechanisms of *Gegen Qinlian* Decoction for the treatment of type 2 diabetes. Liang et al (2014) established a novel network pharmacology approach to analyze traditional herbal formulae, the *Liuwei Dihuang* Pill as a case. These studies were useful to understand the complex action of CHM.

### *3.3 New drug discovery*

The development of new drug plays an important role in the development of pharmaceutical industry and the society. However, with the increasing demands, new drug development is facing great difficulties. Due to the limitations, the hypothesis of highly selective single-target is facing great challenges. In recent years, the network pharmacology has become one of the new strategies for new drug discovery based on single-target drug research. In a review paper, Liu and Du (2010) focused on the basis of the network pharmacology and research progress, including the discussion on development direction and application prospects, the analysis of limitations and problems, and the application of the network pharmacology in new drug development.

A disease is rarely caused by a single virulence gene, but an unbalanced regulatory network arising from dysfunction of multiple genes or their products. Drugs intervene in the occurrence and development of a disease by affecting multiple target points in the disease network and make a synergy effect on each target point in order to achieve the therapeutic effect. Chen and Wang (2013) reported three aspects: the establishment of corresponding disease and drug network, the decomposition of network, and the biological significance of sub-network. Network analysis with high-throughput data provides a new perspective on studying disease pathobiology and pharmacological mechanisms, and brings the forth new ideas on multi-component and multi-target- point pharmacologic mechanisms of TCMs.

Many CHMs are effective to complicated diseases, such as type 2 diabetes mellitus (T2DM). Gu et al (2013) employed the molecular docking and network analysis to elucidate the mechanism of a medicinal composition, which had clinical efficacy for T2DM. They found that multiple active compounds contained in this medicinal composition targeted multiple proteins related to T2DM and the biological network was shifted. The key compounds in the medicinal composition were predicted and some of them were reported in literature. Meanwhile, several compounds such as rheidin A, rheidin C, sennoside C, procyanidin C1, and dihydrobaicalin attracted the attention though no reports about their pharmacological activity against T2DM were previously.

CHM is becoming the mainstream in clinical practice and demonstrates the value of treating and preventing diseases. However, further understanding of botanical drugs is difficult because of its extreme complexity both in chemical components and mechanisms of action. Thus, it is necessary to establish a comprehensive systems approach, which could identify the active ingredients in the crude drugs and their targets as well as understand the biological basis of the pharmacological properties of herbal medicines. Liu et al (2013a) established a novel systematic pharmacology model, which integrated oral bioavailability screening, drug-likeness evaluation, blood-brain barrier permeation, target identification, and network analysis, to investigate the herbal medicine licorice (*Glycyrrhizae Radix*). The comprehensive systematic approach effectively identified 73 bioactive components and 91 potential targets concerning licorice. The 91 targets are closely associated with a series of diseases including respiratory system, cardiovascular system, gastrointestinal system, etc. These targets are further mapped to drug-target and drug-target-disease networks to elucidate the mechanism of this herbal medicine. Their work provides a novel *in silico* strategy to investigate the botanical drugs containing a huge number of components, which has been demonstrated by the well-studied licorice case. This attempt should be helpful for understanding the definite mechanisms of herbal medicines and discovery of new drugs from plants.

Tao et al (2013b) investigated the pharmacological mechanism of huperzine A, which possessed multiple neuroprotective effects on Alzheimer's disease. In their study, proteins from untreated N2a cells (Con group), cells preincubated with huperzine A followed by Aβ (1-42) oligomers treatment (HupA group), and cells treated with Aβ (1-42) oligomers (Aβ group) with five biological replicates in each cohort, were processed in a centrifugal proteomic reactor and quantified by label-free quantitation. A total of 2860 proteins were quantified with high confidence, and 198 proteins were significantly changed ($P < 0.05$) between HupA and Aβ cohorts. The pathway and direct protein-protein

interaction network analysis showed that huperzine A protects N2a cells against Aβ oligomer-induced cell death by down-regulating the cellular tumor antigen p53 (Trp53) expression.

### *3.4 Evaluation of active constituents and drugs*

Changes in drug development pattern bring innovation to drug valuation methods. The pharmacokinetic research of TCM is an important part of TCM modernization and plays a key role in novel drug development. However, the research methods and techniques matching the characteristics of TCM pharmacokinetics research and evaluation system is insufficient. In order to expound the active components and the mechanism of TCM, a paper makes a brief summary of current pharmacokinetic exploration of TCM, metabonomics, and complex network, while putting forward a new strategy based on complex network and metabonomics to study TCM pharmacokinetic (Xiang et al, 2012).

One of the greatest challenges in the modernization of TCM is to identify the active ingredients and ingredient pairs from herbs that produce the therapeutic effects or the adverse. The "prediction and discovery" of the active ingredients and synergistic ingredient pairs in CHM formulae are considered as a major goal of TCM network pharmacology (Li and Zhang, 2013). In order to reveal the correlation between the effect and constituents, Li et al (2012) studied the *Danggui-chuanxiong* herb pair as an example. They evaluated the effect of different prescription proportions and formulae on nourishing and tonifying blood, activating blood circulation and dissolving blood stasis, regulating menstruation, and relieving pain in an all-round way. In the study, the efficacy indexes of the herbal medicines were integrated by using multi-index aggregative index method. Meanwhile, using the artificial neural network, a fitting correlation analysis was made on the component content of the herbal medicines and their integration effect. Consequently, the active constituents of the herbal medicines with different effects and their contributions to the general efficacy was specified, and new ideas and methods of the basic study on complicated active constituents from the herb medicines were provided.

A review paper summarized the research progress of anti-human immunodeficiency virus (HIV) compounds and CHMs (Liu et al, 2012). CHM appears to be a rich source of potentially useful materials for the treatment of HIV infection. Some active components extracted from CHM such as alkaloids, proteins, flavonoids, quercetin, terpenes, lignanoid display anti-HIV effect. Therefore, searching for anti-HIV active constituents with high efficacy and low toxicity from CHM will be an important research direction in the future.

In study of Liu et al (2012), high throughput screening, biochip, and network pharmacology-related technologies provide the new ideas and key methods for active ingredient screening, toxic components exclusion, and molecular mechanisms of CHM research. Cao et al's paper described a sensitive and specific assay for the quantitation of morroniside in rat plasma after ig administration of iridoid glycosides of *Corni Fructus*. Back-propagation (BP) neural network method was first developed for the prediction of pharmacokinetic (PK) parameters of morroniside in *Corni Fructus*, which would be useful to guide the holistic PK study in consistence with the intrinsic theory and characteristics of TCM (Cao et al, 2011). Jiang et al (2005) investigated the effects of four different active ingredients of zedoary (*Curcuma aromatica* Salisb. oil, curcumol, β-elemence, and curcumin) on the gene expression of hepatic stellate cells. Meanwhile, they explored the molecular mechanism of zedoary against hepatic fibrosis on the basis of network pharmacology. Their results showed the possible molecular mechanism of *C. aromatica* oil and curcumol against hepatic fibrosis.

Currently available molecular biomarkers concerning complex diseases probably do not capture the underlying network influences on these diseases. By incorporating assessments of multiple omics approaches in a network context, more rapidly changing of disease biomarkers from genomics, proteomics, or metabolomics may emerge. Demonstration of therapeutic effect in human studies will still be required, while such biomarkers could guide the selection of drugs and their dosages in early stage of drug development (Silverman and Loscalzo, 2013).

### *3.5 Safety and toxicology*

Network toxicology is based on comprehension of "toxicity (side effects) gene target drug," which utilizes the network analysis to speculate and estimate the toxicity and side effects of drugs. It focuses on the toxic reaction of specific components in a complex system and provides the assistance for drug safety evaluation and research.

The concept and framework of network toxicology and network toxicology of TCM have been proposed by Fan et al (2011). The related tools and technologies were briefly introduced, and the prospects for the network toxicology of TCM were forecasted. In their study, network pharmacology method was used to reconstruct the network model to describe the toxicological properties, which offered the valuable information to identify the toxic substances and potential toxicity of known compounds in a complex system.

Cao et al (2012) used the simplified molecular input line entry specification (SMILES) representation-based string kernel, together with the state-of-the-art support vector machine (SVM) algorithm to classify the toxicity of chemicals from the US Environmental Protection Agency Distributed Structure-Searchable Toxicity (DSSTox) database network. In their method, the molecular structure can be directly encoded by a series of SMILES substrings that represent the presence of some chemical elements and different kinds of chemical bonds (double, triple, and stereochemistry) in the molecules. Thus, SMILES string kernel can measure the similarities of molecules by a series of local information hidden in the molecules directly and accurately. Two model validation approaches, five-fold cross-validation and independent validation set, were used to assess the predictive capability of the developed models. The results indicated that SVM based on the SMILES string

kernel could be regarded as a very promising and alternative modeling approach to predict potential toxicity of chemicals.

It is a great demand to evaluate the harmful effect and toxicity of herbs. Nin et al (2011) used multidisciplinary approach and Roussel Uclaf Causality Assessment Method (RUCAM) to trace the causality of Hongkong Herb-Induced Liver Injury (HK-HILI). A team consisting of hepatologist, clinical toxicologist, analytical toxicologist, and TCM pharmacist was established to do the causality assessment based on a protocol for suspected HILI cases. The possibility of the diagnosis on individual case was assessed systematically by hepatologist and clinical toxicologist independently after collecting information from four aspects: (1) clinical course, (2) exclusion of alternative causes, (3) quality of the prescription and herbal product obtained from the examination of the CHM prescriptions and analysis of biological and herb samples, (4) scientific support on comprehensive literature review on both English and Chinese medical database, which subsequently concluded in a consensus meeting held by the multidisciplinary team. The final causality score of each patient was compared with the possibility of causality assessed by RUCAM. Between 2005 and 2007, 48 consecutive patients suspected HILI were enrolled and 21 patients were excluded due to an alternative cause of liver impairment or lack of any information on the herbs taken. Twenty-seven patients were recruited, among them 15 consumed CHM, 10 used proprietary Chinese medicinal products, and two used both. The concordance between hepatologist and clinical toxicologist concerning the causality assessment was moderate. The causality assessment process concluded that the possibility of HILI was "highly probable" in five cases and "probable" in 12 cases, while there were five "highly probable" and 16 "probable" cases assessed by RUCAM. The causality assessment obtained from multi- disciplinary approach and RUCAM also showed moderate agreement. Overall, a multidisciplinary approach using defined algorithms is a scientific approach to assess the causality for HILI and further study is needed to assess its accuracy and applicability.

In addition, the databases such as National Toxicology Program, Comparative Toxicogenomics Database, and Toxicology Data Network are widely used (Davis et al, 2011). Furthermore, the forecasting toxicity softwares including DEREK, Hazard Expert, Prediction System of Carcinogenic Toxicity, and Toxicity Prediction by Computer-Assisted Technology (Lagunin et al, 2009) are other available tools for TCM network toxicology studies.

### *3.6 Quality control*

Published studies support the potential of network pharmacology in the quality control of TCM. These studies help understand how different constituents of a medicinal material and formulation in disease-related network and understand how difference of TCM products in chemical constituents.

As TCM is of great significance，research papers on herbal medicinal products are increasingly published in "Western" scientific journals. Publications are concerned on safety interactions and efficacy. However, quality studies were overlooked time and again. Pelkonen et al (2014) studied the potential reasons for some problems of CHM and proposed some ways forward. If good practice guidelines of evidence-based medicine are followed and relevant controls and outcome measures are scientifically defined, scientific sense can be made when the tested herbal products are authentic, standardized, and quality controlled. As herbal products are complex mixtures, the network pharmacology provides an approach for mechanistic studies emphasizing on networks, interactions, and polypharmacological features behind the action of many drugs, which revolutionizes the study of TCMs. (Pelkonen et al, 2014).

Post-marketing pharmacovigilance of drugs has become one of the most important activities for regulatory authorities. Currently, pharmacovigilance is attracting the attention from government, pharmaceutical industries and the public in China. In order to support high quality pharmaco-epidemiological studies and stimulate innovation benefitting patients and the public, the European Medicines Agency (EMA) organized the European Network of Centers for Pharmacoepidemiology and Pharmacovigilance (ENCePP), which formulated and published the Guide on Methodological Standards in Pharmacoepidemiology as a reference document for the pharmacovigilance activities in the European Union (EU). The introduction of this guidance can provide a useful technical and methodological reference to carry out the pharmacovigilance, especially for safety monitoring of parenterally administered CHM (Tian et al, 2013).

Identification of nontarget compounds in complex mixtures is of significant importance in various scientific fields. On the basis of the universal property, the compounds in complex mixtures can be classified into various analogous families. Using herbal medicine as an example, Wu's study presented a general strategy for the rapid identification of nontarget compounds from complex matrixes. A total of 45 organic acids from Mai-Luo-Ning and *Lonicerae Flos* Injection, and 46 ginsenosides from *Shen-Mai* Injection were successfully identified using this strategy. The quantitative structure retention relationship (QSRR) approach enabled a successful distinction of most isomers. The proposed strategy is expected to be applicable to the identification of nontarget compounds in complex mixtures (Wu, 2013).

Experiential and sensory evaluation is an ancient method that still remains important in the current quality control of CHM. However, sensory evaluation has met with skepticism because it bases on experience and lacks scientific support. In the study of Wang et al (2012), rhubarb was selected to demonstrate how color-based sensory evaluation distinguishes the quality of herbal medicines objectively. The colors of the rhubarb samples, expressed three of the evaluation parameters, R, G, and B values (RGB values) were obtained from different parts and forms of the plant, including the plant's surface, fracture surface, and powder with or without being treated using a color-developing reagent. By comparing the

color of the scale with the tested samples, similar as performing a pH test with indicator paper, subjects without sensory evaluation experience could quickly determine the quality grade of rhubarb. The work illustrates the technical feasibility of the color-based grading of rhubarb quality and offers the references for quantifying and standardizing the sensory evaluation of CHM, foods, and other products.

Patent network of Chinese patent medicines (CPM) is a patent group composed of basic patents and core technologies surrounded by several correlated patents, which is characterized by TCM technologies. Liu et al made an analysis on how Tasly Group Ltd. built a patent network themed on compound *Danshen* preparation products characterized by extract feeds in hope of providing reference for other Chinese pharmaceutical enterprise to establish and improve the key patent networks of TCM (Liu et al, 2013c).

### *3.7 Bioinformatics and systems biology*

In 2002, Kitano pointed out that understanding of a biological system could derive from the insight of four key properties: system structures, system dynamics, control method, and design method (Kitano, 2002). System dynamics is a system behavior over time under various conditions and is understood through metabolic analysis, sensitivity analysis, and dynamic analysis. The control method is used to systematically control the state of the cell, which is modulated to minimize malfunctions and provide the potential therapeutic targets for the treatment of disease. The design method is used to modify and construct the desired biological systems based on definite design principles and simulations, instead of blind trial-and-error. However, the processes of understanding of network structure to investigate the network dynamics and the analysis of dynamics and structure on the basis of network dynamics are overlapping (Kitano, 2002).

Progress in any of the above areas requires breakthroughs in understanding the computational sciences, genomics, and measurement technologies, along with integration of such discoveries with existing knowledge. The progress would lead to an increasing emphasis on hypothesis-driven research in biology (Figure 6). The hypothesis-driven research in systems biology is four parts: technology, genomics, computation, and analysis in the cycle. A set of predictions that can distinguish a correct model from competing models is selected for "wet" experiments. Successful experiments are ones that eliminate inadequate models. Models surviving this cycle are considered to be consistent with existing experimental evidence. Though it is an idealized process of systems biology research, the advancement of research in computational science, analytical methods, measurement technologies, and genomics will gradually transform the biological research to fit the cycle.

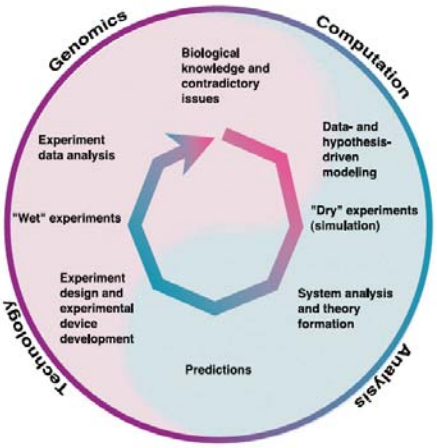


**Figure 6 Hypothesis-driven research in systems biology (Kitano, 2002)**

The idea of systems biology can apply to the research-development and clinical medical practice of chemical and traditional medicines. The most feasible application of systems biology research is to create a detailed model of cell regulation, which focuses on particular signal-transduction cascades and molecules to provide system-level insights into mechanism based drug discovery.

With "omic" data across multiple levels, such as DNA, RNA, protein, metabolomics and so on, tremendous advances have emerged from the bioinformatics and systems biology perspective. Because of an inability to characterize tumors and biological processes in a comprehensive manner, inadequate model systems, and lack of tools dealing with the complexity of biological systems, a positive impact on patient management has been hampered. As bioinformatics and systems biology develops, it is found that cellular functions are not driven by individual genes or proteins but consequence of information integration by complex and dynamic cellular networks (Ram et al, 2012).

GP-TCM is the first EU-funded Coordination Action Consortium associated with TCM research. Fan et al (2012) collected data and drew the comparison of these regulations. Case studies were also used as an example to illustrate the problems involved in registering CHM products worldwide. The experience of global regulation of TCM will provide valuable insights for regulation of other traditional medicine and indigenous medicine (Fan et al, 2012).

Scoparone, an active ingredient isolated from a Chinese medicinal plant *Yinchenhao*, is used in preventing and treating liver damage. As the molecular drug targets associated with the pharmacological effects of scoparone are

mostly unknown, Zhang et al (2013) extended the previous research on *Yinchenhao* through a study on active ingredient and effects. They mapped and evaluated protein-interacting networks and pathways, founding that the hepatoprotective effects of scoparone on liver injury in rats were associated with regulating the expression of six proteins, which is closely related to our protein-protein interaction (PPI) network. Liver fibrosis (LF) is the final stage of liver dysfunction with symptom of diffuse fibrosis. Haptoglobin protein (HP) which can prevent liver damage may be potential molecular targets for early LF diagnostics and therapeutic applications. As protein networks associated with the HP are mostly unknown, Zhang et al (2013a) used a pathological mouse of LF induced by the treatment with carbon tetrachloride for 8 d as a model. In order to better understand the PPI networks in biological context, HP protein was subjected to functional pathway analysis using STRING and Cytoscape software. The results revealed that HP expression associated with fibrosis was up regulated, which suggested that HP responsible for fibrosis may precede the onset and progression of LF (Zhang et al, 2013b).

The herb pair, SMR and NR, has been widely used as a classical formula for cardiovascular diseases (CVDs) at home and abroad. However, the pharmacology of SMR and NR in the herb pair is not thoroughly understood. To compare the mechanisms of SMR and NR at the molecular level on the treatment of CVDs, the study used a systems pharmacology approach, integrating ligand clustering, chemical space, docking simulation, and network analysis. Network analyses revealed that SMR and NR exerted different modes of action between compounds and targets. It is suggested that the method of computational pharmacology is able to intuitively trace out the similarities and differences between two the herbs and their interaction with targets from molecular level (Zheng et al, 2013).

Over expression of drug transporters and deregulation of cellular signaling transduction will result in tumor multidrug resistance (MDR). Xu et al (2013) reported that tanshinone-1, a bioactive ingredient in CHM, directly killed MDR tumor cells and their corresponding parental cells, which was potentiated by the inhibition of secondary activation of signaling networks. Tanshinone-1-induced MDR cell killing depends on the function and expression of drug transporters. The study presents a model for MDR cell killing using a natural original compound, leading to new therapeutic strategies for targeting signaling network(s) in MDR cancers as well as multi-target design.

## 4. Opportunities and challenges on development of network pharmacology in modernization of CMM research

### *4.1 Unique characteristics of TCM network pharmacology*

Pharmaceutically, TCM has an ancient history and unique system, including theory, methodology, prescription, formulation, and medicines. There are differences between TCM and chemical drugs. For TCM, the multiple components *in vivo* are detectable; The number of components is restricted relatively; They could represent the therapeutic effect which could be affected by the combination of traditional medicines in recipe. In addition, the variables of chemical substances are influenced by intrinsic and extrinsic factors such as species, difference, organ specificity, seasonal variation, age, cultivation, harvest, storage, processing methods, and manufacturing practices. Therefore, the difficulty and challenge are much greater in the modernization research of TCMs than the chemical drugs.

Therapeutically, TCM has been practicing holistic views for over 3000 years, and its distinguished feature is treating diseases based on the unique pattern classification. Therefore, Chinese people acknowledge TCM as a great source for disease therapy. Coming strategy for bottleneck of TCM modern research, the innovation of TCM theory is other concept, but is not “one gene, one drug, one disease” compared with chemical drugs in Western medicine.

### *4.2 Challenges on network pharmacology*

#### *4.2.1 Understanding characteristics of holistic view and treatment based on syndrome differentiation*

Due to the features of multi-component, multi-target and synergistic effect in TCM, the effective substances and mechanisms of action are not clear, the qualities of TCM are out of control, and understanding the characteristics is very difficult to the treatment based on syndrome differentiation. It has rich clinical experience thousands of years and demonstrates promising effects to cure complex disease. The network pharmacology is a novel subject based on the construction of multi-layer networks of disease-phenotype-gene-drug to predict the drug targets in a holistic view and promote the efficiency of drug discovery. The theoretical system of network pharmacology is emphasized, and the potential prospect is focused. Methodologically, network pharmacology integrates comprehensive research and systematic assessment, which consistent with the characteristics of holistic view and treatment based on syndrome differentiation in TCM. Thus, the concept and technology of network pharmacology have a powerful ability to explore new research ideas and overcome the problems of CMM modernization study.

Understanding the mechanisms of the pharmacological effects of CHM and its prescriptions are important for their appropriate application. Considering the method and technique characteristic on efficacy of CMM evaluation, the thoughts and methods of network pharmacology to analyze and expound the efficacy and mechanism are proper for CMM. However, it is a recognized problem that the method combines chemical and therapeutic properties with network pharmacology, using a novel approach to evaluate the importance of the targets and ingredients of TCM. The network pharmacology provides a novel strategy to understand the mechanisms of CMM formulae in a holistic way and implies new applications to TCM.

An important task of the modernization of TCM is to elucidate the complex material basis and mechanisms of effect. However, the work was not well done in the past several decades. On the one hand, the action targets of TCM are based on syndrome ("Zheng"), which goes far beyond disease phenotypes but emphasizes the linkages among them, and can not be understood as the diseases targets in the West. While Western medicine is characterized by one drug with one specific target, TCM is a combination of drug targets. On the other hand, CMM contain a variety of components involved in each prescription, and prescription is exactly established on the rational compatibility and subtraction upon the "syndrome" theory. Therefore, CMM constitutes the active substance group, which has a multi-component and multi-target integration effect leading to its terminal pharmacological action based on correspondence of prescription with the syndrome of TCM. The use of effective ingredients of CMM to simplify the study in integration effect of TCM has become a universal research and clinical practice. In fact, the network pharmacology and other interdisciplinary technology may help to understand the holistic and systematic characteristics embodied in TCM in the treatment of complex diseases. Integrating drug targets, disease targets, and clinical biomarkers, network pharmacological study will provide a platform for drug discovery and development of CMM.

#### *4.2.2 Drug discovery and development bottleneck of CMM*

The development of new drug is not only the main driving force for the development of pharmaceutical industry, but also plays a very important role in the social development. However, with the increasing demands, new drug development is facing great difficulties in recent years. The hypothesis of highly selective single-target is meeting the challenges because of its limitations. Network pharmacology has become one of the new strategies for new drug discovery based on single-target drug research in recent years. This paper focused on the basis of network pharmacology and its research progress, discussed its development direction and application prospects, and analyzed its limitations and problems as well. The application of the network pharmacology in new drug development is discussed by comparing its guidelines with those of TCM theory and Effective Components Group hypothesis of CMM.

Based on the network pharmacology, a combinational drug with two or more compounds could offer the beneficial synergistic effects on complex diseases. With the growing understanding of complex diseases, the focus of drug discovery has shifted from the well-accepted "one target, one drug" model to a new "multi-target, multi-drug" model, which aims at systemically modulating multiple targets. New drug discovery strategies compatible with the multi-dimensional complexities of CMMs have been developed. A new drug with multiple compounds can be discovered by CMM pharmacological networks with TCM pattern-based disease molecular networks.

In post-genomic drug discovery, the large-scale integration of genomic, proteomic, signaling and metabonomic data can allow us to construct complex networks of the cell, which provides us with a new framework for understanding the molecular basis of physiological or pathophysiological states. Identification of the interaction between drugs and target proteins plays an important role in drug discovery. Drug, target, and disease spaces can be correlated to study the effect of drugs on different spaces. Their interrelationships can be exploited to design drugs by creating a computational platform that integrates genome-scale metabolic pathway, PPI networks, gene transcriptional analysis in order to build a comprehensive network for multi-target drug discovery. The integrated approach used in systems pharmacology and network pharmacology allows drug action considered in the context of the whole genome to understand drug targets, suggests new targets and approaches for therapeutics, and provides a deeper understanding of the effect and safety of drugs.

#### *4.2.3 Challenges on drug evaluation*

Systems pharmacology and network pharmacology are the emerging fields that integrate systems biology and pharmacology to advance the process of drug discovery-development evaluation. The challenges on drug evaluation are in five aspects. (1) Based on critically examined pharmacology and clinical knowledge, we propose a large-scale statistical analysis to evaluate the efficiency of herbs used in traditional medicines. (2) Studies focus on the exploration of the active ingredients and targets by carrying out complex structure, "-omics" and network-based systematic investigations. (3) Specific informatics methods are developed to infer drug disease connections, with purpose to understand how drugs work on the specific targets and pathways. (4) A new systems pharmacology method is further applied to an integrated systems pharmacology and "-omics" data sets, allowing the systematization of modern and traditional knowledge of TCMs to develop the new drugs for therapy of complex diseases. (5) Key Points in TCMs are featured as abundant bioactive ingredients and multiple targets. Systems pharmacology provides the tools to understand the therapeutic mechanisms of TCMs intervening complex diseases.. Meanwhile, ADME strategies are adopted to visualize the active ingredients and explore the action mechanisms of CMM in the process of drug discovery and development. The strategy of pharmacology and network analyses is devoted to identification and interpretation on the multi-scale mechanisms of drug action, disease association, and even side effects.

CMMs, featured as abundant bioactive ingredients and multiple targets, are considered more effective. It is the combination of drugs that is thought to be the most effective way to counter biological buffering, which allows the reducing dosing of each agent while increasing therapeutic selectivity. Interestingly, TCMs could overcome the shortage of either the long-period toxicity or resistance. Despite the attractiveness of TCM, the clinical evidence that props up the use of most them is still limited, calling for discovery-development evaluation of the methods sufficient to increase the understanding of CMMs.

Combined with the PK and PD evaluations, systems

pharmacology involves the application of systems biology approaches to study the drugs and their targets and effects. Systems pharmacology analysis generally counts on a large number of variables at a genome level to construct the networks for evaluating the drug action and understanding the therapeutic mechanisms. As a major tool, the network analysis basing on widely existed databases permits us to form an initial understanding of the action mechanisms within the context of systems-level interactions. By linking pathways and networks, systems pharmacology is also expected to guarantee the veracity of the predictive PK-PD models of therapeutic efficacy. With the evolution of systems biology and medicine, the pace of new therapeutic development will keep up with the explosion of scientific knowledge, thus facilitating the development of novel drugs. Systems pharmacology and network pharmacology have exhibited great capacity to influence the development and usage of drugs from CMM or CHM.

## References


Abramson DI, Margolin S, 1936. A purkinje conduction network in the myocardium of the mammalian ventricles. *J Anat* 70(2): 250-259.

Arrell DK, Terzic A, 2010. Network systems biology for drug discovery. *Clinical Pharm Thera* 88(1): 120-125.

Barabási AL, Oltvai ZN, 2004. Network biology: Understanding the cell's functional organization. *Nat Rev Genet* 5(2): 101-113.

Boerries M, Eils R, Busch H, 2012. Systems Biology. In: Meyers RA. (ed.) Systems Biology. Boschstr: Wiley-VCH Verlag & Co. KGaA.

Cao DS, Zhao JC, Yang YN, Zhao CX, Yan J, Liu S, Hu QN, Xu QS, Liang YZ, 2012. *In silico* toxicity prediction by support vector machine and SMILES representation-based string kernel. *SAR QSAR Environ Res* 23(1/2): 141-153.

Cao G, Shan Q, Zhang C, Zhang Y, Cai H, Cong X, Cai B, 2011. Pharmacokinetic parameters of morroniside in iridoid glycosides of *Fructus corni* processing based on back-propagation neural network. *Pharm Biol* 49(9): 989-993.

Chen X, Liu MX, Yan GY, 2012. Drug-target interaction prediction by random walk on the heterogeneous network. *Mol Biosyst* 8(7): 1970-1978.

Chen YY, Wang Z, 2013. Application of network analysis in diseases and drug studies. *Chin J Chin Tradit Med* 38(5): 773-776.

Cheng BF, Hou YY, Jiang M, Zhao ZY, Dong LY, Bai G, 2013. Anti-inflammatory mechanism of *qingfei xiaoyan wan* studied with network pharmacology. *Acta Pharm Sin* 48(5): 686-693.

Davis AP, King BL, Mockus S, Murphy CG, Saraceni-Richards CA, Rosenstein MC, Wiegers TC, Mattingly CJ, 2011. The comparative toxicogenomics database: Update 2011. *Nucl Acids Res* 39(1): D1067-D1072.

Fan TP, Deal G, Koo HL, Rees D, Sun H, Chen S, Dou JH, Makarov VG, Pozharitskaya ON, Shikov AN, Kim YS, Huang YT, Chang YS, Jia W, Dias A, Wong VC, Chan K, 2012. Future development of global regulations of Chinese herbal products. *J Ethnopharmacol* 140(3): 568-586.

Fan XH, Zhao X, Jin Y, Shen XP, Liu CX, 2011. Network toxicology and its application to traditional Chinese medicine. *Chin J Chin Med* 36(21): 2920-2922.

Fang Z, Lu B, Liu M, Zhang M, Yi Z, Wen C, Shi T, 2013. Evaluating the pharmacological mechanism of Chinese medicine *Si-Wu-Tang* through multi-level data integration. *PLoS One* 8(11): e72334.

Goh KI, Cusick ME, Valle D, Childs B, Vidal M, Barabási AL, 2007. The human disease network. *Proc Natl Acad Sci USA* 104(21): 8685-8690.

Gu J, Zhang H, Chen L, Xu S, Yuan G, Xu X, 2013. Drug-target network and polypharmacology studies of a traditional Chinese medicine for type II diabetes mellitus. *Comput Biol Chem* 35(5): 293-297.

He M, Cao DS, Liang YZ, Li YP, Liu PL, Xu QS, Huang RB, 2013. Pressor mechanism evaluation for phytochemical compounds using *in silico* compound-protein interaction prediction. *Regul Toxicol Pharmacol* 67(1): 115-124.

Hopkins AL, 2007. Network pharmacology. *Nat Biotechnol* 25(10): 1110-1111.

Hopkins AL, 2008. Network pharmacology: The next paradigm in drug discovery. *Nat Chem Biol* 4(11): 682-690.

Ideker T, Galitski T, Hood L, 2001. A new approach to decoding life: systems biology. *Annu Rev Genomics Hum Genet* 2: 343-372.

Jiang Y, Li ZS, Jiang FS, Deng X, Yao CS, Nie G, 2005. Effects of different ingredients of zedoary on gene expression of HSC-T6 cells. *World J Gastroenterol* 11(43): 6780-6786.

Keiser MJ, Roth BL, Armbruster BN, Ernsberger P, JI John, Shoichet BK, 2007. Relating protein pharmacology by ligand chemistry. *Nat Biotechnol* 25: 197-206.

Keiser MJ, Setola V, Irwin JJ, Laggner C, Abbas AI, Hufeisen SJ, Jensen NH, Kuijer MB, Matos RC, Tran TB, Whaley R, Glennon RA, Hert J, Thomas KL, Edwards DD, Shoichet BK, Roth BL, 2009. Predicting new molecular targets for known drugs. *Nature* 462(7270): 175-181.

Kitano H, 2002. Systems Biology: A brief overview. *Science* 295: 1662-1664.

Lagunin A, Filimonov D, Zakharov A, Xie W, Huang Y, Zhu FC, Shen TX, Yao JH, Poroikov V, 2009. Computer-aided prediction of rodent carcinogenicity by PASS and CISOCPSCT. *QSAR & Combinatorial Sci* 28(8): 806-810.

Li B, Xu X, Wang X, Yu H, Li X, Tao W, Wang Y, Yang L, 2012. A systems biology approach to understanding the mechanisms of action of Chinese herbs for treatment of cardiovascular disease. *Int J Mol Sci* 13(10): 13501-13520.

Li H, Zhao L, Zhang B, Jiang Y, Wang X, Guo Y, Liu H, Li S, Tong X, 2014a. A network pharmacology approach to determine active compounds and action mechanisms of *ge-gen-qin-lian* decoction for treatment of type 2 diabetes. *Evid Based Complement Alternat Med* Article ID 495840.

Li P, Chen J, Wang J, Zhou W, Wang X, Li B, Tao W, Wang W, Wang Y, Yang L, 2014b. Systems pharmacology strategies for drug discovery and combination with applications to cardiovascular diseases. *J Ethnopharmacol* 151(1): 93-107.

Li S, Fan TP, Jia W, Lu A, Zhang W, 2014c. Network pharmacology in traditional Chinese medicine. *Evid Based Complement Alternat Med* Article ID 138460.

Li S, Zhang B, 2013. Traditional Chinese medicine network pharmacology: theory, methodology and application. *Chin J Nat Med* 11(2): 0110-0120.

Li WX, Tang YP, Shang EX, Guo JM, Huang MY, Qian DW, Duan JA, 2012. Analysis on correlation between general efficacy and chemical constituents of danggui-chuanxiong herb pair based on artificial neural network. *Chin J Chin Tradit Med* 37(19): 2935-2942.

Liang X, Li H, Li S, 2014. A novel network pharmacology approach to analyse traditional herbal formulae: The *Liu-Wei-Di-Huang* pill as a case study. *Mol Biosyst* 10(5): 1014-1022.

Liu AL, Du GH, 2010. Network pharmacology: New guidelines for drug discovery. *Acta Pharm Sin* 45(12): 1472-1477.

Liu CX, Xiao PG, 1992. Introduction to Chinese materia medica. Beijing: Peking Medical University and Peking Union Medical College Press.

Liu CX, Xiao PG, Li DP, 2000. *Modern Research and Application of Chinese Medicinal Plants*. Hong Kong: Hong Kong Medical Publishers.

Liu CX, Yang L, Chen Y, 2012. *Research Thinking and Practice on Pharmacokinetics of Traditional Chinese Medicines*. Beijing: Science Press.

Liu H, Wang J, Zhou W, Wang Y, Yang L, 2013a. Systems approaches and polypharmacology for drug discovery from herbal medicines: An example using licorice. *J Ethnopharmacol* 146(3): 773-793.

Liu J, Pei M, Zheng C, Li Y, Wang Y, Lu A, Yang L, 2013b. A systems-pharmacology analysis of herbal medicines used in health improvement treatment: predicting potential new drugs and targets. *Evid Based Complement Alternat Med* Article ID 938764.

Liu Q, Zhang Z, Fang L, Jiang Y, Yin X, 2012. Application of network pharmacology and high through-put technology on active compounds screening from traditional Chinese medicine. *Chin J Chin Tradit Med* 37(2): 134-137.

Liu W, Zou WJ, Wang PL, Xu X, Lei HM, 2013c. Analysis on patent network of compound *Danshen* extracts built by Tasly. *J China Mater Med* 38(1): 133-135.

Liu ZM, Yang YS, Wang XL, Wen RX, 2006. Recent progress on anti-HIV research of traditional Chinese medicine and components. *Chin J Chin Tradit Med* 31(21): 1753-1758.

McCarthy AA, 2003. Microbia: engineering microbial network biology. *Chem Biol* 10(2): 99-100.

Nin CT, Cheung WI, Ngan T, Lin J, Lee KW, Tat PW, Leung VK, Mak T, Tse ML, 2011. Hong Kong Herb-Induced Liver Injury Network (HK-HILIN). Causality assessment of herb-induced liver injury using multidisciplinary approach and Roussel Uclaf Causality Assessment Method (RUCAM). *Clin Toxicol* (Phila) 49(1): 34-39.

Normile D, 2003. Asian medicine. The new face of traditional Chinese medicine. *Science* 299(5604): 188-190.

Paolini GV, Shapland RH, van Hoorn WP, Mason JS, Hopkins AL, 2006. Global mapping of pharmacological space. *Nat Biotechnol* 24(7): 805-815.

Pelkonen OI, Xu QH, Fan TP, 2014. Why is research on herbal medicinal products important and how can we improve its quality? *J Tradit Complement Med* 4(1): 1-7.

Qiu J, 2007. "Back to the future" for Chinese herbal medicines. *Nat Rev Drug Discov* 6: 506-507.

Ram PT, Mendelsohn J, Mills GB, 2012. Bioinformatics and systems biology. *Mol Oncol* 6:147-154.

Shi SH, Cai YP, Cai XJ, Zheng XY, Cao DS, Ye FQ, Xiang Z, 2014. A network pharmacology approach to understanding the mechanisms of action of traditional medicine: *Bushenhuoxue* formula for treatment of chronic kidney disease. *PLoS One* 9(3): e89123.

Silverman EK, Loscalzo J, 2013. Developing new drug treatments in the era of network medicine. *Nature* 93(1): 26-28.

Simon Z, Peragovics A, Vigh-Smeller M, Csukly G, Tombor L, Yang Z, Zahoránszky-Kohalmi G, Végner L, Jelinek B, Hári P, Hetényi C, Bitter I, Czobor P, Málnási-Csizmadia A, 2012. Drug effect prediction by polypharmacology-based interaction profiling. *J Chem Inf Model* 52(1): 134-145.

Sun H, Zhang A, Yan G, Han Y, Sun W, Ye Y, Wang X, 2013. Proteomics study on the hepatoprotective effects of traditional Chinese medicine formulae Yin-Chen-Hao-Tang by a combination of two-dimensional polyacrylamide gel electrophoresis and matrix-assisted laser desorption/ionization-time of flight mass spectrometry. *J Pharm Biomed Anal* 75: 173-179.

Tao W, Xu X, Wang X, Li B, Wang Y, Li Y, Yang L, 2013a. Network pharmacology-based prediction of the active ingredients and potential targets of Chinese herbal *Radix Curcumae* formula for application to cardiovascular disease. *J Ethnopharmacol* 145(1): 1-10.

Tao Y, Fang L, Yang Y, Jiang H, Yang H, Zhang H, Zhou H, 2013b. Quantitative proteomic analysis reveals the neuroprotective effects of huperzine A for amyloid beta treated neuroblastoma N2a cells. *Proteomics* 13(8): 1314-1324.

Tian F, Liao X, Xie YM, 2013. Translation and introduction of guide to methodological standards in Pharmacoepidemiology. *Chin J Chin Trad Med* 38(18): 2949-2957.

Wang JB, Zeng LN, Zang QC, Gong QF, Li BC, Zhang XR, Chu XH, Zhang P, Zhao YL, Xiao XH, 2012. Colorimetric grading scale can promote the standardization of experiential and sensory evaluation in quality control of traditional Chinese medicines. *PLoS One* 7(11): e48887.

Wang LH, Jiang ZS, Chen ZF, 1991. Inhibitory effects of Chinese medicines on SOS responses in *E. coli* and their mechanism. *Genet Acta* 18(1): 90-96.

Wishart DS, Knox C, Guo AC, Shrivastava S, Hassanali M, Stothard P, Chang Z, Woolsey J, 2006. DrugBank: A comprehensive resource for *in silico* drug discovery and exploration. *Nucl Acids Res* 34(supp 1): D668-D6672.

Wolkenhauer O, 2001. Systems biology: The reincarnation of systems theory applied in biology? *Brief Bioinform* 2(3): 258-270.

Wu L, Gong P, Wu Y, Liao K, Shen H, Qi Q, Liu H, Wang G, Hao H, 2013. An integral strategy toward the rapid identification of analogous nontarget compounds from complex mixtures. *J Chromatogr A* 1303: 39-47.

Xiang Z, Cai XJ, Zeng S, 2012. Thoughts and exploration on pharmacokinetic study of herbal medicines based on metabonomics and complex network. *Acta Pharm Sin* 47(5): 558-564.

Xu L, Feng JM, Li JX, Zhu JM, Song SS, Tong LJ, Chen Y, Yang XY, Shen YY, Lian FL, Li YP, Lin DH, Ding J, Miao ZH, 2013. Tanshinone-1 induces tumor cell killing, enhanced by inhibition of secondary activation of signaling networks. *Cell Death Dis* 4: e905.

Xue T, Roy R, 2003. Studying traditional Chinese medicine. *Science* 300(5620): 740-741.

Yildirim MA, Goh KI, Cusick ME, Barabasi AL, Vidal M, 2007. Drug-target network. *Nat Biotechnol* 25(10): 1119-1126.

Zhang A, Sun H, Sun W, Ye Y, Wang X, 2013a. Proteomic identification network analysis of haptoglobin as a key regulator associated with liver fibrosis. *Appl Biochem Biotechnol* 169(3): 832-846.

Zhang A, Sun H, Wu G, Sun W, Yuan Y, Wang X, 2013b. Proteomics analysis of hepatoprotective effects for scoparone using MALDI-TOF/TOF mass spectrometry with bioinformatics. *OMICS* 17(4): 224-229.

Zheng CS, Xu XJ, Ye HZ, Wu GW, Xu HF, Li XH, Huang SP, Liu XX, 2013. Computational pharmacological comparison of *Salvia miltiorrhiza* and *Panax notoginseng* used in the therapy of cardiovascular diseases. *Exp Ther Med* 6(5): 1163-1168.