Network Systems Underlying Traditional Chinese Medicine Syndrome and Herb Formula

Shao Li*

MOE Key Laboratory of Bioinformatics and Bioinformatics Division, TNLIST / Department of Automation, Tsinghua University, Beijing 100084, China

Abstract: Traditional Chinese Medicine (TCM) is characterized by regulating the integrity of the human body and has accumulated thousand-year experience in the use of Herb Formula (“Fu-Fang” in Chinese) for managing complicated TCM Syndrome (“ZHENG” in Chinese). In recent years, there has been increasing concern about the application of bioinformatics and systems biology approaches for deciphering the scientific basis and the systematic features of TCM. Based on the new trends in such an interdisciplinary field, which we termed TCM systems bioinformatics (TCMSB), we propose for the first time a map of “Phenotype network-Biological network-Herb network” with an attempt to uncover the network systems underlying, and identify network biomarkers for, TCM Syndrome and Herb Formula. This multilayer map can serve as a start point towards the systematic interpretation of TCM theory and practice, and give promise to bridge the gap between the ancient TCM and the coming systems biology-based medicine in both system and molecular levels. Moreover, TCMSB approaches, which combine the use of computational modeling and experimental studies, may not only help catch the traditional features of TCM in view of complex biological systems and lead to the step by step modernization for TCM, but may also elude new concept for the future integrative medicine and systems medicine.

Keywords: Traditional Chinese medicine systems bioinformatics, Map of phenotype network-biological network-herb network, network biomarker, TCM syndrome, Herb formula, Systems medicine.

INTRODUCTION

Traditional Chinese medicine (TCM) is a whole medical system with its own rich practice over 3000 years. Unlike allopathic Western Medicine (WM), TCM can be characterized as holistic which emphasizes on regulating the integrity of the human body, applies multiple natural therapeutic methods to improve health, and holds “ZHENG” (TCM syndrome) as the key therapeutic principle and “Fu-Fang” (Herb Formula, also termed as “Fang Ji”) as the typical treatment (Fig. 1A). As one of the main items of Complementary and Alternative Medicine, the practice of TCM has risen dramatically in recent decades [1]. However, the study of TCM still encounters serious challenges due to the almost irreconcilable differences from the conventional WM [2]. TCM uses ZHENG and ZHENG-oriented Fu-Fang treatment as the core, whereas conventional WM uses DISEASE and DISEASE-oriented treatment as the core. The fundamental discrepancy between TCM and WM may root in the inconsistency of the holism approach and the reductionist approach in methodology, as well as the ZHENG concept and DISEASE concept in practice. Consequently, there is still the lack of appropriate methods for understanding TCM in both the systems and molecular levels.

On the other hand, along with the “Omics” revolution advances in modern life science, bioinformatics and systems biology methods attempt to integrate multi-dimensional and various types of data and have ushered in a new era of the medical and biological sciences. For example, for uncovering the genotype-phenotype relationship in human disease, current systems biology has a tendency to retrieve and model the network underlying complex disease ranged from the whole human disease [3-5] to a given disease [6, 7]. Thus, the coming era of systems biology offers a golden opportunity to uncover the essential principles of biological systems and in-depth understanding of system behaviors [8], which makes it possible for the future preventive, predictive and personalized medicine [9].

Since the goals of the systems biology-based medicine potentially overlap with the rules held in TCM (Fig. 1A), whether systems biology can bring TCM and modern medicine together has become an issue of growing concern [10-13]. The present availability of huge amounts of biological data and various computational methods leads to not only the explorations of biological topics at the systems level, but also the changes from reductionist paradigm to systems paradigm [14-16]. Facing the unprecedented opportunities and challenges, bioinformatics and systems biology approaches are expected to open the way to a new convergence of TCM and WM in both concept and methodology. Accordingly, researches in such a new interdisciplinary field, here we termed as TCM Systems Bioinformatics (TCMSB), are emerged and give promise to bridge the gap between TCM, an ancient systematic medicine, and the future systems biology-based medicine, which is still in its infancy, in system- and molecular- levels. Therefore, it is necessary to capture the novel concepts and developments in the bioinformatics and systems biology approaches for making sense of TCM.

Based on the advance of current systems biology and TCMSB researches, we further propose a novel map of the “Phenotype network-Biological network-Herb network”
(PN-BN-HN) (Fig. 1B) and a new concept of “network biomarker” (Fig. 2B). In this map, phenotype can be operationally interpreted as the detectable outward manifestations of human disorders (such as clinical symptoms and signs of DISEASE and TCM ZHENG) or drug effects, and thus “Phenotype network” refers to the potential interrelationships among them. “Biological network” refers to genetic, regulatory, metabolic, and protein–protein interactions among them. “Herb network” refers to the synergistic pair-wise or multi-component relationship among herb combinations or their components derived from TCM Fu-Fang. The systems biology studies such as the identification of network biomarkers for TCM ZHENG and the network-based studies for TCM Fu-Fang are highlighted in the PN-BN-HN map, through which we can seek some agreements between Chinese and Western Medicine in concept and in methodology.

UNDERSTANDING TCM FROM BOTH SYSTEMS AND MOLECULAR LEVELS

The term of “systems biology” is novel but the idea of “system” in medicine is centuries-old. TCM searches for patterns of disharmony to regain the “Yin-Yang” balance and treats the human body as a whole, essentially in a systematic viewpoint. Bell and Koithan [17] claimed that the concepts of networks and nonlinear dynamical complex systems matched well with the holistic TCM, whereas the reductionistic model matched with the isolated local organ, cell, and molecular mechanistic perspectives of conventional medicine. Two medical systems can be integrated and some common language and conceptual models can be found by the growing reliance on complex systems thinking and systems biology for cancer research [17].

There is evidence that both TCM concept and practice may possess of potential biological basis, which is also helpful for gaining new insight into the system behavior of a biological process or disease. For instance, the well known Yin-Yang principle represents the transfer between opposition and interdependence. Several studies demonstrated that certain molecular behaviors are paralleled to the ancient Yin-Yang principle. Hunter considered protein kinases and phosphatases as the Yin and Yang of protein phosphorylation and signaling, where the processes reversibly controlled by protein phosphorylation require not only a protein kinase but also a protein phosphatase [18]. Given the fact that the antioxidant activity and polyphenolic contents of Yin tonic TCM herbs are about six times more than that of Yang tonic TCM herbs, Ou et al. proposed that the Yin-Yang balance is similar to antioxidation-oxidation balance with Yin representing antioxidation and Yang as oxidation [19]. Lu et al. conceived a “Yin and Yang” model in neurotrophin activity for understanding the role of neurotrophins in a wide range of cellular processes [20]. Zhang illustrated the paradoxical roles and interplay of IFN-γ in inflammation and autoimmune diseases by the Yin and Yang map, where Yang denotes an inflammatory process and the Yin denotes the reduction of inflammation and activates a regulatory process [21].

![Diagram](image_url)

**Fig. (1).** A. ZHENG-based traditional Chinese medicine (TCM), DISEASE-based conventional Western Medicine, and the future systems biology-based, preventive, predictive and personalized medicine. B. TCMSB attempts to transfer the rich experience of TCM into a proposed map of “Phenotype network-Biological network-Herb network”, which shows promise to make traditional Chinese medicine compatible with the future systems biology-based medicine.
Despite the new biological extensions of the universal concept in TCM, many researches agreed that isolating single factor is hardly enough to interpret the molecular basis of TCM features [22, 23]. Thus, a major goal of current TCM researches is to develop new scientific methodologies for testing the traditional features and principles in TCM, such as ZHENG and Fu-Fang [12, 13]. To achieve this goal, systems biology, especially the novel computational and detection approaches, is urgently required for analysis of large and complex datasets and accurate detection of proteins as well as metabolites [13]. Although many fundamental problems remain unaddressed and enormous challenges will be encountered, some of the pilot studies in such a new cross-discipline have been conducted for elucidating TCM ZHENG and Fu-Fang (Table 1), demonstrating that systems biology could find applications in managing the complexity of TCM.

**PHENOTYPE NETWORK-BIOLOGICAL NETWORK: A SYSTEMATIC VIEW FOR DISEASE ASSOCIATION AND TCM ZHENG**

“ZHENG” (TCM syndrome), which has a role comparable to the “DISEASE” in conventional WM, is therapeutic basic concept in TCM for understanding the disorder of the human homeostasis and guiding application of Chinese herbs [24]. ZHENG can be expressed as a certain clinical phenotype profile such as symptom combination. Historically, ZHENG is the abstraction from the comprehensive analysis of clinical information gained by four diagnostic methods, namely observation, listening, questioning and pulse feeling. In the recent decades, more and more modern medical diagnosis information has been introduced in ZHENG so that the ZHENG-related “phenotype” is largely extended. A comparative study on the clinical observations of WM and TCM in diagnosing a same disease, rheumatoid arthritis, reported that both medical systems paid attention to disease locations and measurements, while TCM ZHENG observation attached more importance to environmental factors and generally symptoms [25]. Till now, many clinical studies are available to investigate the scientific basis for various symptom combination of ZHENG. It has shown that the non-disease-specific symptom combinations may be associated with the progress and outcome of patients with many diseases such as severe acute respiratory syndrome [26], rheumatoid arthritis (RA) [27] and chronic gastritis [28]. Moreover, according to the rich experience of “phenotype association” in ZHENG, the same disease in WM may be diagnosed as different ZHENGs and thus subjected to different herb treatments, while different diseases with the same ZHENG will share the same herb treatment. For example, a classical TCM Fu-Fang named Liu-Wei-Di-Huang pill is prescribed to restore Kidney-Yin deficiency syndrome (Shen-Yin-Xu ZHENG) that refers to dozens of diseases with overlapping clinical features.

The ZHENG-based therapeutic principle highlights the interrelationships among different diseases, which agrees well with the latest idea that most of human diseases are dependent of each other [29]. Recently, a noticeable progress for recognizing genotype-phenotype relationship reveals that

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Categories</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZHENG (TCM Syndrome)</td>
<td>Cold syndrome (Cold ZHENG); Hot syndrome (Hot ZHENG)</td>
<td>Li et al. 2007 [33]; Xiao et al. 2006 [39]; Yang et al. 2007 [41]</td>
</tr>
<tr>
<td>Kidney-Yang deficiency syndrome (Shen-Yang-Xu ZHENG); Kidney deficiency syndrome (Shen-Xu ZHENG)</td>
<td>Shen et al. 2007 [37]; Qiu et al. 2008 [52]; Zhou et al. 2007 [40]</td>
<td></td>
</tr>
<tr>
<td>Blood Stasis syndrome (Xue-Yu ZHENG)</td>
<td>Ma et al. 2008 [38]</td>
<td></td>
</tr>
<tr>
<td>TCM Fu-Fang</td>
<td>Synergistic effect and herb pair analysis</td>
<td>Williamson 2001 (Review) [58]; Patwardhan and Gautam 2005 (Review) [11]; Adams et al. 2006 [61]; Li &amp; Wu 2006 (Patent) [69]; Wang et al. 2008 [62]; Ung et al. 2007 [59]</td>
</tr>
<tr>
<td></td>
<td>Herb target biological network</td>
<td>Chen et al. 2006 [51]; Ehman et al. 2007 [55]; Li et al. 2007 [33]; Li 2007 (Review) [57]; Yue et al. 2008 [53]; Cheng et al. 2008 [54]</td>
</tr>
<tr>
<td>Selected TCM related PN-BN-HN studies*</td>
<td>PN-BN analysis</td>
<td>Lage et al. 2007 [4]; Wu et al. 2008 [5]</td>
</tr>
<tr>
<td></td>
<td>Drug-BN-phenotype analysis</td>
<td>Zhang et al. 2008 [44]</td>
</tr>
</tbody>
</table>

*PN-BN-HN: Phenotype network-Biological network-Herb network.
different diseases sharing similar clinical manifestations (phenotypes) may often result from abnormalities in functionally related genes (genotypes), for example, participating in a common pathway or sharing the same functional module [30, 31]. Based on the assumption that phenotypically similar diseases may be caused by functionally related genes, Lage et al. [4] created a phenotype-interactome network by integrating disease phenotype similarity information, known gene-phenotype association and human protein-protein interaction network. Wu et al. [5] further established a CIPHER method to infer global phenotype-genotype association and measure the concordance between the human phenotype network and underlying biological network. This method can robustly uncover causative genes with high accuracy for many phenotypes or even the genetically uncharacterized phenotypes, explore gene cooperative behavior in complex diseases, and produce a draft genetic landscape from 8919 genes and 1126 phenotypes, which reveals the global modular organization of human phenotype–genotype relationships (Fig. 2A).

The systematic exploitation of phenotype relationships shifts the “one gene, one disease” paradigm to the “multi-genes, multi-diseases” view [32]. This new trend may not only reach the association of different diseases and then facilitate the study of systems biomedicine, but also provide ways to better understand the holistic rule held in TCM ZHENG. Just as different genes can cause similar diseases, the same genes and gene families may contribute to a range of different diseases [32], the principle of ZHENG in TCM also treat the same disease as different ZHENG while treat different diseases as the same ZHENG. Therefore, these aspects suggest that ZHENG-related phenotype associations and DISEASE-related phenotype relationships both can be attributed to the dysfunction of biological networks, and it is necessary to figure out the system properties and biological mechanisms underlying TCM ZHENG.

TCM ZHENG RELATED BIOLOGICAL NETWORK IN THE CONTEXT OF THE NEURO-ENDOCRINE-IMMUNE SYSTEM

TCM ZHENG takes a holistic view for capturing the dissonant patterns of patients, with the eight guiding principles as Yin and Yang, Exterior and Interior, Cold and Hot, and Deficiency and Excess. Here, Cold ZHENG (Cold syndrome, resulting from Yin-preponderance or Yang-deficiency) and Hot ZHENG (Hot syndrome, resulting from Yang-preponderance or Yin-deficiency) are signs of the Yin-Yang imbalance and manifest two distinct symptom profiles including, but not limited to, the changes of temperature (See [33] for details). Both typical ZHENGs are widely applied in the treatment of patients with various inflammation, infection and immune disorders such as rheumatoid arthritis and chronic gastritis. Accordingly, many Chinese herbs are categorized as either Cold-natured or Hot-natured for restoring the corresponding Yin-Yang balance.

To understand the biological basis of TCM ZHENG, recent works indicated that the neuro-endocrine-immune (NEI) system could be a possible bridge between TCM ZHENG and biological systems. NEI acts as a pivot in modulating host homeostasis and naturally optimizing health through communications among chemical messengers (CMs) such as hormone, cytokine and neurotransmitter [34, 35]. Three aspects make NEI an ideal context for exploring the molecular basis of ZHENG and connecting TCM ZHENG to WM DISEASE. First, the NEI system acts as the host homeostasis mediator during the course of various physiological and pathological processes [34, 35]. Second, patients with different ZHENGs such as Hot ZHENG, Yang-deficiency ZHENG and Xue-Yu ZHENG (Blood stasis syndrome) are found to experience abnormal NEI functions [33, 36-38]. Third, NEI represents an existed empirical system in modern biomedicine, which has shown the most significant modularity in human phenotype-genotype association as well [5] (Fig. 2A).

However, a dilemma faced by current researches on ZHENG is the conflict between reductionism methods and the holism subject, for example, establishing experimental animal models for ZHENG. Since it is difficult to explain the complex molecular basis for ZHENG by reductionism means, we reason that the systems biology approaches may open up the possibility to elucidate such a complex issue. The approaches mainly include the computational way to build models for ZHENG, make testable predictions, generate hypothesis for the experimental way, and adjust models by comparison with experimental results.

With this understanding, Li et al. [33] investigated Cold ZHENG and Hot ZHENG in the context of the NEI system and identified the network biomarkers for both ZHENGs. Using the symptom profiles of Cold ZHENG and Hot ZHENG, two types of literature-derived networks (NEI gene network and NEI CM network) were constructed for each ZHENG. By network analysis and topological comparison, interesting patterns were detected: hormones are predominant in the Cold ZHENG network, immune factors are predominant in the Hot ZHENG network, and both ZHENG networks are connected by neurotransmitters (Fig. 2B). Moreover, gene and pathway investigation on Cold / Hot ZHENG-related diseases validated the distinct ZHENG patterns [33]. Such findings in Cold / Hot ZHENG network are partly supported by a following gene expression investigation of peripheral CD4+ cell in RA patients with Cold or Hot ZHENG [39], and also obtain evidence from a gene expression study for Kidney-Yang deficiency syndrome (Shen-Yang-Xu ZHENG) in rat models [37]. Kidney-Yang deficiency syndrome shares a common Yang-deficiency state with Cold ZHENG. The later study [37] detected a strong positive correlation between Yang-deficiency state and gene expressions of hormones as well as neurotransmitters: various neurotransmitter genes are significantly down-regulated, followed with those of growth and sex hormone related genes. Moreover, epimedium flavonids, a Hot-natured herbal extract, could not only reverse the expression level of above genes, but also significantly up-regulate the gene expression of thyroid stimulating hormone. Meanwhile, gene network associated with Kidney-Yang deficiency syndrome is also addressed by an approach integrating both TCM and modern biomedical literature [40].

Recently, Yang et al. [41] identified differentially expressed genes and gene modules enriched in Cold ZHENG and suggested that it might be caused by the physiological imbalance and/or the disorder of metabolite processes, which
are closely related to NEI function [42]. NEI also participate in the disorder of Blood stasis syndrome, evidenced by a gene expression profile measurements, which found that inflammatory reaction and immune response genes and pathways were predominant in venous bloods of sixteen patients with such ZHENG [38]. Thus, recent works of ZHENG suggest that the thousand-year-old concept of TCM ZHENG may have a network system, e.g. NEI-metabolism system, as its molecular basis. For transferring the conceptual NEI to the systematic NEI network, a specific database named dbNEI [43] is established and a multilayer network for drug-NEI-disease is reconstructed [44], which will benefit the further exploration of NEI’s function in both WM and TCM.

NEI also plays a critical function in genetic-environmental interactions for complex diseases [45]. In this aspect, TCM considers three kinds of conditions, namely seasonal, local and the patient’s individual features, to catch the complex relationship between human body and natural / social environment. The three conditions are closely associated with ZHENG and something like the novel viewpoint of “network medicine” in which biological network, disease network and the social network are integrated [29]. For evaluating such interactions, Kang et al. provided an entropy model to interpret TCM life systems and nicely simulated the evolution of life entropies of Cold and Hot individuals using a real dataset from North China [46]. For the future directions, it is necessary to make quantitative and dynamic analysis for ZHENG-related biological network as well as human-environmental interactions, which will enable a systemic understanding of TCM ZHENG’s functions and properties responsible for various disorders.

**HERB NETWORK-PHENOTYPE NETWORK: THE COMPLEX RELATIONSHIP BETWEEN TCM **

**FU-FANG AND ZHENG**

In practice, TCM makes use of more than 100,000 Fu-Fang for the tailored and holistic treatment of patients suffering from various ZHENG. Herbs categorized to different “nature and flavor properties” are hereby prescribed in the manner of herb pairs or Fu-Fang. So far the components and their structures in numbers of Chinese herbs have been identified [47]. Recently a “herbalome” project is launched for identifying the components of herbal preparations used as medications for centuries in China [48]. New drugs, such as Artemisinin and arsenic trioxide, have been successfully discovered from the bioactive compounds in TCM using
modern technologies of extracting, separating and purifica-
tion. At the same time, researchers found that many herbal
extracts lose their activities when the extracts are fraction-
ated into individual chemical components [23, 49]. Most
importantly, a large number of TCM Fu-Fang are mixtures
of many herbs and serve as ZHENG-oriented prescriptions
for restoring patients’ Yin-Yang balance (Fig. 3A). The “ZHENG-oriented effect” and “action mechanism of com-
plex components” are two greatest challenges existing in
current researches on TCM Fu-Fang.

Recently, the systems biology approach including differ-
ent Omics technologies shows the potential to accelerate the
modernization of TCM and revolutionize natural product
research [50]. Chen et al. [51] documented a metabonomic
study for screening the aristolochic acid-induced nephroto-
xicity in rats. They detected that the administration of aris-
tolochic acid from medicinal herb can lead to certain pertur-
bations of the metabolic pathways, such as accelerating ho-
mocysteine formation and the folate cycle, while decreasing
arachidonic acid biosynthesis, and thus cause lesions of the
metabolic network. Qiu et al. [52] employed GC-MS-based
metabolic profiling to investigate the metabolic responses to
Herba Cistancheus intervention in a rat model of the hydro-
cortisone-induced Kidney-deficiency syndrome (Shen-Xu
ZHENG) and a systemic recovery was detected. By using
proteomics technology and computer-automated estimation,
Yue et al. [53] identified a possible network associated with
the effect of Ganoderma acid D, one of the major components
in Ganoderma triterpenes, on HeLa human cervical carci-
noma cells. Cheng et al. [54] conducted a DNA microarray
research as well as Gene set enrichment analysis and net-
work analysis for a Fu-Fang, San-Huang-Xie-Xin-Tang, and
its herbal components on the gene expression profiles in
HepG2 cells. Results indicated that this Fu-Fang and its
components displayed a unique anti-proliferation pattern via
p53 signaling, p53 activated, and DNA damage signaling
pathways in HepG2 cells, and most genes were regulated by
p53. Thus, the Omics technologies and the system response
analysis make it possible to reveal the action mechanism of
Fu-Fang. Moreover, Ehrman et al. [55] performed a random
forest and self-organizing maps analysis for evaluating the
distribution patterns of 8411 compounds from 240 Chinese
herbs in relation to the herbal categories of TCM and sug-
gested that ethnopharmacological data could contribute to
pharmaceutical prospecting from Chinese herbs.

For addressing the Fu-Fang and ZHENG relationship, Li
et al. [33] further conducted an experiment on the basis of
the Cold ZHENG and Hot ZHENG networks. As shown in
Fig. (3B), RA can be divided into subcategories of Cold / Hot
ZHENG and treated by Hot / Cold Fu-Fang respecti-
vely, the computational results [33] demonstrated that both
ZHENG networks are scale-free and the hub nodes are be-
lieved to play a key role in the network. Therefore, the
experiment was designed to assess the effects of Cold / Hot Fu-
Fang on the hub nodes in corresponding ZHENG networks
by using a rat model of collagen-induced arthritis (CIA), the
widely-used model for RA [56]. Interestingly, experimental
results showed that the Hot Fu-Fang tends to affect the hub
nodes in the Cold ZHENG network and the Cold Fu-Fang
tends to affect the hub nodes in the Hot ZHENG network,
which verified the computational findings of Cold / Hot
ZHENG-related network and accorded well with the TCM
therapeutic philosophy of “Warming the Cold and Cooling
the Hot” for restoring Yin-Yang balance [33]. Therefore, the
research progresses enable us to hypothesize that the net-
work-based disease or ZHENG is fit for multi-targeted inter-
vention and therapy just like TCM Fu-Fang. And the relation-
ship between Fu-Fang and ZHENG, as well as the
Fu-Fang’s action mechanism could be transferred into a net-
work context [57].

SYNERGISTIC EFFECT OF TCM FU-FANG

There is growing evidence that synergistic combinations
of compounds can be more effective than the summed effec-
tiveness of the individual agents [49]. TCM Fu-Fang is ca-
ble of systemically controlling of various disorders
through the synergistic interactions of herb pairs for mutual
enhancement, assistance, and restraint [11, 58, 59]. Evidence
is now accumulating [58, 60] to support the occurrence of
synergistic interactions from components within a single
herb, especially different herbs in a Fu-Fang. Adams et al.
[61] detected the synergistic, additive and antagonistic in-
teractions between different combinations of six herb extracts
against the viability of prostate cancer cell lines. By using
artificial intelligence methods in both datasets of 394 TCM
herb pairs and 2470 non-TCM herb pairs, a work from Ung
et al. [59] suggested that traditionally defined herbal proper-
""
the multi-target effects of Fu-Fang on receptor, enzyme, ion channel, signaling pathway, and eventually, DISEASE or ZHENG related biological networks.

**HERB NETWORK-BIOLOGICAL NETWORK: MULTI-TARGET ACTION MECHANISM OF FU-FANG**

Many complex diseases, as well as TCM ZHENG, are closely related to the deregulation of biological networks. Correspondingly, there is a tendency of treating complex disease as the operation of perturbed pathways and networks [49]. Csermely et al. [70] also suggested that based on network models, even partial inhibition of a small number of targets could be more efficient than the complete inhibition of a single target. Thus, the multi-component therapeutics is believed to be more suitable to control the network-based complex disease or ZHENG, and the rich-experienced TCM Fu-Fang could serve as a huge resource of empirical multi-component treatments and provide fertile ground for current systems-based drug development [71, 72]. In the meantime, TCM Fu-Fang is also helpful for probing the complex biological responses to various diseases and producing new therapeutic clews in a systematic and resourceful manner.

The reductionist approach, one-target, one-drug paradigm, is hard to realize the network complexity and pathway redundancy underlying complex disease or ZHENG. If a mechanism targeted by a drug or multi-component therapeutics could be expressed as a network, and all elements of this network need to interact for the function of the targeted mechanisms, the network efficiency can be employed as a measure for drug efficiency [70]. In such a network-based systematic analysis paradigm, the first requirement is to define a network for a targeted pathological process. For example, angiogenesis, the process of generating new capillary blood vessels, is a key process contributed to various disorders especially solid tumors, vascular and rheumatoid disease [73]. Several works are performed to uncover the molecular mechanism and biological pathways associated with angiogenesis from a viewpoint of network. Abdollahi et al. [74] revealed a global network pattern for the “angiogenic switch” by connecting known angiogenesis-related genes with previously unknown signaling components, and believed that the angiogenesis network is susceptible to the attack of multiple or broad-spectrum inhibitors or to the targeted removal of the identified angiogenic “hub” nodes. Li et al. [6] reconstructed angiogenesis network using a literature mining and microarray analysis approach based on the hypothesis that if a co-cited gene-pair is co-expressed, they may be more inclined to interact with each other [6]. By

**Fig. (3). Herb network-Phenotype network relations: the complex relationship between TCM Fu-Fang and ZHENG. A. TCM ZHENG represents the disharmony of the human body and the “Yin-Yang” balance can be restored by the corresponding Fu-Fang. B. The network-based strategy for understanding TCM Fu-Fang. The upper figure denotes the synergistic effect and multi-target action mechanism of Fu-Fang. Here, DISEASE and ZHENG are liable to be combined in the network level [6, 33], and an alkaloid combination deriving from a Cold Fu-Fang, Qing-Luo-Yin, has anti-angiogenesis synergistic effect and can regulate both ZHENG and angiogenesis networks [69]. The bottom figure shows a case study for elucidating the relationship between Cold / Hot Fu-Fang and corresponding ZHENG [33].**
comparison between angiogenesis network and TCM ZHENG network [33], it is shown that disease or pathological process (e.g. angiogenesis) and ZHENG (e.g. Cold / Hot ZHENG) can be merged in the network level and their overlapping pathways are identified (Fig. 3B), suggesting a multipathway targeted-therapy for angiogenesis related disorders and providing a common base to evaluate the multi-target action mechanism of Fu-Fang. On this network basis, QLY as well as its synergistic combination is found to be able to modulate the overlapping and cross-talk pathways, such as MAPK and NF-κB activated by TNF-alpha, the top node in ZHENG related network, in a multi-target attack manner [33, 57]. The multi-target action against network-based DISEASE or ZHENG may account in part for the “emergence” of the synergistic effect of TCM Fu-Fang.

Although current studies only reach a few aspects of TCM Fu-Fang in effective substance and in the mechanism of action, we can deduce two meaningful consequences. Firstly, the network-based approach has potential to make sense of the integrated effect of TCM Fu-Fang. Secondly, the synergistic effect of Fu-Fang and herbal combinations may “emerge” from the network interactions of their effective targets. With these understandings, the traditional relation among herbs in Fu-Fang could be transformed into the Biological network-targeted synergistic pair-wise or multi-component relation and an “Herb network” in the PN-BN-HN map could be eventually formed, which will benefit the coming combinational as well as individualized therapies.

CONCLUSION

When traditional Chinese medicine meets systems biology in the 21st Century, a new leaf may be turned in the relationship of ZHENG-based TCM and DISEASE-based WM. TCMSB approaches may help catch the traditional features and principles especially ZHENG and Fu-Fang of TCM in view of complex biological systems. Meanwhile, the rich-experienced TCM may also provide a cross-reference for the development of systems biology-based medicine. On this account, we proposed a map of “Phenotype network (including phenotype relationship integrated both DISEASE and ZHENG)-Biological network-Herb network (derived from synergistic or multi-component relation of Fu-Fang)” (Fig. 3B) as a new start point towards the systematic interpretation of TCM theory and practice. The multi-dimensional network interactions in such a map are expected to be gradually figured out by the interdisciplinary researches with the combination and communication of computational modeling and experimental studies. Particular emphasis will be continuously placed on the connection of multilayer network features as well as the identification of network biomarkers associated with DISEASE, ZHENG and TCM Fu-Fang (Fig. 1). Hopefully, the integration of both ancient TCM and future systems biology-based medicine could be achieved in the context of the proposed PN-BN-HN map. Moreover, TCMSB approaches show promise to reach the underlying complex biological system for TCM and thus help to design a tailored diagnosis and treatment for patients in the future, lead to the step by step modernization of TCM, and educe new concepts in the coming integrative medicine and systems medicine.

ACKNOWLEDGEMENTS

Thank all team members and collaborators for their participation in TCMSB works. This work is supported by the 863 program of China (No. 2006AA02Z311), the NSFC (Nos. 30873464, 90709013 and 60721003), and the National TCM Project Application in the 11th Five-Year Period, China (No. 2006BA108B05-05).

REFERENCES
