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Systems pharmacology strategies for drug discovery and combination with applications to cardiovascular diseases

Peng Li [a,b,1], Jianxin Chen [c,1], Jinan Wang [a,b], Wei Zhou [a,b], Xia Wang [a,b], Bohui Li [a,b], Weiyang Tao [a,b], Wei Wang [c,*], Yonghua Wang [a,b,**], Ling Yang [d]

[a] Center of Bioinformatics, Northwest A&F University, Yangling 712100, Shaanxi, China
[b] College of Life Sciences, Northwest A&F University, Yangling 712100, Shaanxi, China
[c] Beijing University of Chinese Medicine, Beijing 100029, China
[d] Laboratory of Pharmaceutical Resource Discovery, Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Dalian, Liaoning, China

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ABSTRACT

Ethnopharmacological relevance: Multi-target therapeutics is a promising paradigm for drug discovery which is expected to produce greater levels of efficacy with fewer adverse effects and toxicity than monotherapies. Medical herbs featuring multi-components and multi-targets may serve as valuable resources for network-based multi-target drug discovery.
Materials and methods: In this study, we report an integrated systems pharmacology platform for drug discovery and combination, with a typical example applied to herbal medicines in the treatment of cardiovascular diseases.
Results: First, a disease-specific drug–target network was constructed and examined at systems level to capture the key disease-relevant biology for discovery of multi-targeted agents. Second, considering an integration of disease complexity and multilevel connectivity, a comprehensive database of literature-reported associations, chemicals and pharmacology for herbal medicines was designed. Third, a large-scale systematic analysis combining pharmacokinetics, chemogenomics, pharmacology and systems biology data through computational methods was performed and validated experimentally, which results in a superior output of information for systematic drug design strategies for complex diseases.
Conclusions: This strategy integrating different types of technologies is expected to help create new opportunities for drug discovery and combination.

1. Introduction

The past decade has seen an intense focus on multi-target drugs and combinatorial therapies that modulate the activities of the targets to achieve therapeutic efficacies, particularly in complex diseases, such as HIV, cancer and diabetes disease (Home et al., 2009; Lennox et al., 2009; Galsky and Vogelzang, 2010). Compared to drugs that modulate single target, multi-target agents might be more effective due to the synergistic action or negative regulation of resistance (Jia et al., 2009; Chan and Loscalzo, 2012). Indeed, the concept of combinatorial therapies has been practiced in traditional medical treatments for thousands of years, which often use botanical mixtures characterized as multi-component and multi-function to treat disease (Qiu, 2007; Kong et al., 2009; Verpoorte et al., 2009; Cheung, 2011). In development, considerable knowledge has been accumulated concerning clinical efficacy and safety of herbal concoctions in targeting complex diseases. However, how to develop new synergistic combinations against multiple targets which must be based on a rational and systematic drug design strategy is still a big challenge.

The exploration of drug combinations is usually dependent on high-throughput screening procedure which tests large number of combinations in the cell-based assays, following by the investigation of the underling synergistic details. Some major types of synergistic drug pairs were explored experimentally

Abbreviations: ADME, Absorption, distribution, metabolism and elimination; CVD, Cardiovascular diseases; TTD, Therapeutic Target Database; DT network, Drug–target network; TT network, Target–target network; DD network, Drug–drug network; OB, Oral bioavailability; TS, Tanimoto similarity; RSM, *Radix Salviae Miltiorrhizae*; CT, *Carthamus tinctorius*; FC, *Fructus Cartaegi*; DSH, RSM, FC and CT; A5L, Arachidonate 5-lipoxygenase; B1AR, beta-1 adrenergic receptor; CDK2, Cyclin dependent kinase 2; CgmpA, cGMP-inhibited 3′,5′-cyclic phosphodiesterase A; ERB, Estrogen receptor beta; MAPK14, Mitogen-activated protein kinase14; M2, Muscarinic acetylcholine receptor 2; NOSE, Nitric-oxide synthase endothelial; PPAR, Peroxisome proliferator-activaed receptor gamma; PGS, Prostaglandin g/h sythase1.

* Corresponding author at: Beijing University of Chinese Medicine, Beijing 100029, China. Tel./fax: +86 10 6428 6283.
** Corresponding author at: Northwest A&F University, College of Life Sciences, Center of Bioinformatics, Yangling, Shaanxi 712100, China. Tel./fax: +86 29 8709 2262.
E-mail addresses: wangwei@bucm.edu.cn, yh_wang@nwsuaf.edu.cn (W. Wang).
[1] Contributing equally to this work.

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(Cokol et al., 2011). However, this "compound to target" approach is highly inefficient if without a *priori* knowledge of the synergistic effects of the combination drugs. Large-scale experimental drug synergy screens have found that synergistic drug pairs are rare (4–10%) (Borisy et al., 2003; Zhang et al., 2007; Cokol et al., 2011). This is especially difficult for herbal medicines, as their combinations usually contain considerable numbers of chemical compounds, which will make it unfeasible to largely uncover the specific mechanism of action underlying such multicomponent synergy associated with the interacting targets, pathways, and even diseases. Alternatively, a systems strategy follows the "target to compound" direction, which is based on systems pharmacology to explore not only the molecular mechanism of a particular disease but also the underlying relationships among distinct phenotypes, might result in the development of more efficient molecules than the currently favored single-target drugs (Zimmermann et al., 2007; Barabasi et al., 2011).

Systems pharmacology involves the application of omics and systems biology technologies, combined with the pharmacokinetics and pharmacodynamics evaluations, to the study of drugs and their targets and effects (Kohl et al., 2010; Sorger et al., 2011; Uzuner et al., 2012). Systems pharmacology analysis generally counts on profiles of genomics, transcriptomics, proteomics, and metabolites metabolomics/metabonomics to construct networks for evaluating the drug action and understanding the therapeutic mechanisms. As a major tool, the network analysis based on widely existed databases permits us to form an initial understanding of the action mechanisms within the context of systems-level interactions. These technologies allow information-rich and high-throughput molecular observations, expected to link them to biologically and clinically relevant functions using a systems biology approach (Barabasi et al., 2011). For example, genome-scale metabolic networks constructed either in cancer or microbial pathogens have result in the identification of some novel drug targets and their synergies, and consequently effective antimicrobials or anticancer drug combinations (Folger et al., 2011; Kim et al., 2011). Consistently, systems pharmacology approach may be especially suitable for addressing and investigating herbal medicines in a holistic manner (Uzuner et al., 2012). Within this paradigm, we have developed an integrated model, which combines oral bioavailability prediction, multiple drug targets prediction, network pharmacology techniques, to investigate the mechanisms for the well-known herbal recipe Compound Danshen Formula (CDF) (Li et al., 2012b).

In this work, we propose for the first time a systems-pharmacological strategy for systematic pursuit of optimal drug combinations. The proposed methodology can include, but not limited to herbal medicine investigations. This strategy aids to drug discovery from three categories: (1) systematically identify and understand the pharmacological information for relevant diseases; (2) utilize disease associated target space to screen out effective herbal molecules and (3) combine biological network analysis and experimental validation to discover novel drug combinations. Presently, different types of data, such as the physiological, biochemical and genomic information have been collected to build the model which is based on an array of computational and experimental approaches including the machine learning method, network analysis and pharmacological analysis. In the course of this presentation, we will highlight a key study on the CVD, which is an extremely complex system involved in many genes, proteins and pathways with sufficient experimental validations. The accomplishment of this model will demonstrate the power of the combined approach for enhancing our understanding of molecular interconnectedness and its potential regulation in the control of complex diseases.

2. Materials and methods

2.1. Protocol

In order to find novel efficacious herbs or herbal combinations, we have constructed an integrated systems-pharmacological model by ADME screening, reverse drug targeting and network analysis to revalue various herbs. This tactic is based on the existing pathological and therapeutic information as well as the documented clinical and experimental data for botanical drugs, travelling the path of "target to compound" as follows (Fig. 1):

(1) a large-scale collection of CVD-associated targets and building of drug–target network;
(2) building of database for medical herbs and screening of candidate compounds with pharmacokinetic evaluation;
(3) reverse drug-targeting for potential active compounds;
(4) network construction for herbs and exploration of novel herbal combinations;
(5) experimental validation by *in vitro* and *in vivo* model.

2.2. Building of CVD-associated drug–target networks

We collect the CVD-associated targets by two ways. First, by data mining and web search from DrugBank database (http://www.drug.bank.ca/), a total of 170 small molecule drugs and their 193 targets were extracted. These drugs include 15 antithrombotic agents, 3 antihemorrhagics, 1 antianemic preparations, 5 blood substitutes and perfusion solutions, 35 cardiac therapy, 16 antihypertensives, 16 diuretics, 6 peripheral vasodilators, 16 vasoprotectives, 15 beta blocking agents, 13 calcium channel blockers, 18 agents acting on the renin-angiotensin system and 14 lipid modifying agents. Second, as complement to the dataset, other 233 targets are further obtained by a large literature retrieve (Cases and Mestres, 2009), with 704 CVD relevant drugs from Therapeutic Target Database (TTD) (Zhu et al., 2011). Finally, by deleting the overlapped and abundant targets, a total of 769 drugs and 372 targets associated with CVD were compiled and indexed by DrugBank ID and UniProt ID number, respectively.

The first drug–target network (DT network) was constructed based on the reference set of drug–target interactions connecting the 769 drugs to 372 protein targets. From the bipartite DT network graph, we generate two biologically relevant network projections: target–target network and drug–drug network. In the target–target network (TT network), nodes are proteins, and two proteins are connected if they are both targeted by at least one common drug. In the complementary drug–drug network (DD network), nodes represent drugs, and two drugs are connected to each other if they share at least one target protein. All networks were generated and visualized by Cytoscape 2.8.1.

2.3. Building of database for herbs and druglikeness evaluation

2.3.1. Database construction

We manually collected 510 medical herbs registered in Chinese pharmacopoeia with more than 31,000 ingredients and built an integrated herbal database TCM^{SP} (http://tcmspnw.com). Glycosides in medicinal herbs are usually metabolized to liberate aglycone by intestinal bacteria (Nemeth et al., 2003), thus these metabolites were also added into the database. In order to eliminate the ineffective compounds in herbs, all compounds were evaluated by two druglikeness indices, i.e., oral bioavailability (OB) and Tanimoto similarity (TS).