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Transcriptome inference and systems approaches to polypharmacology and drug discovery in herbal medicine

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ABSTRACT

Ethnopharmacological relevance: Herbal medicine is a concoction of numerous chemical ingredients, and it exhibits polypharmacological effects to act on multiple pharmacological targets, regulating different biological mechanisms and treating a variety of diseases. Thus, this complexity is impossible to deconvolute by the reductionist method of extracting one active ingredient acting on one biological target.

Aim of the study: To dissect the polypharmacological effects of herbal medicines and their underling pharmacological targets as well as their corresponding active ingredients.

Materials and methods: We propose a system-biology strategy that combines omics and bioinformatical methodologies for exploring the polypharmacology of herbal mixtures. The myocardial ischemia model was induced by Ameroid constriction of the left anterior descending coronary in Ba-Ma miniature pigs. RNA-seq analysis was utilized to find the differential genes induced by myocardial ischemia in pigs treated with formula QSKL. A transcriptome-based inference method was used to find the landmark drugs with similar mechanisms to QSKL.

Results: Gene-level analysis of RNA-seq data in QSKL-treated cases versus control animals yields 279 differential genes. Transcriptome-based inference methods identified 80 landmark drugs that covered nearly all drug classes. Then, based on the landmark drugs, 155 potential pharmacological targets and 57 indications were identified for QSKL.

Conclusion: Our results demonstrate the power of a combined approach for exploring the pharmacological target and chemical space of herbal medicines. We hope that our method could enhance our understanding of the molecular mechanisms of herbal systems and further accelerate the exploration of the value of traditional herbal medicine systems.

1. Introduction

During the past decade, a growing body of post-genomic biology (as reflected by the acquisition of high-throughput genomic, transcriptomic, proteomic, and metabolomic data) has revealed a far more complex portrait of disease pathogenesis, featured by redundancy and multifunctionality. Obviously, this disease complexity resists traditional efforts that attempt to identify a single gene or pathway to treat the disease. Thus, the pharmaceutical industry has given more attention to polypharmacology in recent years: a new area that utilizes multi-target drugs and combinatorial therapies to modulate the disease molecular network in a combinatorial/systems-based fashion to achieve therapeutic efficacies, particularly in complex diseases, such as HIV, cancer and diabetes (Galsky and Vogelzang, 2010; Home et al., 2009; Lennox et al., 2009). It was found that compared to drugs that modulate a single target, multi-target agents might be more effective due to the synergistic action or negative regulation of resistance pathways (Chan and Loscalzo, 2012; Jia et al., 2009).

Indeed, the concept of polypharmacology has been practiced in traditional medicine systems, such as herbal medicine, for thousands of years (Cheung, 2011; Kong et al., 2009; Qiu, 2007; Verpoorte et al., 2009). Herbal medicine is a cost-effective medical system that differs in substance, methodology and philosophy from modern medicine, and it plays an important role in health maintenance. It is characterized by the use of mixtures of several herbs into a single formula, in which the pharmacological activities of one single herb is either potentiated or prolonged, and/or its adverse effects reduced, by the addition of other herbs. However, the concept and applications of herbal medicine are mostly derived from the accumulation of empirical evidence and subsequent deduction to form a series of complex theories, which

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obviously prevents an herbal medicine system from being recognized by the modern scientific community (Schmidt et al., 2007). Thus, there is an urgent need for a systematic tool that could be used to translate herbal evidence into modern biochemical and biological meanings to dispel the irreconcilable differences between traditional herbal medicine and modern science.

Conventionally, following modern scientific approaches for drug discoveries, drug development from herbs uses a separation, purification, and structure elucidation methodology trying to identify discrete entities in herbal preparations and to find their corresponding biological activities. However, the complexity of herbal medicine presents unique experimental and theoretical challenges for this slow and troublesome process, even if sophisticated high-throughput screening technology was utilized. First, most medicinal herbs contain dozens to thousands of constituents, but only a fraction of them are effective. Second, a certain ingredient might act on several relevant or irrelevant biological targets but sufficient knowledge of only a few of these pathways are known. Third, there is still a lack of clear understanding of how multiple ingredients act on multiple targets to produce synergistic effects. Obviously, to truly understand an herbal medicine system, all the active ingredients of the herbs or herbal formulae should be taken into consideration simultaneously to link the components to their molecular targets and corresponding functions and further to specific diseases.

During the past decade, researchers have developed a set of systems biology strategies for systematically uncovering the molecular mechanisms related to the therapeutic efficacy of herbal medicines and the discovery of active herbal ingredients (Gu and Chen, 2013; Li et al., 2012, 2010, 2011; Sharma and Sarkar, 2012). These works have laid a foundation for a more comprehensive understanding of the pharmacological basis (Wang et al., 2012a) and combinatorial effects of herbal medicine (Yao et al., 2013). Most of these studies follow the strategy of collecting the chemical ingredients of herbs or herbal formulas of interest, and then the potential targets of these herbs are identified on a proteome-wide scale with classical computational ligand-based target prediction methods. However, this “herb to compound to target” approach is highly inefficient without a priori knowledge of the set of molecular targets that the herbs act on. It is a near-impossible task to identify the specific therapeutic targets from the considerable predicted targets based on both theoretical and experimental perspectives. Alternatively, a systems strategy following the “bioactivity profiling inference” methodology (Schenone et al., 2013), which involves the application of omics and systems biology technologies, and which has been successfully applied to predict therapeutic targets and effects for many small-molecules, might afford possibilities for exploring not only the molecular mechanisms but also the underlying chemistries of herbal medicine. This method does not require any prior information of the agents being analyzed, and thus there is no need to initially parse the complex chemical ingredients of a particular herbal medicine.

In general, bioactivity profiling methods are based on the principle that agents with the same biological mechanisms will have similar behaviors across different biological assays. Thus, targets or mechanisms of a new agent can be assessed for similar patterns of performance as compared to molecules with known mechanisms (called landmark compounds) to elucidate the activity of the new agent. Among the most promising biochemical patterns to use is the gene expression profile, which can be used to discover “connections” among drugs, pathways and diseases (Iorio et al., 2010). Generally, this method involves datasets that contain the transcriptome data of drugs such as the Connectivity Map (CMap) which includes 1309 small molecules and their gene expression profiles (http://www.broadinstitute.org/cmap/) (Lamb et al., 2006).

In this work, we integrate the gene expression profiling approach into a systems-biology strategy for dissecting the polypharmacological effects and the underling active ingredients of herbal medicine. We use the Qishenkeli (QSKL) formula as an example, which is a traditional herbal mixture that has long been used for the routine treatment of cardiovascular disease in China (Qiu et al., 2014; Wang et al., 2012b, 2012c). It consists of six herbs: *Astragalus propinquus* Schischkin root and rhizome (APS), *Salvia miltiorrhiza* Bunge root and rhizome (SMB), *Lonicera japonica* Thunb flower (LGT), *Aconitum carmichaelii* Debeaux lateral root (ACD), *Glycyrrhiza glabra* L. root and rhizome (GGL), and *Scrophularia ningpoensis* Hemsl root (SNH). The proposed methodology includes four principle components: (1) transcriptome-based inference methods were used to find landmark drugs that have similar gene signatures to the query formula; (2) these landmark drugs were used to infer potential therapeutic targets of the herbal formula; (3) based on the structures of the landmark compounds and therapeutic targets, computational ligand-target interaction methods were utilized to explore the potential active ingredients of the herbal medicine; (4) systematic analysis was performed to the dissect mechanisms of the formula for treating specific diseases (Fig. 1).

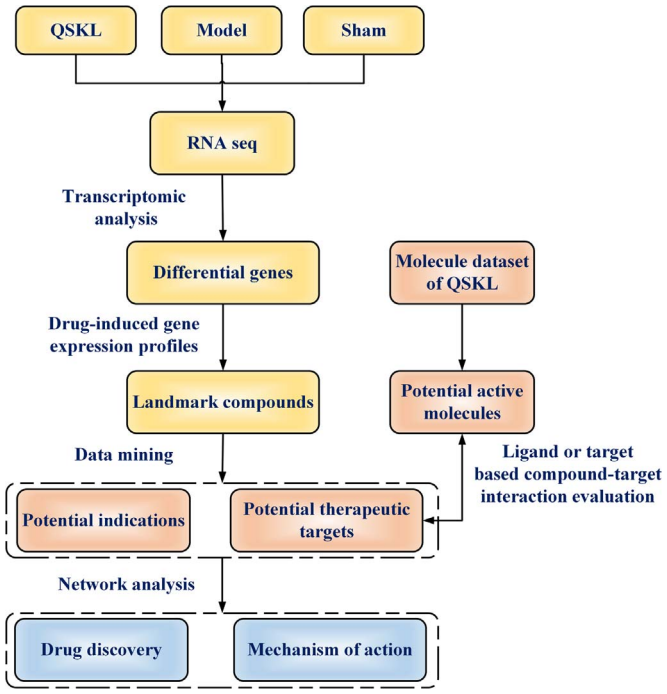

Fig. 1. Systems-biology strategy for dissection of polypharmacological effects and the underling pharmacological targets and active ingredients of herbal medicine..

We hope that our method could be an effective strategy for exploring the mode of action and the biochemical underpinnings of clinically effective formulae at the computational and experimental levels, to further accelerate the exploration of the value of traditional herbal medicine system.

2. Materials and methods

2.1. Preparation of the QSKL formula

The QSKL formula was manufactured by the School of Chinese Materia Medica, Beijing University of Chinese Medicine (Beijing, China). The six herbs APS, SMB, LGT, ACD, GGL and SNH were acquired from the Beijing Tongrentang Drugstore (Beijing, China). The composition of the QSKL formula was 460 g APS, 230 g SMB, 160 g LGT, 160 g SNH, 140 ACD, and 90 g GGL. The preparation process was as follows: the raw herbs were first boiled in water for 2 h at two times atmospheric pressure. Then, the decoctions were merged and filtered and were concentrated by heating under low-atmospheric pressure conditions. The concentrates were extracted with 95% ethanol for 24 h, and further concentrated to form a paste. The pastes were dried under reduced pressure, smashed and then sieved to form the final product.