

Invited Mini Review

Systems pharmacology approaches in herbal medicine research:
a brief reviewMyunggyo Lee^{1,#}, Hyejin Shin^{2,#}, Musun Park³, Aeyung Kim⁴, Seongwon Cha³ & Haeseung Lee^{1,*}¹Department of Pharmacy, College of Pharmacy, Pusan National University, Busan 46241, ²Korean Medicine (KM) Convergence Research Division, Korea Institute of Oriental Medicine, Daejeon 34054, ³Korean Medicine (KM) Data Division, Korea Institute of Oriental Medicine, Daejeon 34054, ⁴Korean Medicine (KM) Application Center, Korea Institute of Oriental Medicine, Daegu 41062, Korea

Herbal medicine, a multi-component treatment, has been extensively practiced for treating various symptoms and diseases. However, its molecular mechanism of action on the human body is unknown, which impedes the development and application of herbal medicine. To address this, recent studies are increasingly adopting systems pharmacology, which interprets pharmacological effects of drugs from consequences of the interaction networks that drugs might have. Most conventional network-based approaches collect associations of herb-compound, compound-target, and target-disease from individual databases, respectively, and construct an integrated network of herb-compound-target-disease to study the complex mechanisms underlying herbal treatment. More recently, rapid advances in high-throughput omics technology have led numerous studies to exploring gene expression profiles induced by herbal treatments to elicit information on direct associations between herbs and genes at the genome-wide scale. In this review, we summarize key databases and computational methods utilized in systems pharmacology for studying herbal medicine. We also highlight recent studies that identify modes of action or novel indications of herbal medicine by harnessing drug-induced transcriptome data. [BMB Reports 2022; 55(9): 417-428]

CURRENT TRENDS IN SYSTEMS PHARMACOLOGY
FOR HERBAL MEDICINE RESEARCH

Medicinal herbs have been used extensively for the treatment of various ailments in ancient medical traditions of China (traditional Chinese medicine, TCM), Korea (traditional Korean me-

dicine), Japan (Kampo medicine), India (Ayurveda), Indonesia (Jamu), North America (phytotherapy), and Europe (herbalism) (1). Standardized herbal extracts (hereafter referred to as herbs) or herbal formulae that blend several herbs into a single formula are composed of a variety of bioactive chemical compounds. They provide a fertile ground for modern drug development with therapeutic leads. Antimalarial quinine and artemisinin, antipyretic analgesic aspirin, and arsenic trioxide for leukemia are examples of modern drugs originally used in traditional medicine (2). In general, pharmacological effects of herbal medicine are achieved by their active ingredients that simultaneously modulate multiple biomolecules in the human body via an additive or synergistic manner. This multi-component nature of herbal medicine has been considered advantageous over single-target drugs for treating complex multifactorial disorders such as cancer and nervous system disease (3, 4). Although therapeutic effects of herbal medicine have been clinically verified in traditional settings for thousands of years, its unknown mode of action on the human body hinders its application and development.

Along with great progress in systems biology, systems pharmacology or network pharmacology approaches have been introduced to decipher complex mechanisms of action (MOAs) of drugs in networks of biomolecules that interact with the drugs (5-7). These approaches have been extensively applied to explore pharmacological effects of multi-component herbal medicine acting on multiple targets of disease from a holistic perspective (8). A typical network pharmacology approach starts from constructing a network in which a node represents either an herb, herbal ingredient, or target protein/gene and an edge indicates link between herb and its constituent compound or interaction between compound and its target (Fig. 1). By associating targets with biological functions or diseases, an herb-compound-target-function/disease network is constructed and leveraged to study the MOA of an herbal treatment. Protein-protein interaction (PPI) network could be further employed to interpret the synergistic effect of herbal medicine by analyzing interactions among target proteins (9, 10). As this network-based approach is solely based on the network constructed, a significant factor influencing subsequent analysis is the reliability of connections (herb-compound, compound-target, and target-dis-

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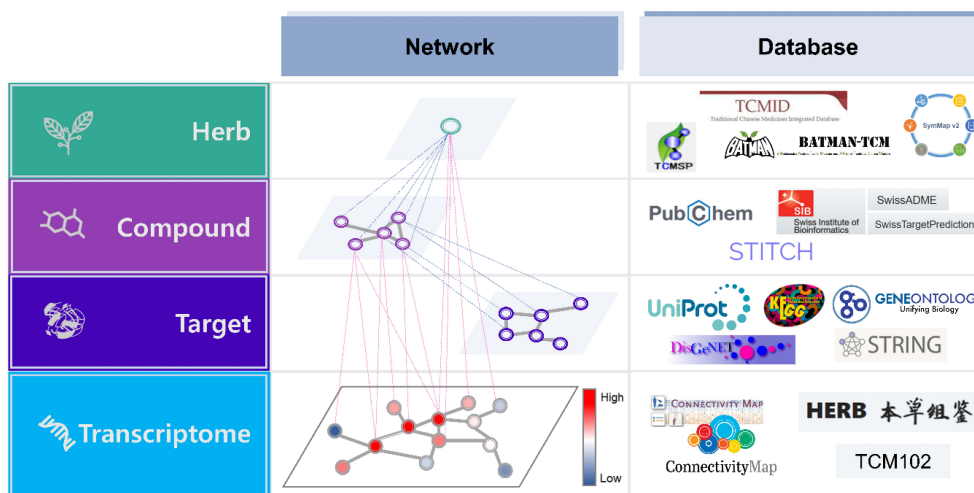


Fig. 1. Resources for network pharmacology in herbal medicine research. Left shows typical forms of an herb-compound-target network (blue edges) and herb-gene or compound-gene network (pink edges) used in network pharmacology research. Right shows public databases frequently utilized to construct networks for herbal medicine research.

ease) in the network. However, establishing reliable connections requires laborious tasks such as identifying herbal ingredients and their molecular targets (11). Thus, most network pharmacology studies only cover a fraction of herbal compounds quantified and rely on target annotations based on *in silico* prediction. Moreover, although effects of compounds on targets depend on biological systems perturbed (e.g., tissue or cell), most of the available information on compound-target interactions have been obtained by high-content screenings in cell-free systems. In addition, even if reliable associations between compounds and targets are available, it is difficult to identify downstream genes affected by the targets to exert a therapeutic effect.

One emerging alternative to address these issues is leveraging drug-induced transcriptome data. The gene expression profile induced by herbal treatment reflects genome-wide effects of multi-component herbs in a certain biological system, thereby providing comprehensive and reliable associations between herbs and genes (Fig. 1) (12). A typical application of drug-induced transcriptome data was first introduced systematically by the connectivity map (CMap) (13). CMap currently provides large-scale gene expression profiles before and after treatment with ~33,000 small molecule compounds in 230 human cell lines and periodically releases additional data (14). It has been widely utilized in research to retrieve drug repositioning candidates and to elucidate drug's MOA in modern and herbal medicine (15, 16). With a similar concept, drug-induced transcriptome data from MCF-7 breast cancer cell line treated with each of 102 TCM components (TCM102) have been published (17), enabling researchers to explore the activity of TCM ingredients at the molecular and cellular levels (18, 19). As more and more herbal medicine studies conducting high-throughput

transcriptome profiling have been published, numbers of gene expression data sets of herbs/ingredients generated in various experimental settings have been accumulated. To integrate these data, Fang S and colleagues have collected transcriptome data sets of 20 herbs and 152 ingredients and built an organized database, HERB, a high-throughput experiment- and reference-guided database of TCM (20). As demonstrated by the increasing demand for these key resources, analyzing transcriptome data of herbal treatment can efficiently uncover novel associations between herbal medicine and modern drugs, genes, and diseases, which in turn can encourage the application and development of herbal medicine.

Systems pharmacology approaches using network-based methods or drug-induced transcriptome data are increasingly adopted pivotally in herbal medicine research. In this review, we summarize key databases and computational methods used in systems pharmacology for studying herbal medicine. We also highlight the recent application of pharmaco-transcriptomics in herbal medicine research.

PUBLIC DATABASES USED IN SYSTEMS PHARMACOLOGY FOR HERBAL MEDICINE RESEARCH

Systems pharmacology approaches for herbal medicine usually start with integrating current knowledge on different types of data (including herbs, compounds, targets, pathways, and diseases) and organizing them into a network. Typically, in the network, a node represents an herb, compound, target, pathway, or disease and an edge represents an interaction between nodes. Numerous databases provide information on the nodes or edges, each database containing data of different scopes and evidence levels. These databases can be divided into four types: herb-

related (HRDB), compound-related (CRDB), target-related (TRDB), and disease-related (DRDB) databases (Table 1). Information on herbal properties and herbal ingredients can be obtained from HRDBs, such as TCMID (21), TCMSP (22), BATMAN-TCM (23), and SYMMAP (24). Most HRDBs provide not only information on herbal ingredients, but also ingredient-related targets, pathways, and diseases. For this reason, HRDBs have been mainly utilized for network pharmacology analysis of herbal medicine. The CRDB includes databases for compounds, compound-target interaction (CTI), and compound-induced transcriptome data. PubChem (25) and SwissADME (26) contain information on physicochemical descriptors, pharmacokinetic properties, and ADME (absorption, distribution, metabolism, and excretion) parameters of compounds, which can be used to calculate drug-

likeness values of herbal compounds. SwissTargetPrediction (27), STITCH (28), and Therapeutic Target Database (TTD) (29) provide known or predicted CTI information. Although high-throughput targeted assays have been developed to screen for drug targets, it is a fairly arduous task to identify binding targets on a genome-wide scale, in some cases for hundreds of herbal ingredients. Therefore, some CRDBs additionally provide information on CTIs predicted by using machine learning (30) or similarity (31) based on structures of compounds and targets. Among TRDBs, Uniprot (32) and GeneCards (33) provide information on the sequences and functional roles of proteins/genes. KEGG (34), Reactome (35), and gene ontology (36) provide sets of genes classified by their biological functions. These databases are utilized to perform functional enrichment analysis, such as

Table 1. Public databases widely used in herbal medicine research

Type	Database	Numbers of available data	Website or reference
Herb-related database	TCMID (version 2.0)	46,929 TAM prescriptions 8,159 herbs/43,413 compounds	http://www.megabionet.org/tcmid/
	TCMSP (version 2.3)	501 herbs/13,144 compounds 3,311 targets/837 diseases 29,384 HC pairs/84,260 CT pairs 2,387 TD pairs	https://old.tcm-sp-e.com/tcm-sp.php
	SYMMAP (version 2.0)	698 herbs/26,035 compounds 20,965 targets/14,086 diseases 2,518 TAM symptoms 1,148 MM symptoms	http://www.symmap.org/
Compound-related database	PubChem (2021)	111 million compounds 278 million substances 295 million bioactivities	https://pubchem.ncbi.nlm.nih.gov/
	STITCH (v5.0)	430,000 compounds 9,600,000 proteins	http://stitch.embl.de/
	CMap	33,000 compounds/230 cell lines	https://clue.io/
	TCM102	102 compounds 1 cell line (MCF-7)	(17)
	HERB	7,263 herbs/28,212 compounds 12933 targets/49,258 phenotypes 6,164 gene expression profiles	http://herb.ac.cn/
Target-related database	UniProt (2020.04)	292,000 proteins (190 million sequences)	https://www.uniprot.org/
	KEGG (2022.03.24)	551 biological pathways	https://www.genome.jp/kegg/
	Gene Ontology (2022.03.22)	7,838,790 gene sets involved in biological process, molecular function, and cellular components	http://geneontology.org/
	STRING (v11.5)	24,584,628 proteins 5,090 organisms	https://string-db.org/
Disease-related database	DisGeNet (v7.0)	21,671 genes/30,170 diseases 1,134,942 gene-disease associations	https://www.disgenet.org/ (41)
	OMIM (2022.05.02)	16,730 genes/6,378 phenotypes	https://www.omim.org/ (42)
	Human Phenotype Ontology (2022.04)	4,791 genes/10,274 phenotypes	https://hpo.jax.org (43)

TAM, traditional Asian medicine; MM, modern medicine.

GSEA (gene set enrichment analysis) (37). In addition, STRING (38) and Human Protein Reference Database (39) provide known PPI information. PPI information can be integrated with CTI to construct a target network in which potential drug targets are identified as interacted protein modules in the network (40). DRDB provides collections of genes and variants associated with diseases. DRDB is also widely used because one of the ultimate goals of systems pharmacology is to predict and evaluate therapeutic effects of drugs by exploring the relationship between drugs and diseases.

COMPUTATIONAL APPROACHES FOR STUDYING HERBAL MEDICINE

With the introduction of polypharmacology, the paradigm of drug research has shifted from single-target to multi-target strategies, revealing the potential of multicomponent herbal medicines to treat a variety of multifactorial disorders (44–46). In line with this, various computational approaches have been introduced to identify targets, indications, and/or synergistic combinations of herbal medicines (Table 2).

Prediction of pharmacological targets

Network-based methods have been most widely applied to predict potential targets of herbs or herbal formulae. For example, Wang et al. (47) have predicted synergistic targets of four herbs, *Radix Astragali Mongolici* (RAM), *Radix Puerariae Lobatae* (RPL), *Radix Ophiopogonis Japonici* (ROJ), and *Radix Salviae Miltiorrhiza* (RSM) in cardiovascular diseases (CVD). They selected bioactive compounds of the four herbs by considering compounds' drug-likeness and oral bioavailability. Targets of the compounds were then predicted based on their structural and physicochemical properties via a machine learning method (30). Finally, synergistic effects of RSM and other herbs were interpreted from target proteins shared by herbs in the herb-compound-target network. For another example, Li et al. (48) have investigated pharmacological targets of an herbal formula Qishenkeli (QSKL) by analyzing QSKL-induced transcriptome data of myocardial ischemia pig model. QSKL is a traditional herbal mixture used for routine treatment of CVD in China. It consists of six herbs: *Astragalus propinquus* Schischkin root and rhizome, *Salvia miltiorrhiza* Bunge root and rhizome, *Lonicera japonica* Thunb flower, *Aconitum camichaelii* Debeaux lateral root, *Glycyrrhiza glabra* L. root and rhizome, and *Scrophularia*

Table 2. Computational approaches for studying herbal medicine

Reference	Prediction type	<i>In silico</i> tools utilized	Data sources utilized
Wang et al., 2012 (47)	Herb-target interactions	Ligand-target interaction prediction (30)	TCMSP (22), DrugBank (64), PharmGKB (65), TTD (29)
Li et al., 2017 (48)	Herb-target interactions	Ligand-target interaction prediction (30)	TCMSP, DrugBank, CMap
Wang et al., 2019 (49)	Herb-target interactions	node2vec, KNN, SVM, RF, LR, DT, GBDT	HIT (66), Chinese pharmacopoeia, SIDER (67), MalaCards (68), DrugBank, SemMedDB (69), Zhou et al. (70), STRING (38)
Zhao et al., 2021 (50)	Herb-target interactions	GNN (71), meta relation-based attention mechanism	HeNetRW (72), YaTCM (73), TCMIP (74)
Keum et al., 2016 (51)	Herb-target interactions	BLM (53), SVM	DrugBank, TCMID (21), TCM-ID (75), KTKP (http://www.koreantk.com), KAMPO (http://kamipo.ca), ChemSpider (http://www.chemspider.com/)
Yoo et al., 2018 (55)	Indications of herbal compounds	RWR, hierarchical clustering	OMIM (42), KTKP, MeSH, TCMID, TCMSP, TCM@Taiwan (76), TCM-ID, KAMPO
Yoo et al., 2020 (56)	Indications of herbal compounds	RWR, DNN	MeSH, OMIM, KTKP, TCMID, COCONUT (77), FooDB (http://foodb.ca/), DrugBank, CTD (78), MATADOR (79), STITCH (28), TTD, BioGrid (80)
Kim et al., 2019 (58)	Indications of herbal compounds	LR, RF, SVM	DrugBank, OMIM, SIDER, OFFSIDES (81), STITCH, UniProt (82), DGIdb (83), HPO (84), DisGeNet (85), KTKP, TCMID, TCM-ID, KAMPO, BindingDB (86), MATADOR
Li et al., 2014 (60)	Effective combination of herbs	Ligand-target interaction prediction (30)	DrugBank, TTD, TCMSP
Wang et al., 2021 (63)	Synergistic MOA of herbs	Network proximity measure (87)	TCMID, STITCH, Cheng et al. (87)

KNN, K-Nearest Neighbor; SVM, support vector machine; RF, Random forest; LR, Logistic Regression; DT, Decision Tree; GBDT, Gradient Boosting Decision Tree; GNN, Graph Neural Network; BLM, Bipartite Local Model; RWR, Random walk with restart; DNN, Deep Neural Network; GTB, Gradient Tree Boosting; OMIM, Online Mendelian Inheritance in Man; MOA, mechanism of action.

ningpoensis Hemsl root. Based on the hypothesis that drugs with similar drug-induced expression patterns share the same MOA, they virtually screened drugs with expression patterns similar to QSKL from CMap database. Known targets of these drugs were collected from DrugBank and considered as potential pharmacological targets of QSKL. As a result, MOA of QSKL was interpreted from a QSKL-drug-target network that included 18 drugs within the ATC (anatomical therapeutic chemical) cardiovascular system category and their known target genes such as CPT-1 and CPT-2 involved in cardiac energy metabolism.

Several machine learning methods have also been employed to predict herb-target interactions (49-51). Wang *et al.* (49) have presented a network integration pipeline HTINet (Herb-Target Interaction Network) for herb-target prediction. HTINet firstly constructs a heterogeneous network by integrating five types of nodes (herb, target, drug, disease, symptom) and edges as their corresponding interactions from diverse data sets. From the network, feature representations of herb and protein nodes are extracted using a network embedding algorithm called node2vec (52). Finally, a classification model to predict herb-target interactions is built by applying a supervised learning method K-Nearest Neighbor (KNN), which learns rules from the known relationships between herbs and targets based on their features. In a similar way, Zhao *et al.* (50) have proposed a herb-target prediction method called HGNA-HTI (Heterogeneous Graph Neural Network with Attention Mechanism for Prediction of Herb-Target Interactions), which has three parts: i) constructing a heterogeneous herb-target network from diverse data sets, ii) learning feature representations of herbs and targets using an attention mechanism with respect to the topological structure of the network, and iii) predicting interactions between herbs and targets by link prediction based on features. As another example, Keum *et al.* (51) have employed classification models that predict the interactions between targets and herbal compounds by utilizing information on approved drugs, target proteins, and known interactions thereof. They used chemical structural similarities of drugs and sequence similarities of proteins as feature representations of drugs and targets, respectively. Similarities of drugs and proteins were calculated using the Open Babel fingerprint and the Smith-Waterman algorithm, respectively. Using these features, a bipartite local model (53) was trained for predicting drug-target associations. Prediction models were generated separately depending on functional classes of target proteins, including G-protein-coupled receptors (GPCRs), enzymes, transporters, receptors, and other proteins. A list of herbal compounds was obtained from TCMID, TCM-ID, KTKP, and KAMPO. Chemical structure information was taken from ChemSpider. Lastly, target proteins of herbal compounds were predicted by applying the constructed classification models.

Prediction of pharmacological indications

Active compounds stemming from medicinal herbs are appealing in modern drug development due to their high efficacies and low toxicities (2, 54). However, new therapeutic opportu-

nities for numerous herbal compounds are yet to be identified. In this section, we will review a few studies applying state-of-the-art machine learning methods to prioritize therapeutically effective herbal compounds for several diseases (55-58).

Yoo *et al.* (55) have introduced a network-based method for identifying pharmacological effects of herbal compounds based on phenotype-related associations. They applied a random walk with restart (RWR) algorithm to generate phenotype vectors of herbs based on associations between known efficacies of herbs and thousands of phenotypes in a phenotypic network. The phenotypic network represents the hierarchical relationship of the Unified Medical Language System (UMLS) which provides integrated information for various phenotypic terms. Hierarchical clustering of phenotype vectors was conducted to extract herb clusters with similar efficacies. In an herb cluster, significantly enriched herbal compounds were selected using Fisher's exact test. These compounds were considered to have pharmacological effects that the herb cluster had. The same group further applied a deep neural network approach to predict medicinal uses of herbal compounds based on molecular and chemical features of approved drugs (56). They extracted feature representations of approved drugs and herbal compounds using three types of data: i) latent knowledge of drugs obtained by text mining of scientific literature, ii) molecular interactions of drugs in the PPI network, and iii) chemical properties, including physicochemical properties, lipophilicity, pharmacokinetics, and drug-likeness. A deep learning model was then trained based on extracted features and verified indication information of drugs. Using the trained model, potential medicinal uses of herbal compounds were predicted based on extracted features of compounds. As another example, Kim *et al.* (58) have applied several classification algorithms to predict new indications for herbal compounds. They hypothesized that similar drugs could treat similar diseases. They used similarities for both drug and disease aspects as predictive features to predict novel drug-disease associations. Drug-drug similarity was calculated using four types of data (chemical structure, side-effects, gene ontology, and targets) and disease-disease similarity was calculated using three types of data (phenotypes, human phenotype ontology, and gene ontology). As a training data set, information on drugs, diseases, and drug-disease associations were obtained from DrugBank, Online Mendelian Inheritance in Man (OMIM), and a previous study (59), respectively. Prediction models were constructed using three machine learning algorithms: logistic regression, random forest, and support vector machine algorithms. They showed that the random forest approach achieved the best performance upon cross-validation and external validation using independent dataset (58).

Prediction of synergistic combinations

Synergy is one of the major advantages of multi-component herbal medicine. Network-based strategies enable us to efficiently explore herb combinations and to better infer the mechanisms of synergistic action of herbs or herbal compounds. For exam-

ple, Li et al. (60) have proposed an effective herbal combination for CVD from CVD-associated compound-target networks. They collected information on known targets related to CVD from DrugBank, TTD, and a previous study (61) and predicted their potential ligands via a machine learning method (30). Based on this prediction, a bipartite network of compound-target was built. To find effective herbs for CVD, ligand compounds in the network were mapped to herbs that contained corresponding compounds using TCMSP database. Considering that herbal formulae consist of multiple herbs with different roles (62), *Radix Salviae Miltiorrhizae* (RSM) was first selected with expectation of its dominant role in CVD treatment as it was associated with many CVD-related targets and pathways. Li et al. (60) have hypothesized that if two herbs affect the same functional pathways, they would have pharmacological synergy against a disease. Therefore, additional herbs acting on the same pathways as those of RSM were recruited as follows: *Carthamus tinctorius* (CT) and *Fructus Cartaegi* (FC). Finally, a mixture of RSM, CT, and FC was proposed as a novel herb combination for the treatment of CVD. Therapeutic effects of the mixture were further validated in a mouse model of myocardial infarction.

As another example, Wang et al. (63) have proposed a network-based method to infer the molecular mechanisms of synergistic interactions of herbs. Considering that traditional herbal formulae were completed based on the principle of herb compatibility, they assumed that herb pairs in the existing herbal formulae would be more synergistic than not in the herbal formulae. To examine this assumption, they developed a network proximity model quantifying the degree of synergistic herb interactions. Firstly, information on herbal ingredients and their targets related to all herb pairs within herbal formulae were collected from TCMID and STITCH, respectively. This information was integrated into the human PPI network, resulting in a network consisting of three levels of interactions, including herb-ingredient, ingredient-target, and target-target for each herb pair. Wang et al. then measured the network proximity of each set of targets of a given herb pair in the PPI network and showed that commonly used herb pairs tended to affect proteins at shorter distances in the PPI network compared to random herb pairs. In addition, they found that ingredients located at the center of the herb PPI network played an important role in their synergy with other herbs. It implies that this network proximity model is feasible to prioritize active ingredients producing combinational effects of herb pairs.

TRANSCRIPTOME-BASED ELUCIDATION OF MOLECULAR TARGET AND/OR MOA OF HERBAL MEDICINE

Herbal prescriptions are combinations of herbal formulas for treatment, meaning that various ingredients in these formulas have the potential to affect multiple genes and biological pathways. In recent decades, systems pharmacology studies

applying transcriptome analysis after treatment with herbal medicines *in vitro* or *in vivo* have elucidated the mechanisms of herbal effect in various diseases (88). In this section, we will look at several studies investigating the molecular targets and MOA of herbal medicines using transcriptome data.

Si-Wu-Tang (SWT) (Samul-tang in Korean, Shimotsu-to in Japanese) is one of the most popular herbal prescriptions consisting of four herbs including *Paeonia Radix*, *Ligusticum Rhizoma*, *Rehmannia Radix*, and *Angelica Radix* at a 1:1:1:1 ratio. It has been clinically applied for centuries in Asia to treat symptoms of hematological disorders such as anemia, menstrual irregularities, dysmenorrhea, and menopausal syndrome (89-97). In recent years, an increasing number of studies have elucidated the MOA of SWT by analyzing SWT-induced changes in gene expression. Wen et al. (91) have reported the MOA and therapeutic evidence of SWT using SWT-induced transcriptome data of MCF-7 cells and CMap data for the first time. Gene expression profiles of SWT-treated MCF-7 cells were significantly matched with those of estradiol-treated MCF-7 cells in the CMap database, suggesting that SWT might have potential phytoestrogenic effects. Pathway enrichment analysis using differentially expressed genes (DEGs) of SWT-treated datasets revealed that *Nrf2*-regulated genes, antioxidant genes, and chemopreventive inducible genes were affected by SWT treatment, but not by estradiol or ferulic acid in MCF-7 cells. The potential role for phytoestrogen of SWT was further confirmed by whole genome microarray profiling and by estrogen-responsive element luciferase reporter assay in MCF-7 cells (92), suggesting the potential of SWT as an estrogen receptor modulator. Another *in silico* analysis (93) predicted SWT's new targets, FOS, JUN, and CAPS3 based on SWT-induced transcriptome data and herb-target information obtained from TCMID database. These studies provide insights into the understanding of complex actions of SWT for gynecological diseases. Antioxidative and estradiol-regulating effects of SWT may help ameliorate ovarian follicular maldevelopment associated with ovulatory and menstrual disorders known to cause infertility (94). Recently, protective effects of SWT on ovarian function and oocyte maturation against cyclophosphamide (CP, a chemotherapy drug)-induced chronic ovarian dysfunction and maternal aging have been demonstrated using transcriptome analysis in an *in vivo* mouse model (95, 96). DEGs by SWT treatment in CP-treated mice ovaries were involved in ovarian follicle development, binding of sperm to zona pellucida (Zp), and microtubule nucleation (95). Among downregulated DEGs, oocyte-specific genes such as fertilization-related genes (*Zp2*, *Zp3*, *Nlrp5*), ovarian follicle development-related genes (*Bmp15*, *Oas1d*), and oocyte maturation-related genes (*Obox1*) were restored to normal levels after SWT administration. Additionally, further enrichment analysis using Target-Scan, a tool for sequence-based microRNA-target predictions, identified microRNA binding sites of DEGs. Three microRNAs (miR-200b-3p, miR-665-3p, and miR-667-3p) had binding sites on either *Bmp15* or *Oas1* mRNA and their expression levels were restored in SWT plus CP-treated mice. Kim and You have

also investigated the influence of SWT on ovarian reserve and fertilization in aged mice (> 40-week-old) (96, 97). They performed functional enrichment analysis of transcriptome data obtained from ovulated ovaries and revealed that RAS signaling pathway-related genes were restored to normal levels as those in young mice after SWT administration to aged mice (96). SWT administration also restored miR-223-3p, which could interact with embryo implantation-related genes (*Hal*, *Acp5*) in the uterus of aged mice (97). This transcriptome analysis result was consistent with the phenotypic findings of the protective effect of SWT on the increase of ovarian reserve, ovulated mature oocytes, and pregnancy rate in aged mice (96, 97).

Tao-Hong-Si-Wu Decoction (THSWD), a traditional herbal medicine, is composed of six herbs (*Prunus persica*, *Carthamus tinctorius*, *Rehmannia glutinosa*, *Angelica sinensis*, *Paeonia lactiflora*, and *Ligustium chuanxiong*). It has been used for clinical treatment of gynecological and cerebrovascular diseases (98, 99). Duan et al. (99) have performed transcriptome profiling of a rat stroke model treated with THSWD and functional enrichment analysis of DEGs induced by THSWD. From enriched functional terms, cell cycle, complement and coagulation cascades, and neuroactive ligand-receptor interactions were suggested as potential therapeutic targets of THSWD for intracerebral hemorrhage-induced neurological deficits.

Compound Kushen Injection (CKI) is an approved Chinese patented drug in adjuvant treatment for chemotherapy. It consists of extracts of two herbs, Kushen (*Sophora flavescens*) and Baituling (*Heterosmilax chinensis*). Qu et al. (100) have leveraged CKI-induced transcriptome data of MCF-7 cells and revealed that CKI primarily downregulates most genes functioning in the cell cycle in a similar degree to a chemotherapeutic agent, 5-fluorouracil (5-FU). Interestingly, although CKI exerted anticancer effects on MCF-7 cells, the expression of *TP53*, a pro-apoptotic gene, was decreased by CKI treatment but increased by 5-FU. From this observation, they proposed that CKI might induce MCF-7 cell apoptosis via a p53 independent pathway. The anticancer effect of CKI was also confirmed via transcriptome analysis using single herb extracts in another breast cancer cell model, MDA-MB-231 (101). CKI has complementary effects on cancer cells as Kushen perturbs cell cycle regulation whereas Baituling activates the immune system. Further transcriptome analysis and cell migration assay revealed that CKI could control cancer metastasis in multiple cancer cell lines of colon (HT-29, SW-480, DLD-1), brain (U87-MG, U251-MG), and breast (MDA-MB-231) (102). To identify core CKI response genes in cancer cells, further investigation was conducted to compare MCF-7 CKI-DEGs to Hep G2 (liver cancer) CKI-DEGs and MDA-MB-231 CKI-DEGs by Cui et al. (103). They found that eight components of CKI obtained from BATMAN tools potentially interacted with 52 core genes in integrated DEGs in multiple cancer cell lines.

Feifukang (FFK) is a pulmonary rehabilitation mixture comprising eight herbs for protecting lung function: *Astragalus membranaceus*, *Codonopsis pilosula*, *Ophiopogon japonicus*,

Schisandra chinensis, *Panax notoginseng*, *Bulbus fritillariae thunbergii*, *Rhizoma anemarrhenae*, and *Glycyrrhiza uralensis* (104, 105). Li et al. (105) have evaluated anti-pulmonary fibrosis effect of FFK in a bleomycin (BLM)-induced pulmonary fibrosis mouse model and explored targets of FFK by analyzing transcriptome data of FFK-treated mice. FFK has potential anti-fibrotic effects by significantly reducing collagen fiber formation in BLM-induced lung fibrosis. Functional enrichment analysis of DEGs in FFK-treated mice showed that the JAK-STAT signaling pathway was significantly downregulated by FFK. qRT-PCR and western blot analysis of *JAK1*, *STAT3*, and *ADAM17* as representative genes confirmed the effect of FFK in regulating lung fibrosis through JAK-STAT signaling pathway.

Paeoniae Radix (PR), a root of the plant *Paeonia lactiflora*, is a key material in many herbal treatments. It is known to supply blood, prevent sweating, regulate menstruation, and relieve pain (106). Baek et al. (107) have identified a novel anticancer mechanism and activity of PR by analyzing transcriptome data derived from lung cancer cells treated with PR extracts or its ingredients. They performed a series of GSEA based on dose-dependent PR-induced transcriptome profiles of lung cancer cells and showed that PR and its two ingredients, hederagenin and oleanolic acid, exerted anticancer effects on lung cancer by downregulating the Aurora B pathway. The synergistic MOA of PR was further interpreted via an integrated PR extracts-compounds-target genes network in the Aurora B pathway.

These individual studies on specific herbal formulae or an herb have laid the basis for developing efficient strategies to systematically infer MOA of herbal medicines at the molecular level, which may rationalize and modernize herbal medicines ultimately.

TRANSCRIPTOME-BASED IDENTIFICATION OF NOVEL INDICATIONS FOR HERBAL COMPOUNDS

Another great advantage of obtaining drug-induced transcriptome data of herbs/ingredients is that novel indications of herbal medicine can be rapidly screened computationally by a systems-based approach. A systems-based approach involves modulating a list of abnormally expressed genes in disease, in contrast to a traditional target-based approach which involves modulating the molecular state of one single protein (108). This approach was first designed and introduced to the public by CMap to link drugs and diseases (13). It defines a set of abnormally expressed genes in a disease, termed a disease signature, and queries it in the CMap reference database. It then searches for drugs that inversely regulate the expression of the disease signature, that is, those that decrease the expression of upregulated disease genes and increase the expression of downregulated disease genes. These drugs are considered candidates for reversing the diseased state back to the normal state.

Several studies using this approach have demonstrated its applicability to drug repositioning of herbal compounds (109,

110). Luo *et al.* (109) have identified flavonoid luteolin as a therapeutic agent for ischemic stroke (IS) via inhibiting *MMP9* and activating PI3K/Akt signaling pathways. Flavonoids are common constituents of plants used in traditional herbal medicine to treat a wide range of diseases (111). Luteolin was predicted by CMap analysis finding compounds that could regulate the expression of IS signature obtained from transcriptome data of IS patients. Further *in vivo* experiments demonstrated that luteolin reduced the infarct volume in a rat model of IS. Similarly, Liu *et al.* (110) have utilized the CMap database to seek compounds that could mimic transcriptional changes induced by a variety of interventions to reduce endoplasmic reticulum (ER) stress based on the observation that increased ER stress could develop leptin resistance and lead to obesity. As a result, they identified celastrol, a pentacyclic triterpene extracted from roots of *Thunder of God Vine* plant as a promising agent for treating obesity by increasing leptin sensitivity. They showed that celastrol could reduce ER stress and increase leptin sensitivity to reduce body weights of obese mice.

TCM102 database is also widely utilized in research to discover new indications of herbal compounds based on the systems-based approach (18, 19). For example, Li *et al.* (18) have identified several vasodilators from TCM102 database. They first generated two gene signatures that involved in positive and negative regulation of blood vessel diameter by using databases GO and SEEK (search-based exploration of expression compendia) (112). Compounds that inversely regulated the expression of these two gene signatures were then screened by performing GSEA on drug-induced transcriptome data in TCM102. Top 10 candidate compounds were tested for their vasorelaxant effects on vascular tension of constricted thoracic aortic rings in a rat model. Among these 10 compounds, ferulic acid exhibited the strongest vasorelaxant effect, and others also induced relaxation significantly. Finally, mechanisms of six compounds (ferulic acid, borneol, daidzin, magnolol, chenodeoxycholic acid, and artemisinin) were inferred from one integrated network representing pathways involving signature genes specifically regulated by these six compounds. As another example, Wang *et al.* (19) have utilized TCM102 database for the evaluation of phillyrin as an anti-cardiac fibrosis agent. Phillyrin was of interest because it was one of the major active ingredients of *Forsythia suspensa*, an herbal medicine used as an anti-inflammatory and antipyretic drug (113). They first analyzed transcriptome data sets of human heart diseases and constructed a cardiac fibrosis (CF)-related gene functional module (CFGM), which had a set of genes containing three known CF markers, *Postn*, *Ddr2*, and *Pdgfra*, and their co-expressed genes. They found that treatment with phillyrin reduced expression levels of most CFGM members from phillyrin-induced transcriptome data in TCM102. They hypothesized that drugs decreasing the expression of CFGM had the potential to treat CF by inhibiting the core pathological process of CF. Cardio-protective and anti-CF effects of phillyrin were further validated in a myocardial infarction rat model.

These systems-based approaches have been mainly conducted by screening desired compounds using well-organized databases, such as CMap and TCM102. However, since these databases only contain data on small molecule compounds, herbs or herbal formulae are inevitably excluded from the screening. The expanded database including a variety of medicinal herbs would offer clues to identify evidence-based connections between herbs and diseases, hence spurring the application and development of herbal medicines.

CLOSING REMARKS

Systems pharmacology approach is increasingly adopted and developed in a wide range of modern drug development processes to better understand molecular MOA of drugs in the human body. Although this approach would also lend itself to herbal medicine research, its practical application and development are relatively slow. The main reason is that the data on herbs and herbal medicines themselves are insufficient for systems pharmacology approach to directly utilize. Whereas for modern drugs, CMap alone provides drug-induced transcriptome data for ~40,000 small molecules, and furthermore, the Library of Integrated Network-Based Cellular Signatures (LINCS) project is continuously generating drug-related multi-omics data sets including proteome, epigenome, and metabolome data for a comprehensive understanding of drug MOA. The advantage of such large-scale data is that we can rapidly utilize them to repurpose existing drugs in urgent situations. For example, several approved drugs have been proposed as candidates for clinical intervention to combat rapidly emerging diseases such as COVID-19 through *in silico* screening using CMap data (114, 115). Accordingly, the establishment of a well-organized drug-related database (e.g., drug-induced transcriptome data) for standardized herbs or herbal medicines should pave the way for advancement of herbal medicine research.

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CONFLICTS OF INTEREST

The authors have no conflicting interests.

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