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Metabolomics and Its Potential in Drug Discovery and Development From TCM

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ABSTRACT

Metabolomics, an omic science in systems biology, is the comprehensive profiling of metabolic changes occurring in living systems and has been widely used in the modern research of traditional Chinese medicine (TCM). TCM is a complex medical science, which reflects rich philosophical dialectical thought, puts the human body into a large system for observation and keeps human in a healthy status. For TCM aroused great interest in the whole world, herbs and Chinese medical formulae (CMF) as treatment methods have also been widely attention. Metabolomics represents a powerful way that provides a dynamic drawing of the phenotype of biological systems via the research of endogenous metabolites, and its methods are similar to those of TCM. This review summarizes the advantages of metabolomics, highlight the key role of biomarkers for drug discovery and development of TCM.

Key words: Metabolomics, traditional Chinese medicine, formulae, biomarker, drug discovery

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INTRODUCTION

Traditional Chinese medicine (TCM), embodies traditional Chinese culture and philosophical principles as a complex medical science, reflects affluent dialectical thought, puts the human body into a large system for health protection and disease control[1]. TCM, depending on natural products, as the ancient medicine has been used for thousands of years in Asia and attracted worldwide interest[2]. In the past, "one disease-one target-one drug" and "one drug fits all" modes have been used in the treatment of human diseases, and the modes have been transformed to search for combination therapies currently[3]. Formulae, the most common clinical practice of TCM is herb combination, is composed of several types of medicinal herbs or minerals[4]. Generally, the integrated formulae consist of four elements: the monarch-which is the major role in the formulae, the minister-which strengthens the availability of the monarch herb, the assistant-which helps the monarch and minister components reach their target positions, and the servant-which can decrease the side effects and/or raise the effectiveness of the whole formulae[5]. The synergistic effect of various herbs and constituents were seen as the therapeutic efficacy of TCM. As the combinatorial therapeutic strategies over a millennium, formulae minimize adverse reactions or perfect the therapeutic efficacy[6]. It is supposed that, at least in some formulae, multiple components could impact on multiple targets and express synergistic therapeutic efficacies[7]. Amount of compounds have been isolated from TCM, and most of these resources have not been characterized for pharmacological research program with the purpose of new drugs development already[5]. TCM has a long history and been accepted as a valuable resource and methods in China. TCM studies have made hopeful progress in the aspects of medicinal chemistry, pharmacology and TCM preparations[8]. At present, many single extracting effective components from Chinese medicinal plants have been successfully developed into new drugs[9,10]. However, TCM is also facing serious challenges or problems. Due to the features and advantages of TCM are complex and difficult to understand, the insufficient modern scientific research lower the position of TCM and restrict the development of TCM in the abroad.

Metabolomics is defined as "the quantitative measurement of the dynamic multiparametric metabolic response of living systems to pathophysiological stimuli or genetic modification"[11]. Metabolomics is a new techniques of the post-genomic era, together with genomics, transcriptomics and proteomics, collectively constitutes the 'Systems Biology'[12,13]. Actually, metabolomics is the study of the comprehensive characterization of the small molecule (<1 kDa) metabolites, including lipids, amino acids, peptides, nucleic acids, organic acids, vitamins, thiols and carbohydrates in biological systems to measure perturbations in response to physiological challenges, toxic insults or disease processes[14]. It is to say that metabolomics is a novel and practical subject to evaluate the relationship between the endogenous and exogenous metabolite concentrations and function of body fluids and cells. Metabolomics has been widely used to investigate miscellaneous metabolic features of control, pathological, and drug-administrated courses, as well as to probe into the mechanism of drug intervention. It is well accord with the complete and systematic feature of TCM, and usually

involves multi-ingredient, multi-target and multi-pathway treatments[15]. Metabolomics has become an important tool in the research field of life sciences and is also widely used for innovative drug discovery and provides powerful methods for the essence and function of herbal compound recipe in TCM research[4]. In addition, metabolomics is an ideal tool for connecting TCM and molecular pharmacology, revealing the interacting mechanism between herbal medicine and organism in part[16, 17]. Its property is consistent with the wholistic methods underlying the practice of TCM, indicating that it is considered to have the potential to promote the innovation of TCM research.

ADVANTAGE OF METABOLOMICS

It is necessary to supply qualitative and quantitative descriptions of the emergent properties of the holistic system for the study of biological systems in a whole manner[1]. Systems biology stages studies focused on the complex interactions of integrated living systems, accenting the complete system including the structure and dynamics of cellular and organismal function rather than characteristics of isolated parts[18]. Metabolomics has many potential applications and benefits for the research of complex systems. As a functional level tool, metabolomics is employed to investigate the complex interactions between metabolites and the regulatory role metabolites afford through interaction with genes, transcripts, and proteins[1], and represent appealing candidates to understand phenotype of disease. As small molecule profiling technology combined with chemometrics emerge, metabolomics has quickly become an important method to measure a whole spectrum of endogenous metabolites in cells, biofluids, or tissues for mechanistic studies of diseases[19–21]. Technology development is a powerful force that pushes the progress of scientific knowledge[22]. The application of techniques almost spans the whole process of drug discovery and development. Nuclear magnetic resonance (NMR), high-performance liquid chromatography/mass spectrometry(HPLC/MS), ultra-performance liquid chromatography/mass spectrometry (UPLC/MS) coupled with tandem mass spectrometry (MS/MS) and gas chromatography/mass spectrometry (GC/MS), have been employed as the most common technologies in metabolomics studies recently[23–25]. These analytical platforms could capacitate separation, detection, characterization and quantification of such metabolites and related metabolic pathways[26]. Data obtained from these analytical techniques are in association with multivariate data analysis frequently, such as principal components analysis (PCA), partial least least squares (PLS), and hierarchical cluster analysis (HCA)[27–29]. Comprehensive software XCMS, which has been developed at The Scripps Research Institute is used to analyze complex MS-based metabolomics datasets[30,31]. There are other commonly used metabolomics programs for mass spectral analysis, such as MZmine[32], MetAlign[33] and MathDAMP[34].

Urine, blood plasma or serum as the most commonly biological sample are used for metabolomics studies. Due to its characteristics and simple collection methods noninvasively, urine is the most suitable biofluid for metabolomic analysis even in small babies. The use of noninvasive techniques is a fundamental requirement, urine and plasma as easily collected body fluid clearly makes these samples suitable for large-scale study. New insights and opportunities should be given for the drug discovery and development process and even for understanding drug toxicology by the advisable use of 'omics' data certainly[35]. Metabolomics has been applied in many fields, and drug discovery and development from TCM has become an area of considerable interest in metabolomics.

METABOLOMICS IN DRUG DISCOVERY AND DEVELOPMENT FROM TCM

1. Pharmacological studies on single herbs and active ingredients

Metabolomics method was commonly used in pharmacological research, particularly in TCM. Changed endogenous biomarkers and pathways detected by metabolomics may afford evidence to a deep understanding of drug action mechanisms and drug discovery. Yinchenhao (YCH, *Art*emisia annua L) as a famous Chinese herbal medicines, has efficient clinical usage for centuries to release from liver diseases in Asia. An UPLC/ESI-Q-TOF/MS method combined with pattern recognition and pathway analysis on potential biomarkers was established, five different potential biomarkers and three pathways were provided evidence to assess the hepatoprotective outcomes and possible mechanisms of Yinchenhao on ANIT-induced liver injury[36]. After YCH treatment, the changes in metabolic profiling were recovered to their baseline values according to the score plots. Notably, potential pharmacological activities of YCH by regulating multiple disordered pathways back to their normal state, that is associated with biochemistry test evaluation. Poria cocos epidermis (Fulingpi, FLP) as an ancient TCMs is usually used for the treatment of chronic kidney disease (CKD) in China. Zhao et al using UPLC Q-TOF/HSMS/MSE combine with PLS-DA identified 19 metabolites as potential biomarkers of chronic kidney disease, 10 markers were reversed to the control level in FLP-treated. Moreover, CKD were ameliorated by intervening in some main metabolic pathways influenced by FLP treatment[37]. Anti-blood deficiency mechanism of *Angelica sinensis* (AS) were investigated by metabolomics based on GC-MS, potential biomarkers in plasma and splenic tissue and related 5 metabolic pathways were detected[38]. Using mass spectrometry based metabolomics to study the metabolic changes in APC gene mutations induced colon cancer and the therapeutic mechanism of nutmeg[39].

Scoparone, an principle bioactive component of Yinchenhao, the effect against carbon tetrachloride-induced liver injury was studied by metabolomics[40]. Curcumin, a important constituent of Curcuma longa L, is generally known for its anti-hyperlipidemia effect. The intervention effect of curcumin on hyperlipidemia mice induced by high-fat diet (HFD) feeding were investigated by NMR and MS based urine metabolomics. 35 identified biomarkers proved that

curcumin treatment can partially restore the metabolic disturbance induced by HFD[41]. D-glucaro-1,4-lactone, a specific inhibitor of β-glucuronidase, was first detected in LiuWeiDiHuang pills (LWPs) through metabonomic strategy, reveal the effect of LWPs in decreasing the activity of intestinal β-glucuronidase and exerting an inhibitory effect on rat liver lysosomal fraction[42]. Puerarin, a bioactive constituents isolated from the root of *Pueraria lobata* (Willd.), have ameliorating effects on blood stasis. 15 and 10 potential biomarkers as well as its corresponding metabolic pathways were found through ^{1}H NMR-based plasma and urinary metabonomic approach which was applied to investigate the therapeutic effects of puerarin on blood stasis and its underlying mechanisms[43]. Wu et al analyzed the overall lipid profiles of hypothyroidism in rat cerebellum and screened out 23 potential lipid biomarkers, illustrates the lipid metabolomics is cogent in giving a complementary view to the pathophysiology of hypothyroidism and affords a worthy tool for systematic study of the therapeutic effects of Sini decoction on hypothyroidism at lipid level[44]. The hepatoprotective mechanism of *Angelica sinensis* polysaccharides (ASP) was probed through biochemical parameters combined with GC–MS based metabolomics and chemometrics. 9 potential biomarkers in the liver homogenate and 10 potential biomarkers in the plasma were considered to be in answer to hepatoprotective effects of ASP[45].

2. Metabolomic dissection for studying CMF

CMF (prescription) is a key issue in TCM and the premise on the study of material basis for TCM. The therapeutical effect of TCM is generally attributed to the cooperation mechanism between variety herbs and ingredients[46]. YinChenHaoTang (YCHT), a famous TCM formula recorded in 'Shanghan Lun', consists of *Artemisia* annua L., *Gardenia* jasminoides Ellis, and *Rheum* Palmatum L., achieve an efficacy in treating jaundice and liver injury syndrome. To probe into the molecular mechanisms is critical, because of its accurate mechanism and drug candidates are still complicated. HPLC-UV was used for controlling quality of medical formula YCHT, 15 representative general fingerprint peaks were determined as well as identified chemical constituents of YCHT *in vivo*[47,48]. Forty-five compounds in YCHT and twenty-one compounds *in vivo* were detected by the established UPLC-MS method.

In order to evaluate metabolomic characters of the alcohol-induced hepatotoxicity and the YCHT-intervention effects, UPLC/ESI-QTOF-MS was used to analyze urinary samples from control, alcohol- and YCHT-treated rats. As a result, compared with urine of control rats, ceramide (d18:1/25:0) was elevated and ions m/z 155.3547 and 708.2932 were at a lower concentration. And the related sphingomyelin signaling pathway providing further support for alcohol hepatotoxicity and the intervention effects of YCHT[49]. The three active components from YCHT including 6,7-dimethylesculetin (D), geniposide (G), and rhein (R), combination produces a stronger synergistic effect than any one or two of the three individual compounds by hitting multiple targets[5]. ^{1}H-NMR based urine metabolomics profiles were established to clarify the anti-depressant effect and action mechanism of XiaoYaoSan, 8 metabolites were found to be used as potential biomarkers for depression diagnosis or antidepressant evaluation and the detection of the mechanism of depression[50].

Modified Sinisan displayed prevention and therapy effects of dimethylnitrosamine-induced liver injury through partially regulating the perturbed pathways, including phenylalanine, tyrosine and tryptophan biosynthesis, phenylalanine metabolism, tryptophan metabolism, retinol metabolism and tyrosine metabolism[51]. Liu et al explored a HPLC-LTQ–Orbitrap/MS method to investigate the targeted metabolomics in the hypothalamus tissue of yeast-induced pyrexia rats, 0furthermore, the pathophysiology of the disease due to the biochemical changes in the hypothalamus during the febrile response was clarified[52]. Using spike-in method coupled with UHPLC-LTQ-Orbitrap MS for plasma metabolomics analyzed acute myocardial ischemia (AMI) rats and intervenion effect of Danqi Tongmai tablet, 19 potential biomarkers in rat plasma were identified and 10 related pathways were disturbed in the early stages of AMI development[53]. Yao et al using HPLC/QTOF-MS-based plasma and urine metabolomics combined with chemometric analysis investigated carbon tetrachloride-induced liver injury and assess hepatoprotective effects as well as possible mechanisms of Erzhiwan[54]. Urine metabolomic approach combining molecular docking analysis were utilized to profile psoriasis patients with Blood Stasis Syndrome metabolic changes, assess the efficacy and action mechanism of the Optimized YinXieLing formula[55]. HuangLianJieDu Decoction (HLJDD) is a representative antipyretic and detoxifying recipe with anti-inflammatory activity in TCM. A NMR-based integrative metabolomics approach was applied to evaluate the therapeutic effect of HLJDD. The result revealed that HLJDD could moderate stroke rats suffering from the ischemia/reperfusion injury by ameliorating the disturbance in several pathways, moderating the oxidative stress from reactive oxygen species and the inflammatory damage, and retrieving the destructed osmoregulation[56–58]. Chen et al using GC/MS and UPLC/MS/MS coupled with Ingenuity Pathway Analysis to go into the metabolic profile of cardiac metabolic characteristics in rats with doxorubicin-induced cardiomyopathy, and the therapeutic mechanism of Shengmai Injection[59].

3. Processing effects of TCM

TCM herbal processing approaches, namely ''Paozhi'', refers to the process of making medicine from raw materials of Chinese herbal medicine, and the main purpose is to strengthen therapeutic efficacy, lessen toxicity and side effect, convenient storage and convenient use[60,61]. ''Fuzi'', the lateral root of Aconitum carmichaelii Debx, has been utilized to relieve joint pain and treat rheumatic diseases for centuries with narrow therapeutic ranges, toxicological risk, but high frequency usage[62]. The processed products including Yanfuzi (YFZ), Heishunpian (HSP) and Baifupian (BFP) have got the toxicity reduced. UPLC-Q-TOF-HDMS based metabolomic analysis detected the metabolome of Fuzi and its

processed products, nineteen metabolite biomarkers were identified and the underlying regulations of Paozhi-perturbed metabolic pathways were discussed[63–64]. Toh *et al* developed a metabolomics platform based on UHPLC-TOF-MS coupled with PCA and PLS-DA for profiling of raw and steamed Panax notoginseng and a correlation between the duration of steaming and the maximum production of bioactive ginsenosides were established[65]. In a similar case, the study was accomplished to identify chemical markers for discriminating between raw and processed *Radix Rehmanniae* samples[66] .

4. Metabolomics provides a means of toxicity studies of TCM

TCM medicines safety is a global concern, and metabolomics is widely used in the toxicity research of TCM[67]. The dried root of Kansui (*Euphorbia kansui* L.) is an effective TCM that is widely applied to treat with edema, ascites, and asthma[68]. On the side, Kansui can induce toxic symptoms such as stomachache, diarrhea, dehydration, respiratory failure and toxicity to liver and kidney if used improperly. Tang et al studied the metabolites changes of urine to acquiring the comprehensive biochemical signature of rats treated with Kansui, using ^{1}H-NMR spectroscopy with PCA. Combined with the histopathology examination and clinical biochemistry assay, the correlation between biochemical changes and different administration doses and periods were investigated in detail to elucidate the toxicity of Kansui by metabonimic analysis[69]. Zhang et al analyzing metabolomics profiling coupled with western blot showed the opposite effects of Acanthopanax senticosus Harms on central nervous system in physiology with those in pathology, which may cause potential neurotoxicity. The result facilitated understanding the safe applicationof AS in the clinic[70,71]. Dong *et al* using a ^{1}H NMR-based metabolomics approach combined with linical chemistry, electrocardiographic recordings, and histopathological evaluation investigated the cardiotoxicity of Venenum Bufonis. The results proved that Venenum Bufonis induced oxidative stress, mitochondrial dysfunction, and energy metabolism perturbations were linked with the cardiac damage[72].

5. TCM identification, quality control and effective component screening

Aim of metabolomics is to analysing comprehensively metabolites in a biological sample, and it has huge potential for directly clarifying plant metabolic process[73]. The efficacy of TCM is mainly based on the synergistic effect of multi-targeting, multi ingredient formulation, but modern pharmacology and drug development usually focused on a single chemical entity. The quality and contents of the active ingredients of the herb are varied, and the change depends on the species, parts of herbaceous plant, agrarian geographical region as well as planting period. Metabolomics can be effectively applied for the quality control of plant extracts[74].

In the Chinese Pharmacopoeia 2010, only two *Aconitum* species including *Aconitum kusnezoffii* Reichb and *Aconitum carmichaelii* Debx are recorded. Using UPLC–QTOF–HDMS coupled with pattern recognition analyses, the two species were distinguished successfully[74]. Moreover, the NMR spectra combined with PCA were used to differentiate between Artemisia annua and Artemisia afra on the basis of phenylpropanoids including caffeic acid, chlorogenic acid, dicaffeoyl quinic acid, and ferulic acid[75]. Moreover, *Eleutherococcus senticosus* and its counterfeit detection were rapidly identificated via NIR coupled with PCA, DA, SIMCA, and PLS-DA[76]. Using pattern recognition including PCA and HCA as well as SIMCA and a BP-ANN aided fingerprint analysis to identify and distinguish the secondary metabolites between Epimedium wushanense and Epimedium koreanum. The SIMCA method failed to identify one sample, whereas BP-ANN precisely predicted the whole test set[77].

Zhang *et al* identified and quantified the different chemical constituents of the roots, leaves, stems, and seeds of *P. tenuifolia* using metabolomics, and 22 marker compounds were detected, and 7 triterpenoid saponins with significant differences among the different tissues were found. Combination of RtPCR, the excellent genetic traits in the triterpenoid saponin biosynthesis pathway were explored[78]. NMR-based metabolomic linked with analysis pattern recognition methods were applied to analyse extraction of 24 leaf samples which divided into six locations from the tip of the stem in each of four strains. Twenty-four extracts from mulberry leaf showed independent spectra by ^{1}H NMR[79].

Asian and American ginsengs are widely used medicinal materials and health products. Metabolomics provides a holistic analysis of the unique chemical fingerprint and all the metabolites of a specific organism . Chen et al investigated 17 Asian and 21 American ginseng samples using UPLC-Q/TOF-MS/MS and detected all of the peaks, the potential characteristic chemical components between Asian and American ginsengs were identified, the diversity of contents in forest samples and sun-dried ginsengs of Asian as well as wild and cultivated samples of American were also discovered[80]. By ^{1}H-NMR detector, Song et al characterize the holistic metabolic profile of Peucedani Radix (Qianhu), and found the markers to distinguish Qianhu from different districts[81]. Pan et al used ^{1}H-NMR, combination with PCA and HCA to detect *Lamiophlomis rotata* metabolomics and successfully discriminate samples from three different locations[82]. Furthermore, the essential oils of the Cinnamon Cortex specimens obtained from different localities have been analyzed via GC-MS[83].

6. Future prospects

As an independent discipline of the post gene era, metabolomics provides new ideas and new means for drug discovery and development from TCM, and has broad prospects for development[84–88]. To understand the role of metabolomics in pharmaceutical research correctiy, will provide ideas for the development of many subjects[89,90]. The generation and development of metabolomics is based on high flux, high-resolution, and sensitive analysis technology as well as massive data processing and graph recognition technology

for supporting. The main concern of metabolomics is the interaction of the overall metabolite, their functions in the biosystem, and factors that affect health, can be reflected in the metabolic group. The characteristics of systematicness and integrality of metabolomics study are consistent with TCM theory in nature, and it is predicted that the metabolomics is most suitable for the global concept of TCM[4].

CONCLUSIONS

Metabolomics is facing challenges and opportunities in drug discovery and development from TCM. It provide a comprehensive profile of all the metabolites present in a biological sample and this property is in according with the holistic thinking of TCM. Metabolomics has brought great opportunities for toxicity detection advanced and biomarkers discovery, a 'top-down' strategy adopted by metabolomics reflect the function of organisms from terminal symptoms of the metabolic network and to understand metabolic changes of a global system caused by interventions in a integral condition. The development of TCM can be expected promote by metabolomocs, especially in the understanding of Chinmedomics. Overall, it is indicated that the metabolomics in drug discovery and development of TCM will lead to better understanding and greater opportunities.

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COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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