Metabolomics: Towards Understanding Traditional Chinese Medicine

Authors

Aihua Zhang, Hui Sun, Zhigang Wang, Wenjun Sun, Ping Wang, Xijun Wang

Affiliation

Heilongjiang University of Chinese Medicine, National TCM Key Lab of Serum Pharmacochimistry, Harbin, China

Key words

- metabolomics
- biomarkers
- traditional Chinese medicine
- systems biology
- NMR
- molecular markers
- LC-MS
- metabolic diseases

Abstract

Metabolomics represent a global understanding of metabolite complement of integrated living systems and dynamic responses to the changes of both endogenous and exogenous factors and has many potential applications and advantages for the research of complex systems. As a systemic approach, metabolomics adopts a “top-down” strategy to reflect the function of organisms from the end products of the metabolic network and to understand metabolic changes of a complete system caused by interventions in a holistic context. This property agrees with the holistic thinking of Traditional Chinese Medicine (TCM), a complex medical science, suggesting that metabolomics

has the potential to impact our understanding of the theory behind the evidence-based Chinese medicine. Consequently, the development of robust metabolomic platforms will greatly facilitate, for example, the understanding of the action mechanisms of TCM formulae and the analysis of Chinese herbal (CHM) and mineral medicine, acupuncture, and Chinese medicine syndromes. This review summarizes some of the applications of metabolomics in special TCM issues with an emphasis on metabolic biomarker discovery.

Abbreviation

LC: liquid chromatography

Introduction

In the last two decades, metabolomics are considered important tools to be applied and utilized to understand the biology of an organism and its response to environmental stimuli or genetic perturbation. Metabolomics (a flowchart is shown in **Fig. 1**) was originally proposed as a method of functional genomics [1], but its utility extends well beyond that – it is useful whenever an assessment of changes in metabolite levels is needed. Metabolomics (or metabonomics) is used for assessing responses to environmental stress [2,3], comparing mutants [4] and different growth stages [18,19], for drug [5] and natural products [22] discovery, toxicology [6,7], nutrition [8–12], genetic manipulation [13], cancer [14–17], and diabetes [20,21]. Indeed, metabolomics is the study of global metabolite profiles in a system (cell, tissue, or organism) under a given set of conditions and has its roots in early metabolic profiling studies, but is now a rapidly expanding field of scientific research, which has justifiably taken its place alongside genomics, transcriptomics, and

proteomics as one of the latest and most exciting “-omic” sciences [23]. The global metabolite profiling involves measuring low molecular-weight metabolites (<1 kDa) in complex biofluids/tissues to study perturbations in response to physiological challenges, toxic insults, or disease processes [24]. Metabolites are the end products of cellular regulatory processes and can be regarded as the ultimate response of biological systems to control genetic or environmental changes [25]. A deeper understanding of global perturbations in biochemical pathways in complex diseases could provide valuable insights about the mechanisms of disease. In addition, metabolomics has the potential to enable mapping of early biochemical changes in disease and hence provide an opportunity to develop predictive biomarkers that can trigger earlier interventions. TCM, a complex medical science, reflects traditional Chinese culture and philosophical principles, embodies rich dialectical thought, places the human body into a large system for observation and adjusts humans to remain in a healthy status. Entering the 21st century, TCM is getting

received August 1, 2010
revised October 14, 2010
accepted October 21, 2010

Bibliography

DOI <http://dx.doi.org/10.1055/s-0030-1250542>
Published online November 5, 2010
Planta Med 2010; 76:
2026–2035 © Georg Thieme
Verlag KG Stuttgart · New York ·
ISSN 0032-0943

Correspondence

Prof. PhD. Xijun Wang
National TCM Key Lab
of Serum Pharmacochimistry
Heilongjiang University
of Chinese Medicine
Heping Road 24
Harbin 150040
China
Phone: + 86 451 82 11 08 18
Fax: + 86 451 82 11 08 18
phar_research@hotmail.com

Fig. 1 Flowchart of metabolomic analysis.

more and more popular in the whole world for improving health conditions of human beings and preventing or healing diseases, and is especially showing great advantages in early and combination intervention, personalized therapy, etc. The international community has attached increasing importance to TCM [26]. However, like almost all other ethnopharmacologies, TCM faces severe challenges and suffers from insufficient scientific research owing to the lack of modern and technologic approaches; this restricts the development of TCM in the world. Chinese medicine is not yet an integral part of the standard healthcare system in Western countries due to a lack of scientific evidence for its efficacy and safety as well as a language and cultural barrier [27]. Fortunately, metabolomics adopts a “top-down” strategy to reflect the function and metabolic changes of complete organisms caused by interventions in a holistic context. This property coincides with the holistic thinking of TCM and has the potential to impact our understanding of Chinese medicine theory. Recently, a wide range of metabolomic analytical techniques are widely used in the modern research of TCM. Metabolomic analysis will facilitate the modernized study of TCM syndromes, provide an in-depth understanding of the TCM theory, help predict the disease on-set and achieve a comprehensive evaluation of clinical efficacy, safety, and action mechanisms of TCM formulae. Metabolomics combined with TCM methodologies will provide a new pathway and methodology for the study of complicated systems theory of TCM and its modernization. Especially the advances in the high-throughput and comprehensive research technologies and the idea of metabolomics provides new strategies for the analysis of active components in the formulae of TCM *in vivo*. Furthermore, the initiatives of metabolomics may pave a new way to explain the action mode of TCM in light of modern sciences and so contribute to establish a new technique platform for evaluating the efficacy of the TCM formulae. It is believed that with the further development of metabolomic analytical techniques, especially multi-analysis techniques, metabolomics will greatly promote TCM research and be beneficial to its modernization as well as establish international standards for it. Thus, we draw attention to applicable approaches of using metabolomic techniques to resolve special TCM issues and focus particularly on the potential of metabolomics to contribute to biomarker discovery in TCM research.

Advantages of Metabolomics

▼ The study of biological systems in a holistic manner (systems biology) is increasingly being viewed as a necessity to provide qualitative and quantitative descriptions of the emergent properties of the complete system. Systems biology performs studies focused on the complex interactions of system components, emphasizing the whole system rather than the individual parts. Metabolomics is one functional level tool being employed to investigate the complex interactions of metabolites with other metabolites but also the regulatory role metabolites provide through interaction with genes, transcripts, and proteins (e.g., allosteric regulation). Technological developments are the driving force behind advances in scientific knowledge. Recent advances in the two analytical platforms of mass spectrometry (MS) and nuclear magnetic resonance (NMR) spectroscopy have driven forward the discipline of metabolomics [28–32]. Technological advances in NMR and mass spectrometry have opened a new chapter in biochemistry by using metabolomics as an approach to study metabolism and its regulation in relation to genetic, disease, and environmental factors. NMR is one of the most commonly used technologies in metabolomics research, providing detailed information on the molecular structure and probing metabolite molecular dynamics and mobility [33]. The high selectivity of mass spectrometry with low-detection limits makes mass spectrometry an ideal tool for metabolomic applications. It requires separation of the metabolic components using either gas chromatography after chemical derivatization or ultra-performance liquid chromatography (UPLC). UPLC/MS is often used to obtain the largest possible biochemical profile information subset. It is a sensitive tool that can be used to characterize, identify and quantify a large number of compounds in a biological sample where metabolite concentrations might cover a broad range of information with regard to disease pathophysiology [34–36]. All metabolomic studies result in complex multivariate datasets that require visualization software as well as chemometric and bioinformatic methods for interpretation. The application of software tools for the analysis of the information contained in a database can identify the signature of a disease and predict its risk and progression.

One of the major benefits of metabolomics in the study of disease and drug therapy is that metabolic profiling can usually be achieved using urine or plasma samples. The accessibility of urine and plasma clearly makes these samples ideal for large-scale re-

search [37]. Metabolomics data can be analyzed with a range of statistical and machine-learning algorithms. These algorithms can be classified within two major classes: unsupervised and supervised [38]. They can be useful in the identification of biomarkers [39,40]. Examples of unsupervised methods that have been routinely used in analyzing molecular fingerprinting data include principal component analysis (PCA) and self-organizing maps [41].

Metabolomics has become useful in many medicine areas as an aid to disease diagnosis or staging and as a tool to predict or monitor treatment response or toxicity. Considerable interest in the field of metabolomics is that of personalized health care, whereby an individual's drug treatment is tailored so as to achieve maximal efficacy while avoiding adverse drug reactions. Another more recent approach has been to use metabolomics to predict the metabolism of a dosed substance based on a pre-dose metabolic profile [42]. Metabolomics holds a comprehensive and non-invasive analysis of metabolic biomarkers that could detect early-stage disease, identify residual disease post-surgery and help to monitor treatment response. Additionally, metabolomics can be seen as bridging the gap between genotype and phenotype, providing a more comprehensive view of how cells function, as well as identifying novel or striking changes in specific metabolites [25,43]. In the future, metabolomics may enable us to develop new approaches for improving the health conditions of human beings and preventing or healing diseases, and is especially showing great advantages in early and combination intervention, personalized therapy, etc. [44,45].

Challenges for TCM

TCM has been practiced for thousands of years and the written documents showed sophisticated theories for TCM even in its early forms although these theories were established on a philosophical basis and clinical experiences rather than science perhaps owing to the fact that this medical system had been developed well before modern science was born. Like almost all other traditional medicines, the practice of TCM suffers, at present, from insufficient modern scientific research. For example, many treatments of TCM are still practiced in their original form, and although they have been effective in the therapy of many conditions, especially chronic ones, they lack the necessary definition of a molecular mechanism and sometimes even of a molecular

basis. Prescribed medicines in TCM are normally mixtures of a number of plants, and their composition is not well defined. There have been many recent attempts to address these issues but most of them were still based on the "reductionism" philosophy. Therefore, there is a clear urgency of scientific research in terms of quality control, clinical efficacy, and molecular mechanisms of traditional medicines, which undoubtedly demands a significant amount of analytical power and effort.

Metabolomic Applications of TCM

Metabolomic evaluation of formulae

Wang et al. evaluated metabolomic characters of the hepatotoxicity induced by alcohol and the intervention effects of Yin Chen Hao Tang (YCHT), a classic traditional Chinese medicine formula composed of *Flos Artemisiae*, *Gardeniae Jasminoidis*, and *Fructus* and *Radix et Rhizoma Rhei* for the treatment of jaundice and liver disorders in China [46]. The greatest difference in metabolic profiling was observed in alcohol-treated rats compared with control and YCHT-treated rats (● Fig. 2). The number of positive ions, m/z 664.3126 (9.00 min), was elevated in the urine of alcohol-treated rats, whereas ions with m/z 155.3547 (10.96 min) and 708.2932 (9.01 min) were at a lower concentration compared with those in the urine of control rats. However, the number of these ions was not statistically different between control and YCHT-treated rats (● Fig. 3). The ion with m/z 664.3126 was found to correspond to ceramide (d18:1/25:0), providing further support for an involvement of the sphingomyelin signaling pathway in alcohol hepatotoxicity and the intervention effects of YCHT (● Fig. 4). More recently, Wang et al. explored the excretion pattern of low molecular mass metabolites in the male Wistar-derived rat model of kidney yin deficiency induced with thyroxine and reserpine as well as the therapeutic effect of Liu Wei Di Huang Wan (LW), one of the most important Chinese formulas consisting of six herbs including *Rehmannia glutinosa* Libosch. (family: Scrophulariaceae), *Cornus officinalis* Sieb. (family: Cornaceae), *Dioscorea opposita* Thunb. (family: Dioscoreaceae), *Alisma orientale* (G. Samuelsson) Juz (family: Alismataceae), *Poria cocos* (Schw.) Wolf (family: Polyporaceae), and *Paeonia suffruticosa* Andrews (family: Paeoniaceae), and widely used in eastern Asia for treating kidney yin deficiency in China [47]. The results showed that the changes in metabolic profiling were restored to their baseline values after treatment with LW according to the PCA

Fig. 2 Resulting score plots from PCA of reversed-phase UPLC-ESI-TOF-MS data obtained from (A) control rats, (B) alcohol-treated rats, (C) YCHT-treated rats urine samples collected from day 7. Symbols: control rat (□), alcohol-treated rat (×), and YCHT-treated rat (+).

Fig. 3 Resulting loadings plot from PCA of reversed-phase UPLC-ESI-TOF-MS data obtained from control rats, alcohol-treated rats, and YCHT-treated rats urine samples collected from day 7.

Fig. 4 The proposed metabolic pathway for explanation of the relationship between alcohol hepatotoxicity and the increased content of ceramide (d18:1/25:0) in urine.

score plots (● Fig. 5), indicating 20 ions (8 in the positive mode, 14 in the negative mode, and 2 in both) as “differentiating metabolites” (● Fig. 6). PCA score plots separated urine samples into different blocks, and samples subjected to the same treatment were located on the same trajectory, indicating that treatments have greatly disturbed the normal urine metabolic profiles of rats. There were different phenotypes of metabolites based on HPLC-UV urinary profiling after administration of LW pills or carageenan-stimulated inflammation model, and those could be conveniently discriminated by PCA. In addition, the results also showed that LW Pills could restore the metabolite network that was disturbed by inflammation, which would be a proof of the therapeutic efficacy of LW pills on inflammation from a metabolomics study [48]. A urinary metabolomics method based on the UPLC-MS was used to evaluate the efficacy and study the action mechanism of Xindi soft capsules, consisting of sea buckthorn flavanoids (e.g., quercetin, kaempferol, and isorhamnetin), which is a TCM preparation against blood stasis [49]. Compound Dan-

shen tablets, a herbal (*Salvia miltiorrhiza* Bge.) compound preparation, presented protective effects on myocardial ischemia by reversing potential biomarkers to sham levels, especially for the four metabolites in the pathway of purine metabolism (hypoxanthine, xanthine, inosine, and allantoin) [50]. Damage of mitochondria, disorder of energy metabolism, and osmoregulation were observed in a cyclophosphamide-caused blood deficiency model by an NMR-based metabolomics method. Siwutang, a novel prescription consisting of *Paeonia lactiflora* Pall, *Angelica sinensis*, *Rehmannia glutinosa*, and *Rhizoma Chuanxiong* could improve these effects [51].

Metabolomic analysis of CHM

CHM has long been used for disease prevention and therapy in China and is becoming increasingly important in the West [52]. Herbal medicine has been the source of many drugs used in modern therapeutics, and particularly in the case of anticancer drugs, more than 50% originally came from natural products. However,

Fig. 5 PCA score plots of urine samples collected from different treatment groups of rats (a) in negative ESI mode and (b) in positive ESI mode. Key: ■ Control; ■ LW; ■ Model 1; ■ Model 2; ■ Sanbu; ■ Sanxie.

due to the painstaking way of conventional lead-finding, the attention towards CHM has been deviated in the last decades. A new strategy for the detection of active compounds is necessary to get natural product research out of its stalemate. Metabolomics, with its holistic approach and the possibility it provides for the simultaneous detection of all sorts of metabolites, has the potential to be instrumental for this new approach. A combined GC/MS and LC/MS metabolic profiling strategy indicates that *Tripterygium wilfordii* Hook. f. caused a time-dependent toxic effect at a high dose as revealed by the perturbed metabolic regulatory network involving disorders in energy metabolism, elevated amino acid, and choline metabolism pathways, as well as altered structure of gut flora [53]. Urinary metabolic perturbations associated with liver toxicity induced by Huang-yao-zi (root of *Dioscorea bulifera* L.) were studied using NMR to determine the correlations between metabolomic profiling and histopathologic/biochemical observations and to discover biomarkers for liver toxicity, indicating metabolic changes observed in urine samples in response to Huang-yao-zi treatment. In addition, the mechanism associated with oxidative injury of hepatic mitochondria was investigated [54]. HPLC-MS/MS-based metabolomics method was used to find the possible biomarker of Rhizoma Coptidis

in rat urine. 169 kinds of biomarkers were found. The result was consistent with the pharmacological effects of R. Coptidis, such as anti-inflammatory, anticonvulsant, and energy metabolism inhibition [55]. Berberine might play a pivotal role in the treatment of type 2 diabetes through downregulating the high level of free fatty acids. Comprehensive metabolomic measurements are potentially very useful for studying the mechanisms of action of traditional Chinese medicines [56]. Combined NMR and LC-DAD-MS analyses reveal comprehensive metabolomic variations for three phenotypic cultivars of *Salvia miltiorrhiza* Bunge [57]. NMR-based metabolomics is an attractive method for nonselective and comprehensive analysis of *Ginkgo* extracts, which are very complex mixtures prepared from raw leaf extracts by a series of extraction and prepurification steps [58]. *Ginkgo biloba* leaves exert multidirectional lipid-lowering effects on the rat metabolome, including limitation of the absorption of cholesterol, inactivation of HMGCoA, and favorable regulation of profiles of essential polyunsaturated fatty acid [59]. Recently, changes of metabolites in rat urine after treatment with *Aristolochia fangchi* decoction were studied by the metabolomic method [60]. High-dose *Aristolochia fangchi* can induce nephrotoxicity, and its seriousness corresponds to the duration of ad-

Fig. 6 Component loadings in (a) negative ESI mode and (b) positive ESI mode.

ministration. *Aristolochia fangchi* may also have toxicity on the liver. The results suggested that this metabolomic approach is a promising methodology for the rapid *in vivo* screening of nephrotoxicity associated with ingesting multi-ingredient medicinal herb supplements [61]. Aristolochic acids, naturally present in *Aristolochia* plant species that have been used in CHM containing a mixture of varying herb species, were identified by UPLC-MS-based methodology [62]. A LC/MS metabolomics approach was applied to characterize the aging of rats and the antiaging effect of total flavones of *Epimedium*, a traditional Chinese medicine, indicating that aging could be characterized by changes of lipid metabolism and accumulation of free radicals. The antiaging effects of total flavones of *Epimedium* might be due to the intervention on lipid metabolism and its property of antioxidation [63]. Phenotype of aging at different levels demonstrates a common age-dependent trend. *Epimedium* flavanoids can reverse this age-dependent change at different levels in a synchronous manner [64]. The total flavones of *Epimedium* administration can markedly influence the aging process and exert antiaging effects, which might due to the melioration of pyruvate metabolism and oxidative phosphorylation [65]. Significant differences in endogenous metabolite profiles were observed in the intervention rats, and the abnormality of metabolism recovered towards the normal level after administration with *Epimedium brevicornum* extract.

Four active constituents of *Epimedium brevicornum* Maxim were found in the blood circulation of kidney-deficient rats and two of the metabolites in the urine. It suggests that the metabolomic approach is a potentially powerful tool to explore the therapeutic basis and to clarify the possible action mechanism of TCM herbs [66].

Metabolic changes in Wistar rats caused by *Aconitum* alkaloids aconitine, mesaconitine, and hypaconitine, which are the main toxic components of the traditional herbal medicine Fu Zi (*Aconitum carmichaelii* Debx.) were investigated by means of integrated analysis of two metabolomic approaches. Metabolites with significant changes or with a tendency to change in the aconitine and mesaconitine groups were dissimilar, suggesting a possible difference in the acute toxicity mechanisms of these alkaloids [67]. A metabolomic investigation of intoxication with *Aconitum* sp. alkaloids was carried out: they can cause metabolic disorders in rats. The toxicity and corresponding mechanism of hypaconitine was different from those of aconitine and mesaconitine, based on the differences of perturbed metabolic patterns between groups [68]. The effect mechanism and potential biomarkers of the toxicity of Hei-Shun-Pian, the processed lateral root of *Aconitum carmichaelii* Debx. (Ranunculaceae), on the metabolic profile of rats, suggests a toxic effect of Hei-Shun-Pian on the rat heart in a dose-dependent manner [69]. *Artemisia afra*

has been used as an infusion to treat malaria throughout the southern parts of Africa, in much the same way as the antimalarial plant *Artemisia annua* in China. Liu et al. had used metabolomics to investigate the ethnopharmacological use of *Artemisia afra* with NMR spectroscopy and multivariate data analysis [70]. Their findings show that there is no *in vitro* activity, and a list of the identified metabolites causing the metabolic differences is presented. Artemisinin has been proven to be an effective antimalarial compound, especially for chloroquine-resistant and cerebral malaria. In order to get new clues about artemisinin biosynthesis, metabolic profiling by GC and GC-MS was applied to compare the secondary metabolites of two *Artemisia annua* L. genotypes. It could be shown that there were clear differences in terpenoids and artemisinin metabolism between different growth stages and genotypes [71].

Metabolite profiling of five medicinal *Panax* herbs, including *Panax ginseng* (Chinese ginseng), *Panax notoginseng* (Sanchi), *Panax japonicus* (Rhizoma Panacis Majoris), *Panax quinquefolium* L. (American ginseng), and *P. ginseng* (Korean ginseng), was performed using UPLC-MS and a multivariate statistical analysis technique. PCA of the analytical data showed that the five *Panax* herbs could be separated into five different groups of phytochemicals. The chemical markers such as ginsenoside Rf, 20(S)-pseudoginsenoside F11, malonyl ginsenoside Rb1, and ginsenoside Rb2, accountable for such variations, were identified through the loadings plot of PCA, tentatively by the accurate mass of TOF/MS, and partially by a reference standard [72]. Six different types of ginseng roots from China and Korea could be easily differentiated by NMR-based metabolomics [73]. Pharmacodynamic effects of the ginsenoside Rg3 on the urine metabolomes of healthy and liver-tumor-bearing rats have been investigated. Seventeen biomarker candidates including three apolar metabolites were detected for global analysis of highly complex biosamples [74]. This approach may not only increase the number of discovered biomarkers but consequently improve the comprehensive information on metabolic changes.

Liang et al. used LC/MS to analyze 16 saponins simultaneously, and the developed methodology could effectively break the application bottleneck on the quantitative analysis of multi-component LC/MS data and be applied widely in related fields for multi-component analysis, especially in CHM research [75]. Concurrently, a number of metabolites involved in glucose metabolism, citric acid cycle, and amino acid metabolism were affected immediately after the intake of green tea, and the proposed approach provided a more comprehensive picture of the metabolic changes after intake of green tea in human urine [76]. Interestingly, green and black tea intake had a different impact on endogenous metabolites in urine and plasma. Green tea intake caused a stronger increase in urinary excretion of several citric acid cycle intermediates, which suggests an effect of green tea flavanols on human oxidative energy metabolism and/or biosynthetic pathways [77]. The metabolic strategy has shown its potential in optimization of harvest time and chemical markers screening of tangerine peels, herbal materials of two coupled traditional Chinese medicines, *Pericarpium Citri Reticulatae* and *Pericarpium Citri Reticulatae Viride*, which will open a wide perspective in the analysis of "coupled TCMs" [78]. Major metabolite of both arecoline and arecaine, N-methylinpecotic acid, is a novel metabolite arising from carbon-carbon double-bond reduction. Another unusual metabolite found was the monoacylglyceride of arecaine. It was shown which role is played by these uncommon metabolites in the toxicology of arecoline and arecaine [79]. Evocarpine,

from the Chinese herb *Evodia rutaecarpa*, is not transported by p-gp and showed only slight toxicity at the highest test concentration of 30 μ M [80]. *Evodia rutaecarpa* has changed the endogenous metabolites of rats and can provide the base for the further research on the interpretation of drug property [81]. Molecular compositions of rosemary (*Rosmarinus officinalis* L.) extracts and their dependence on extraction solvents, seasons, and drying processes were systematically characterized using NMR spectroscopy and multivariate data analysis. Results showed that the rosemary metabolome was dominated by 33 metabolites including sugars, amino acids, organic acids, polyphenolic acids, and di-terpenes, among which quinate, cis-4-glucosyloxycinnamic acid, and 3,4,5-trimethoxyphenylmethanol were found in rosemary for the first time [82]. It can be concluded that the metabolomics are a potentially powerful tool to explore the therapeutic basis of TCM herbs.

Metabolomics in the study of mineral medicine

An important traditional Chinese mineral medicine, *Cinnabar*, has been widely used as a Chinese patent medicine ingredient for sedative therapy, induced disturbance in energy metabolism, amino acid metabolism and gut microflora environment, as well as slight injury in the liver and kidney, which might indirectly result from cinnabar induced oxidative stress [83]. The time-dependent biochemical variations induced by realgar were achieved using pattern recognition methods. They illustrated the high reliability of the NMR-based metabolomic approach for the study of biochemical effects induced by mineral medicine [84].

Metabolomics in acupuncture

Chinese acupuncture, handed down from the ancient times, still survives in clinical practice nowadays. At present, acupuncture has attracted extensive attention in the domestic and overseas circles in the TCM field. Numerous experimental studies have demonstrated that acupuncture can correct various metabolic disorders such as hyperglycemia, overweight, hyperphagia, hyperlipidemia, inflammation, altered activity of the sympathetic nervous system, and insulin signal defect, all of which contribute to the development of insulin resistance [85]. In addition, acupuncture has the potential to improve insulin sensitivity. These studies have revealed the mechanisms responsible for the beneficial effects of acupuncture, though further investigations are warranted. Functional dyspepsia acupuncture-treated patients showed significantly changed levels of leucine/isoleucine, lactate, and glucose, and a slightly changed lipids level towards those of the healthy controls, demonstrating the therapeutic effects of acupuncture on the relief of functional dyspepsia symptoms; this is a NMR-based metabolomic approach in the study of biological effects of acupuncture [86].

Metabolomics in Chinese medicine syndromes

Metabolomics, the analysis of the metabolite profile in body fluids or tissues, is being applied to the analysis of a number of different diseases as well as being used in following responses to therapy. There is good reason to think that metabolomics will find particular utility in the investigation of disease, which may have tissue specific and systemic components. Metabolomic analysis can inform us about ocular or other body fluids and can therefore provide new information on pathways and processes involved in these responses.

Metabolomics has been used in several animal models of human disease. A recent study was conducted by Qiu et al. to investigate

the metabolic responses to Herba Cistanches intervention in a rat model of hydrocortisone-induced “kidney-deficiency syndrome”. It revealed that Herba Cistanches caused a systemic recovery from hydrocortisone-induced metabolic perturbation in rats [87]. The TCM concepts of “Xinxueyuzheng (heart blood stasis obstruction pattern)” and “Qiyinliangxuzheng (qi and yin deficiency pattern)” for myocardial ischemia rat models were constructed by Yan et al. Endogenous metabolites in rat plasma were identified, such as valine, serine, threonine, ornithine, hydroxyproline, lysine, 2-hydroxybutanoic acid, 3-hydroxybutanoic acid, galactofuranose, and inositol. These compounds were indicated as the potential biomarkers, suggesting that the two above-mentioned patterns are involved in dysfunction, oxidative stress, energy metabolism, and amino acid metabolism [88]. Chen et al. described the metabolomic study of a biochemical modification *in vivo* induced by a high dose of hydrocortisone, which led to a unique pathologic condition similar to the “kidney deficiency syndrome”, an early stage of obesity and diabetes in TCM [89]. In the study, 24-h urine was collected pre-dose and at days 1, 3, 7, and 10 post-dose after rats were injected with hydrocortisone at 1.5 mg/100 g. The acquired data were transferred into Matlab to be processed using PCA. Results indicated clear and consistent biochemical changes following hydrocortisone intervention. It suggested that metabolomic approach could be used as a potentially powerful tool to investigate the biochemical changes of certain physiopathologic conditions such as metabolic syndrome, as an early diagnostic means.

Luo et al. determined the changes of the plasma metabolic phenotype in rats with chronic restraint stress (rats with syndrome of liver qi stagnation and spleen deficiency) [90]. Metabolomic analysis of rat plasma revealed different metabolic spectra between stress and control groups, which were consistent with alterations of *in vivo* metabolisms in rats under stress stimuli. Compared with the control group, rats with repeated stress displayed significant changes in spectral peak shapes of acetate, lactate, tyrosine, and low-density lipoprotein. These altered metabolites can be used as biomarkers of syndrome of liver qi stagnation and spleen deficiency. Therefore, metabolomics is an approach with good development prospects for studies of TCM syndromes.

Conclusion and Future Perspective

In this review, we delineate and discuss metabolomic approaches to resolve special TCM issues. Metabolomics, a new but rapidly growing field, has the potential to impact our understanding of TCM theory and molecular mechanisms of disease. Facing the complicated life phenomenon, metabolomics will be a powerful means to the research of a complex system theory and modernization of TCM. So that application of metabolomics for TCM will facilitate the understanding of the intrinsic quality of TCM syndromes and the evaluation of the therapeutic effects of its formulae, Chinese herbal and mineral medicine, as well as acupuncture, and explore the therapeutic basis and the possible action mechanisms of TCM. Combined metabolomics and TCM methodologies will also provide a new pathway and methodology for TCM and its modernization. Overall, with the further development of metabolomic analytical techniques, especially multi-analysis techniques, we eagerly expect that metabolomics will greatly pro-

mote TCM research and establish international standards, being beneficial to its modernization.

Acknowledgements

This work was supported by grants from the National Program on Key Basic Research Project of China [Grant No. 2005CB523406]. We are grateful for the constructive and critical comments on the manuscript from the reviewers and the Planta Medica editorial team.

Reference

- 1 Oliver SG, Winson MK, Kell DB, Baganz F. Systematic functional analysis of the yeast genome. *Trends Biotechnol* 1998; 16: 373–378
- 2 Workentine ML, Harrison JJ, Weljie AM, Tran VA, Stenroos PU, Tremaroli V, Vogel HJ, Ceri H, Turner RJ. Phenotypic and metabolic profiling of colony morphology variants evolved from *Pseudomonas fluorescens* biofilms. *Environ Microbiol* 2010; 12: 1565–1577
- 3 Pedersen KS, Kristensen TN, Loeschcke V, Petersen BO, Duus JØ, Nielsen NC, Malmendal A. Metabolomic signatures of inbreeding at benign and stressful temperatures in *Drosophila melanogaster*. *Genetics* 2008; 180: 1233–1243
- 4 Johansen KK, Wang L, Aasly JO, White LR, Matson WR, Henchcliffe C, Beal MF, Bogdanov M. Metabolomic profiling in LRRK2-related Parkinson's disease. *PLoS One* 2009; 4: e7551
- 5 Catchpole GS, Beckmann M, Enot DP, Mondhe M, Zywicki B, Taylor J, Hardy N, Smith A, King RD, Kell DB, Fiehn O, Draper J. Hierarchical metabolomics demonstrates substantial compositional similarity between genetically modified and conventional potato crops. *PNAS* 2005; 102: 14458–14462
- 6 Nicholson JK, Lindon JC, Holmes E. ‘Metabonomics’: understanding the metabolic responses of living systems to pathophysiological stimuli via multivariate statistical analysis of biological NMR spectroscopic data. *Xenobiotica* 1999; 29: 1181–1189
- 7 Mally A, Amberg A, Hard GC, Dekant W. Are 4-hydroxy-2(E)-nonenal derived mercapturic acids and (1)H NMR metabolomics potential biomarkers of chemically induced oxidative stress in the kidney? *Toxicology* 2007; 230: 244–255
- 8 Stewart D, McDougall GJ, Sungurtas J, Verrall S, Graham J, Martinussen I. Metabolomic approach to identifying bioactive compounds in berries: advances toward fruit nutritional enhancement. *Mol Nutr Food Res* 2007; 51: 645–651
- 9 German JB, Roberts MA, Watkins SM. Genomics and metabolomics as markers for the interaction of diet and health: lessons from lipids. *J Nutr* 2003; 133: 2078S–2083S
- 10 Schnackenberg LK, Beger RD. Monitoring the health to disease continuum with global metabolic profiling and systems biology. *Pharmacogenomics* 2006; 7: 1077–1086
- 11 Pexa A, Boeger RH, Henle T, Schwedhelm E, Deussen A. Effects of moderate hyperhomocysteinaemia induced by 4 weeks methionine-enriched diet on metabolite profile and mesenteric artery function in rats. *Br J Nutr* 2008; 99: 993–999
- 12 Astle J, Ferguson JT, German JB, Harrigan GG, Kelleher NL, Kodadek T, Parks BA, Roth MJ, Singletary KW, Wenger CD, Mahady GB. Characterization of proteomic and metabolomic responses to dietary factors and supplements. *J Nutr* 2007; 137: 2787–2793
- 13 Urakami K, Zangiacomi V, Yamaguchi K, Kusuhara M. Quantitative metabolome profiling of *Illicium anisatum* by capillary electrophoresis time-of-flight mass spectrometry. *Biomed Res* 2010; 31: 161–163
- 14 Urayama S, Zou W, Brooks K, Tolstikov V. Comprehensive mass spectrometry based metabolic profiling of blood plasma reveals potent discriminatory classifiers of pancreatic cancer. *Rapid Commun Mass Spectrom* 2010; 24: 613–620
- 15 Kim HK, Wilson EG, Choi YH, Verpoorte R. Metabolomics: a tool for anti-cancer lead-finding from natural products. *Planta Med* 2010; 76: 1094–1102
- 16 Sreekumar A, Poisson LM, Rajendiran TM, Khan AP, Cao Q, Yu J, Laxman B, Mehra R, Lonigro RJ, Li Y, Nyati MK, Ahsan A, Kalyana-Sundaram S, Han B, Cao X, Byun J, Omenn GS, Ghosh D, Pennathur S, Alexander DC, Berger A, Shuster JR, Wei JT, Varambally S, Beecher C, Chinnaiyan AM. Metabo-

- lomic profiles delineate potential role for sarcosine in prostate cancer progression. *Nature* 2009; 457: 910–914
- 17 Chan EC, Koh PK, Mal M, Cheah PY, Eu KW, Backshall A, Cavill R, Nicholson JK, Keun HC. Metabolic profiling of human colorectal cancer using high-resolution magic angle spinning nuclear magnetic resonance (HR-MAS NMR) spectroscopy and gas chromatography mass spectrometry (GC/MS). *J Proteome Res* 2009; 8: 352–361
 - 18 Wang H, Ma C, Ma L, Du Z, Wang H, Ye H, Li G, Liu B, Xu G. Secondary metabolic profiling and artemisinin biosynthesis of two genotypes of *Artemisia annua*. *Planta Med* 2009; 75: 1625–1633
 - 19 Martins AM, Camacho D, Shuman J. A systems biology study of two distinct growth phases of *Saccharomyces cerevisiae* cultures. *Curr Genomics* 2004; 5: 649–663
 - 20 Lanza IR, Zhang S, Ward LE, Karakelides H, Raftery D, Nair KS. Quantitative metabolomics by H-NMR and LC-MS/MS confirms altered metabolic pathways in diabetes. *PLoS One* 2010; 5: e10538
 - 21 Bao Y, Zhao T, Wang X, Qiu Y, Su M, Jia W. Metabonomic variations in the drug-treated type 2 diabetes mellitus patients and healthy volunteers. *J Proteome Res* 2009; 8: 1623–1630
 - 22 Lawaetz AJ, Schmidt B, Staerk D, Jaroszewski JW, Bro R. Application of rotated PCA models to facilitate interpretation of metabolite profiles: commercial preparations of St. John's Wort. *Planta Med* 2009; 75: 271–278
 - 23 Goodacre R, Vaidyanathan S, Dunn WB, Harrigan GG, Kell DB. Metabolomics by numbers: acquiring and understanding global metabolite data. *Trends Biotechnol* 2004; 22: 245–252
 - 24 Want EJ, Wilson ID, Gika H, Theodoridis G, Plumb RS, Shockcor J, Holmes E, Nicholson JK. Global metabolic profiling procedures for urine using UPLC-MS. *Nat Protoc* 2010; 5: 1005–1018
 - 25 Fiehn O. Metabolomics – the link between genotypes and phenotypes. *Plant Mol Biol* 2002; 48: 155–171
 - 26 Lao YM, Jiang JG, Yan L. Application of metabonomic analytical techniques in the modernization and toxicology research of traditional Chinese medicine. *Br J Pharmacol* 2009; 157: 1128–1141
 - 27 Zhao J. Publishing Chinese medicine knowledge as linked data on the web. *Chin Med* 2010; 5: 27
 - 28 Beckonert O, Coen M, Keun HC, Wang Y, Ebbels TM, Holmes E, Lindon JC, Nicholson JK. High-resolution magic-angle-spinning NMR spectroscopy for metabolic profiling of intact tissues. *Nat Protoc* 2010; 5: 1019–1032
 - 29 Sobolev AP, Testone G, Santoro F, Nicolodi C, Iannelli MA, Amato ME, Ianniello A, Brosio E, Giannino D, Mannina L. Quality traits of conventional and transgenic lettuce (*Lactuca sativa* L.) at harvesting by NMR metabolic profiling. *J Agric Food Chem* 2010; 58: 6928–6936
 - 30 Straadt IK, Young JF, Bross P, Gregersen N, Oksbjerg N, Theil PK, Bertram HC. NMR-based metabonomic investigation of heat stress in myotubes reveals a time-dependent change in the metabolites. *J Agric Food Chem* 2010; 58: 6376–6386
 - 31 Straadt IK, Young JF, Petersen BO, Duus JØ, Gregersen N, Bross P, Oksbjerg N, Bertram HC. Metabolic profiling of heat or anoxic stress in mouse C2C12 myotubes using multinuclear magnetic resonance spectroscopy. *Metabolism* 2010; 59: 814–823
 - 32 Kamleh MA, Dow JA, Watson DG. Applications of mass spectrometry in metabolomic studies of animal model and invertebrate systems. *Brief Funct Genomic Proteomic* 2009; 8: 28–48
 - 33 Moolenaar SH, Engelke UF, Wevers RA. Proton nuclear magnetic resonance spectroscopy of body fluids in the field of inborn errors of metabolism. *Ann Clin Biochem* 2003; 40: 16–24
 - 34 Field D, Sansone SA, Collis A, Booth T, Dukes P, Gregurick SK, Kennedy K, Kolar P, Kolker E, Maxon M, Millard S, Mugabushaka AM, Perrin N, Remacle JE, Remington K, Rocca-Serra P, Taylor CF, Thorley M, Tiwari B, Wilbanks J. Megascience. 'Omics data sharing. *Science* 2009; 326: 234–236
 - 35 Fiehn O. Extending the breadth of metabolite profiling by gas chromatography coupled to mass spectrometry. *Trends Anal Chem* 2008; 27: 261–269
 - 36 Tolstikov VV, Fiehn O, Tanaka N. Application of liquid chromatography-mass spectrometry analysis in metabolomics: reversed-phase monolithic capillary chromatography and hydrophilic chromatography coupled to electrospray ionization-mass spectrometry. *Methods Mol Biol* 2007; 358: 141–155
 - 37 Bollard ME, Stanley EG, Lindon JC, Nicholson JK, Holmes E. NMR-based metabonomic approaches for evaluating physiological influences on biofluid composition. *NMR Biomed* 2005; 18: 143–162
 - 38 Kell DB. Metabolomics and systems biology: making sense of the soup. *Curr Opin Microbiol* 2004; 7: 296–307
 - 39 Sajda P. Machine learning for detection and diagnosis of disease. *Annu Rev Biomed Eng* 2006; 8: 537–565
 - 40 Shin H, Markey MK. A machine learning perspective on the development of clinical decision support systems utilizing mass spectra of blood samples. *J Biomed Inform* 2006; 39: 227–248
 - 41 Patterson AD, Li H, Eichler GS, Krausz KW, Weinstein JN, Fornace Jr AJ, Gonzalez FJ, Idle JR. UPLC-ESI-TOFMS-based metabolomics and gene expression dynamics inspector self-organizing metabolomic maps as tools for understanding the cellular response to ionizing radiation. *Anal Chem* 2008; 80: 665–674
 - 42 Clayton TA, Lindon JC, Cloarec O, Antti H, Charuel C, Hanton G, Provost JP, Le Net JL, Baker D, Walley RJ, Everett JR, Nicholson JK. Pharmacometabonomic phenotyping and personalized drug treatment. *Nature* 2006; 440: 1073–1077
 - 43 Roessner U, Bowne J. What is metabolomics all about? *Biotechniques* 2009; 46: 363–365
 - 44 Rosenson RS. New technologies personalize diagnostics and therapeutics. *Curr Atheroscler Rep* 2010; 12: 184–186
 - 45 McClay JL, Adkins DE, Isern NG, O'Connell TM, Wooten JB, Zedler BK, Dasika MS, Webb BT, Webb-Robertson BJ, Pounds JG, Murrelle EL, Leppert MF, van den Oord EJ. (1)H nuclear magnetic resonance metabolomics analysis identifies novel urinary biomarkers for lung function. *J Proteome Res* 2010; 9: 3083–3090
 - 46 Wang X, Lv H, Sun H, Liu L, Yang B, Sun W, Wang P, Zhou D, Zhao L, Dou S, Zhang G, Cao H. Metabolic urinary profiling of alcohol hepatotoxicity and intervention effects of Yin Chen Hao Tang in rats using ultra-performance liquid chromatography/electrospray ionization quadrupole time-of-flight mass spectrometry. *J Pharm Biomed Anal* 2008; 48: 1161–1168
 - 47 Wang P, Sun H, Lv H, Sun W, Yuan Y, Han Y, Wang D, Zhang A, Wang X. Thyroxine and reserpine-induced changes in metabolic profiles of rat urine and the therapeutic effect of Liu Wei Di Huang Wan detected by UPLC-HDMS. *J Pharm Biomed Anal* 2010; 53: 631–645
 - 48 Xie B, Gong T, Gao R, Liu J, Zuo J, Wang X, Zhang Z. Development of rat urinary HPLC-UV profiling for metabonomic study on Liuwei Dihuang pills. *J Pharm Biomed Anal* 2009; 49: 492–497
 - 49 Zhao X, Zhang Y, Meng X, Yin P, Deng C, Chen J, Wang Z, Xu G. Effect of a traditional Chinese medicine preparation Xindi soft capsule on rat model of acute blood stasis: a urinary metabolomics study based on liquid chromatography-mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci* 2008; 873: 151–158
 - 50 Lv Y, Liu X, Yan S, Liang X, Yang Y, Dai W, Zhang W. Metabolomic study of myocardial ischemia and intervention effects of Compound Danshen Tablets in rats using ultra-performance liquid chromatography/quadrupole time-of-flight mass spectrometry. *J Pharm Biomed Anal* 2010; 52: 129–135
 - 51 Wang M, Rang W, Zhang Q, Huo C, Ma Z, Wang Y, Yan X, Gao Y. NMR-spectroscopy-based metabonomic approach to analysis of Siwutang, a novel prescription, treated blood deficiency in mice. *Zhongguo Zhong Yao Za Zhi* 2010; 35: 630–634
 - 52 Vlietinck A, Pieters L, Apers S. Legal requirements for the quality of herbal substances and herbal preparations for the manufacturing of herbal medicinal products in the European union. *Planta Med* 2009; 75: 683–688
 - 53 Chen M, Ni Y, Duan H, Qiu Y, Guo C, Jiao Y, Shi H, Su M, Jia W. Mass spectrometry-based metabolic profiling of rat urine associated with general toxicity induced by the multiglycoside of *Tripterygium wilfordii* Hook. f. *Chem Res Toxicol* 2008; 21: 288–294
 - 54 Liu Y, Huang R, Liu L, Peng J, Xiao B, Yang J, Miao Z, Huang H. Metabolomics study of urine from Sprague-Dawley rats exposed to Huang-yao-zi using (1)H NMR spectroscopy. *J Pharm Biomed Anal* 2010; 52: 136–141
 - 55 Xu G, Ma X, Zhang Q, Li B, Huang L, Yu R, Liu H. Study of metabonomics on pharmacological action appraisal *Rhizoma coptidis* in rats. *Zhongguo Zhong Yao Za Zhi* 2009; 34: 1845–1847
 - 56 Gu Y, Zhang Y, Shi X, Li X, Hong J, Chen J, Gu W, Lu X, Xu G, Ning G. Effect of traditional Chinese medicine berberine on type 2 diabetes based on comprehensive metabonomics. *Talanta* 2010; 81: 766–772
 - 57 Dai H, Xiao C, Liu H, Hao F, Tang H. Combined NMR and LC-DAD-MS analysis reveals comprehensive metabonomic variations for three phenotypic cultivars of *Salvia miltiorrhiza* Bunge. *J Proteome Res* 2010; 9: 1565–1578

- 58 Agnolet S, Jaroszewski JW, Verpoorte R, Staerk D. H NMR-based metabolomics combined with HPLC-PDA-MS- SPE-NMR for investigation of standardized *Ginkgo biloba* preparations. *Metabolomics* 2010; 6: 292–302
- 59 Zhang Q, Wang GJ, AJY, Wu D, Zhu LL, Ma B, Du Y. Application of GC/MS-based metabolomic profiling in studying the lipid-regulating effects of *Ginkgo biloba* extract on diet-induced hyperlipidemia in rats. *Acta Pharmacol Sin* 2009; 30: 1674–1687
- 60 Liang Q, Ni C, Xie M, Zhang Q, Zhang YX, Yan XZ, Yang MJ, Peng SQ, Zhang YZ. Nephrotoxicity study of *Aristolochia fangchi* in rats by metabolomics. *Zhong Xi Yi Jie He Xue Bao* 2009; 7: 746–752
- 61 Chen M, Su M, Zhao L, Jiang J, Liu P, Cheng J, Lai Y, Liu Y, Jia W. Metabolomic study of aristolochic acid-induced nephrotoxicity in rats. *J Proteome Res* 2006; 5: 995–1002
- 62 Jacob SS, Smith NW, Legido-Quigley C. Assessment of Chinese medicinal herb metabolite profiles by UPLC-MS- based methodology for the detection of aristolochic acids. *J Sep Sci* 2007; 30: 1200–1206
- 63 Yan S, Wu B, Lin Z, Jin H, Huang J, Yang Y, Zhang X, Shen Z, Zhang W. Metabolomic characterization of aging and investigation on the anti-aging effects of total flavones of *Epimedium*. *Mol Biosyst* 2009; 5: 1204–1213
- 64 Huang JH, Shen ZY, Wu B. Effect and mechanism of *Epimedium* flavonoids for aging retardation from viewpoint of transcriptomics and metabolomics. *Zhongguo Zhong Xi Yi Jie He Za Zhi* 2008; 28: 47–50
- 65 Wu B, Yan S, Lin Z, Wang Q, Yang Y, Yang G, Shen Z, Zhang W. Metabolomic study on ageing: NMR-based investigation into rat urinary metabolites and the effect of the total flavone of *Epimedium*. *Mol Biosyst* 2008; 4: 855–861
- 66 Li F, Lu X, Liu H, Liu M, Xiong Z. A pharmaco-metabolomic study on the therapeutic basis and metabolic effects of *Epimedium brevicornum* Maxim. on hydrocortisone-induced rat using UPLC-MS. *Biomed Chromatogr* 2007; 21: 397–405
- 67 Sun B, Li L, Wu S, Zhang Q, Li H, Chen H, Li F, Dong F, Yan X. Metabolomic analysis of biofluids from rats treated with Aconitum alkaloids using nuclear magnetic resonance and gas chromatography/time-of-flight mass spectrometry. *Anal Biochem* 2009; 395: 125–133
- 68 Sun B, Wu S, Li L, Li H, Zhang Q, Chen H, Li F, Dong F, Yan X. A metabolomic analysis of the toxicity of Aconitum sp. alkaloids in rats using gas chromatography/mass spectrometry. *Rapid Commun Mass Spectrom* 2009; 23: 1221–1228
- 69 Li L, Sun B, Zhang Q, Fang J, Ma K, Li Y, Chen H, Dong F, Gao Y, Li F, Yan X. Metabolomic study on the toxicity of Hei-Shun-Pian, the processed lateral root of *Aconitum carmichaelii* Debx. (Ranunculaceae). *J Ethnopharmacol* 2008; 116: 561–568
- 70 Liu NQ, Cao M, Frdrich M, Choi YH, Verpoorte R, van der Kooy F. Metabolomic investigation of the ethnopharmacological use of *Artemisia afra* with NMR spectroscopy and multivariate data analysis. *J Ethnopharmacol* 2010; 128: 230–235
- 71 Wang H, Ma C, Ma L, Du Z, Wang H, Ye H, Li G, Liu B, Xu G. Secondary metabolic profiling and artemisinin biosynthesis of two genotypes of *Artemisia annua*. *Planta Med* 2009; 75: 1625–1633
- 72 Xie G, Plumb R, Su M, Xu Z, Zhao A, Qiu M, Long X, Liu Z, Jia W. Ultra-performance LC/TOF MS analysis of medicinal *Panax* herbs for metabolomic research. *J Sep Sci* 2008; 31: 1015–1026
- 73 Kang J, Lee S, Kang S, Kwon HN, Park JH, Kwon SW, Park S. NMR-based metabolomics approach for the differentiation of ginseng (*Panax ginseng*) roots from different origins. *Arch Pharm Res* 2008; 31: 330–336
- 74 Wang Y, Wang J, Yao M, Zhao X, Fritsche J, Schmitt-Kopplin P, Cai Z, Wan D, Lu X, Yang S, Gu J, Hring HU, Schleicher ED, Lehmann R, Xu G. Metabolomics study on the effects of the ginsenoside Rg3 in a beta- cyclodextrin-based formulation on tumor-bearing rats by a fully automatic hydrophilic interaction/reversed-phase column-switching HPLC-ESI-MS approach. *Anal Chem* 2008; 80: 4680–4688
- 75 Liang Y, Kang A, Xie T, Zheng X, Dai C, Hao H, AJ, Sheng L, Xie L, Wang GJ. Influence of segmental and selected ion monitoring on quantitation of multi-component using high-pressure liquid chromatography-quadrupole mass spectrometry: Simultaneous detection of 16 saponins in rat plasma as a case. *J Chromatogr A* 2010; 1217: 4501–4506
- 76 Law WS, Huang PY, Ong ES, Ong CN, Li SF, Pasikanti KK, Chan EC. Metabolomics investigation of human urine after ingestion of green tea with gas chromatography/mass spectrometry, liquid chromatography/mass spectrometry and (1)H NMR spectroscopy. *Rapid Commun Mass Spectrom* 2008; 22: 2436–2446
- 77 Van Dorsten FA, Daykin CA, Mulder TP, Van Duynhoven JP. Metabolomics approach to determine metabolic differences between green tea and black tea consumption. *J Agric Food Chem* 2006; 54: 6929–6938
- 78 Yi LZ, Yuan DL, Liang YZ, Xie PS, Zhao Y. Fingerprinting alterations of secondary metabolites of tangerine peels during growth by HPLC-DAD and chemometric methods. *Anal Chim Acta* 2009; 649: 43–51
- 79 Giri S, Idle JR, Chen C, Zabriskie TM, Krausz KW, Gonzalez FJ. A metabolomic approach to the metabolism of the areca nut alkaloids arecoline and arecaidine in the mouse. *Chem Res Toxicol* 2006; 19: 818–827
- 80 Adams M, Mahringer A, Kunert O, Fricker G, Efferth T, Bauer R. Cytotoxicity and p-glycoprotein modulating effects of quinolones and indoloquinazolines from the Chinese herb *Evodia rutaecarpa*. *Planta Med* 2007; 73: 1554–1557
- 81 Zhang Q, Xu G, Wu L, Ma X, Zeng Z, Huang L, Yu R, Liu H. Preliminary study of metabolomics on aqueous extract of *Evodia rutaecarpa* in sprague-dawley rats. *Zhongguo Zhong Yao Za Zhi* 2010; 35: 99–102
- 82 Xiao C, Dai H, Liu H, Wang Y, Tang H. Revealing the metabolomic variation of rosemary extracts using 1H NMR spectroscopy and multivariate data analysis. *J Agric Food Chem* 2008; 56: 10142–10153
- 83 Wei L, Liao P, Wu H, Li X, Pei F, Li W, Wu Y. Toxicological effects of cinnabar in rats by NMR-based metabolic profiling of urine and serum. *Toxicol Appl Pharmacol* 2008; 227: 417–429
- 84 Wei L, Liao P, Wu H, Li X, Pei F, Li W, Wu Y. Metabolic profiling studies on the toxicological effects of realgar in rats by (1)H NMR spectroscopy. *Toxicol Appl Pharmacol* 2009; 234: 314–325
- 85 Liang F, Koya D. Acupuncture: is it effective for treatment of insulin resistance? *Diabetes Obes Metab* 2010; 12: 555–569
- 86 Wu Q, Zhang Q, Sun B, Yan X, Tang Y, Qiao X, Chen Q, Yu S, Liang F. (1)H NMR-based metabolomic study on the metabolic changes in the plasma of patients with functional dyspepsia and the effect of acupuncture. *J Pharm Biomed Anal* 2010; 51: 698–704
- 87 Qiu Y, Chen M, Su M, Xie G, Li X, Zhou M, Zhao A, Jiang J, Jia W. Metabolic profiling reveals therapeutic effects of Herba Cistanches in an animal model of hydrocortisone-induced “kidney-deficiency syndrome”. *Chin Med* 2008; 3: 3
- 88 Yan B, AJ, Hao H, Wang G, Zhu X, Zha W, Liu L, Guan E, Zhang Y, Gu S, Huang Q, Zheng Y. Metabolomic phenotype and identification of “heart blood stasis obstruction pattern” and “qi and yin deficiency pattern” of myocardial ischemia rat models. *Sci China C Life Sci* 2009; 52: 1081–1090
- 89 Chen M, Zhao L, Jia W. Metabolomic study on the biochemical profiles of a hydrocortisone-induced animal model. *J Proteome Res* 2005; 4: 2391–2396
- 90 Luo HG, Ding J, Yue GX, Chen JX. Metabolomic study of syndrome of liver qi stagnation and spleen deficiency in rats. *Zhong Xi Yi Jie He Xue Bao* 2007; 5: 307–313