REVIEW

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Recent highlights of metabolomics for Traditional Chinese Medicine

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Systems biology is an emerging science of the 21st century and has developed in recent years from a technology-driven enterprise to a new strategic tool in life sciences as well as its method and design resemble those of traditional Chinese medicine (TCM), a holistic approach to health that attempts to bring the body, mind and spirit into harmony. The technology platforms of systems biology, especially metabolomics could provide useful tools for facilitating drug discovery and development of TCM. Metabolomes of medicinal herbal medicine are particularly a valuable natural resource for the evidence-based TCM. Metabolomics adopts a 'top-down' strategy to reflect the function of organisms from terminal symptoms of metabolic network and understand metabolic changes of a complete system caused by interventions in holistic context. Its property consists with the holistic thinking of TCM, may beneficially provide an opportunity to scientifically express the meaning of evidence-based Chinese medicine, will greatly benefit both drug discovery and development for TCM research. Some successful metabolomic applications in important TCM field related to drug discovery and development from natural sources aims at raising the potential of metabolomics in reducing the gap between TCM and modern drug discovery demand, highlight the key role of biomarkers for drug discovery and development of traditional oriental medicine.

1. Introduction

Traditional Chinese Medicine (TCM) is regarded as intellectual experience that humans have been accumulating since time immemorial for the prevention and treatment of diseases along with skillful application of natural medicines. As an ancient medical system, it has been used in China and other Asian countries for thousands of years (Normile 2003). It is based on a holistic view of treatment which is believed to cure human diseases through establishment of equilibrium in the different elements of human life, the body, the mind, the intellect and the soul. Through persistent attempts and practice for generations, people have accumulated profound experience in disease prevention, diagnosis, and treatment, and formed a whole theoretical system of medicine and therapy. As one of the most important parts in complementary and alternative medicine, TCM should play the key role in the formation of personalized medicine. It has developed its own coherent theories with regard to aetiology, diagnosis, and treatment of disease and has been accepted by the academic community and patients as superior and unique value in the treatment of multiple organ system disorders, in particular chronic diseases and metabolic syndromes (Greensfelder 2000; Bian et al. 2011). Also, it has accrued a myriad of valuable clinical observations, some of which have provided the basis for some successful conventional medicines. Artemisinin (Qinghaosu), for example, is an extract that is prepared from the Qinghao plant (*Artemisia annua*) and has been used by TCM practitioners for 1500 years; it is now a very promising antimalarial drug (Klayman 1985). As TCM is attractive as a pool chemical compounds and natural products for medicinal use, increasing attention is being paid to the scientific evaluation of TCM. It is characterized as holism (concept of global and system thinking) with emphasis on the integrity of the human body and the close relationship between human and its social and natural environment. There are abundant systematic concepts of health and the wisdom of life-cultivation in TCM, not only including the connotation of modern health, but also with many of its own characteristics. The unique features of TCM for maintaining health consist of prevention, emotion regulation, obeying nature. TCM focuses on health maintenance and in the treatment of disease, emphasizes on enhancing the body's resistance to diseases and showes great advantages in early intervention, personalized medicine and combination therapies *etc.* (Xue 2003; Song et al. 2007). In contrast to the reductionist approach of Western medicine based on modern anatomy, physiology, pathology, pharmacology as well as cell and molecular biology, TCM uses a unique system and individualised and holistic approach to describe health and disease, based on the philosophy of Yin-Yang balance and an emphasis on global functions (Stone 2008).

China's extensive experience in the use of TCMs in disease therapy indicates that TCM preparations are effective, with few or no side-effects. There are more than 10000 traditional medicines in use for disease therapy in China. Some of their therapeutic effects in disease have been confirmed by recent clinical studies.

A large number of compounds have been isolated from TCMs and most of these resources have not yet been characterized for pharmacological research program aimed at the development of new drugs (Zhang et al. 2011). There are several obstacles for lead compound discovery from TCM. The key issue is that suffers from insufficient modern scientific research, that not only lowering the position of TCM also restricting the development of TCM in the abroad. One of the reasons for such failure is that the methodology used in the TCM research follows basically the path of partitioned reductive analysis, which is unable to capture practically the characteristics of TCM scientific system, such as the holistic and dynamic nature of diseases, and the interaction among various biological components. Fortunately, the advances in molecular medicine and biotechnology provide new tools to alternative approaches to the understanding of TCM action and mechanism. Systems biology, a new science of the 21st century, thus becomes practically available and resembles TCM in many aspects such as study method and design (van der Greef J et al. 2007). The introduction of the concept of systems biology, enabling the study of living systems from a holistic perspective based on the profiling of a multitude of biochemical components, opens up a unique and novel opportunity to reinvestigate natural products (Li et al. 2009; Dharia et al. 2010). Systems biology has developed in recent years from a technology-driven enterprise to a new strategic tool in life sciences, particularly for innovative drug discovery and drug development. Combining the ultimate in systems phenotyping with in-depth investigations of biomolecular mechanisms will enable a revolution in our understanding of disease pathology and will advance translational medicine, combination therapies, integrative medicine, and personalized medicine. Adoption of the systems biology approach would help a lot in exploring the the modernization of Chinese herbal medicine. Its technological platforms, such as genomics, proteomics and metabolomics, provide powerful tools for the essence and the function of herbal compound recipe (Nicholson et al. 2008). Compared with other molecular biological methods, such as genomics and proteomics, metabolomics method is more direct and more concise, especially for providing an effective method for the study of TCM therapy in preventing and treating diseases (Blow 2008). As a systemic approach, metabolomics adopts a 'top-down' strategy to reflect the function of organisms from terminal symptoms of metabolic network and understand metabolic changes of a complete system caused by interventions in holistic context (Ji et al. 2010; Wang et al. 2011; Prestele et al. 2010). Metabolomics offers a practical approach to measuring the metabolic end points that link directly to whole system activity and metabolic profiles are determined by both host genetic and environmental factors (Sreekumar et al. 2009). Its property is in concert with the the global efficacy of TCM, suggesting that metabolomics has the potential to impact our understanding of the theory behind the evidence-based Chinese medicine (Zhang et al. 2010a). Marker metabolites can be therapeutic targets as well (Arakaki et al. 2008; Holmes et al. 2008). It opens up the possibility of studying the effect of complex mixtures, such as those used in TCM, in complex biological systems; abridging it with molecular pharmacology (Wang et al. 2005; Zhang et al. 2012). This approach is considered to have the potential to revolutionize TCM research and to advance the development of scientific based herbal medicine. The value of metabolomics in the understanding of TCM has been recognized in basic and clinical studies of TCM, and would be further explored along with the increasing worldwide distribution of TCM.

Metabolomics, as "the quantitative measurement of the dynamic multiparametric metabolic response of living systems to pathophysiological stimuli or genetic modification", is a new '-omics' science in post-gene time. Its has been applied in in many fields such as responses to environmental stress, toxicology, nutrition, studying global effects of genetic manipulation, cancer, comparing different growth stages, diabetes, gut functional ecology, disease diagnosis, drug metabolism and natural product discovery. Metabolomics is a relatively new field of 'omics' technology that is primarily concerned with the global or system-wide characterization of small molecule metabolites using technologies such as nuclear magnetic resonance, liquid chromatography and/or mass spectrometry. Its unique focus on small molecules and the physiological effects of small molecules aligns the field of metabolomics very closely with the aims and interests of many researchers in the pharmaceutical industry. Because of its conceptual and technical overlap with many aspects of pharmaceutical research, metabolomics is now finding applications that span almost the full length of the drug discovery and development pipeline, from lead compound discovery to post-approval drug surveillance. One area of considerable interest in the field of metabolomics is that of drug discovery and development, whereby metabolic profiling of biofluids and tissues can provide a panoramic view of abundance changes in endogenous metabolites in monitoring cellular responses to perturbations of drug treatments, has played increasingly important roles in pharmaceutical research and development. Precise identification and accurate quantification of metabolites facilitate downstream pathway and network analysis for the discovery of clinically accessible and minimally invasive biomarkers of drug efficacy. Metabolomics holds the promise of a comprehensive, non-invasive analysis of metabolic biomarkers that could detect early-stage disease, identify residual disease post-surgery, and help to monitor treatment response and detect early treatment toxicity (Manna et al. 2010; Lindon et al. 2004). Metabolomics apporoaches as pillars of the potential bridge between Chinese and Western medicine, may beneficially influence and provide an opportunity to explain the theoretical meaning of evidence-based chinese medicine. In many fields of medicine there is a growing interest in characterizing diseases at molecular level with a view to developing an individually tailored therapeutic approach (Shaham et al. 2010). Metabolomics is a novel area that promises to contribute significantly to the characterization of various disease phenotypes and to the identification of personal metabolic features that can predict response to therapies. The metabolomic approach enables a comprehensive overview of the metabolites, leading to the characterization of the metabolic fingerprint of a given sample. These metabolic fingerprints can then be used to distinguish between different disease phenotypes and to predict a drug's effectiveness and/or toxicity and has appeal for the study of diseases. In the holistic and systemic context, metabolomics have a convergence with TCM, which could overcome the one-sidedness and singleness of TCM and avoid the complication and tedious processes (Kell et al. 2006). Chinese medicine has a wealth of experience and metabolomics has a substantial research potential, the integration of the two aspects will bring a great enhancement of our knowledge of disease (Gallagher et al. 2009; Lee et al. 2009). This review explores some of the most interesting or successful applications of metabolomics as they relate to TCM research and development. Specific examples are given that show how metabolomics can be used to facilitate lead compound discovery from TCM, to improve biomarker identification and to monitor drug metabolism and post-approval drug monitoring. These examples show that metabolomics potentially offer drug researchers and drug regulators an effective, inexpensive route to addressing many of the riskier or more expensive issues associated with the discovery, development and monitoring of TCM.

2. Superiorities of metabolomics

Metabolomics enables the parallel assessment of the levels of a broad range of metabolites and has been shown to have a great impact on investigation of physiological status, diagnosing diseases, measuring the response to treatment, discovering biomarkers, identifying perturbed pathways due to disease or treatment, functional genomics. Technological developments are the driving force behind advances in scientific knowledge. A variety of analytical metabolic profiling tools are used routinely, are also currently under development, and include proton nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry with a prior online separation step such as high-performance liquid chromatography (HPLC), ultra-performance liquid chromatography (UPLC), or gas chromatography (Shroff et al. 2009; Godzien et al. 2011). 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 very efficient in biochemical investigations, as the analysis is nondestructive, nonselective, cost effective, and relatively sensitive. The high selectivity of mass spectrometry with low-detection limits makes mass spectrometry an ideal tool for metabolomic applications. The application of either HPLC or UPLC online with MS within the metabonomic research has become increasingly popular, particularly when combined with exact mass measurements, which allow elemental compositions to be determined (Cooks et al. 2006; Tolstikov et al. 2007). Data generated by these analytical techniques are often combined with multivariate data analysis, i.e., pattern recognition, for respectively generating and interpreting the metabolic profiles of the investigated samples. The most commonly used biological samples for metabolomics studies are urine, blood plasma or serum. Because of its characteristics and simple non-invasive methods of collection, urine is particularly suited for metabolomic analysis even in small babies. The use of non-invasive techniques is an essential requirement, the accessibility of urine and plasma clearly makes these samples ideal for large-scale research.

Considerable interest in the field of metabolomics is that of personalised healthcare, 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 of a pre-dose metabolic profile (Clayton et al. 2006). A deeper understanding of global perturbations in biochemical pathways in complex diseases could provide valuable insights into the mechanisms of disease. 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. Biomarkers are objectively measured characteristics that are indicators of normal biological processes, pathogenic processes, or responses to therapeutic interventions (Griffiths et al. 2010). It includes not only small molecules that are the products and intermediates of metabolism but also carbohydrates, peptides and lipids, many of which may also derive from diet or may be altered in disease. The identification of biomarkers is of growing interest in the fields of drug discovery, biological monitoring and the study of disease disorders (Kotłowska et al. 2011). 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 (Fiehn et al. 2002). In the future, metabolomics may enable us to develop new approaches in medicine that will be predictive, preventative, and personalized. It will be a strategy or major opportunity for the discovery and development of new therapeutic as well as preventative drugs, achieving the ability to determine a probabilistic health history for each individual.

3. Metabonomics in drug R&D

Historically, TCM has been a rich source of lead molecules in drug discovery, based on their capability to create unique and diverse chemical structures. However, it is also true that the vast number of metabolites typically present in TCMs and their huge dynamic range results in the loss of many possibly bioactive natural compounds, becoming an inextricable obstacle for drug development (Yuliana et al. 2011). Recently, new strategies which favour a holistic approach as opposed to the traditional reductionist methods used previously, have been introduced with the purpose of overcoming the bottlenecks in TCM research. This approach is based on the application of new technologies, allows a systematic study of a complex mixture such as a phytochemical preparation, which can be linked to observations obtained through biological testing systems without the need for isolating active principles. This may put drug discovery from natural products back in the limelight again (Walgren et al. 2004; Bluestone et al. 2010). Metabonomics involves the determination of multiple metabolites simultaneously in biofluids, tissues and tissue extracts and brings a different and complementary perspective to biomedical research and therapeutic development. Metabolites are measurable, directly responsible for health, and changeable through intervention. By definition, the concentrations of metabolites are the direct reflection of metabolism, and by measuring changes in metabolite concentrations, the full range of biochemical effects induced by a therapeutic intervention can be determined. It is useful in virtually all aspects of biomedical research, and it is likely to evolve into an essential component of the drug discovery and development process (Lindon et al. 2007; Smith et al. 2010; Lee et al. 2010).

In recent years, quantitative metabolomics has played increasingly important roles in pharmaceutical research and development. Metabolic profiling of biofluids and tissues can provide a panoramic view of abundance changes in endogenous metabolites to complement transcriptomics and proteomics in monitoring cellular responses to perturbations such as diseases and drug treatments (Xu et al. 2009). Precise identification and accurate quantification of metabolites facilitate downstream pathway and network analysis using software tools for the discovery of clinically accessible and minimally invasive biomarkers of drug efficacy. Metabolite abundance profiles are also indicative of biochemical phenotypes, which can be used to identify novel quantitative trait loci in genome-wide association studies. Metabolomics is a relatively new field of 'omics' technology that is primarily concerned with the global or system-wide characterization of small molecule metabolites using technologies such as nuclear magnetic resonance, liquid chromatography and/or mass spectrometry (Watkins et al. 2002). Its unique focus on small molecules and the physiological effects of small molecules aligns the field of metabolomics very closely with the aims and interests of many researchers in TCM. Because of its conceptual and technical overlap with many aspects of TCM research, metabolomics is now finding applications that span almost the full length of the drug discovery and development pipeline, from lead compound discovery to post-approval drug surveillance. Developing a new drug is a tedious and expensive undertaking (Koul et al. 2011; de Bono et al. 2010; Mazzone et al. 2008; Kaiser et al. 2010). Metabolomics technologies facilitate the systematic characterisation of a drug target's physiology, thereby helping to reduce the typically high attrition rates in discovery projects, and improving the overall efficiency of TCM research processes. So, in complex disor-

ders like cancer or neurodegenerative diseases, which are rooted in relatively subtle and multimodal dysfunction of important physiologic pathways, drug discovery programs based on the concept of high affinity/high specificity compounds ('one-target, one-disease'), which still dominate the pharmaceutical industry increasingly turn out to be unsuccessful (Fischer et al. 2005). Despite improvements in rational drug design and high throughput screening methods, the number of novel, single-target drugs fell much behind expectations during the past decade and the treatment of "complex diseases" remains a most pressing medical need (Davies et al. 2009; Evason et al. 2005; Schrattenholz et al. 2008).

Metabolomics technologies may also help to identify drug-drug interactions that influence either the disease state or the proposed therapy. Efficacy biomarkers are used to assess whether target modulation has occurred. They are used for the characterization of disease models and to assess the effects and mechanism of action of lead candidates in animal models. Biomarkers are used to screen compounds in pre-clinical studies for target organ toxicities as well as later on in development during clinical trials (Wishart et al. 2008). Complementary approaches such as proteomics and genomics can be used in conjunction with metabolomics throughout the drug development process to create more of a unified, systems biology approach. Metabolomics is the untargeted analysis of metabolic composition in a biological sample, and is principally aimed at biomarker discovery. The frequent use of noninvasive biofluid analysis in metabonomics is suited to the clinic and facilitates dynamic monitoring. Analytical protocols for metabolic biomarkers are potentially robust because a metabolite is the same chemical entity irrespective of its origin, facilitating 'bench-to-bedside' translational research. Metabonomics can make an impact at several points in the drug-development process: target identification; lead discovery and optimization; preclinical efficacy and safety assessment; mode-of-action and mechanistic toxicology; patient stratification; and clinical pharmacological monitoring. The future goals for metabonomics are the validation of existing biomarkers, in terms of mechanism and translation to man, together with a focus on characterizing the individual ('personalized healthcare') (Fitzgerald et al. 2010; Fidock et al. 2010). Metabolomics, including both targeted and global metabolite profiling strategies, is fast becoming the approach of choice across a broad range of sciences including systems biology, drug discovery, molecular and cell biology, and other medical and agricultural sciences. New analytical and bioinformatics technologies and techniques are continually being created or optimized, significantly increasing the crossdisciplinary capabilities of this new biology. The metabolomes of medicinal plants are particularly a valuable natural resource for the evidence-based TCM. Comparative metabolomics platforms are evolving into novel technologies for monitoring disease development, drug metabolism, and chemical toxicology (Shyur et al. 2008; Keun et al. 2007). An efficient multidisciplinary marriage of these emerging metabolomics techniques with biotechnology will greatly benefit both drug discovery and development of TCM research.

4. Metabolomic dissection of Chinese medicine formula

The complexity of medicine suggests that treatment protocols should be carefully designed, and the construction of a prescription is an art in fighting disease. Increasing evidence demonstrates that, in treating illnesses, treatment regimens containing multiple herbs with distinct but related mechanisms can usually amplify the therapeutic efficacies of each agent, leading to maximal therapeutic efficacy with minimal adverse effects (Wang et al. 2008a; Zhang et al. 2009a). Interestingly, combination therapy has been advocated over thousands of years by prescriptions called formula in TCM. Typically, formula consist of several types of medicinal herbs or minerals, in which one represents the principal component, and others serve as adjuvant ones to assist the effects or facilitate the delivery of the principal component. It is believed that multiple components could hit multiple targets and exert synergistic therapeutic efficacies. The therapeutical effect of TCM usually attributed to the synergism mechanism among multiple herbs and constituents, which was named as 'formula compatibility' of TCM (Park et al. 2002). However, the precise mechanisms of formula remain to be addressed by using molecular approaches, thus hampering the modernization of TCM. Studies on formula compatibility/synergism principles are essential, and metabolomics is considered as one of the available and ideal means in this process. The rapid development of metabolomics, especially the advances in the high-throughput and comprehensive research technologies provide new strategies in the analysis of active components of the formula. The integrative approach of metabolomics is in line with the holistic concept and practices of TCM. The promise of metabolomics, a new "omics" technique, to validate effect of Chinese medicines and the compatibility of Chinese formulas has been appreciated and performed.

Wang et al. (2008b) 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 Fructus* and *Radix et Rhizoma Rhei* for treatment of jaundice and liver disorders in China. The greatest difference in metabolic profiling was observed from alcohol-treated rats compared with the control and YCHT-treated rats. The positive ions m/z 664.3126 was elevated in urine of alcohol-treated rats, whereas, ions m/z 155.3547 and 708.2932 were at a lower concentration compared with that in urine of control rats, however, these ions did not indicate a statistical difference between control rats and YCHT-treated rats. The ion m/z 664.3126 was found to correspond to ceramide, providing further support for an involvement of the sphingomyelin signaling pathway in alcohol hepatotoxicity and the intervention effects of YCHT.

With the increasing pace of life, many young people begin to suffer from Kidney yin deficiency syndrome, a common disease in China, especially for old people. Liuwei Dihuang Wan (LW), one of the most important Chinese patent medicines consists of six herbs including *Radix Rehmanniae preparata, Fructus Macrocarpii, Rhizoma Dioscoreae oppositae, Poria, Rhizoma Alismatis* and *Cortex Moutan Radicis*, has been widely used clinically for treatment of diseases with the sign of kidney yin insufficiency. More recently, Wang et al. (2010a) adopted ultra-performance liquid chromatography-mass spectrometry (UPLC-MS) to investigate the metabolic profiling of rats with kidney yin deficiency induced by thyroxine and reserpine. It could been shown that the changes in metabolic profiling were restored to their baseline values after treatment with LW according to the PCA score plots, indicating 20 ions as "differentiating metabolites". There were different phenotypes of metabolites based on HPLC-UV urinary profiling after administration of LW, and those could be conveniently discriminated by PCA. In addition, the results also indicated that LW could restore the metabolite network that disturbed by inflammation, which would be a proof of therapeutic efficacy of LW to inflammation by metabolomics study (Xie et al. 2009).

Urinary metabolomics based UPLC-MS was used to evaluate the efficacy and mechanism of Xindi soft capsule, consisting of sea buckthorn flavonoids (e.g., quercetin, kaempferol and isorhamnetin), which is a TCM preparation to blood stasis (Zhao et al. 2008). With pattern recognition analysis (PCA and PLS-DA) of urinary metabolites, a clear separation of acute blood stasis

model group and healthy control group was achieved, the dose groups were located between acute blood stasis model group and healthy control group showing a tendency of recovering to healthy control group, high dose and middle dose were more effective than low dose. Some significantly changed metabolites like cholic acid, phenylalanine and kynurenic acid have been found and identified and used to explain the mechanism. It revealed that the metabolomics method is a valuable tool in the action mechanism of Chinese TCM preparation.

Compound Danshen Tablets, an 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) (Lv et al. 2010). Based on the symptoms and characteristics of patients and guided by the theories of TCM, formula are designed to contain a combination of different kinds of plants or minerals to improve clinical efficacy.

Siwutang, a classic TCM formula, treated cyclophosphamide induced "blood deficiency" model by NMR-based-metabolomics method (Wang et al. 2010b). When mice were dosed with Siwutang for 7 days, cyclophosphamide caused "blood deficiency" model were reversed in PCA. It could been shown that Siwutang can improve therapeutical and pharmacological effects. Furthermore, the initiatives of metabolomics may pave a new way to explain the formula compatibility and contribute to the establishment of a new technique platform for evaluating the efficacy of TCM formula.

5. Metabolomic analysis of Chinese herbal medicine

Chinese herbal medicine (CHM) has long been used for disease prevention and therapy in China and are becoming increasingly important in the West (Vlietinck et al. 2009; Huang et al. 2011). 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, 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 (Chen et al. 2008). 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, mechanism associated with oxidative injury of hepatic mitochondria was investigated (Liu et al. 2010). HPLC-MS/MS-based metabolomics method was used to find the possible biomarker of *Rhizoma Coptidis* in rat urine. The result was consistent with pharmacological effects of *R. Coptidis*, such as antiinflammatory, anticentral nerve and energy metabolism inhibition (Xu et al. 2009).

Berberine might play a pivotal role in the treatment of type 2 diabetes through down-regulating the high level of free fatty acids. Comprehensive metabolomic measurements are potentially very useful for studying the mechanisms of action of traditional Chinese medicines (Gu et al. 2010). Combined NMR and LC-DAD-MS analysis reveals comprehensive metabolomic variations for three phenotypic cultivars of *Salvia miltiorrhiza Bunge* (Dai et al. 2010). NMR-based metabolomics was an attractive method for non-selective and comprehensive analysis of *Ginkgo* extracts, which are very complex mixtures prepared from raw leaf extracts by a series of extraction and prepurification steps (Agnolet et al. 2010). *Ginkgo biloba* leaves exert multi-directional lipid-lowering effects on the rat metabonome, including limitation of the absorption of cholesterol, inactivation of HMGCoA and favorable regulation of profiles of essential polyunsaturated fatty acid (Zhang et al. 2009b).

Recently, changes of metabolites in rat urine after treatment with *Aristolochia fangchi* decoction were studied by a metabolomic method (Liang et al. 2009). High-dose *Aristolochia fangchi* can induce nephrotoxicity and its seriousness is corresponding to the duration of administration. *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 (Chen et al. 2006). 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 (Jacob et al. 2007). A LC/MS metabolomics approach was applied to characterize the aging of rats, and the anti-aging 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 anti-aging effects of total flavones of *Epimedium* might be due to the intervention on lipid metabolism and its property of anti-oxidation (Yan et al. 2009). 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 (Huang et al. 2008). The total flavones of *Epimedium* administration can markedly influence the ageing process and shows anti-ageing effects, which might be due to the melioration of pyruyate metabolism and oxidative phosphorylation (Wu et al. 2008). 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 (Li et al. 2007).

Metabolic changes in Wistar rats caused by the *Aconitum* alkaloids aconitine, mesaconitine, and hypaconitine which are the main toxic components of 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 (Sun et al. 2009). A metabolomic investigation of intoxication with *Aconitum sp*. alkaloids was carried out. *Aconitum sp*. alkaloids 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 (Sun et al. 2009). The effect mechanism and potential biomarkers of the toxic effects 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 rat heart in a dose dependent manner (Li et al. 2008). *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. (2010) have used metabolomics to investigate the ethnopharmacological use of *Artemisia afra* with NMR spectroscopy and multivariate data analysis. The findings show that there are no *in vitro* activities and lists the identified metabolites causing the metabolic differences. 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 been shown that there were clear differences in terpenoids and artemisinin metabolism between different growth stages and genotypes (Wang et al. 2009). Metabolite profiling of five medicinal Panax herbs including *Panax ginseng* (Chinese ginseng), *P. notoginseng*, (Sanchi), *P. japonicus* (Rhizoma Panacis Majoris), *P. quinquefolium L.* (American ginseng), and *P. ginseng* (Korean ginseng) were performed using UPLC-MS and 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 gisenoside Rb1, and gisenoside Rb2 accountable for such variations were identified through the loadings plot of PCA, and were identified tentatively by the accurate mass of TOF/MS and partially verified by reference standard (Xie et al. 2008). Six different types of ginseng roots from China and Korea could be easily differentiated by NMR-based metabolomics (Kang et al. 2008). Pharmacodynamic effects of the ginsenoside Rg3 on the metabonome in urine 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 (Wang et al. 2008). It may not only increase the number of discovered biomarkers but consequently improve the comprehensive information on metabolic changes. Liang et al. (2010) used LC/MS to analyze of 16 saponins simultaneously and the developed methodology could effectively break the application bottleneck on the quantitative analysis of multi-component with LC/MS, and would be applied widely in related fields for multi-component analysis, especially in CHM research. 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 (Law et al. 2008). 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 (Van Dorsten et al. 2006). 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 have a wide perspective in the analysis of "coupled TCMs" (Yi et al. 2009). The major metabolite of both arecoline and arecaidine, *N*-methylnipecotic acid, is a novel metabolite arising from carbon-carbon double-bond reduction. Another unusual metabolite found was the monoacylglyceride of arecaidine. It was shown which role is played by these uncommon metabolites in the toxicology of arecoline and arecaidine (Giri et al. 2006). 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 microM (Adams et al. 2010). *Evodia rutaecarpa* has changed the endogenous metabolites of rats and can provide the base for the further research on the interpretation of drug property (Zhang et al. 2010b). 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 metabonome was dominated by 33 metabolites including sugars, amino acids, organic acids, polyphenolic acids, and diterpenes, among which quinate, cis-4-glucosyloxycinnamic acid, and 3,4,5-trimethoxyphenylmethanol were found in rosemary for the first time (Xiao et al. 2008). It can be concluded that the metabolomics is a potentially powerful tool to explore the therapeutic basis of TCM herbs.

6. Conclusion and future perspectives

Systems biology is a new territory in life science and represents the future of biomedicine in the 21st century (Hood et al. 2004; Nicholson et al. 2003). Its applicable potentiality in medicine is infinite and will have significant impact on traditional medicine, clinical research and drug development. Metabolomics focuses on interactions of molecular assembly (global metabolite analysis) and their functions in the body system, and all the factors affecting one's health can be reflected by metabolome. The integral and systematic study of metabolomics is in agreement with TCM theory in nature, therefore it may be one of the best methods to study the complex system of TCM. The rapid growth of the metabolomics field provides an array of new tools for the integration of TCM with modern technology, and is potentially advancing the progress of modernization and internationalization of TCM. In fact, a systems view on how we live will be crucial to make progress in the world we live in and will have an enormous impact on the future medicine or, more importantly, personalized medicine. The acceptance of metabolomics in the scientific domain of TCM has been very fast and is expected to grow even more rapidly. Consequently, metabolomics is the best to fit the holistic concept of multi-targets and systems of TCM theory. By analyzing and verifying the specific markers of a disease, metabolomics enables us to better understand pathological processes, and substance metabolic pathways. In addition, metabolomics can assist to discover early biomarkers of diseases. Compared with traditional diagnostic methods, even little changes of metabolites can help to detect early pathologic changes more sensitively.

For drug discovery of traditional medicines, we find that these medicines have more implications for drug discovery than just providing new chemical entities. The history of traditional medicines indicates that they depended more on the combination of natural agents than on screening new agents to find new remedies. This phenomenon suggests that shifting the current drug discovery paradigm from 'finding new-entity drugs' to 'combining existing agents' may be helpful for overcoming the 'more investment, fewer drugs' challenge. TCM have been a rich source of lead drug discovery, based on their capability to create unique and diverse chemical structures. In recent years, metabolomics has played increasingly important roles in TCM research and development. Precise identification and accurate quantification of metabolites facilitate downstream pathway and network analysis for the discovery of clinically accessible and minimally invasive biomarkers of drug efficacy. Metabolomic technologies facilitate the systematic characterisation of a drug target's physiology, thereby helping to reduce the typically

high attrition rates in discovery projects, and improving the overall efficiency of TCM research processes. Metabolomics, including both targeted and global metabolite profiling strategies, is fast becoming the approach of choice across a broad range of sciences including systems biology, drug discovery, molecular and cell biology, and other medical and agricultural sciences. The metabolomes of TCM are particularly a valuable natural resource for evidence-based TCM. Recent studies suggest that metabolomics-based principles for drug discovery and development of TCM will lead to a greater understanding and even greater opportunities. We urgently desiderate strengthening modernization studies of TCM and believe that the comprehensive metabonomic approach is a potentially powerful tool to explore the therapeutic basis and to clarify the possible action mechanism of TCM. It is conceivable that the application of technologies developed in metabonomics in the basic and therapeutic research of Chinese medicine will eventually lead to the reconciliation and integration of TCM and contemporary medicine. Overall, integration of metabolomics-based principles into TCM would make it possible to explore drug discovery and development of TCM, the molecular mechanism of therapeutic effects of herbal medicine with multiple targets, might be the direction to enable a revolution for future health care. It seems that to create a brighter future of drug discovery,also perhaps it is time to embrace the arrival of 'TCM-OMICS' era in Chinese medicine research.

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References

Arakaki AK, J Skolnick, JF McDonald (2008) Marker metabolites can be therapeutic targets as well. Nature 456: 443.

Adams M, Mahringer A, Kunert O, Fricker G, Efferth T, Bauer R (2007) Cytotoxicity and p-glycoprotein modulating effects of quinolones and indoloquinazolines from the Chinese herb Evodia rutaecarpa. Planta Med 73: 1554–1157.

Agnolet S, Jaroszewski JW, Verpoorte R, Staerk D (2010) NMR-based metabolomics combined with HPLC-PDA-MS- SPE-NMR for investigation of standardized Ginkgo biloba preparations. Metabolomics 6: 292–302.

Beckonert O, Coen M, Keun HC, Wang Y, Ebbels TM, Holmes E, Lindon JC, Nicholson JK (2010) High-resolution magic-angle-spinning NMR spectroscopy for metabolic profiling of intact tissues. Nat Protoc 25: 1019–1032.

Blow N (2008) Metabolomics: Biochemistry's new look. Nature 455: 697–700.

Bluestone JA, Herold K, Eisenbarth G (2010) Genetics, pathogenesis and clinical interventions in type 1 diabetes. Nature 464: 1293–1300.

Bian Q, Jia K, Liu SF, Shu B, Liang QQ, Zhou CJ, Zhou Q, Wang YJ (2011) Inhibitory effect of YQHYRJ recipe on osteoblast differentiation induced by BMP-2 in fibroblasts from posterior longitudinal ligament of mice. Pharmazie 66: 784–790.

Chen M, Su M, Zhao L, Jiang J, Liu P, Cheng J, Lai Y, Liu Y, Jia W (2006) Metabonomic study of aristolochic acid-induced nephrotoxicity in rats. J Proteome Res 5: 995–1002.

Chen M, Ni Y, Duan H, Qiu Y, Guo C, Jiao Y, Shi H, Su M, Jia W (2008) Mass spectrometry-based metabolic profiling of rat urine associated with general toxicity induced by the multiglycoside of Tripterygium wilfordii Hook. f. Chem Res Toxicol 21: 288–294.

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 (2006) Pharmaco-metabonomic phenotyping and personalized drug treatment. Nature 440: 1073–1077.

Cooks RG, Ouyang Z, Takats Z, Wiseman JM (2006) Detection technologies. Ambient mass spectrometry. Science 311: 1566–1570.

Dai H, Xiao C, Liu H, Hao F, Tang H (2010) Combined NMR and LC-DAD-MS analysis reveals comprehensive metabonomic variations for three phenotypic cultivars of Salvia Miltiorrhiza Bunge. J Proteome Res 9: 1565–1578.

Davies IW, Welch CJ (2009) Looking forward in pharmaceutical process chemistry. Science 325: 701–704.

Dharia NV, Chatterjee A, Winzeler EA (2010) Genomics and systems biology in malaria drug discovery. Curr Opin Investig Drugs 11:131–138.

de Bono JS, Ashworth A (2010) Translating cancer research into targeted therapeutics. Nature 467: 543–549.

Evason K, Huang C, Yamben I, Covey DF, Kornfeld K (2005) Anticonvulsant medications extend worm life-span. Science 307: 258–262.

Fidock DA (2010) Drug discovery: Priming the antimalarial pipeline. Nature 465: 297–298.

Fiehn O (2002) Metabolomics–the link between genotypes and phenotypes. Plant Mol Biol 48: 155–171.

Fischer HP (2005) Towards quantitative biology: integration of biological information to elucidate disease pathways and to guide drug discovery. Biotechnol Annu Rev 11: 1–68.

Fitzgerald G (2010) Drug development needs a new brand of science. Nature 468: 869.

Holmes E, Loo RL, Stamler J, Bictash M, Yap IK, Chan Q, Ebbels T, De Iorio M, Brown IJ, Veselkov KA, Daviglus ML, Kesteloot H, Ueshima H, Zhao L, Nicholson JK, Elliott P (2008) Human metabolic phenotype diversity and its association with diet and blood pressure. Nature 453: 396–401.

Gallagher WM, Tweats D, Koenig J (2009) Omic profiling for drug safety assessment: current trends and public-private partnerships. Drug Discov Today 14: 337–342.

Giri S, Idle JR, Chen C, Zabriskie TM, Krausz KW, Gonzalez FJ (2006) A metabolomic approach to the metabolism of the areca nut alkaloids arecoline and arecaidine in the mouse. Chem Res Toxicol 19: 818–827.

Greensfelder L (2000) Alternative medicine. Herbal product linked to cancer. Science 288: 1946.

Griffiths WJ, Koal T, Wang Y, Kohl M, Enot DP, Deigner HP (2010) Targeted metabolomics for biomarker discovery. Angew Chem Int Ed Engl 49: 5426–5445.

Godzien J, Ciborowski M, Angulo S, Ruperez FJ, Paz Martínez M, Señorans FJ, Cifuentes A, Ibañez E, Barbas C (2011) Metabolomic approach with LC-QTOF to study the effect of a nutraceutical treatment on urine of diabetic rats. J Proteome Res 10: 837–844.

Gu Y, Zhang Y, Shi X, Li X, Hong J, Chen J, Gu W, Lu X, Xu G, Ning G (2010) Effect of traditional Chinese medicine berberine on type 2 diabetes based on comprehensive metabonomics. Talanta 81: 766–772.

Hood L, Heath JR, Phelps ME, Lin B (2004) Systems biology and new technologies enable predictive and preventative medicine. Science 306: 640–643.

Huang JH, Shen ZY, Wu B (2008) Effect and mechanism of Epimedium flavanoids for aging retardation from viewpoint of transcriptomics and metabonomics. Zhongguo Zhong Xi Yi Jie He Za Zhi 28: 47–50.

Huang L, Zhao H, Huang B, Zheng C, Peng W, Qin L (2011) Acanthopanax senticosus: review of botany, chemistry and pharmacology. Pharmazie. 66: 83–97.

Jacob SS, Smith NW, Legido-Quigley C (2007) Assessment of Chinese medicinal herb metabolite profiles by UPLC-MS- based methodology for the detection of aristolochic acids. J Sep Sci 30: 1200–1206.

Ji H, Ehrlich LI, Seita J, Murakami P, Doi A, Lindau P, Lee H, Aryee MJ, Irizarry RA, Kim K, Rossi DJ, Inlay MA, Serwold T, Karsunky H, Ho L, Daley GQ, Weissman IL, Feinberg AP (2010) Comprehensive methylome map of lineage commitment from haematopoietic progenitors. Nature 467: 338–342.

Kaiser J (2010) National Institutes of Health. A government niche for translational medicine and drug development. Science 330: 1462–1463.

Kang J, Lee S, Kang S, Kwon HN, Park JH, Kwon SW, Park S (2008) NMR-based metabolomics approach for the differentiation of ginseng (Panax ginseng) roots from different origins. Arch Pharm Res 31: 330–336.

Kell DB (2006) Systems biology, metabolic modelling and metabolomics in drug discovery and development. Drug Discov Today 11: 1085–1092.

Keun HC, Athersuch TJ (2007) Application of metabonomics in drug development. Pharmacogenomics 8: 731–741.

Klayman DL (1985) Qinghaosu (artemisinin): an antimalarial drug from China. Science 228: 1049–1055.

Koul A, Arnoult E, Lounis N, Guillemont J, Andries K (2011) The challenge of new drug discovery for tuberculosis. Nature 469: 483–490.

Kotłowska A, Sworczak K, Stepnowski P (2011) Urine metabolomics analysis for adrenal incidentaloma activity detection and biomarker discovery. J Chromatogr B Analyt Technol Biomed Life Sci 879: 359–363.

Law WS, Huang PY, Ong ES, Ong CN, Li SF, Pasikanti KK, Chan EC (2008) Metabonomics 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 22: 2436–2446.

Lee SY, Kim HU, Park JH, Park JM, Kim TY (2009) Metabolic engineering of microorganisms: general strategies and drug production. Drug Discov Today 14: 78–88.

Lee W, Jiang Z, Liu J, Haverty PM, Guan Y, Stinson J, Yue P, Zhang Y, Pant KP, Bhatt D, Ha C, Johnson S, Kennemer MI, Mohan S, Nazarenko I, Watanabe C, Sparks AB, Shames DS, Gentleman R, de Sauvage FJ, Stern H, Pandita A, Ballinger DG, Drmanac R, Modrusan Z, Seshagiri S, Zhang Z (2010) The mutation spectrum revealed by paired genome sequences from a lung cancer patient. Nature 465: 473–477.

Li F, Lu X, Liu H, Liu M, Xiong Z (2007) A pharmaco-metabonomic study on the therapeutic basis and metabolic effects of Epimedium brevicornum Maxim on hydrocortisone-induced rat using UPLC-MS. Biomed Chromatogr 21: 397–405.

Li L, Sun B, Zhang Q, Fang J, Ma K, Li Y, Chen H, Dong F, Gao Y, Li F, Yan X (2008) Metabonomic study on the toxicity of Hei-Shun-Pian, the processed lateral root of Aconitum carmichaelii Debx. (Ranunculaceae). J Ethnopharmacol 116: 561–568.

Li JW, Vederas JC (2009) Drug discovery and natural products: end of an era or an endless frontier? Science 325: 161–165.

Liang Q, Ni C, Xie M, Zhang Q, Zhang YX, Yan XZ, Yang MJ, Peng SQ, Zhang YZ (2009) Nephrotoxicity study of Aristolochia fangchi in rats by metabonomics. Zhong Xi Yi Jie He Xue Bao 7: 746–752.

Liang Y, Kang A, Xie T, Zheng X, Dai C, Hao H, A J, Sheng L, Xie L, Wang GJ (2010) 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 1217: 4501–4506.

Lindon JC, Holmes E, Bollard ME, Stanley EG, Nicholson JK (2004) Metabonomics technologies and their applications in physiological monitoring, drug safety assessment and disease diagnosis. Biomarkers 9: 1–31.

Lindon JC, Holmes E, Nicholson JK (2007) Metabonomics in pharmaceutical R&D. FEBS J 274: 1140–1151.

Liu NQ, Cao M, Frédérich M, Choi YH, Verpoorte R, van der Kooy F (2010) Metabolomic investigation of the ethnopharmacological use of Artemisia afra with NMR spectroscopy and multivariate data analysis. J Ethnopharmacol 128: 230–235.

Liu Y, Huang R, Liu L, Peng J, Xiao B, Yang J, Miao Z, Huang H (2010) Metabonomics study of urine from Sprague-Dawley rats exposed to Huang-yao-zi using (1)H NMR spectroscopy. J Pharm Biomed Anal 52: 136–141.

Lv Y, Liu X, Yan S, Liang X, Yang Y, Dai W, Zhang W (2010) 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 52: 129–135.

Manna SK, Patterson AD, Yang Q, Krausz KW, Li H, Idle JR, Fornace AJ Jr, Gonzalez FJ (2010) Identification of noninvasive biomarkers for alcohol-induced liver disease using urinary metabolomics and the Pparanull mouse. J Proteome Res 9: 4176–4188.

Mazzone M, Carmeliet P (2008) Drug discovery: a lifeline for suffocating tissues. Nature 453: 1194–1195.

Nicholson JK, Wilson ID (2003) Opinion: understanding 'global' systems biology: metabonomics and the continuum of metabolism. Nat Rev Drug Discov 2: 668–676.

Nicholson JK, Lindon JC (2008) Systems biology: Metabonomics. Nature 455: 1054–1056.

Normile D (2003) Asian medicine, the new face of traditional Chinese medicine. Science 299: 188–190.

Park AJ (2002) What's in a Name? A systematic review of the nomenclature of Chinese medical formulae. J Chin Med 30: 419–427.

Prestele J, Hierl G, Scherling C, Hetkamp S, Schwechheimer C, Isono E, Weckwerth W, Wanner G, Gietl C (2010) Different functions of the C3HC4 zinc RING finger peroxins PEX10, PEX2, and PEX12 in peroxisome formation and matrix protein import. Proc Natl Acad Sci U S A 107: 14915–14920.

Schrattenholz A, Soskić V (2008) What does systems biology mean for drug development? Curr Med Chem 15: 1520–1528.

Shaham O, Slate NG, Goldberger O, Xu Q, Ramanathan A, Souza AL, Clish CB, Sims KB, Mootha VK (2010) A plasma signature of human mitochondrial disease revealed through metabolic profiling of spent media from cultured muscle cells. Proc Natl Acad Sci U S A 107: 1571–1575.

Shroff R, Rulísek L, Doubsky J, Svatos A (2009) Acid-base-driven matrix-assisted mass spectrometry for targeted metabolomics. Proc Natl Acad Sci U S A 106: 10092–10096.

Shyur LF, Yang NS (2008) Metabolomics for phytomedicine research and drug development. Curr Opin Chem Biol 12: 66–71.

Smith SB, Qu HQ, Taleb N, Kishimoto NY, Scheel DW, Lu Y, Patch AM, Grabs R, Wang J, Lynn FC, Miyatsuka T, Mitchell J, Seerke R, Désir J, Eijnden SV, Abramowicz M, Kacet N, Weill J, Renard ME, Gentile M, Hansen I, Dewar K, Hattersley AT, Wang R, Wilson ME, Johnson JD, Polychronakos C, German MS (2010) Rfx6 directs islet formation and insulin production in mice and humans. Nature 463: 775–780.

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 (2009) Metabolomic profiles delineate potential role for sarcosine in prostate cancer progression. Nature 457: 910–914.

Song HT, Zhang Q, Wang HJ, Guo XJ, Chen DW, He ZG (2007) Preparation of the traditional Chinese medicine compound recipe Shuxiong sustained-release capsules by multiparticulate time-controlled explosion technology. Pharmazie 62: 372–377.

Stone R (2008) Biochemistry. Lifting the veil on traditional Chinese medicine. Science 319: 709–710.

Sun B, Li L, Wu S, Zhang Q, Li H, Chen H, Li F, Dong F, Yan X (2009) Metabolomic analysis of biofluids from rats treated with Aconitum alkaloids using nuclear magnetic resonance and gas chromatography/time-of-flight mass spectrometry. Anal Biochem 395: 125–133.

Sun B, Wu S, Li L, Li H, Zhang Q, Chen H, Li F, Dong F, Yan X (2009) A metabolomic analysis of the toxicity of Aconitum sp. alkaloids in rats using gas chromatography/mass spectrometry. Rapid Commun Mass Spectrom 23: 1221–1228.

Tolstikov VV, Fiehn O, Tanaka N (2007) 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 358: 141–155.

Van Dorsten FA, Daykin CA, Mulder TP, Van Duynhoven JP (2006) Metabonomics approach to determine metabolic differences between green tea and black tea consumption. J Agric Food Chem 54: 6929–6938.

van der Greef J, Martin S, Juhasz P, Adourian A, Plasterer T, Verheij ER, McBurney RN (2007) The art and practice of systems biology in medicine: mapping patterns of relationships. J Proteome Res 6: 1540–1559.

Vlietinck A, Pieters L, Apers S (2009) Legal requirements for the quality of herbal substances and herbal preparations for the manufacturing of herbal medicinal products in the European union. Planta Med 75: 683–688.

Wang M, Lamers RJ, Korthout HA, van Nesselrooij JH, Witkamp RF, van der Heijden R, Voshol PJ, Havekes LM, Verpoorte R, van der Greef J(2005) Metabolomics in the context of systems biology: bridging traditional Chinese medicine and molecular pharmacology. Phytother Res 19:173–182.

Walgren JL, Thompson DC (2004) Application of proteomic technologies in the drug development process. Toxicol Lett 149: 377–385.

Watkins SM, German JB (2002) Metabolomics and biochemical profiling in drug discovery and development. Curr Opin Mol Ther 4: 224–228.

Wang Y, Wang J, Yao M, Zhao X, Fritsche J, Schmitt-Kopplin P, Cai Z, Wan D, Lu X, Yang S, Gu J, Häring HU, Schleicher ED, Lehmann R, Xu G (2008) Metabonomics 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 80: 4680–4688.

Wang H, Ma C, Ma L, Du Z, Wang H, Ye H, Li G, Liu B, Xu G (2009) Secondary metabolic profiling and artemisinin biosynthesis of two genotypes of Artemisia annua. Planta Med 75: 1625–1633.

Wang L, Zhou GB, Liu P, Song JH, Liang Y, Yan XJ, Xu F, Wang BS, Mao JH, Shen ZX, Chen SJ, Chen Z (2008a) Dissection of mechanisms of Chinese medicinal formula Realgar-Indigo naturalis as an effective treatment for promyelocytic leukemia. Proc Natl Acad Sci U S A 105: 4826–4831.

Wang X, Lv H, Sun H, Liu L, Yang B, Sun W, Wang P, Zhou D, Zhao L, Dou S, Zhang G, Cao H (2008b) Metabolic urinary profiling of alcohol hepatotoxicity and intervention effects of Yin Chen Hao Tang in rats using ultra-performance liquid chromatography/electrospray ionization quadruple time-of-flight mass spectrometry. J Pharm Biomed Anal 48: 1161–1168.

Wang P, Sun H, Lv H, Sun W, Yuan Y, Han Y, Wang D, Zhang A, Wang X (2010a) 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 53: 631–645.

Wang M, Rang W, Zhang Q, Huo C, Ma Z, Wang Y, Yan X, Gao Y (2010b) NMR-spectroscopy-based metabonomic approach to analysis of Siwutang, a novel prescription, treated blood deficiency in mice. Zhongguo Zhong Yao Za Zhi 35: 630–634.

Wang X, Sun H, Zhang A, Sun W, Wang P, Wang Z (2011) Potential role of metabolomics apporoaches in the area of traditional Chinese medicine: As pillars of the bridge between Chinese and Western medicine. J Pharm Biomed Anal 25: 173–182.

Wishart DS (2008) Applications of metabolomics in drug discovery and development. Drugs R D 9: 307–322.

Wu B, Yan S, Lin Z, Wang Q, Yang Y, Yang G, Shen Z, Zhang W (2008) Metabonomic study on ageing: NMR-based investigation into rat urinary metabolites and the effect of the total flavone of Epimedium. Mol Biosyst 4: 855–861.

Xiao C, Dai H, Liu H, Wang Y, Tang H (2008) Revealing the metabonomic variation of rosemary extracts using 1H NMR spectroscopy and multivariate data analysis. J Agric Food Chem 56: 10142–10153.

Xie G, Plumb R, Su M, Xu Z, Zhao A, Qiu M, Long X, Liu Z, Jia W (2008) Ultra-performance LC/TOF MS analysis of medicinal Panax herbs for metabolomic research. J Sep Sci 31: 1015–1026.

Xie B, Gong T, Gao R, Liu J, Zuo J, Wang X, Zhang Z (2009) Development of rat urinary HPLC-UV profiling for metabonomic study on Liuwei Dihuang Pills. J Pharm Biomed Anal 49: 492–497.

Xu EY, Schaefer WH, Xu Q (2009) Metabolomics in pharmaceutical research and development: metabolites, mechanisms and pathways. Curr Opin Drug Discov Devel 12: 40–52.

Xu G, Ma X, Zhang Q, Li B, Huang L, Yu R, Liu H (2009) Study of metabonomics on pharmacological action appraisal Rhizoma coptidis in rats. Zhongguo Zhong Yao Za Zhi 34: 1845–1847.

Xue T, Roy R (2003) Studying traditional Chinese medicine. Science 300: 740–741.

Yan S, Wu B, Lin Z, Jin H, Huang J, Yang Y, Zhang X, Shen Z, Zhang W (2010) Metabonomic characterization of aging and investigation on the anti-aging effects of total flavones of Epimedium. Mol Biosyst 5: 1204–1213.

Yi LZ, Yuan DL, Liang YZ, Xie PS, Zhao Y (2009) Fingerprinting alterations of secondary metabolites of tangerine peels during growth by HPLC-DAD and chemometric methods. Anal Chim Acta 649: 43–51.

Yuliana ND, Khatib A, Choi YH, Verpoorte R (2011) Metabolomics for bioactivity assessment of natural products. Phytother Res 25: 157–169.

Zhang QY, Mao JH, Liu P, Huang QH, Lu J, Xie YY, Weng L, Zhang Y, Chen Q, Chen SJ, Chen Z (2009a) A systems biology understanding of the synergistic effects of arsenic sulfide and Imatinib in BCR/ABL-associated leukemia. Proc Natl Acad Sci U S A 106: 3378–3383.

Zhang Q, Wang GJ, A JY, Wu D, Zhu LL, Ma B, Du Y (2009b) Application of GC/MS-based metabonomic profiling in studying the lipid-regulating effects of Ginkgo biloba extract on diet-induced hyperlipidemia in rats. Acta Pharmacol Sin 30: 1674–1687.

Zhang A, Sun H, Wang Z, Sun W, Wang P, Wang X (2010a) Metabolomics: towards understanding traditional Chinese medicine. Planta Med 76: 2026–2035.

Zhang Q, Xu G, Wu L, Ma X, Zeng Z, Huang L, Yu R, Liu H (2010b) Preliminary study of metabonomcs on aqueous extract of Evodia rutaecarpa in sprague-dawley rats. Zhongguo Zhong Yao Za Zhi 35: 99–102.

Zhang A, Sun H, Wang P, Han Y, Wang X (2012) Modern analytical techniques in metabolomics analysis. Analyst 137: 293–300.

Zhao X, Zhang Y, Meng X, Yin P, Deng C, Chen J, Wang Z, Xu G (2008) Effect of a traditional Chinese medicine preparation Xindi soft capsule on rat model of acute blood stasis: a urinary metabonomics study based on liquid chromatography-mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci 873: 151–158.

Zhang Y, Li PP (2011) Assessment of shikonin for potential estrogenic activity by dual-luciferase reporter based bioluminescent measurements *in vitro*. Pharmazie 66: 141–143.