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Oriental Medicine

Integration of multi-omics in investigations on the mechanisms of action of Chinese herbal medicine interventions in metabolic diseases

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Competing interests

The authors declare no conflicts of interest.

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Abbreviations

CHMs, Chinese herbal medicines; TCM, traditional Chinese medicine; DM, diabetes mellitus; HPLC, high-performance liquid chromatography; MS, mass spectrometry; T2DM, type-2 diabetes mellitus; UHPLC-QTOF-MS, ultra-high performance liquid chromatography-quadrupole time-of-flight mass spectrometry; NAFLD, nonalcoholic fatty liver disease; NP, network pharmacology; IL, interleukin; PCR, polymerase chain reaction.

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Abstract

The rapid development of bioinformatics has provided novel approaches and methods for exploring the mechanisms of disease treatment via Chinese herbal medicines. Compound Chinese herbal medicines formulas have complex compositions and are characterized by their multiple constituents and diverse array of biological targets. Therefore, the mechanisms of action of most compound Chinese herbal medicines formulas cannot be adequately explained using a single pathway. Omics technologies describe high-throughput-based analytical and detection techniques, which include transcriptomics, proteomics, and metabolomics and provide multilayer parameter information that can be integrated to characterize the overall relationships involved in the therapeutic effects of Chinese herbal medicines formulas. Through their combination with network biology and drug effect networks, omics technologies also enable investigations into the mechanisms of disease treatment in traditional Chinese medicine. The integration of multiple omics technologies is in line with the concept of holism in traditional Chinese medicine and provides an approach for combining modern science and technology with traditional Chinese medicine theories. In recent years, omics technologies have been widely used to elucidate the mechanisms of action of Chinese herbal medicines. The latest studies employing multi-omics integration for investigating the mechanisms of action of Chinese herbal medicines interventions in metabolic diseases have devoted greater attention to in-depth explorations of disease pathogenesis. This paper provides a review of the following multi-omics technologies, which are used in research on the treatment of common metabolic diseases (e.g., type-2 diabetes mellitus, nonalcoholic fatty liver disease): network pharmacology combined with metabolomics, 16S rRNA sequencing combined with transcriptomics, 16S rRNA sequencing combined with metabolomics, and 16S rRNA sequencing combined with network pharmacology and metabolomics.

Keywords: omics technology; Chinese herbal medicine; metabolic disease; mechanism of action

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Highlights

This article reviews the research progress of multi-omics studies on the mechanism of Chinese herbal medicines in the intervention of metabolic diseases. Traditional Chinese medicine has shown good efficacy in the treatment of metabolic diseases. It is urgent to explain the complexity of molecules at multiple levels through omics technologies such as multi-omics technology, and to provide a scientific basis for the treatment of diseases with traditional Chinese medicine.

Background

Omics technologies refer to a set of high-throughput-based analytical and detection techniques used in modern biological research that include transcriptomics, proteomics, and metabolomics. The concept of holism in traditional Chinese medicine (TCM) views the human body as an integrated whole and emphasizes the unity of humans with the natural and social environments. Despite their recent emergence, omics technologies are in line with the age-old concept of holism in TCM due to their comprehensive, systematic, and dynamic nature. A large amount of evidence has been gathered since the recent adoption of omics technologies by researchers to promote objective syndrome differentiation in TCM, and the application of omics in the field of TCM has produced significant results and attracted widespread attention. The Chinese herbal medicines (CHMs) used in TCM have complex compositions and are characterized by multiple components and diverse targets. Therefore, the holistic effects of TCM in disease treatment using CHMs cannot be explained by a single pathway. The utilization of integrated omics approaches may enable investigation of the mechanisms of action of CHMs from multiple perspectives and aspects, which will further promote the development of clinical applications of TCM and provide novel directions for future research in TCM.

In the present study, we reviewed and summarized the literature relating to the integration of multi-omics in investigations into the mechanisms of action of CHM interventions in metabolic diseases. Our results revealed that the omics technologies commonly used in TCM research include transcriptomics, proteomics, metabolomics, and 16S rRNA sequencing. Transcriptomics, a discipline involving the comprehensive study of all gene transcriptions and transcriptional regulation patterns within cells, serves as a powerful tool for high-throughput gene expression profile analysis. Proteomics enables the understanding of the composition and patterns of change of proteins in cells or organisms through the analysis of protein expression, protein structure, and protein-protein interactions. Metabolomics can be used to study the types and numbers of metabolites within organisms and the patterns of change of metabolites under the influence of internal and external factors. It may also reflect patterns of dynamic changes of metabolites in organisms when group indicator analysis is performed through information modeling and system integration. 16S rRNA sequencing enables the accurate identification of the types and abundance of gut microbes (Figure 1).

However, current research findings indicate that the use of omics technologies is insufficient for gaining a more complete understanding of the complex compositions of CHMs. This may be explained by two reasons. First, limitations exist in single omics analyses, leading to the inability to perform comprehensive and adequate investigations into the mechanisms of action of CHMs in disease treatment. Second, although significant progress has been achieved in the integration of multi-omics in research into the treatment of diseases with CHMs, the relevant studies lack adequate depth and mainly involve the deduction of possible biological processes and metabolic routes based on the measurement of gene and protein expression regulated by CHMs or changes in metabolites. Therefore, there is a need to develop new approaches for enabling comprehensive analyses and in-depth research. The utilization of multiple omics technologies enables the multidimensional explanation of molecular complexities, which can be used to achieve a holistic understanding of the relationships between human health and disease and provide a scientific basis for the use of TCM in disease treatment.

TCM has been used in the treatment of metabolic diseases for thousands of years. For instance, Liuwei Dihuang pill (National Medical Products Administration of China approval number: 20180402), which consists of Shudihuang (cooked *Rehmanniae radix*), Jiuyurou (wine-treated *Corni fructus*), Mudanpi (*Moutan cortex radicis*), Shanyao (*Dioscoreae rhizoma*), Fuling (*Poria cocos*), and Zexie (*Alismatis rhizoma*), is a classic compound formula commonly used in the clinical treatment of diabetes mellitus (DM). Studies have found that Liuwei Dihuang pill significantly decreases DM patients' fasting glucose and urine albumin to creatinine ratio, but the underlying mechanisms have not been investigated in depth. Therefore, there is an urgent need to utilize metabolomics and other methods to observe the differences in metabolites, which will contribute to the elucidation of the mechanisms of action of Liuwei Dihuang pill. The results of an in vitro experiment revealed that the comparison of differences in metabolism between the model and control groups using metabolomics methods enabled the confirmation of the therapeutic effects of Liuwei Dihuang pill in DM and diabetes-associated renal failure [1]. Huanglian Jiedu decoction is a well-known heat-clearing prescription consisting of Huangqin (*Scutellariae radix*), Huanglian (*Coptidis rhizoma*), Huangbai (*Phellodendri* cortex), and Zhizi (*Gardeniae fructus*). Since its first documentation in the ancient TCM medical book *Handbook of Prescriptions for Emergency* (written by Hong Ge, Eastern Jin Dynasty, 317–420 C.E.), Huanglian Jiedu decoction has remained widely used in clinical practice. In one study, neuroprotective compounds in a crude extract of Huanglian Jiedu decoction were screened and identified through living cell biospecific extraction coupled with high-performance liquid chromatography (HPLC)-quadrupole/electrostatic field orbitrap high-resolution tandem mass spectrometry (MS), which demonstrated that the combination of the aforementioned techniques is an effective approach for the rapid screening of potential bioactive components in CHMs [2]. In another study, researchers explored the effects of Huanglian Jiedu decoction on Alzheimer's disease using a plasma metabolomics technique based on ultra-high performance liquid chromatography-quadrupole time-of-flight mass spectrometry (UHPLC-QTOF-MS), and found that the decoction exerted certain preventive effects against Alzheimer's disease [3].

Based on the recent literature, it is apparent that studies involving the integration of multi-omics in investigations into the mechanisms of action of CHM interventions in metabolic diseases have devoted increasing attention to the in-depth exploration of disease pathogenesis. This paper provides a review of the following multi-omics technologies used in research on the treatment of common metabolic diseases (e.g., type-2 diabetes mellitus (T2DM), nonalcoholic fatty liver disease (NAFLD)): network pharmacology (NP) combined with metabolomics; 16S rRNA sequencing combined with transcriptomics; 16S rRNA sequencing combined with metabolomics; and 16S rRNA sequencing combined with NP and metabolomics.

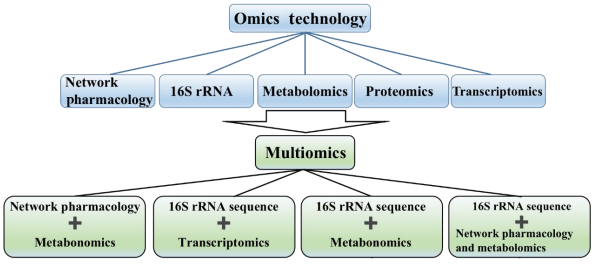

Figure 1 Omics and multi-omics

Omics technologies

Network pharmacology

NP is an emerging discipline that is used to analyze biological system networks and select specific signal nodes for multi-target molecular drug design [4]. A biological network consists of various topological parameters such as nodes, edges, and degrees. Nodes may represent units such as genes, proteins, CHM compounds, or disease phenotypes. Edges represent the connections between units and may indicate protein-protein interactions, compound-target interactions, or transcriptional regulation. The degree of a node is the number of edges incident to the node, and reflects the relationships between that node and other nodes; high-degree nodes are generally regarded as central nodes [5]. Network construction in TCM research typically includes the screening of bioactive compounds and the discovery of targets to realize the visualization of the "drug-gene-target-disease" network [6]. Jiangzhi Ligan decoction is an empirical clinical formula comprised of Zexie (*Alismatis rhizoma*), Danshen (*Salviae miltiorrhizae radix et rhizoma*), Juemingzi (*Cassiae semen*), Yujin (*Curcumae radix*), Haizao (*Sargassum*), and Heye (*Nelumbinis folium*). Tang et al. identified 82 active components in Jiangzhi Ligan decoction and 53 targets involved in NAFLD via network analysis [7]. The targets were mainly related to insulin resistance, oxidative stress, and inflammatory responses in NAFLD, and participated in the PI3K/Akt signaling pathway, inflammasome pathway, interleukin (IL)-10 signaling pathway, and T-cell signaling pathway. These results enabled a preliminary investigation of the potential mechanisms of Jiangzhi Ligan decoction intervention in NAFLD. Liuwei Dihuang pill is documented in the *Key to Therapeutics of Children's Diseases* (a key TCM pediatrics medical book, written by Yi Qian, 1119 C.E.). By adopting a systematic NP approach, He et al. successfully identified 45 active components in Liuwei Dihuang pill that were relevant to the treatment of T2DM [8]. Among these active components, ten components with higher predictive power (including quercetin, kaempferol, stigmasterol, diosgenin, garcinone B, and isofucosterol) exerted anti-inflammatory, anti-oxidative, and β-cell damage-reducing effects. Further investigation revealed that Liuwei Dihuang pill could play a role in the treatment of T2DM and its complications (namely atherosclerosis and nephropathy) through the AGE-RAGE, TNF, and NF-κB signaling pathways. Nie et al. used a combination of NP and molecular docking methods to validate the therapeutic effects of the classic formula Chaihu Shugan powder, which contains the CHM ingredients Chenpi (*Citri reticulatae* pericarpium), Chaihu (*Bupleuri radix*), Chuanxiong (*Chuanxiong rhizoma*), Xiangfu (*Cyperi rhizoma*), Zhike (*Aurantii fructus*), and Shaoyao (*Paeoniae radix*) [9]. The screening results indicated that peroxisome proliferator-activated receptor gamma, farnesoid X receptor, peroxisome proliferator-activated receptor-alpha, retinoic acid receptor-a, and peroxisome proliferator-activated receptor delta were the genes targeted by Chaihu Shugan powder. After intervention with Chaihu Shugan powder in a rat model of NAFLD, improvements were observed in terms of body weight, hepatic histopathology, and serum and liver lipids. The mRNA levels of peroxisome proliferator-activated receptor gamma, The farnesoid X receptor, peroxisome proliferator-activated receptor-alpha, and retinoic acid receptor-a were also significantly altered. These results indicate that the screening of CHM formulas for bioactive compounds and disease targets by NP and subsequent experimental validation of the screening results may represent a reliable basis for determining the mechanisms of action of CHM interventions in metabolic diseases. Through a combination of UPLC/LTQ-Orbitrap-MS and NP, Wang et al. identified chemical compounds in an aqueous extract of Jiegeng (*Platycodonis radix*) and predicted the key chemical components and potential mechanisms of action of *Platycodonis radix* in the treatment of NAFLD [10]. Their results revealed that *Platycodonis radix* contained 13 active components, interacted with 278 drug targets, 1,536 disease targets, and 83 common targets, and participated in the PI3K-AKT, insulin resistance, THF-a, IL-17, JAK-STAT, and T-cell receptor signaling pathways. Experimental validation indicated the presence of differential expression of key target genes.

16S rRNA sequencing

16S rRNA gene sequencing is an important technology for the study of microbial community structures and diversity [11]. After the extraction of total microbial DNA, primers are designed based on the conserved regions of 16S rRNA gene sequences for the amplification of these 16S rRNA gene segments by polymerase chain reaction (PCR), and the amplified fragments are subsequently sequenced. This enables the classification and identification of microbes based on differences in hypervariable region sequences, which makes it possible to determine the composition of gut microbiota. The human gut microbiota plays essential roles in protecting gut cells from damage, processing energy and nutrients, and promoting cell growth. Dysbiosis may impair vital functions and result in pathological states. Therefore, the utilization of 16S rRNA gene sequencing to analyze the state of the gut microbiota is of utmost importance for the prevention of dysbiosis-induced body damage. Jiangan Xiaozhi decoction is an empirical formula for the clinical treatment of NAFLD and consists of Fuling (*Poria cocos*), Zexie (*Alismatis rhizoma*), Baizhu (*Atractylodis macrocephalae rhizoma*), Huangqi (*Astragali radix*), and Chaihu (*Bupleuri radix*). The results of 16S rRNA analysis performed by Liao et al. revealed that Jiangan Xiaozhi decoction impacted the diversity of gut microbiota, decreasing the *Firmicutes/Bacteroidetes* ratio and increasing the relative abundance of probiotics such as *Alloprevotella*, *Lactobacillus,* and *Turicibacter* species. Subsequent gut permeability evaluations indicated that the expressions of ZO-1 and occludin in the colon were increased after Jiangan Xiaozhi decoction treatment [12]. Kang Shuai Lao pill (National Medical Products Administration of China approval number: 20171020) is a famous CHM formulation based on a court prescription of the Ming Dynasty and consists of Dihuang (*Rehmanniae radix*), Renshen (*Ginseng radix et rhizoma*), Gouqi (*Lycii fructus*), and Maidong (*Ophiopogonis radix*). Through the use of 16S rRNA gene sequencing, Gong et al. found that Kang Shuai Lao pill could improve high-fat diet -induced obesity, glucose tolerance disorder, and gut dysbiosis [13]. In the gut, Kang Shuai Lao pill corrected the increased abundance of *Firmicutes* and *Proteobacteria*, increased the *Firmicutes/Bacteroidetes* ratio, and decreased the overabundance of *Bacteroidetes* caused by high-fat diet.

Metabolomics

Metabolomics refers to the study of all low-molecular-weight metabolites within a certain organism or cell over a specific physiological period. It is a branch of systems biology aimed towards information modeling and system integration based on group indicator analysis and enabled by the adoption of high-throughput testing and data processing methods. The concept of metabolomics was first introduced by Nicholson and coworkers in 1999, and mainly includes clinical metabolomics, pharmacometabolomics, lipidomics, and fluxomics [14]. Deng et al. adopted an UHPLC-MS-based untargeted lipidomics approach to analyze hepatic lipid alterations and used the SIRT1-selective inhibitor EX 527 to inhibit SIRT expression in the liver [15]. Shenling Baizhu powder, a CHM composed of Baibiandou (*Lablab album semen*), Baizhu, Fuling, Gancao (*Glycyrrhizae radix et rhizoma*), and Jiegeng (*Platycodonis radix*), has demonstrated protective effects against NAFLD. Lipidomics analysis showed that 30 lipid species were effectively regulated by Shenling Baizhu powder administration in rats fed a high-fat diet, and pathway analysis indicated that glycerophospholipid metabolism and glycerolipid metabolism were potential target pathways involved in the mechanisms of Shenling Baizhu powder's effects against NAFLD. The classic TCM compound formula Liuwei Dihuang pill is currently commonly used in the clinical treatment of DM. Huang et al. adopted a metabolomics approach coupled with gas chromatography-mass spectrometry and a random forest algorithm to determine the metabolic differences between members of the model and control groups and to observe the therapeutic effects of Liuwei Dihuang pill in DM and diabetes-associated renal failure [16]. Their results indicated that certain metabolites such as 3-hydroxybutyric acid, citric acid,

hexadecanoic acid, and octadecanoic acid exhibited significant differences between members of the control and model groups, and treatment with Liuwei Dihuang pill resulted in a significant decrease in fasting blood glucose and the urine albumin to creatinine ratio. These results suggest that Liuwei Dihuang pill may exert protective effects against diabetes-associated renal failure. The patented TCM drug Zishen Jiangtang pill (National Medical Products Administration of China approval number: 20170509) is composed of Huangqi (*Astragali radix*), Shudihuang (cooked *Rehmanniae radix*), Yinyanghuo (*Epimedii* herba), and Sanqi (*Notoginseng radix et rhizoma*), and is widely used in clinical practice for the treatment of T2DM. Chen et al. utilized a UHPLC-Orbitrap/MS-based metabolomics tool to reveal the potential mechanisms of Zishen Jiangtang pill in diabetic mice and found that 24 metabolites among the 26 potential biomarkers in serum samples of the model mice were driven back to control-like levels after treatment with Zishen Jiangtang pill [17]. Subsequent analysis of the metabolic pathways suggested that glutathione metabolism, steroid hormone biosynthesis, and glycerophospholipid metabolism were all closely associated with DM. Therefore, it can be concluded that Zishen Jiangtang pill assists in blood glucose control by regulating the metabolism of phospholipids, including phosphatidylcholines, lysophosphatidylcholines, and phosphatidylinositol. Zheng et al. employed serum metabolomics to investigate the effects of the ethnic drug Buzang Tongluo decoction on metabolic pathways in diabetic mice with hindlimb ischemia [18]. Serum samples were subjected to untargeted metabolomics analysis by UPLC-MS, a metabolic network was built by integrating metabolite data with the Gene Expression Omnibus dataset (GSE3313), and quantitative PCR was used to confirm the key target genes. The results showed that Buzang Tongluo decoction led to a remarkable reversal of altered metabolite levels in the sera of diabetic mice with hindlimb ischemia. It also significantly corrected the previously present down-regulation of vascular endothelial growth factor receptor 2 and endothelial nitric oxide synthase or up-regulation of IL-4-induced 1 and cytochrome P450 family 1 subfamily B member 1 at the mRNA level, which are key regulatory genes involved in the metabolic pathways of glutamate and tryptophan.

Proteomics

Proteomics is a discipline aimed at elucidating the expression and functional patterns of all proteins expressed in cells of various organisms. It involves the determination of the expression, modes of existence (types of modifications), structures, functions, and interactions of proteins [19]. Lo et al. found that oral administration of the aqueous extract of Gualou (*Trichosanthes kirilowii*) elicited hypoglycemic effects in mice in a dose-dependent manner [20]. An abundant novel *Trichosanthes kirilowii* protein was further identified through a proteomic approach, and docking analysis revealed that *Trichosanthes kirilowii* protein interacted with the insulin receptor and activated the kinase activity of the insulin receptor. In addition, *Trichosanthes kirilowii* protein enhanced glucose clearance in diabetic mice in a dose-dependent manner. Tang Luo Ning is an empirical clinical formula consisting of Huangqi (*Astragali radix*), Shanzhuyu (*Corni fructus*), Gouqi (*Lycii fructus*), and Danshen (*Salviae miltiorrhizae radix et rhizoma*). A study by Zhang et al. showed that Tang Luo Ning blood glucose levels and mitigated diabetic symptoms in model rats, including response time to cold or hot stimuli and nerve conduction velocity [21]. Compared to the normal group, there were 388 differentially expressed proteins in the Tang Luo Ning group, 445 in the alpha lipoic acid group, and 451 in the model group; compared to the model group, there were 275 differentially expressed proteins in the Tang Luo Ning group and 251 in the alpha lipoic acid group. As compared to the model group, mitochondrial complex III expression was significantly lower while glutathione peroxidase and peroxidase were higher in the Tang Luo Ning group. As compared to the alpha lipoic acid group, mitochondrial complex III expression was higher and mitochondrial complex IV expression was lower in the Tang Luo Ning group. Hugan Qingzhi decoction is an empirical clinical formula commonly used in the prevention and treatment of NAFLD. It is mainly composed of Zexie (*Alismatis rhizoma*), Shanzha (*Crataegi fructus*), Sanqi (*Notoginseng radix et rhizoma*), Heye (*Nelumbinis folium*), and Gouqi (*Lycii fructus*). In a study by Yao et al., it was found that Hugan Qingzhi decoction exerted protective effects on the livers of rats with high fat diet-induced NAFLD, and proteomics analysis revealed that phytanoyl-CoA 2-hydroxylase, acyl-CoA synthetase 1 long chain, hemopexin, alpha-1-acid glycoprotein (ORM1), fatty acid binding protein 4, soluble sulphotransferase 2a1 (Sult2a1), and argininosuccinate synthase 1 were targets for the treatment of NAFLD by Hugan Qingzhi decoction [22].

Genomics and transcriptomics

Genomics involves the study of genetic diversity and gene expression and functions, which includes the composition and changes of base sequences, DNA methylation, and chromatin modifications. Since its advent, it has been widely applied in the field of TCM research. Commonly used genomic technologies include quantitative analytical techniques such as real-time fluorescence-based quantitative PCR, high-throughput sequencing technologies such as whole-exome capture and sequencing, and single-cell sequencing, in which novel technological breakthroughs have recently been achieved. Genomic technologies enable the simultaneous testing of tens of thousands of genes and have significant advantages such as high-throughput processing capabilities, high comprehensiveness and precision, and the ability to perform analyses at the microscopic level. Therefore, they serve as important tools for precision medicine in the modern era. Transcriptomics is the comprehensive study of gene transcription and transcriptional regulation patterns in cells. In short, it involves the investigation of gene expression at the RNA level, with the transcriptome defined as the sum total of the RNA that can be transcribed from a live cell. Therefore, transcriptomics serves as a key method for the investigation of cell phenotype and function. In a study by Chang et al., the Illumina sequencing platform was used for transcriptome sequencing of the pancreas of diabetic rats and for measuring the expression levels of target genes [23]. Results of their gene classification analysis using eggNOG revealed that 24.25% of genes were of unknown function, which comprised the largest class of genes. Other gene functions were, in descending order of relative proportion, energy conversion, amino acid transport and metabolism, nucleotide transport and metabolism, carbohydrate transport and metabolism, coenzyme transport and metabolism, and lipid transport and metabolism. The Kyoto Encyclopedia of Genes and Genomes enrichment analysis showed that Huangqi Liuyi decoction played a therapeutic role in the treatment of T2DM through four types of metabolic pathways, namely environmental information processing, cellular processes, organismal systems, and human diseases. Qushi Huayu decoction is an empirical clinical formula consisting of Huzhang (*Polygoni cuspidati rhizoma et radix*), Jianghuang (*Curcumae longae rhizoma*), Yinchen (*Artemisiae scopariae* herba), Dihuang (*Rehmanniae radix*), and Zhizi (*Gardeniae fructus*). Xin et al. utilized transcriptome gene chip technology to determine transcriptome expression profiles of liver tissues of mice belonging to the normal, NAFLD model, and Qushi Huayu decoction treatment groups, so as to compare the expression of differentially expressed genes among the three groups and analyze the functions and relevant signaling pathways of these genes [24]. Their results indicated that Qushi Huayu decoction may play a role in NAFLD intervention through immune, metabolic, and cancer signaling pathway-related genes, and through signaling pathways (e.g., extracellular matrix-receptor interactions, mitogen-activated protein kinases signaling pathway, and PI3K-AKT signaling pathway).

Multi-omics integration

Omics technologies such as genomics, proteomics, metabolomics, and transcriptomics can be used to describe vital processes/activities within different layers of cells. Data obtained through different omics technologies can be integrated on the basis of complex pathways and network connections. By exhibiting the regulatory relationships

among these data through organization, statistical analysis, and calculations, the mechanisms of action of CHMs in disease treatment may be elucidated [25].

NP combined with metabolomics

NP can be used to analyze the relationships among drugs, targets, metabolic pathways, and diseases through the construction of network models. This enables prediction of the effects of active components on certain key targets and their pathways. Metabolomics based on HPLC-MS may aid in the discovery of biomarkers in biological systems and can be used to focus on changes in molecular compounds, thereby providing an experimental basis for investigating the metabolic mechanisms of CHMs in diseases. The classical TCM formula Huanglian decoction originated from the *Treatise on Cold Pathogenic Diseases* (written by Zhongjing Zhang, Eastern Han Dynasty, 25–220 C.E.) and is composed of Huanglian (*Coptidis rhizoma*), Gancao (*Glycyrrhizae radix et rhizoma*), Ganjiang (dried *Zingiberis rhizoma*), Guizhi (*Cinnamomi* ramulus), and Renshen (*Ginseng radix et rhizoma*). A metabolomics study in rats with T2DM conducted by Pan et al. revealed that Huanglian decoction regulated biomarkers such as cytosine, L-carnitine, betaine, phenylalanine, glucose, citrate, phenylpyruvate, and hippuric acid in glyoxylate and dicarboxylate metabolism, phenylalanine metabolism, and the tricarboxylic acid cycle [26]. Huanglian decoction promotes blood glucose control by regulating gene and protein expression levels of glucose transporter 4, insulin receptor, and mitogen-activated protein kinase 1 to interfere with glyoxylate and dicarboxylate metabolism, which is consistent with the prediction results obtained by NP. Meng et al. adopted a combination of NP and metabolomics to investigate the mechanisms of action of Jowiseungki-tang in the treatment of mice with high fat diet-induced obesity [27]. Results of their metabolomics analysis showed that Jowiseungki-tang strongly inhibited the expression of inducible nitric oxide synthase, inflammatory proteins, and cyclooxygenase-2 in the pancreas, stomach, and liver tissues, and reduced hepatic steatosis and pancreatic hyperplasia. Based on NP analysis, it was deduced that the functional targets of Jowiseungki-tang modulated cofactor-, coenzyme- and fatty acid-bonding, insulin resistance, and the inflammatory response, thereby regulating phosphatase binding and activation of signaling pathways of mitogen-activated protein kinases, phosphatidylinositol 3-kinases/protein kinase B, protein kinase C, and receptor for advanced glycation end products. Results of metabolomics analysis revealed that the contents of medium- and long-chain fatty acids and energy metabolites in the pancreases of the mice were significantly altered. In a study by Wang et al., NP was combined with metabolomics to explore the mechanisms of action of Shenyan Kangfu tablet (Tianjin Tongrentang Pharmaceutical Co., Ltd., Tianjin, China, batch number: IP17396) in the treatment of diabetic nephropathy and to perform a qualitative study on the chemical components of Shenyan Kangfu tablet and search for potential targets through NP based on the characterized chemical components [28]. Subsequently, the metabolic profiles of urine samples of the model mice were analyzed using UHPLC-QTOF-MS, and biomarkers were identified using multivariate statistical analysis. Finally, the results obtained by metabolomics and NP were jointly analyzed. When the corresponding targets of the qualitatively identified components of Shenyan Kangfu tablet were searched for in the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform, a total of 36 potentially active components and 160 targets related to diabetic nephropathy were obtained. 38 biomarkers were found through metabolomics based on UHPLC-QTOF-MS. Joint analysis of the metabolomics and NP results revealed the presence of two overlapping targets, namely hexokinase 2 and maltase glucoamylase. This means that these two targets were not only targets related to pathways involving potential biomarkers in metabolomics, but were also disease-drug intersection targets identified by NP.

16S rRNA sequencing combined with transcriptomics

Transcriptomics is typically utilized for the dynamic representation of transcription status in the entire genome. However, studies based solely on transcriptomics are unable to provide a complete characterization of biological systems. With the combination of transcriptomics with 16S rRNA sequencing, intersecting pathway analysis can be performed on the results obtained using the two omics technologies. Consequently, issues stemming from the inability of a single omics technology to comprehensively reflect biological characteristics are effectively resolved and the mechanisms of action of drugs can be investigated in depth, which confers greater credibility to the obtained results. Han et al. combined liver transcriptomics with 16S rRNA gene sequencing to investigate the mechanisms of action of Simiao powder in the treatment of NAFLD [29]. Liver transcriptomics was used to enrich differentially expressed genes and to predict regulatory pathways after gene set enrichment analysis, and 16S rRNA gene sequencing was used to determine microbial composition. The results demonstrated that Simiao powder downregulated the biosynthesis of fatty acids, stimulated the insulin secretion pathway, and significantly altered the gut microbiota composition, with a particular increase in the proportion of *Akkermansia muciniphila*. Du et al. characterized a spleen transcriptome by RNA sequencing and investigated gut microbiota composition by 16S rRNA analysis [30]. Results indicated that berberine inhibited interphotoreceptor retinoid-binding protein-induced experimental autoimmune uveitis, which was associated with significant changes in the spleen transcriptome and intestinal microbiota composition.

16S rRNA sequencing combined with metabolomics

16S rRNA sequencing enables accurate identification of the types and abundance of microbes in the gut, and metabolomic techniques provide unique and novel approaches for disease and pharmacodynamic characterization which assist in systematic analyses of changes in metabolites. Therefore, the combination of 16S rRNA sequencing and metabolomics analysis allows for the mechanisms of disease treatment by CHMs to be explained at the gut microbiota and metabolic levels [31]. The classical TCM formula Dachaihu decoction, which originated from the *Essentials from the Golden Cabinet* (written by Zhongjing Zhang, Eastern Han Dynasty, 25–220 C.E.), consists of Chaihu (*Bupleuri radix*), Huangqin (*Scutellariae radix*), Dahuang (*Rhei radix et rhizoma*), Zhishi (*Aurantii fructus* immaturus), Banxia (*Pinelliae rhizoma*), Baishao (*Paeoniae radix* alba), Dazao (*Jujubae fructus*) and Shengjiang (*Zingiberis rhizoma* recens). Cui et al. employed a combination of 16S rRNA sequencing and untargeted metabolomics to investigate the effects of Dachaihu decoction on the microbiota and serum metabolites in NAFLD model rats [32]. Their results indicated that Dachaihu decoction may alleviate NAFLD through the regulation of gut microbiota and the improvement of metabolic pathways related to the regulation of amino acids, glycerophospholipids, and glycine/serine/threonine. In a study by Li et al., 16S rRNA sequencing was combined with liver metabolomics to explore the mechanisms of action of Yinchenhao decoction in the treatment of NAFLD [33]. It was found that Yinchenhao decoction attenuated the imbalances in the diversity of *Firmicutes*, *Bacteroidetes*, *Actinomycetes*, and *Pseudomonadota* present in NAFLD model rats, with significant back-regulation exerted on 16 species. Thirteen liver metabolites related to treatment were identified, and the three metabolic pathways with the highest degree of involvement were glycerophospholipid metabolism, purine metabolism, and glutathione metabolism. Mutual relationships were also found between gut microbial species with strong correlations and potential biomarkers.

16S rRNA sequencing combined with NP and metabolomics

16S rRNA sequencing enables accurate determination of the types and abundance of microbes in the gut, NP involves comprehensive pharmacological and bioinformatics analyses through the prediction of potential targets, and metabolomics allows for the analysis of active metabolites of the various components of CHM that exert therapeutic effects after their absorption into the bloodstream. The combination of 16S rRNA sequencing, NP, and metabolomics may provide a means for

achieving a comprehensive and systematic understanding of the complex processes by which diseases are treated with CHMs. Gao et al. designed the Qijian mixture, a conceptual TCM formula using four CHM ingredients, namely Huangqi (*Astragali radix*), Guijianyu (*Euonymi* ramulus), Huanglian (*Coptidis rhizoma*), and Gegen (*Puerariae radix*), which were selected through data analysis [34]. The effects of Qijian mixture on T2DM were subsequently evaluated using a combination of metabolomics, gut microbiota and NP. A ^{1}H-NMR-based metabolomics approach was adopted to determine changes in metabolites in the Qijian mixture treatment group. Fifty-five Qijian mixture-related proteins and 4 Qijian mixture-related signaling pathways were explored, including galactose metabolism, valine, leucine, and isoleucine degradation metabolism, aminoacyl-tRNA biosynthesis metabolism, and alanine, aspartate and glutamate metabolism pathways. Principal coordinate analysis of gut microbiota revealed that treatment with Qijian mixture led to a profound enrichment of *Bacteroidetes*. In addition, the NP paradigm showed that Qijian mixture exerted its therapeutic effects through the TP53, AKT1, and peroxisome proliferator-activated receptor-alpha proteins. The classical TCM formula Baihu Jia Renshen decoction originated from the *Treatise on Cold Pathogenic Diseases* and is composed of Zhimu (*Anemarrhenae rhizoma*), Shigao (*Gypsum fibrosum*), Gancao (*Glycyrrhizae radix et rhizoma*), Gengmi (*Oryzae semen*), and Renshen (*Ginseng radix et rhizoma*). Meng et al. evaluated the mechanism of action of Baihu Jia Renshen decoction in the improvement of symptoms in mice with diabetic nephropathy through a combined metabolomics, gut microbiota, and NP approach [35]. A metabolomics test using serum led to the identification of profoundly altered metabolites in the Baihu Jia Renshen decoction treatment group. Thirty-six Baihu Jia Renshen decoction-related proteins and four Baihu Jia Renshen decoction-related signaling pathways were explored, including valine, leucine, and isoleucine biosynthesis, nicotinate and nicotinamide metabolism, tryptophan metabolism, and alanine, aspartate, and glutamate metabolism pathways. Results of Principal coordinate analysis showed that treatment with Baihu Jia Renshen decoction significantly affected gut microbiota composition. In addition, NP analysis revealed that Baihu Jia Renshen decoction exerted its effects through PI3K/Akt and mitogen-activated protein kinases-related protein targets.

Conclusion and outlook

Omics technologies are novel disciplines in life science research that involve the study of the constitution of all components (e.g., genes and proteins) in a biological system and the interactions of these components under specific conditions. In omics technologies, systematic analytical methods are adopted, and the study subject is viewed as a single, integrated entity, which is consistent with the holistic principle underlying TCM and which is also in agreement with the approaches employed in TCM research. Through genomics, proteomics, metabolomics, and other high-throughput omics platforms, a massive amount of data and information can be comprehensively acquired for subsequent processing via bioinformatics and mathematical methods to identify pertinent patterns and elucidate the hidden secrets of organisms. As the majority of CHMs have complex compositions and interact with diverse targets, the holistic approach of TCM and specific mechanisms of action of CHMs cannot be adequately explained by single pathways. Therefore, the integration of omics in studies and the utilization of comprehensive multi-omics analyses for multi-perspective and multi-faceted exploration of mechanisms of action of CHMs is beneficial for promoting the widespread application of CHMs in clinical practice and is of great significance in terms of driving the modernization of TCM.

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