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Academic Exploration

Integration of Gut Microbiota and Metabolomics for Chinese Medicine Research: Opportunities and Challenges*

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ABSTRACT Chinese medicines (CM) are gaining more attentions from all over the world. However, there are a large body of questions to be answered because of the chemical complexity of CM and intricate molecular reactions within human body. In recent years, gut microbiota and metabolomics have emerged as two cynosures in deciphering the mechanisms of how our body is functioning. Since gut microbiota and host is a closely interrelated system, paying attention only to gut microbiota or metabolites may omit the interplays among CM, gut microbiota, and hosts. To systemically study these interplays, a network understanding of CM components, gut microbiota, metabolites of gut microbiota, metabolites in human body is necessary. Although there are some obstacles impeding the application of this integrative approach, the potential areas for implementation of the integrative approach is vast. These areas include, but not limited to, elucidating the mechanisms of CM at system level, screening bioactive compounds in CM, and guiding quality control of CM.

KEYWORDS gut microbiota, metabolomics, Chinese medicine, herbal medicines

In China and its surrounding countries, both chemical drugs and CM are extensively used in clinic. Compared with chemical drugs, CM has advantages in treating chronic diseases such as fatigue.[1] In addition, CM exhibit several unique characteristics. Many CM compounds exhibit poor oral bioavailability. For example, the oral bioavailability of berberine is less than 1%,[2] however, it still possesses strong cholesterol-lowering effects in clinic.[3] The material bases of the therapeutic effects of most CM remain unknown, and this conundrum between low bioavailability and efficacy of CM has perplexed researchers for a long time.[4,5] Meanwhile, CM are usually used as formulas that contain multiple herbs and numerous compounds acting in a synergistical and holistic fashion to treat diseases.[6] Correspondingly, it is almost impossible to use a few targets to completely describe the mechanisms of CM. As a result, the active compounds and mechanisms of CM are usually unknown. The ambiguity of active compounds also makes it rather difficult to set proper quality standards for CM. This status quo of CM has limited the rational use of CM in clinic and acceptance of CM in the world.

Gut Microbiota, a New Frontier to Understand CM

Gut microbiota, or intestinal microbiota, gut flora, intestinal flora, is a large and diverse group of microorganisms that dwell in the gastrointestinal tract of human body. Gut microbiota can metabolize intestinal contents and produce a variety of new metabolites that contribute to the homeostasis of host. The interplay between gut microbiota and human body can also shape the development of diseases that includes, but not limited to, allergy,[7] hypertension,[8] colitis,[9] type 2 diabetes mellitus, and non-alcoholic fatty liver disease.[10] Most CM are administrated orally to achieve the therapeutic effects, they will inevitably contact with gut microbiota. As a result, gut microbiota could influence the therapeutic and toxic effects of CM by changing the structure of CM compounds.[11,12] Gut microbiota could transform ginsenosides such as ginsenoside Rb1, Rc, Rb2, Rb3, and Rd to compound K, a metabolite that exhibits stronger anti-tumor effects

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than its parent compounds.[13] In addition, some studies also demonstrated the indispensable role of gut microbiota in achieving the therapeutic effects of CM. Using horizontal fecal microbiota transplantation, antibiotic treatment, and 16S rDNA-based microbiota analysis, Wu, et al[14] found out that *Parabacteroides goldsteinii* plays a predominant role in the anti-obesity effects of *Hirsutella sinensis* polysaccharides. Because of the important role of gut microbiota in maintenance of health and achievement of the biological effects of CM, gut microbiota has become a new frontier to study CM.

Metabolomics, a Useful Tool for Studying CM

Metabolomics has raised as a hot topic in life science over the past decade. So far, a battery of hyphenated analytical platforms for metabolomics have been fully developed such as gas chromatography, high-performance liquid chromatography, ultra-performance liquid chromatography (UPLC), capillary electrophoresis combined with nuclear magnetic resonance spectroscopy or mass spectrometry.[15] Based on experimental purposes, metabolomics can be classified into targeted and untargeted metabolomics. According to the sample types, metabolomics can be classified as plant metabolomics, plasma metabolomics, fecal metabolomics, and food metabolomics, etc.

As a tool in the post-genomics era, metabolomics is an omics approach to comprehensively analyze all the metabolites in a biological system, and to elucidate the complex interaction of components.[16,17] By analyzing various biological matrices such as urine, plasma, feces, or biopsies from host, microbes, and their co-metabolism, metabolomics enables personalized health care, identification of risk biomarkers, discovery of new targets, and characterizing of microbial metabolism.[16,18] Sharing similar holistic thinking of CM by comprehensively characterizing the function and metabolic changes of whole organisms, metabolomics is believed to have great potential to expand our understanding on CM application.[19] In the past decade, researchers have used this method to study CM and yielded fruitful achievement in areas such as quality control, bioactive component screening, and mechanism study of CM.[20,21] Undoubtably, the work on metabolomics has and will continue to help us understand and use CM.

Need for Integration of Gut Microbiota and Metabolomics

Over the past two decades, a diverse range of metabolites associated with gut microbiota have been identified. These metabolites can be broadly grouped into 3 groups: (1) produced by gut microbiota directly from dietary components (and drugs); (2) secreted by host and modified by gut microbiota; (3) synthesized by gut microbiota *de novo*.[22,23] Metabolites such as indole derivatives and polyamines are produced by gut microbiota from dietary components; metabolites such as secondary bile salts and taurine are secreted by host and modified by gut microbiota; metabolites such as ATP and polysaccharide A are *de novo* synthesized metabolites.[22,24] For CM, the interactions between CM and gut microbiota lead to the change of the content of gut microbiota metabolites such as short chain fatty acids (SCFAs), and transformation of CM compounds to metabolites of different bioactivates and toxicities.[25] These metabolites act as shuttling information that links gut microbiota and host, and gut microbiota could thereupon act up target organs to modulate the homeostasis of host.[24] Correspondingly, integrating the gut microbiota and metabolomics is crucial and necessary for comprehensive understanding of the interactions between gut microbiota and hosts at system level.[16,26]

Besides, paying attention only to metabolites or gut microbiota may omit the associative or causal relationship between CM compounds, gut microbiota, and the response of human body. Although genetic analysis of gut microbiota is invaluable for understanding the contribution of microorganisms to hemostasis or progress of diseases, it offers nothing about the actual activity of the microbial community, or how it interacts with the hosts at system level.[16] Similarly, paying attention only to gut microbiota could not answer the question of what compounds are associated with the curative effects of CM. Holistic profiling of metabolites could provide direct functional readout of cellular state and are easier to correlate with phenotype.[27] However, it cannot directly trace which kind of microorganisms should be responsible for the changing of metabolites considering the large number of metabolites and microorganisms. Therefore, paying attention only to gut microbiota or metabolomics would omit important information in other dimensions and the association between CM compounds, gut microbiota, and human body.

Furthermore, compared with single dataset generated from gut microbiota analysis or metabolomics, multiple datasets of different kind of information could help infer missing information.[28] Due to the limitation of extraction methods, detection parameters and types of equipment, many omics techniques such as UPLC-quadrupole-time of flight mass spectrometry (UPLC/Q-TOF-MS) based metabolomics are inherently flawed. Incorporating different detection methods, such as plant metabolomics and fecal metabolomics, could infer the missing metabolites in fecal samples. Similarly, integrating data information derived from different molecular types could enhance the statistical power considering that the data information from one molecular level can adjust the data information from another level.[28] Therefore, integration of gut microbiota and metabolomics is a necessary approach to investigate the interplay among CM, gut microbiota, and human body. Examples beyond CM research area using this integrative approach has allowed identification of beneficial and detrimental gut microorganisms and metabolites.[29-38] This integrative idea has been embraced and practiced in CM by some researchers with some certain successful cases, guiding us to explore deeper into this ancient medical system.[39-43]

Currently, integration of other types of omics techniques such as genomics, transcriptomics, and proteomics has been widely practiced to study the mechanisms of how our body functioning in life science. In CM research area, other omics techniques such as transcriptomics and proteomics are also used in CM research. It seems that integration of these techniques is necessary for studying the mechanisms of CM at system level. Du, et al[44] integrated metabolomics and transcriptomics to study the mechanisms of Baoyuan Decoction (保元汤). However, data generated in those studies are derived from animals or humans, but not gut microbiota, which is different from the data discussed here (generated from both gut microbiota and hosts). In fact, the human body and gut microbiota are two different types of (interrelated) life entities each with their own genes, protein, and metabolites. Correspondingly, on the one hand, integration of genomics, transcriptomics, and proteomics can be used to study the two entities separately;[45,46] on the other hand, integration of information from both systems is needed to study the interplay between the two entities. Since metabolites is the main compounds that link the gut microbiota and hosts, we believe that integration of gut microbiota (mainly taxonomic information) and metabolomics is fundamentally needed to study CM although other types of integration such as integration of human proteomics and gut microbiota can be used. In addition, unlike genes and proteins whose functions are susceptible to epigenetic regulation and post-translational modifications, metabolites are a series of small molecules that provide direct functional readout of cellular state and are easier to correlate with phenotype.[27] Therefore, integration of gut microbiota and metabolomics is more practical and meaningful as compared with other types of integration.

The method to integrate the datasets of gut microbiota and metabolites is undoubtably the core issue of the integrative approach considering the complexity and the enormity of data obtained from different resources. And the development of algorithms and integrating platforms specialized for integrating information of gut microbiota and metabolites are still in inceptive stage.[47] Nevertheless, progress in development of techniques used for integration of those data has been made. Noecker, et al[48] introduced a framework to integrate the metabolomic variation with community composition of gut microbiota using taxonomic, genomic, and metabolic information. McHardy, et al[49] used Spearman correlations to identify the microbiota-metabolite relationships. In an effect to examine the bacterial fermentation in respiratory tract, Whiteson, et al[50] used linear models to identify the explanatory ability of *Streptococcus*, *Rothia* and *Pseudomonas* on 2,3-butanedione concentration in cystic fibrosis patients. Using non-metric multidimensional scaling analysis, Antharam, et al[29] identified 63 gut bacteria that were associated with high levels of fecal coprostanol and two gut bacteria were correlated with low fecal coprostanol levels. In fact, although there are only a few specialized methods for integrating the data derived from gut microbiota and metabolomics, a relatively large number of integrating methods with the ability to integrate different types of datasets have been developed, and these methods can be applicable to other omics data with minor modifications.[47,51] In practice, a good understanding of the integrating algorithms is crucial for correct application of the integrative approach.

According to the aim of most CM studies, we

present here a scheme for integration of information from gut microbiota and metabolites (Figure 1). Layer 1 is the information of constitutional compounds of CM obtained by plant metabolomics. Layer 2 is the gut microbiota information that mainly describe the taxonomic profiles of gut microbiota obtained from 16S rRNA sequencing or other types of information such as gene information obtained from whole-metagenome sequencing. Layer 3 is the metabolite information of gut microbiota obtained by fecal metabolomics. Layer 4 is the metabolite information of human body obtained by plasma/serum metabolomics or other types of metabolomics such as urine metabolomics. Choosing appropriate information at different layers according to the aim of study is the first step toward integration process. For example, integration of information at layers 1, 2, and 3 is needed to reveal the influence of CM on metabolism of gut microbiota. To holistically reveal the mechanisms of a CM compound at system level, integration of information at layer 2–4 is needed.

Application Fields of Integrative Approach

It's easy to imagine that the direct application fields of the integration approach are screening bioactive compounds and paving the way towards better understanding of the mechanism of CM at system via construction of networks linking the CM compounds, gut microbiota, and hosts at molecular level. In practice, the application fields might stretch beyond these two fields. Focusing on gut microbiota and metabolites, we depict the possible fields that involve the integrated approach (Figure 1, lower panel). Here we take the possible application field, quality control of CM, which has long been an important issue, as an example. Traditional approach for quality control of CM emphasizes the content of a certain compounds in CM products or the similarity of chemical fingerprint.[52] However, this approach seems to omit the fact that those detected compounds

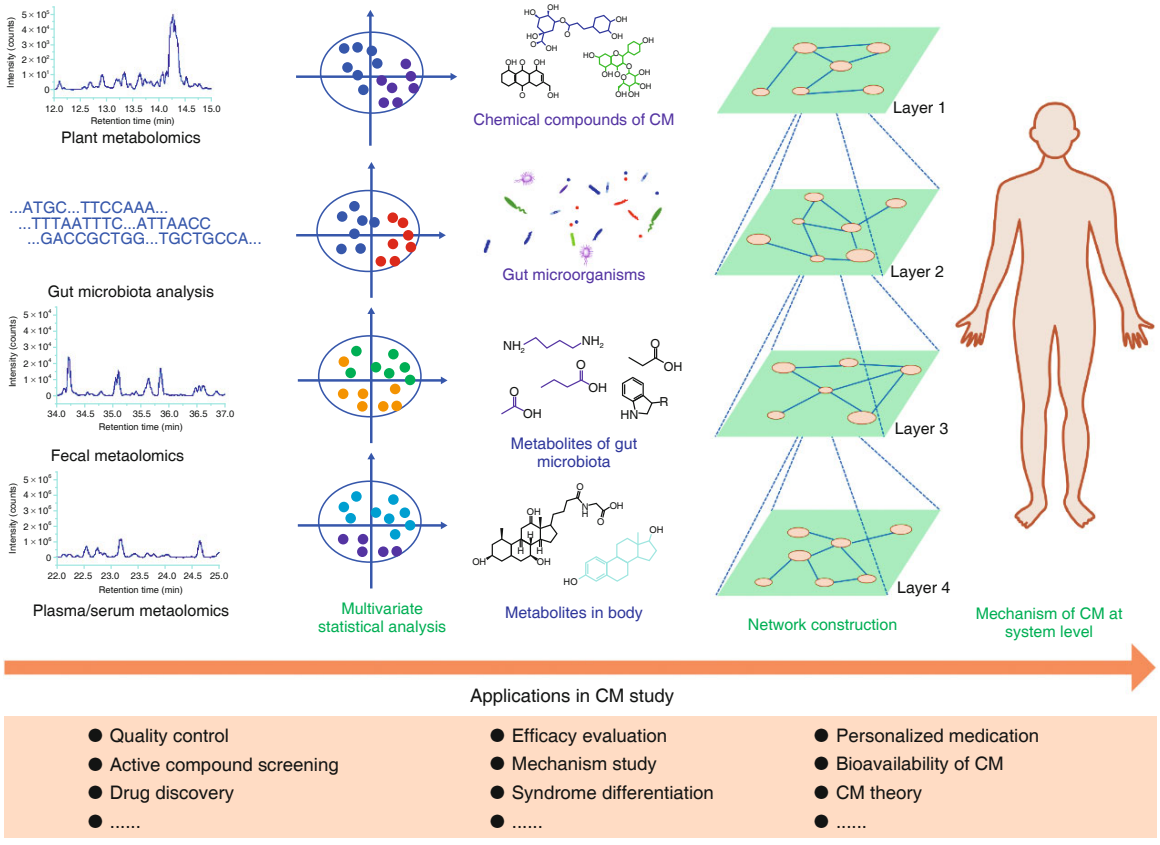

Figure 1. Integration of Gut Microbiota and Metabolomics at Different Layers Reveals the Material Bases and Mechanisms of CM at System Level

Notes: Using multivariate statistical analysis, each detection method generate key elements such as chemical compounds in CM and taxonomic information of gut microbiota that are useful for integration. In practice, different networks might be constructed according to the aim of study, such as plant metabolites-gut microbiota-gut microbiota metabolites network and plant metabolites–gut microbiota network. The integrative approach can be used broadly in CM research areas that include, but not limited to, active compound screening, quality control and mechanism study of CM

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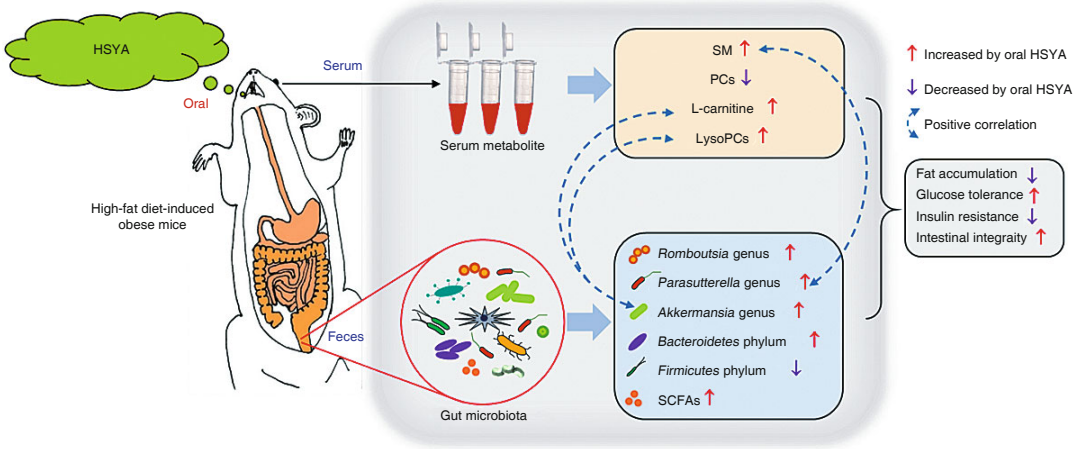

Figure 2. Combination of Serum Metabolomics and Gut Microbiota Reveals the Effect of HSYA in Obese Mice
Notes: SCFAs: short-chain fatty acids; SM: sphingomyelin; PC: phosphatidylcholine; HSYA: hydroxysafflor yellow A

might not be the active compounds *in vivo* and the active compounds might be the compounds that are transformed by gut microbiota.[53] Therefore, a possible solution to address this issue is to find out the gut microbiota transformed active compounds by integrating fecal metabolomics and plasma/serum metabolomics. And the precursor of gut microbiota transformed active compounds such as the aforementioned compounds ginsenosides can be used to control the quality of CM. Similarly, other possible application fields of this approach could be envisioned.

Our previous study has used this strategy to study the mechanism of hydroxysafflor yellow A (HSYA) on obese mice.[40] HSYA is a water-soluble monomer isolated from *Carthamus tinctorius* L. We used untargeted UPLC-Q-TOF/MS to study the serum metabolites, targeted GC-MS to study the SCFAs in caecal content, and 16S rRNA sequencing method was used to study the gut microbiota. Targeted metabolomics showed that HSYA increases SCFAs production in high fat diet-fed mice, and untargeted metabolomics showed that HSYA increases lysophosphatidylcholines, L-carnitine and sphingomyelin, and decreases phosphatidylcholines in obese mice. Sequencing of gut microbiota showed that HSYA increases SCFAs-producing bacteria, including genera *Butyricimonas* and *Alloprevotella*. To further analyze the relationship between serum metabolites and gut microbiota, Spearman's correlation coefficient was used to obtain a correlation matrix. We found out that 5 bacterial strains, including phylum *Verrucomicrobia*, genera *Akkermansia*, *Butyricimonas*, *Parasutterella*, and *Romboutsia*, are positively correlated with 8 lysoPCs. The correlation between bacterial strains and metabolites provide a reference for further researching the mechanisms of HYSA at system level (Figure 2).

Challenges of Integrative Approach

Although integration of metabolomics and gut microbiota give us a more holistic description of the reactions within a biological system, integration of different kind of datasets from two distinctive data-types remains a core issue to be tackled.[54,55] The first reason for the challenges is lies in the actions within/between gut microbiota and metabolites themselves. For instance, the change of gut microbiota and metabolites in body might happen in different time-scale, and the content change of metabolites might be resulted not from the interaction among gut microbiota, hosts, and medicines, but from the interactions between different gut microbiota species. The second reason is associated with the algorithms used to integrate the datasets. Pearson and Spearman correlations are traditional correlation methods that enable pairwise correlation between different omics datasets, however, it is prone to generate false positive correlations because of high dimensionality datasets. In fact, because the datasets usually contain too many of the metabolites and microorganisms, it would be inevitable to generate significant correlations by chance.[56] Furthermore, even though the correlations between the datasets were founded, it is still necessary to establish the causal or correlative relationship. Whatever challenges we would face, the potential for integration of gut microbiota and

metabolomic data is promising with the development of algorithms and cautious measures were taken.

Comments on Future Studies

Targeted or untargeted, it is a choice that necessitates full consideration before initiation of metabolomic analysis. Although metabolomics aims at depicting metabolite landscape in a given system, in practice, metabolomics is divided into two strategies: targeted and untargeted metabolomics. Targeted metabolomics focus on the concentration of a set of chemically characterized and biochemically annotated metabolites,[57] while untargeted metabolomics is the global profiling of metabolites as many as possible without a priori knowledge of metabolites.[58] Untargeted metabolomics offers an unbiased method to examine the metabolites even though it is not possible to gain all metabolites simultaneously because of metabolite recovery and that some metabolites remain unannotated in databases.[59] Targeted metabolomics can obtain the exact concentrations of metabolites identified by untargeted metabolomics and thus act as an analytical validation role.[60] Therefore, according to the classes of interest metabolites and the aim of studies, the choice of targeted or untargeted metabolomics (or both methods), needs to be considered before initiation of work.

Further validation is needed to strengthen the integrative results. It has been noticed that many integrative algorithms generate false results across multivariate data sets especially in huge data. Since the integrative approach is highly depend on statistical analysis, the results can be false positive. Correspondingly, a further validation step is a requisite to strengthen the integrative results. In this step, germ-free animal, gnotobiotic animal (mono-colonized or with defined microbial communities), culturomics (a culturing approach for the identification of bacterial species),[61] and fecal microbiota transplantation can be used. Using fecal transplantation and antibiotic treatment, Wu, et al[14] proved that *Parabacteroides goldsteinii* is the predominant bacteria that responsible for anti-obesity effects of polysaccharides isolated from *Hirsutella* sinensis. By *in vitro* culturing, *in vivo* animal studies, and targeted metabolomics, Wang, et al[62] found out that berberine could improve energy metabolism by increasing butyrate and the abundance of butyrate-producing bacteria. These studies provided excellent examples for researchers to validate the integrative results.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author Contributions

Peng C conceived and proposed the idea; Feng WW designed, wrote and revised the manuscript; Liu J and Cheng H wrote and revised the manuscript.

REFERENCES

1. Luo C, Xu X, Wei X, Feng W, Huang H, Liu H, et al. Natural medicines for the treatment of fatigue: bioactive components, pharmacology, and mechanisms. Pharmacol Res 2019;148:104409.
2. Chen W, Miao YQ, Fan DJ, Yang SS, Lin X, Meng LK, et al. Bioavailability study of berberine and the enhancing effects of TPGS on intestinal absorption in rats. Aaps Pharmscitech 2011;12:705-711.
3. Kong W, Wei J, Abidi P, Lin M, Inaba S, Li C, et al. Berberine is a novel cholesterol-lowering drug working through a unique mechanism distinct from statins. Nat Med 2004;10:1344-1351.
4. Chen F, Wen Q, Jiang J, Li HL, Tan YF, Li YH, et al. Could the gut microbiota reconcile the oral bioavailability conundrum of traditional herbs? J Ethnopharmacol 2016;179:253-264.
5. Zhang F, He F, Li L, Guo L, Zhang B, Yu S, et al. Bioavailability based on the gut microbiota: a new perspective. Microbiol Mol Biol Rev 2020;84:e00072-19.
6. Yue SJ, Liu J, Feng WW, Zhang FL, Chen JX, Xin LT, et al. System pharmacology-based dissection of the synergistic mechanism of Huangqi and Huanglian for diabetes mellitus. Front Pharmacol 2017;8:694.
7. Simonyté Sjödin K, Hammarström ML, Rydén P, Sjödin A, Hernell O, Engstrand L, et al. Temporal and long-term gut microbiota variation in allergic disease: a prospective study from infancy to school age. Allergy 2019;74:176-185.
8. Marques FZ, Mackay CR, Kaye DM. Beyond gut feelings: how the gut microbiota regulates blood pressure. Nat Res Cardiol 2018;15:20-32.
9. Zhu W, Winter MG, Byndloss MX, Spiga L, Duerkop BA, Hughes ER, et al. Precision editing of the gut microbiota ameliorates colitis. Nature 2018;553:208-211.
10. Danneskiold-Samsøe NB, Barros HD, Santos R, Bicas JL, Cazarin CB, Madsen L, et al. Interplay between food and gut microbiota in health and disease. Food Res Int 2019;115:23-31.
11. Feng W, Ao H, Peng C, Yan D. Gut microbiota, a new

frontier to understand traditional Chinese medicines. Pharmacol Res 2019;142:176-191.
12. Feng W, Liu J, Ao H, Yue S, Peng C. Targeting gut microbiota for precision medicine: focusing on the efficacy and toxicity of drugs. Theranostics 2020;10:11278-11301.
13. Wang HY, Qi LW, Wang CZ, Li P. Bioactivity enhancement of herbal supplements by intestinal microbiota focusing on ginsenosides. Am J Chin Med 2011;39:1103-1115.
14. Wu TR, Lin CS, Chang CJ, Lin TL, Martel J, Ko YF, et al. Gut commensal *Parabacteroides goldsteinii* plays a predominant role in the anti-obesity effects of polysaccharides isolated from *Hirsutella sinensis*. Gut 2019;68:248-262.
15. Jacob M, Lopata AL, Dasouki M, Abdel Rahman AM. Metabolomics toward personalized medicine. Mass Spectrom Rev 2019;38:221-238.
16. Nicholson JK, Lindon JC. Metabonomics. Nature 2008;455:1054-1056.
17. Wishart DS. Metabolomics for investigating physiological and pathophysiological processes. Physiol Rev 2019;99:1819-1875.
18. Vernocchi P, Del Chierico F, Putignani L. Gut microbiota profiling: metabolomics based approach to unravel compounds affecting human health. Front Microbiol 2016;7:1144.
19. Zhang A, Sun H, Wang Z, Sun W, Wang P, Wang X. Metabolomics: towards understanding traditional Chinese medicine. Planta Med 2010;76:2026-2035.
20. Hu C, Xu G. Metabolomics and traditional Chinese medicine. Trend Anal Chem 2014;61:207-214.
21. Zhang AH, Sun H, Yan GL, Han Y, Zhao QQ, Wang XJ. Chinmedomics: a powerful approach integrating metabolomics with serum pharmacochemistry to evaluate the efficacy of traditional Chinese medicine. Engineering 2019;5:60-68.
22. Postler TS, Ghosh S. Understanding the holobiont: how microbial metabolites affect human health and shape the immune system. Cell Metabolism 2017;26:110-130.
23. Feng W, Ao H, Peng C. Gut microbiota, short-chain fatty acids, and herbal medicines. Front Pharmacol 2018;9:1354.
24. Nicholson JK, Holmes E, Kinross J, Burcelin R, Gibson G, Jia W, et al. Host-gut microbiota metabolic interactions. Science 2012;336:1262-1267.
25. Xu J, Chen HB, Li SL. Understanding the molecular mechanisms of the interplay between herbal medicines and gut microbiota. Med Res Rev 2017;37:1140-1185.
26. Knight R, Vrbanac A, Taylor BC, Aksenov A, Callewaert C, Debelius J, et al. Best practices for analysing microbiomes. Nat Rev Microbiol 2018;16:410-422.
27. Patti GJ, Yanes O, Siuzdak G. Metabolomics: the apogee of the omics trilogy. Nat Rev Mol Cell Biol 2012;13:263-269.
28. Choi H, Pavelka N. When one and one gives more than two: challenges and opportunities of integrative omics. Front Genet 2012;2:105.
29. Antharam VC, McEwen DC, Garrett TJ, Dossey AT, Li EC, Kozlov AN, et al. An integrated metabolomic and microbiome analysis identified specific gut microbiota associated with fecal cholesterol and coprostanol in *Clostridium difficile* infection. PLoS One 2016;11:e0148824.
30. De Filippis F, Pellegrini N, Vannini L, Jeffery IB, La Storia A, Laghi L, et al. High-level adherence to a Mediterranean diet beneficially impacts the gut microbiota and associated metabolome. Gut 2016;65:1812-1821.
31. Dumas ME, Barton RH, Toye A, Cloarec O, Blancher C, Rothwell A, et al. Metabolic profiling reveals a contribution of gut microbiota to fatty liver phenotype in insulin-resistant mice. Proc Natl Acad Sci U S A 2006;103:12511-12516.
32. Francavilla R, Calasso M, Calace L, Siragusa S, Ndagijimana M, Vernocchi P, et al. Effect of lactose on gut microbiota and metabolome of infants with cow's milk allergy. Pediatr Allergy Immunol 2012;23:420-427.
33. Sinha R, Ahn J, Sampson JN, Shi J, Yu G, Xiong X, et al. Fecal microbiota, fecal metabolome, and colorectal cancer interrelations. PLoS One 2016;11:e0152126.
34. Spring S, Premathilake H, DeSilva U, Shili C, Carter S, Pezeshki A. Low protein-high carbohydrate diets alter energy balance, gut microbiota composition and blood metabolomics profile in young pigs. Sci Rep 2020;10:1-5.
35. Feng YL, Cao G, Chen DQ, Vaziri ND, Chen L, Zhang J, et al. Microbiome–metabolomics reveals gut microbiota associated with glycine-conjugated metabolites and polyamine metabolism in chronic kidney disease. Cell Mol Life Sci 2019;76:4961-4978.
36. Yu M, Jia H, Zhou C, Yang Y, Zhao Y, Yang M, et al. Variations in gut microbiota and fecal metabolic phenotype associated with depression by 16S rRNA gene sequencing and LC/MS-based metabolomics. J Pharm Biomed Anal 2017;138:231-239.
37. Ni Z, Sun S, Bi Y, Ding J, Cheng W, Yu J, et al. Correlation of fecal metabolomics and gut microbiota in mice with endometriosis. Am J Reprod Immunol 2020:e13307.
38. Xu K, Bai M, Liu H, Duan Y, Zhou X, Wu X, et al. Gut microbiota and blood metabolomics in weaning multiparous sows: associations with oestrous. J Anim Physiol Anim Nutr 2020;104:1155-1168.
39. Li M, Shu X, Xu H, Zhang C, Yang L, Zhang L, et al. Integrative analysis of metabolome and gut microbiota in diet-induced hyperlipidemic rats treated with berberine compounds. J Transl Med 2016;14:237.
40. Liu J, Yue S, Yang Z, Feng W, Meng X, Wang A, et al.

Oral hydroxysafflor yellow A reduces obesity in mice by modulating the gut microbiota and serum metabolism. Pharmacol Res 2018;134:40-50.
41. Luo D, Chen K, Li J, Fang Z, Pang H, Yin Y, et al. Gut microbiota combined with metabolomics reveals the metabolic profile of the normal aging process and the anti-aging effect of FuFang Zhenshu TiaoZhi (FTZ) in mice. Biomed Pharmacother 2020;121:109550.
42. Rong W, Han K, Zhao Z, An J, Li Q, Bi K. The protective effect of *Xanthoceras sorbifolia* Bunge husks on cognitive disorder based on metabolomics and gut microbiota analysis. J Ethnopharmacol 2020;113094.
43. Liu X, Zheng H, Lu R, Huang H, Zhu H, Yin C, et al. Intervening effects of total alkaloids of *Corydalis saxicola* bunting on rats with antibiotic-induced gut microbiota dysbiosis based on 16S rRNA gene sequencing and untargeted metabolomics analyses. Front Microbiol 2019;10:1151.
44. Du Z, Shu Z, Lei W, Li C, Zeng K, Guo X, et al. Integration of metabonomics and transcriptomics reveals the therapeutic effects and mechanisms of Baoyuan Decoction for myocardial ischemia. Front Pharmacol 2018;9:514.
45. Franzosa EA, Hsu T, Sirota-Madi A, Shafquat A, Abu-Ali G, Morgan XC, et al. Sequencing and beyond: integrating molecular 'omics' for microbial community profiling. Nat Rev Microbiol 2015;13:360-372.
46. Romero R, Espinoza J, Gotsch F, Kusanovic JP, Friel LA, Erez O, et al. The use of high-dimensional biology (genomics, transcriptomics, proteomics, and metabolomics) to understand the preterm parturition syndrome. BJOG 2006;113:118-135.
47. Bersanelli M, Mosca E, Remondini D, Giampieri E, Sala C, Castellani G, et al. Methods for the integration of multi-omics data: mathematical aspects. BMC Bioinform 2016;17:S15.
48. Noecker C, Eng A, Srinivasan S, Theriot CM, Young VB, Jansson JK, et al. Metabolic model-based integration of microbiome taxonomic and metabolomic profiles elucidates mechanistic links between ecological and metabolic variation. MSystems 2016;1:e00013-15.
49. McHardy IH, Goudarzi M, Tong M, Ruegger PM, Schwager E, Weger JR, et al. Integrative analysis of the microbiome and metabolome of the human intestinal mucosal surface reveals exquisite inter-relationships. Microbiome 2013;1:1-9.
50. Whiteson KL, Meinardi S, Lim YW, Schmieder R, Maughan H, Quinn R, et al. Breath gas metabolites and bacterial metagenomes from cystic fibrosis airways indicate active pH neutral 2,3-butanedione fermentation. ISME J 2014;8:1247-1258.
51. Huang S, Chaudhary K, Garmire LX. More is better: recent progress in multi-omics data integration methods. Front Genet 2017;8:84.
52. Yang W, Zhang Y, Wu W, Huang L, Guo D, Liu C. Approaches to establish Q-markers for the quality standards of traditional Chinese medicines. Acta Pharm Sin B 2017;7:439-446.
53. Wu X, Zhang H, Fan S, Zhang Y, Yang Z, Fan S, et al. Quality markers based on biological activity: a new strategy for the quality control of traditional Chinese medicine. Phytomedicine 2018;44:103-108.
54. Gomez-Cabrero D, Abugessaisa I, Maier D, Teschendorff A, Merkenschlager M, Gisel A, et al. Data integration in the era of omics: current and future challenges. BMC Syst Biol 2014;8:I1.
55. Palsson B, Zengler K. The challenges of integrating multi-omic data sets. Nature Chem Biol 2010;6:787-789.
56. Alyass A, Turcotte M, Meyre D. From big data analysis to personalized medicine for all: challenges and opportunities. BMC Med Genomics 2015;8:1-2.
57. Roberts LD, Souza AL, Gerszten RE, Clish CB. Targeted metabolomics. Curr Protoc Mol Biol 2012;98:30-32.
58. De Vos RC, Moco S, Lommen A, Keurentjes JJ, Bino RJ, Hall RD. Untargeted large-scale plant metabolomics using liquid chromatography coupled to mass spectrometry. Nat Protoc 2007;2:778-791.
59. Zamboni N, Saghatelian A, Patti GJ. Defining the metabolome: size, flux, and regulation. Mol Cell 2015;58:699-706.
60. Johnson CH, Ivanisevic J, Siuzdak G. Metabolomics: beyond biomarkers and towards mechanisms. Nat Res Mol Cell Biol 2016;17:451-459.
61. Lagier JC, Dubourg G, Million M, Cadoret F, Bilen M, Fenollar F, et al. Culturing the human microbiota and culturomics. Nat Rev Microbiol 2018;16:540-550.
62. Wang Y, Shou JW, Li XY, Zhao ZX, Fu J, He CY, et al. Berberine-induced bioactive metabolites of the gut microbiota improve energy metabolism. Metabolism 2017;70:72-84.

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