

CHAPTER 1

INTRODUCTION OF SYSTEMS BIOLOGY IN TRADITIONAL CHINESE MEDICINE (TCM)

1.1 CHARACTERISTICS AND COMPATIBILITY PRINCIPLES OF TRADITIONAL CHINESE MEDICINE (TCM)

1.1.1 Special Features of Diagnosis and Treatment in TCM

Practitioners of TCM have accumulated valuable experiences in learning about life, improving health and fighting disease in its extensive history of production activities and real life practice. Chinese medicine has a unique theoretical system, rich clinical experience, and scientific ways of thinking. Based on natural sciences and the humanities and social sciences alike, it is a notable medical system in the multitudes of traditional medicines worldwide.

Eastern and Western medicines are obviously different in their treatment theories and drug forms (Fig. 1.1). These are the reflections of the differences and specialties between the East and the West. These differences cause great difficulties not only in medical communication, but also in the recognition of TCM by the Western medical system.

TCM has its unique treatment theory and long-term clinical experience, especially for the diagnosis and treatment of many complex chronic diseases. Meanwhile, it also faces the new challenge of inheritance and innovative development. However, the modernization and internationalization of TCM is the trend of the times. On the other hand, the Western medical system has

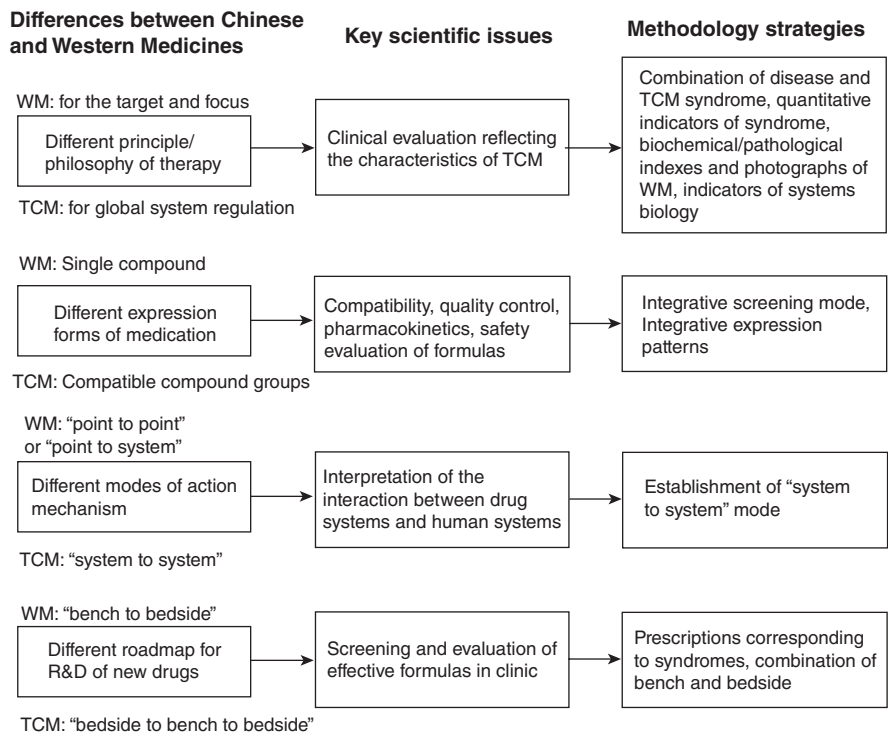


Fig. 1.1 Difference between TCM and Western medicine (WM) systems.

its own problems. Therefore, practitioners of Eastern and Western medicine should learn from each other and make use of others' experiences for reference. Through the conduction of modern research on TCM, for instance, the efficiency of TCM and its scientific value could be proved and further explained by modern scientific language through modern technology and scientific experiments, which will promote the development of TCM and modern medicine and life science as well.

1.1.1.1 Concept of Holism and System Theory The concept of holism is the core idea of TCM theory. TCM has a unique holistic, dynamic, and dialectical theory of the complex phenomena of life and diseases. For instance, it considers the human body to be an organic integrity; the various components of body composition on body structure are indispensable, coordinating in their functions and interacting pathologically with each other. In addition, it advocates "harmony between body and nature," and emphasizes that human activities should be adapted to geographical and seasonal changes, thus maintaining the body's health. Meanwhile, it makes appropriate diagnoses and recommends treatments based on the overall perspective of pathogenesis, location, and potential of diseases, combined with season, geographical aspects, and diet.

As a result, the holistic concept of TCM can be seen in the comprehensive and coordinative functions targeting regulation of many organs of the body. TCM formulas are based on the condition of patients; characteristics of the medication; the taste, functions, and indications of drugs; and relationships of the seven conditions of ingredients in prescription (single effect, mutual assistance, mutual reinforcement, mutual detoxication, mutual antagonism, mutual restraint, and mutual incompatibility). Prescriptions are made according to the principles of compatibility of “*Jun*, *Chen*, *Zuo*, and *Shi*” (roles of ingredients in the prescription with the functions of *Jun*, *Chen*, *Zuo*, and *Shi*, respectively). The mutual restraint, guidance, and synergies in the process of compatibility constitute the overall effect, which is more efficient than the simple combination of each part.^[1] Professor Yongyan Wang, an expert of TCM, has stated that “TCM is not a simple allopath but an integrated regulation from the perspective of multichannels, multilinks, and multifaceted roles in the human body based on TCM formulas and the main procedure of the incidence of disease. Therefore, the human body at different levels, overall, organ, cell, subcell and molecule, can be adjusted effectively.”^[2] Moreover, the holistic concept is reflected in “the unity of medicine and treatment.” TCM formulas originate from the clinical diagnosis and are applied to the clinical diagnosis. Diagnosis, methods, prescription, and drugs are combined through the three steps of “dialectical-legislation-prescription,” and finally unified into a whole. Therefore, “systematic theory” and “holistic” ways of thinking are the essential in TCM theories, which coincide with the mainstream of modern life sciences.

The theory of “*Yin* and *Yang* balance” is the important manifestation of the concept of holism, which guides the development of TCM. In TCM, the human body is considered as a whole unit with the balance of *Yin* and *Yang* keeping a healthy condition. In TCM, we consider a disease to be caused by functional imbalance of *Yin* and *Yang*. There is a dialectical relationship between *Yin* and *Yang*. When *Yin* is too much it will lead to a deficiency of *Yang*, and vice versa (from “A Great Theory on *Yin* and *Yang* of *Su Wen*”). TCM treatment must follow the relationship between *Yin* and *Yang*, which will be regulated until the health recovers. Therefore, TCM medication is very different from the Western type. “Compatibility” is very important in TCM treatment. For example, medication should be adjusted according to the location, time, patient, and syndrome. Such a traditional theory of TCM plays an effective role in clinical diagnosis and treatment. However, its scientific mechanisms still need to be fully understood and validated.

With the changes in the human disease spectrum, the Western medical treatment, which aims at a single target, often fails to fully treat complex diseases. The defects of the reduction of humans to “organs–cells–genes” have become more and more evident. A combination of various interdisciplinary research ideas has also become a biological topic in the forefront of life science research. Therefore, based on the overall concept and system theory ideas, the TCM treatment system has unique advantages in complex chronic disease

diagnosis and treatment. Its theory, technology, and medicine have important practical significance. The development of modern medicine and the influence of life science research will further promote scientific contents and better explanations of TCM.

1.1.1.2 Syndrome Differentiation Treatment and Individualized Treatment Treatment based on syndrome differentiation is characterized in diagnosis and therapy with TCM, in which relevant information about a disease, especially symptoms and signs, at a particular stage, is collected in order to discern the nature of the disease.

In a TCM clinic, doctors collect as many symptoms and signs as possible from the patients by inspection, auscultation and olfaction, interrogation, and palpation of the pulses. However, the body anatomy and biochemical metabolic processes are poorly understood (like a black box in TCM). With thousands of years of clinical practice, functional linkages from the body have been summarized as the Meridian, although it still lacks accurate measurement. After the cause and syndrome of the disease are confirmed, appropriate prescriptions according to previous experience can be used with close observation. The physicians can make some revision on the prescription for the patients according to variation of syndromes and signs.

For individualized treatment, TCM holds that each body is unique due to the physical differences, when confronted by different virus and diseases, various syndromes will naturally form. For the elderly, children, and certain individuals, the dosage will be different from that for others. For instance, the amount of bitter cold gypsum, honeysuckle, and mild *Mahuang* (*Ephedrae Herba*), which have strong effects, should be reduced. Here, the syndrome differentiation and the principle of individual treatment are fully reflected in the course taken.

Therefore, there might be a case for different treatments for the same disease, and the same treatment for different diseases. In recent years, systems biology has discovered that many diseases (especially the complex polygenic diseases) are often attributed to combined results of genetic and environmental factors. There may be a variety of pathogenic factors (mechanisms). As a result, it is possible that the inner basis of individualized treatment principles can be explained in the perspective of systems biology.

1.1.1.3 Theories of Compatibility of Complex Formulas and Prescriptions Corresponding to Syndromes The preparations and usage of TCM are completely different from those of Western medicine. TCM emphasizes “compatibility,” and the prescriptions are adjusted according to the changes of location, time, patients and syndrome, so the body reaches a new balance of *Yin* and *Yang* after the coordination of the overall system. The earliest record available in prescription literature is the *Prescriptions of Fifty-two Diseases* and 13 records of *Huangdi's Inner Classic of Medicine* collected in the spring and autumn period, which summarized the theory of syndrome

differentiation treatment principle and rules, prescriptions, and compatibility, and provided solid theoretical foundation for further development. Zhang Zhongjing of the East Han dynasty wrote the two famous books, *Treatise on Exogenous Febrile Disease* and *Synopsis of Golden Chamber*. Zhang created the syndrome diagnosis theory, combining “theory, principle, formula, and medicine,” and established a close relationship between medical theory and clinical practice, which was regarded as the “ancestor of prescriptions.” *Shen Nong’s Herbal* is the earliest pharmaceutical monograph in China with a total of 365 types of *materia medica*, which proposed medicinal theory of “four properties and five flavors.” It clearly stated that “cold disease should be treated with hot drugs, and vice versa” and the compatibility theory of “seven functional relations for drugs,” providing an important theoretical basis for making prescriptions.

“Prescriptions corresponding to the syndromes” is the principle for TCM formula. For example, *Liuwei Dihuang* pills mainly cure fevers, sweating, weakness at the waist and knees, vertigo, and tinnitus; *Wuling* powders are mainly used in the treatment of urination disorder, vomiting inverse, edema, diarrhea, pale tongue with white and thin fur, and smooth or deep pulse.

“Prescriptions corresponding to the syndromes” not only refers to the interaction between one prescription and one syndrome, but also uses one prescription for many other syndromes and many prescriptions for one individual syndrome. This correspondence may change with time. For instance, when the syndrome changes after the prescription is taken for some time, there would be a gap period between this prescription and the syndrome. At this time, the prescription has to be adjusted based on the syndrome.

1.1.2 Compatibility of Prescriptions

1.1.2.1 Compatibility of “Jun, Chen, Zuo, and Shi” (Monarch, Minister, Assistant, and Guide) in the Prescription The formula of TCM is neither a simple piling up of drugs of the same concoction, nor a simple efficacy pile of various drugs. It is indeed guided by the essence of TCM theory of “concept of holism, syndrome differentiation treatment,” and based on the compatibility principle of “Jun, Chen, Zuo and Shi,” leading to the synergies of drugs, mutual restraint of overall function, and, finally, obtaining attenuated efficiency.

The basic principle of the “Jun, Chen, Zuo, and Shi” was first recorded in *Huangdi’s Inner Classic of Medicine*. The “Jun” herbs are the major ingredients for treatment of the main disease and play a key role in the drug treatment. Their efficacy dominates in the first place. “Chen” herbs are the secondary ingredients, which help Jun herbs in producing therapeutic effect against the main disease and syndrome or for treatment of other diseases. Chen herbs have weaker efficacy compared with the Jun herbs. Zuo herbs are of adjuvant effects, which are divided into three categories according to their functions. These adjuvant drugs can enhance the therapeutic effect of Jun and Chen herbs. Junior drugs are used to eliminate the drug toxicity of Jun and Chen

herbs; anti-adjuvant drugs are contrary to the *Jun* herbs and have enhanced effects in the medication. In the prescription, the efficacy of *Zuo* herbs is weaker than that of *Chen* herbs. *Shi* herbs, the adjusting drugs, play a guiding role in the medication. Moreover, they provide the regulating effect with a minor dosage and mild efficacy.

The *Mahuang* decoction, made from the *Mahuang* (Ephedrae Herba), *Guizhi* (Cinnamomi Ramulus), *Xingren* (Armeniacae amarum Semen) and *Zhigancao* (processed Glycyrrhizae Radix), is the main prescription in the treatment of exogenous cold. When the human body has been bothered by wind chill, sweat excretion is inhibited and the syndrome of no sweat and severe chills can be observed. The function of skin is controlled by the lung, the skin pores are closed, and the lung *qi* cannot be diffused; then cough symptoms appear. In the formula, *Mahuang* can encourage sweat and relieve the exterior to disperse the wind chill, diffuse lung *qi* to relieve cough and panting, and is therefore the *Jun* herb. *Guizhi* can induce sweating and relieve muscle pain, and is the *Chen* herb. *Xingren* can alleviate cough, and is recognized as the *Zuo* herb. *Zhigancao* can adjust the whole features of the herbs in the formula and is the *Shi* herb. With the mutual coordination and restraint of these herbs, the *Mahuang* decoction is used in the treatment of cough and asthma.

1.1.2.2 Unity in Prescription Compatibility The principle of the unity of opposites is the fundamental law of materialist dialectics, which not only runs through the basic theory in TCM, but also plays a guiding role in clinical practice. Ancient physicians attached great importance to the compatibility of heat and chills, *Yin* and *Yang*, reinforcement and reduction for complex syndromes. They emphasized the adjustment of chills and heat, complementary of *Yin* and *Yang*, the combination of nutrition and purgation, and the compatibility of promoting and control.

(1) Combination of cold and heat medication: This kind of treatment can be used for a variety of syndromes. For example, in the formula of *Banxiaxiexin* decoction, *Banxia* (Pinelliae Rhizoma) is pungent and warm and can be used to downbear counterflow to suppress vomiting, as a *Jun* herb. Dried ginger is very hot and can cure cold syndromes. *Huangqin* (Scutellariae Radix) and *Huanglian* (Picrorhizae Rhizoma) are bitter and cold and used against fevers as *Chen* herbs together with dried ginger. These four herbs can keep a balance between cold and heat.

(2) Compatibility complementary of *Yin* and *Yang*: Drugs are used for syndromes in deficiency of *Yin* or *Yang*, or both *Yin* and *Yang*. For example, in the formula of *Shibu* pills, *Lurong* (Cervi Pantotrichum Cornu) and *Fuzi* (Aconiti Lateralis Preparata Radix) are *Jun* herbs and have warming effects for *Yin* and *Yang*. *Wuweizi* (Schisandrae Fructus) is acidic and mild, that helps *Jun* herbs to nourish the kidney. *Shudi* (Rehmanniae Preparata Radix), *Shanzhuyu* (Corni Fructus) and *Shanyao* (Dioscorea opposita) can nourish kidney and essence, increase *Yang* from *Yin*, and as *Chen* herbs together with *Rougui*

(Cinnamomi Cortex) can tonify fire, assist *Yang* and conduct fire back to its origin.

(3) Compatibility of nutrition and purgation medication: It is used for syndromes with intermingled deficiency and excess. For instance, in *Liuwei Dihuang* pills, *Shudi* (Rehmanniae Preparata Radix) can nourish *Yin*, the kidneys and essence as a *Jun* herb. *Shanzhuyu* (Corni Fructus) nourishes the liver and kidneys. *Shanyao* (Dioscorea opposita) tonify spleen and *Yin*, and tonify kidney to control nocturnal emissions as *Chen* herbs with *Shanzhuyu*. The three *Jun* and *Chen* herbs are normally called “Three reinforcing herbs.” *Zexie* (Alisma Rhizoma) can remove dampness and turbidity, and control the overnourishing of *Shudi*. *Danpi* (Moutan Cortex) can clear ministerial fire and restrain the warmth and astringent effects of *Shanzhuyu*. *Fuling* (Poria) can eliminate dampness and it can assist not only *Zexie* to remove turbidity of the kidneys but also *Shanyao* in transporting the essence of food and water. These three herbs are called “Three inducing herbs.”

(4) Compatibility of promoting and controlling medication: It can be used normally with drugs for invigorating blood circulation and eliminating stasis as well as with astringents. For the formula of *Wenjing* decoction, *Wuzhuyu* (Evodiae Fructus) and *Guizhi* (Cinnamomi Ramulus), as *Jun* herbs, can warm the meridian to dissipate cold and smooth the circulation of blood. *Danggui* (Angelicae Sinensis Radix) and *Baishao* (Paeoniae Alba Radix) can nourish blood and astringe *Yin*. *Chuanxiong* (Ligustici Wallachii Rhizoma), *Danpi* (Moutan Cortex) and *Ejiao* (Corii Asini Colla) can promote the circulation of blood and nourish blood and *Yin*. The formula can be used for treatment of menstrual disorders, large amounts of bleeding and even infertility.

The complementary principles of the prescriptions were based on the *Yin* and *Yang* balance of human body, physiology and pathology, therapy and the characteristics of drugs, which have crucial clinical significance.

1.1.2.3 Systematic Theory of TCM Formulas The principles of holism, relevance, time-order and dynamics for TCM formulas are based on the idea of diagnosis and treatment, as well as systematic theory. With the aid of modern systematic theory, the TCM formula combines qualitative with quantitative, macro with micro, and global with local investigation. A new theory of the modern system of TCM formula compatibility will be developed.

(1) Holism principle of TCM formulas: The core idea of systems theory is the concept of holism. In TCM, the concept of holism lies in the fact that drugs with opposite and complementary functions can be used based on compatibility to achieve the best therapeutic effect. For example, in the formula of the *Qingkailing* injection, bile acid and deoxycholic acid, as *Jun* herbs, can clear heat, expel miasma, calm the heart and induce resuscitation. Using a Buffalo horn as a *Chen* herb has an effect of protecting the heart and eliminating fever. *Huangqin* (Scutellariae Radix), *Zhizi* (Garbeniae Fructus), *Jinyinhua*

(*Lonicerae Flos*), and *Banlangen* (*Isatidis Radix*) as *Zuo* herbs can clear heat and detoxify. *Zhenzhumu* (*Margaritifera Usta Concha*) used as a *Shi* herb can calm the nerves. As a result, the combination of these eight ingredients, focusing on the core issue of detoxication, can be used for clearing heat, expelling miasma, calming the heart and inducing resuscitation. In the clinic, this formula has been widely used against fever, coma, stroke, paralysis, unconsciousness, acute hepatitis, upper respiratory tract infection, pneumonia, cerebral thrombosis, and cerebral hemorrhage. This formula follows the principle of holism for TCM formula compatibility.

(2) Relevance principle of TCM formulas: Systematic approaches focus on the linkages between the elements and roles. Drug prescription fully reflects the composition and drug interactions. It is not simply a combination of drugs; rather, it is an optimized composition for the specific disease according to the interrelated functions of different causes, locations, the nature of the sickness, and its developing trend. Its diagnosis and treatment are based on the drug's nature, smell, category, effectiveness, and function to finally achieve the desired therapeutic purposes. For example, in the composition of *Liushen* pills, six ingredients—bezoar, realgar, musk, borneol, pearl myogenic, and *Chansu* (*Venenum Bufonis*)—have different functions individually; however, they interact with each other to achieve the overall function of clearing heat and detoxifying, reducing swelling, stopping pain, and promoting tissue regeneration.

(3) Time-order principle of TCM formulas: The time-order principles of TCM formulas can be found in the use of drugs following the roles of “*Jun*, *Chen*, *Zuo*, and *Shi*.” Without the order of these roles in prescriptions, physicians easily lose their logical thinking processes and the prescription cannot achieve an optimized structure. In addition, the cooking of the drugs in prescription follows an order; otherwise, the best efficacy of the formula cannot be achieved. Principles such as “relieving the exterior syndrome and then catharizing,” “supplying deficiency and then expelling excess,” and “ascending the clear and descending the turbid” are typical time-order principles in TCM formulas.

(4) Dynamic principles of TCM formulas: In TCM theory, the balance (or imbalance) of *Yin* and *Yang* are regarded as an orderly (or disorderly) state. Also, the dynamic nature of the prescription is shown by regulating the imbalance between *Yin* and *Yang* with a medication. Furthermore, the prescription has its orderly structure. Change of composition or drugs leads to different functions and therapeutic effects, which might be suitable for other diseases. For instance, the formula of the *Mahuang* decoction for dispersing lung-*qi* and relieving asthma can also be used against exogenous cold. If plaster, ginger, and jujube are added, the formula is called a *Daqinglong* decoction, which can be used against heat, chills, fever, headache, pain, and irritability as well as cold. Without *Guizhi*, it is a formula called *Sanniu* decoction, which can be used against heavy nasal sound, weak voice, cough, and chest tightness.

1.2 KEY SCIENTIFIC ISSUES IN TCM MODERNIZATION

1.2.1 A Comprehensive Evaluation System under the Guidance of the Concept of Holism

For the diagnosis and treatment of disease, Western medicine mainly pays attention to “patient’s disease,” using subtypes to express the characteristics of the patients at different stages. However, TCM emphasizes “patients with disease,” using different types of symptoms to differentiate the individual characteristics and adjusted prescriptions in clinic. Western medicine diagnosis and treatment tend to have a clear target lesion or organ, and use clinical biochemical laboratory or imaging test results to establish clear diagnostic criteria. In TCM, the diagnosis of symptoms lays comparatively far behind that of Western medicine in objectivity, standardization, and quantity. By reviewing the current TCM clinical approaches, we find it is necessary to combine ancient medical research with the current systematic research to achieve the unity of TCM treatment, dialectical characteristics. The evaluation of human health should be more comprehensive and systematic. A feasible way to integrate Eastern and Western treatment is to coordinate systematic concepts and methods based on quantitative evaluation, TCM (syndrome, behavior, and life quality assessment), Western biochemical pathology (clinical biochemistry, imaging, pathologic anatomy, etc.) and systems biology (genomics, proteomics, metabonomics, etc.) evaluation to establish a comprehensive evaluation indicator system.

1.2.2 Key Scientific Issues That Need to Be Addressed in Modern TCM Research

The traditional theories of characteristics, meridian, and compatibility only show some intuitive and analogism instructions on the efficacy of the prescriptions, which cannot reveal the substance, process of the activity, or the exact mechanisms. The traditional TCM theories cannot indicate the material basis for the mechanism and pathology. It has a great limitation in clinical use although it can guide the clinical application of formulas.

TCM formulas can reflect the concepts of holism and syndrome differentiation and treatment. There are complicated interactions between the active components in the formulas and human body. Therefore, the research on the mechanisms and compatibility of herbs must reflect the interaction between two complex systems of TCM and human body, and create a higher system, based on the holistic features of complex prescriptions.

1.2.2.1 Material Basis of TCM Formulas In China, the material basis of TCM formulas is studied through separating active chemical components from formulas with the model of Western medicine. For a single herb, active ingredients are still unclear. There are three main reasons for this. (1) The chemical

composition of TCM formula is very complicated, and a single herb may have a variety of chemicals. Take licorice as an example; to the best of our knowledge so far, it contains 18 types of flavonoids and terpenoids, 22 species and 14 amino acids, alkaloid, coumarin, cinnamic aldehyde, cellulose, starch, salt and 60 other chemicals. (2) Based on integrity of the prescription, clinical efficacy is evaluated with the overall effect on human body. The multiple and complex targets cannot be easily related to their exact active components. (3) Other issues, such as multiple use of one prescription, adjusted complex prescriptions and syndrome differentiation and treatment, make it difficult to reveal the active chemicals by traditional pharmacological methods.

To illustrate the complexity and the integrity of TCM formulas, it is necessary to add a new concept between raw herbs and effective compounds. That is “effective component groups of TCM formulas.”^[3]

The concept of “effective component groups of TCM formulas” refers to the fact that there is a large group of compounds with similar chemical properties. A complicated TCM formula can be divided into several effective component groups (there may be hundreds of chemical components) to illustrate the chemical substance basis of the compatibility of TCM formula with the aid of pharmacodynamics. As a result, we put forward a research strategy including using modern separation methods to extract effective component groups, fingerprint for qualification, indicate components for quantification, and identify the integrity of effective component groups and pharmacodynamics. Based on modern separation methods, herbs in a formula are divided into different effective component groups such as volatile oils, alkaloids, and flavones, or smaller classes. The qualification of effective chemicals can be achieved using the instruments of high performance liquid chromatography (HPLC), capillary electrophoresis (CE), HPLC coupled with mass spectrometry (HPLC-MS), CE coupled with MS (CE-MS) and chromatographic fingerprints. Several effective or indicator components are selected for quantification. Integrated with the pharmacologic activity, these chemicals are further investigated on mechanisms at four different levels (animal, organs, cells, and subcells, and molecular biology). With the information of the activity and compatibility of these component groups and compounds in *Jun*, *Chen*, *Zuo*, and *Shi* herbs, the material basis of TCM formulas can be explained clearly.

Therefore, the research on TCM formulas should start from the concepts of holism and systems biology. The suitable clinical cases are selected based on clinical efficacy. The quantitative pharmacologic models are established and one or more observational indicators are used to evaluate the activity. Botanical chemical separation methods are employed for extraction and separation. Closely integrated with pharmacologic tests, the chemicals and the relationship among activity, quality, quantity and size can be scientifically investigated. In research principles, the methods of integration of traditional TCM theories and modern science and technology, in vivo and in vitro evaluation, organic and inorganic chemicals, and static and dynamic analysis should be established.

1.2.2.2 Quality Evaluation System The quality of TCM formulas should be illustrated since they have complicated components and treatment. The modern quality evaluation system of TCM preparations should follow the material basis of herbs with effective component groups. The methods of fingerprints and quantification of multiple indicator components not only have the feature of integration but also show precise qualification and quantification, which is a suitable model for quality control and evaluation of TCM herbs.

(1) Qualification with fingerprints: For each effective component group, modern separation and analysis methods such as HPLC are used to get chromatogram containing dozens of chemical components. HPLC-MS (MS), CE-MS and other advanced identification methods are combined to identify the chemical constituents. The molecular structures of the peaks can possibly be studied by MS/MS. HPLC chromatogram can be considered as a fingerprint. Further investigation can be carried out with the comparison of fingerprints of some effective component groups and the original single herb. Novel compounds can be found and the structures and pharmacology of these compounds can be further investigated.

(2) Quantification with indicator components: The fingerprint of each effective component group is analyzed and those effective compounds or special compounds with high content are selected as indicator compounds. In some cases, effective and special compounds are the same. The quantification of indicator compounds must be considered the relationship of their representation and analytical technology for multicomponent quantification.

(3) Multiple types of information in fingerprints: The fingerprint of TCM herbs includes information on chemistry and efficacy. Based on literature describing the chemistry and pharmacology of each herb in TCM formulas and combined with the technological process, types of compounds can be elucidated. By modern separation methods (such as supercritical extraction, macroporous resin column, countercurrent chromatography, and a variety of preparative chromatography), the chemicals can be separated into individual chemical parts (compound groups). A variety of pharmacological experiments can be carried out to confirm the effective component groups and compounds as well as their ratios in content. With the relationship of the variation of compounds (type, number, and content) and efficacy (from experiment or clinic), the fingerprint contains information on chemistry and efficacy. For example, certain fingerprints can indicate pharmacology and clinical efficacy. Therefore, efficacy and quality assurance can be achieved.

1.2.2.3 Evaluation of the Efficiency and Safety of TCM Formulas As remedies used in clinical situations, TCM formulas must be compliant with the primary requirement of “safe, effective, stable, and controllable.” Due to the lack of regulation and convincing scientific data to evaluate on the

effectiveness and safety for most of the TCM, new technology and methods are developed at a slow pace. TCM has had a difficult time to get approved and accepted by the international medical world; therefore, it is important to establish a complete evaluation system on its effectiveness and safety. Evaluation of efficacy on TCM formulas refers to the following two aspects: preclinical and clinical evaluation.

(1) Preclinical evaluation on TCM formulas: At present, animal models with similar clinical syndromes are often used for pharmacological investigation. It is, however, difficult to make an animal compliant with the clinical syndrome as TCM defines, especially for the more complex syndromes. Thus, it is necessary to develop some animal model corresponding to both symptom and disease. Furthermore, the final activity is caused by the compatibility of each herb in the prescription and the traditional investigation method based on single factor analysis cannot obtain entire and systematic information of mechanism. Therefore, the mechanism of TCM formulas is evaluated based on the overall effect.

(2) Clinical evaluation on TCM formulas: In ancient times, the classic TCM books recorded the procedure of the diagnosis and treatment in form of medicinal cases, paying attention to the standard of improvement of clinical syndrome. In recent years, TCM practitioners pay attention to both subjective and objective syndromes. The disease names used in Western medicine are also applied to TCM. Meanwhile, laboratory and physical examinations, especially the former, are also employed to indicate the effect and scientific nature, combined with the evaluation criteria of Western medicine or criteria self-developed by the researchers. However, the evaluation of TCM syndrome is ignored.

Therefore, the evaluation of TCM efficacy should be guided by the theory of syndrome differentiation and treatment based on the concept of holism, as well as by the recent achievement of modern medicine and life sciences. Based on clinical efficacy and animal experiments, comprehensive evaluation system should be established with quantification evaluation indices in TCM (such as syndromology, ethnology, and life quality), pathological and biochemical indices in Western medicine (clinical biochemistry, iconography, anatomy, etc.), as well as the evaluation indices in systems biology (genomics, proteomics, metabonomics, etc.).

At present, toxicity of TCM formulas is mainly evaluated by experiments of effective components, pharmacology and toxicology. For pharmacological and toxicological study, the routine methods for chemical drugs are often used. However, most chemical drugs contain only one single compound, different from TCM formulas. Therefore, different methods and analysis tools should be developed and utilized for evaluation of the safety of TCM formulas.

1.2.3 Main Ideas and Progress of Research on TCM Formulas in Recent Years

1.2.3.1 Compatibility of Effective Component Groups and the Relationship Between Effective Component Groups and Efficacy The compatibility of effective component groups has been widely studied by elucidating the material basis of TCM formulas using advanced techniques. Moreover, the relationships of compatibility, chemical components, and pharmacological effects can be investigated.

In the 1990s, a novel research system for TCM formulas was proposed which can be summarized as “One combination, two basic elucidations, three chemical layers and four pharmacological levels” (“1-2-3-4” system).^[4,5] “One combination” refers to the integration of chemical components and pharmacological investigation. “Two basic elucidations” means the effective components should be elucidated, as well as the efficacy and the mechanism of TCM formula. Based on “three chemical layers” (formula and raw herbs, effective component groups and effective compound mixtures), the research with “four pharmacological levers” (animal, tissues and organs, cells and subcells, molecular biology) can be carried out to illustrate the chemical basis of the compatibility theory of TCM formulas. This research system lays critically on the material basis of TCM formulas, emphasizes the interaction of ancient compatibility theory and modern pharmacological theory and the combination of substance analysis and observation on the pharmacological effect. It has been widely applied to TCM formulas.

New techniques and methods have to be developed to elucidate the effect and relationship of active substances in TCM formulas. The methodology of “effective component groups–efficacy” (analysis of the correlation between effective component groups and efficacy) is one of these effective models combining chemistry, pharmacodynamics, and informatics following the idea that: Under the guidance of TCM theory, the entire complex prescription is properly divided into n effective component groups, global chemome are presented with matrix $n \times k$ where n vectors represent n effective component groups and each vector element represents the number of compounds (k) in each component group (amended with 0 if insufficient). Thus there are $n \times k$ compounds in the global chemome. In the matrix of models, p pharmacological models are used including the animal, tissues and organs, cells and subcells, and systems biology (genomics, proteomics, etc.), each vector element represents the number of indexes (q) in each model (amended with 0 if insufficient). From the matrix ($n \times k$) of global chemome and the matrix ($p \times q$) of models, effective chemome ($m \times j$ matrix) can be obtained based on bioinformatics and experimental research, which is the objective of chemomics. Meanwhile, both the number of active component groups and effective compounds in the effective chemome are smaller than those in the global chemome. Therefore, the conditions of $m < n$ and $j < k$ are required, and irrelative

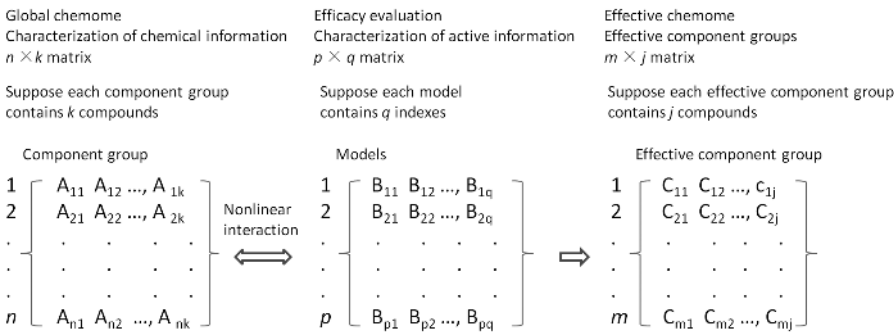


Fig. 1.2 Method to study the relationship between effective chemome and efficacy of TCM formula. A: compounds of component group; B: indicators of model; C: compounds of effective component group.

components are ejected from the global chemome. Chemomics provides a new “omics” approach to develop modernized composite medicine, where the phytochemical composition of a herbal formula with demonstrated clinical efficacy is regarded as a global chemome, which can be simplified successively through bioactivity-guided screening to achieve an optimized chemome with minimal phytochemical composition for further drug development, while maintaining its curative effect for a specific disease. This technique routine is shown as Fig. 1.2.

The chemical information of TCM formulas and effects can be characterized by fingerprints and quantification of multiple indicator compounds. The methodology of “effective component groups–efficacy” in TCM requires pharmaceutical informatics to identify the effective component groups related to efficacy and clarify the roles of fingerprint peaks. This methodology is different from relation investigation between quantification and structure of drugs in Western medicine system. A fingerprint chromatogram of TCM herbs focuses on the characteristics of active compound groups rather than one or more compounds. Moreover, it is different from the methodology of “chromatogram–efficacy,” which confirms the relation between the entire fingerprint chromatogram and efficacy, rather than the relationship between the compounds or groups and the efficacy.

1.2.3.2 Serum Pharmacology and Serum Pharmacochemistry in TCM Drugs The method of serum pharmacology, developed by a Japanese expert, can be defined as an *in vitro* pharmacological test with serum containing medicinal components after oral administration of TCM preparations. In China, some researchers also investigated the methodology of serum pharmacology, such as the relationship between the activity and *in vivo* concentration, administration regime, dosage of serum containing medicinal components, time for blood collection, storage condition and the

impact of serum inactivation on the efficacy. For instance, the *Shuanghuan-glian* formula showed an obvious effect on anti-RSV based on the method of serum pharmacology.^[6]

For traditional in vitro analytical methods, it is difficult to identify and test the pharmacological effect of a number of compounds altogether, and the state analysis on these compounds cannot reveal the dynastic metabolism. However, serum pharmacochemistry focuses on the absorbed components including the metabolites. Through investigation of the components and their metabolites, active components are able to be identified. After the establishment of the fingerprint of serum chemical components and the determination of pharmacological effects, the analysis on active components can be carried out to find the relative active compounds. Several Chinese experts set up a HPLC fingerprint of rat serum after oral administration of *Liuwei Dihuang* pills. Eleven compounds were found in rat serum, four of which were new metabolites and the other seven of which were compounds from the original preparation.

1.2.3.3 Other Research Methods The main idea of the “*fenziyaoxing*” and “*yaoxiaotuanyaoxing*” hypotheses^[46] is that the special structures of molecules are responsible for their bioactivity. Drug components have a variety of molecules and effective groups, leading to multiple targets.^[44–49]

The theory of “TCM molecularomics” argues that the TCM formulas affect the integration of signal molecules with the integration of chemical components, leading to recovery of the signal molecular net and treatment of TCM syndromes.^[47] It starts from a chart of essential syndromes with “syndrome–pathway of effect–signal molecules–molecular net” and investigates the influence of complex prescriptions, effective component groups, and effective compounds to the syndrome. The method is effective to some extent; however, there is no exact conclusion for recognition of signal molecules of TCM syndromes.

The theory of “histological pharmacology of TCM formulas” studies the relationship between the spectrum of main components and their concentrations in the target pathological tissues and the pathological morphology of target tissues as well as the efficacy.

The theory of “metabonomics of TCM formulas” investigates the therapeutic mechanism, targets, criteria of efficacy, toxic models, biological makers, and so on, based on the theory of metabonomics.

The theory of “differential pharmacodynamic serum spectrum” establishes profiles of chemical compounds in serum based on the maximum and minimum efficacy by the chromatographic technique, and then separates the active components by the differentiation method. The components are tested in the animal models with identical TCM syndromes and are identified with further experiments until the active components are confirmed.

The limitations for the aforementioned strategies, however, underline the fact that there are no efficient experimental technical platforms under the

molecular level, and the concept of holism of TCM cannot be systematically expressed. Systems biology from the post-genomic epoch can be implemented into the research on TCM.

1.3 DEVELOPMENT OF SYSTEMS BIOLOGY

1.3.1 Proposal of Systems Biology

Systems biology is a new branch in the field of life sciences, which was first proposed by an American scientist, Leroy Hood, one of the pioneers for the Human Genome Project (HGP).^[7] According to Hood's definition, systems biology investigates all the compositions (gene, mRNA, protein, etc.) in the biological system, and the interactions of these compositions in certain circumstances.^[8] Therefore, different from the original experimental biology focusing on individual genes and proteins, systems biology focuses on global investigations of interactions among many genes, proteins and compositions. Hood pointed out that "systems biology would be the core driving force for medicine and biology in 21st century." Hood and two other scientists founded the first systems biology institute at the end of 1999. Thereafter, systems biology was gradually accepted by scientists, and aroused the attention by a flood of experts in the external field of biological research. In March 2002, a special issue for systems biology was published in the journal *Science*.

1.3.2 Technological Platforms of Systems Biology

With the development of the genomic sequencing, scientists have focused their studies on functional genes. A number of testing platforms, including genomics, transcriptomics, proteomics, metabonomics, interactomics, and phenomics, were developed to study the gene expression and regulation, the structure and function of genes and their metabolites, the interactions between molecules, and the correlation between genotype and phenotype at the genomics level.

(1) Genomics: Genomics includes technical platforms such as gene sequencing, genotyping based on single nucleotide polymorphism (SNP), and epigenomics. Gene sequencing reveals the entire genetic code in each organism, which is the basis of systems biology. Genotyping is now accurate at the level of single ribonucleotide. The haplotype omics research was initiated by the U.S. National Institutes of Health (NIH) in 2001 for determining the SNP of different ethnic groups, which illustrated the susceptibility for diseases, drug answering, and other individual differences for characteristics and behaviors, crucial for development of prospective medicine and individualized medicine.

(2) Transcriptomics: Transcriptomics investigates gene expression profiling at the mRNA level, providing a high-throughput and parallel tool on the

genomics level for determining mRNA abundance using DNA chips. DNA chips are used to determine the gene expression profiling for different cells and tissues, compare the expression profiling between diseased and normal tissues, and determine gene expression profiling for cells at different differentiation and development stages as well as in different environmental conditions. These results are not only adopted to investigate novel gene functions and signal transduction systems and the response to environmental factors in cells and organisms, but are also used to identify and validate the target genes for new medicines.

(3) Proteomics: Proteomics studies the structure, space-time distribution, and function for proteins in cells and organisms, including three branches: proteomics, functional proteomics, and structural proteomics. In recent years, development of two-dimensional LC-MS and protein chip-MS systems has further extended the scope of proteomics. Expression proteomics has been applied to discover and identify protein-labeled molecules at certain physiological and pathological stages, and the protein-labeled molecules can be used in drug discovery, clinical diagnosis, and disease monitoring.

(4) Metabonomics: Metabonomics is another important branch of systems biology besides genomics, transcriptomics, and proteomics. The concept of metabonomics was first derived from metabolic profiling.^[9] The Nicholson group proposed the metabonomics method in 1999, to investigate metabolite changes in organisms under external stimulations, focusing on the changes of small metabolites with molecular weight of less than 1,000 in the metabolic cycle, by response to external stimulations and genetic modifications,^[10] which has been successfully used in disease diagnosis and medicine screening. Fiehn first proposed the concept of metabolomics^[11] and correlated the plant metabolites with gene functions. Since then, many studies on plant metabolomics have been conducted, and the two main branches of metabolomics were developed. Fiehn, Allen, Nielsen, Oliver, Villas-Boas, and others further developed some definitions of metabolomics, which are accepted worldwide.^[12–16] The first level is target analysis, a quantitative study of the substrate and/or products for a target protein; the second level is metabolic profiling, quantitative analysis of relationships between pre-installed metabolites and structures and characteristics in certain metabolic processes, using specialized techniques; the third level is metabolic fingerprinting, qualitative or semi-quantitative analysis of all intracellular and extracellular metabolites; the fourth level is metabolomics/metabonomics, the quantitative study of all metabolites in organisms (which is difficult to achieve).

Metabonomics has been extensively used for the investigation of drug toxicities and mechanisms,^[17–21] microorganisms and plants,^[22] disease diagnosis and animal models,^[23–26] and gene functions.^[27] As a science branch, metabonomics has been developed quickly and applied to many other research aspects including safety evaluation for TCM compositions,^[28] metabolic analysis for

medicines,^[29, 30] toxicogenomics,^[31, 32] nutrigenomics,^[33] pharmacometabonomics,^[34–36] systems of absorption, distribution, metabolism, excretion, and toxicity (systems- ADME/Tox),^[37–39] and so on.

(5) Phenomics: Nowadays, phenomics studies are mainly at the cellular level, since cells, as the basic elements of vital activities, have the major characteristics of live organisms, such as signal transduction, space-time organization, breeding, homeostasis, and the response and adaption to the environmental changes. Cells can be used for studies at a high-throughput genome-wide level.

The major phenomics platforms are cell chips and tissue chips. Cell chips carry out various genetic manipulations for each gene at the genome-wide level, including gene knockout, gene transfer, gene suppression, gene activation, constructing corresponding cell lines, and high-throughput phenotype investigation. Tissue chips are mainly used in research on high-throughput pharmacology, toxicology, and pathology. Phenomics is the terminal of the omics platform for systems biology, implementing the whole process for genome sequence to basic vital activities by genomics, transcriptomics, proteomics, metabonomics, interactomics, and phenomics. Systematic investigations from genomics to phenomics have been conducted in the glycometabolism of colibacillus and yeast. More and more cell chips have been used for the discovery of new medicines and medicine targets and the evaluation of new medicines, which enables development of new drugs from a high-throughput to high-content base.

(6) Computational biology: Integration of experimental and computational results is necessary for explanation and quantitative forecasting in complex biological systems. Computational biology, including knowledge discovery and analysis based on simulation, provides a strong basis for proposing scientific questions, using modeling and theory exploration. Knowledge discovery is also called data exploitation, which extracts the implied law from the huge amount of data and information produced by each experimental platform of systems biology, and forms hypotheses. Analyses based on simulations predict the biologic experiments *in vitro* and *in vivo*, using a hypothesis from computational validation.

Knowledge discovery has been widely used in bioinformatics, as in the prediction of introns, exons, and the stereo chemical structures for encoded proteins based on the nucleotide sequence and gene regulation network by gene expression profiling. These studies are based on heuristic and statistics differential analytical methods. Analysis based on simulation goes in for predicting the system kinetics, and the production and accumulation for a large amount of quantitative data from high-throughput platform provide a solid basis for investigations based on simulation. Computational modeling and analysis provide useful biologic cues and prediction, such as bifurcate analysis for cell cycles, metabolic analysis, and comparison research on biologic tank loop homeostasis. Drug research and development is also an application

hotspot in computational biology. The computational modeling for virtual screening and molecule design, ADME, and studies of drug toxicity is an important tool for research and development of drugs.

1.4 CHEMOMICS INTEGRATED SYSTEMS BIOLOGY

In recent years, tools of systems biology have been applied in TCM investigations by many scholars.^[42, 43] However, systems biology is just taken as a tool to illustrate some theories and regulations of TCM, and the disadvantages are the lack of globalization and systematicness. However, it still has limits explaining some of the theories and rules of TCM, and lacks integration and system. In other countries, significant results of TCM research using biology systems have been explored. However, because they are limited in their understanding of TCM theory, foreign scholars cannot get the essence to the full extent. Understanding the philosophy and scientific theories of TCM in the view of modern science is persuasive. Investigations in systems biology range from “compositions” to “functions.”^[7] Systems biology is unceasingly integrated and developed with the development of technologies. Leroy Hood first integrated genomics, proteomics, and numerology into integrative systems biology. Nicholson proposed the global systems biology by integrating genomics, proteomics, and metabonomics, based on establishment of metabonomics techniques.^[40] More omics have been integrated into systems biology studies in recent years.

Systems biology uses integrative concepts and methods to elucidate the physiology, pathology, and dynamic rules of complex human systems. However, limited to the descriptions for biological internal system (response system) information, current systems biology cannot completely explain externally disturbing information (information such as the intervening chemical information of drugs).^[41] When the external intervening system is relatively simple, for example, a single compound, it is summarized as disturbance point, which is the equivalent of action of “point to system.” But when the external intervening system is a complicated system, for example, TCM formulas, the current systems biology is not yet compatible with the integrated research for the corresponding TCM formulas. Although it can provide methods for quantitative characterization of the syndromes and the biological effects of drugs (efficacy and safety evaluation), which may solve the problem of expressing a “syndrome,” there is no integrated information of “formula” (drug intervening system). Thus it is difficult to reveal the internal relationship between the two systems (the biological response system and the complicated substance system of TCM formula).

Based on our studies on TCM and systems biology, we have proposed a number of new methods of systems biology, suitable for TCM research, called “chemomics integrated systems biology.”^[42]

1.4.1 Definition of Chemomics Integrated Systems Biology

Investigation of the mechanisms of TCM formulas and evaluation of compatibility should consider the integrity of the TCM effect, which in nature manifests the interaction between the two complex systems, TCM (external system) and human (internal system). Only under the guidance of TCM theories and in combination with modern scientific technologies can the interactions between these two systems illustrate TCM compatibility theories, mechanisms, and the substance basis of efficacy. To achieve this goal, two things should be considered: one is the global characteristics for human response system (internal system) during the interference of TCM, and the other is the compatible relationship of TCM formula (external system), which are the problems that should be solved by systems biology and chemomics, respectively. The interactions can be revealed by combining the two systems. The investigations of TCM require establishing a “system to system” (S2S) method corresponding to its characteristics. Therefore, we have proposed that chemomics integrated systems biology (global systems biology) should be developed based on current systems biology (Fig. 1.3). Global systems biology represents the compositions and interactions of drug interference systems using chemomics, delineates the response from biological systems, and further analyzes the interactions between the two systems. It reveals the correlations between the changes in chemomics and space-time response of the biological system (the correlation between formula and syndrome). Moreover, the biological system is studied not only with systems biology information at the molecular level (such as genomics, proteomics, metabonomics, etc.), but also with the information of pharmacology and safety evaluation at the level of animal experiments, organ tissues, cells, subcells, and molecular biology, respectively.

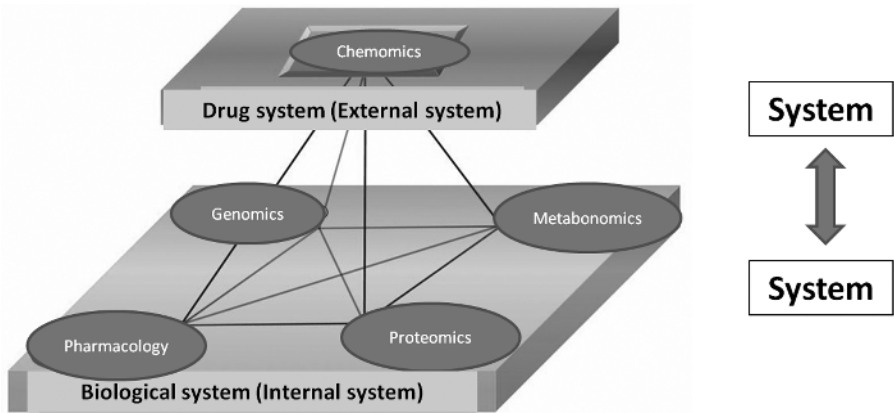


Fig. 1.3 Chemomics integrated systems biology.

1.4.2 Research Mode of Chemomics Integrated Systems Biology

The research system of chemomics integrated systems biology includes the investigation of changes in pharmacodynamic substances, transmigration laws during the process of the drug compatibility, and the component compatibility for prescription by chemomics. It explains rationality on the basis of substances and investigates the corresponding changes of pharmacodynamics and biological effects with the changes of pharmacodynamic substances during the compatibility process of TCM formulas, by chemomics integrated systems biology and the combination of chemomics integrated systems biology with global animals, organ tissues, cells, subcells, and pharmacodynamic studies at the molecular level. The advantages of TCM formulas in efficacy enhancement and toxicity reduction are also being studied. Moreover, the underlying pharmacodynamic mechanisms of TCM formula are investigated, and an evaluation system for TCM is established. The global technical route is shown in Fig. 1.4.

1.4.2.1 Definition of Chemomics Chemomics is an approach used to identify and quantify all of the chemical components in the chemome and to

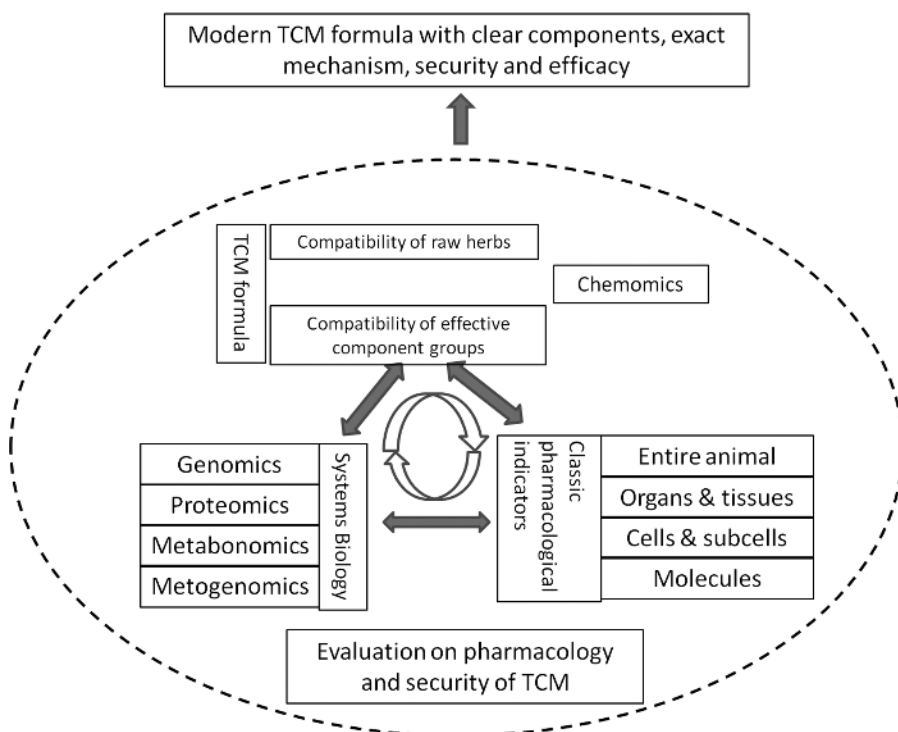


Fig. 1.4 Technical route for applying global systems biology to TCM formula investigation.

understand the relationships between the changes in the chemome and the time-related responses of biological systems, whereas the chemome is the aggregate of all the chemical components among a specified external intervention system interacting with the biological system.^[43] For chemomics of TCM formulas, following the compatibility theories of TCM formulas, a strategy derives from system to part (top-down) and increases step by step, by correlation analysis of chemical information flow and multiparameter biological information flow for chemomics at different levels, to identify the composition of effective chemomes and to reveal the essential pharmacodynamic compositions from the global chemomics, and to illustrate the compatibility theories of TCM formulas based on raw materials, component groups, and compounds. Another strategy is the bottom-up mode. Raw herbs can be also considered as a global chemome, different compositions are screened, and the effective component groups are then recombined as a complex formula, which is the bottom-up mode of TCM chemomics. For a formula, the research mode of chemomics from top to down is recommended, which is derived from TCM formula with global effects and investigates the compatibility of a chemome under the condition of keeping the global compatibility of the formula, using the top-down mode and increasing step by step, by revealing the correlation between formula and effectiveness, to find an effective chemome and effective component groups from a global formula. We think that, on one hand, the effective and reasonable TCM formula compatibility should be retained and explained fully; on the other hand, during the development of compatibility of TCM component groups, the global idea from “top to down” should be emphasized to better manifest the characteristics of TCM formulas, to more easily optimize the compatibility under the guidance of TCM theories, and to reveal the mechanisms of TCM formulas. The two application modes of chemomics are shown in Fig. 1.5.

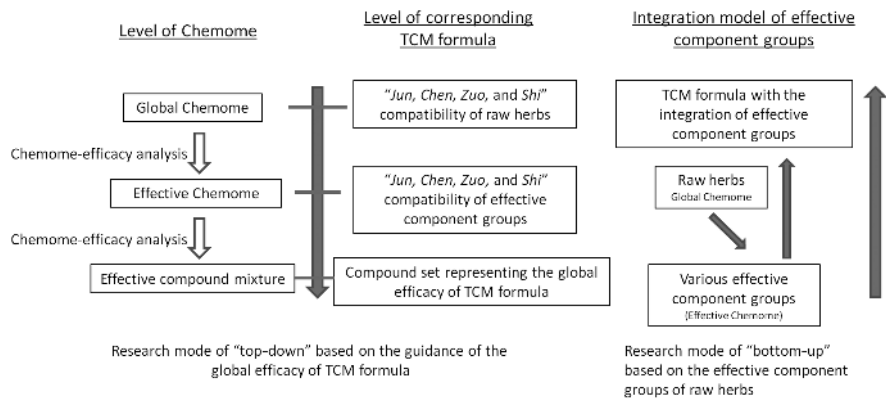


Fig. 1.5 Two application modes of chemomics.

1.4.2.2 Key Technologies of Chemomics Integrated Systems Biology

The key technologies of chemomics integrated systems biology include information access and processing technologies.

In systems biology, including all types of omics research, a great amount of information is obtained by high-throughput information access technologies that are suitable for development of chemomics, genomics, proteomics, and metabonomics, such as new methods like LC-MS/MS and ultra-performance liquid chromatography coupled with quadrupole-timeoflight MS (UPLC-Q/TOF), biological chips with the application of construction analysis for enterobacteria flora (metagenomics research), and the key technologies for TCM formula safety evaluation.

Information integration technologies include the information integrated in one system, as well as between two systems. It integrates bioinformatics and chemometrics technologies and develops correlation analysis, multisource fusion technology, the discovery technology for causality and the theory and method of multivariate statistics, including regression analysis methods of principal component regression (PCR), partial least squares (PLS), and support vector regression (SVR), as well as the classification methods of linear discriminant analysis (LDA), partial least squares discriminant analysis (PLS-DA) and support vector clustering (SVC), and the network theories that construct the interaction mode for two systems.

1.4.2.3 Application of Chemomics to the Compatibility of TCM Formulas

TCM formula compatibility is crucial for TCM's global view, syndrome differentiation, and treatment theories. Chemomics can reveal the compatibility rules of TCM *materia medica* and component groups based on the substances and mechanisms investigation at the molecular level, and TCM formula compatibility investigation can be carried out on formulas and syndrome correspondence.

(1) Combining the formula and syndrome correspondence with TCM formula compatibility is a key point in explaining the theories of TCM formula compatibility. Take the formula of the *Sijunzi* decoction for treatment of splenasthenic syndrome as an example. It is composed of four raw herbs (global chemomics), *Renshen* (Ginseng Radix), *Baizhu* (*Atractylodis Macrocephalae Rhizoma*), *Fuling* (*Poria*) and *Gancao* (*Glycyrrhizae Radix*). Based on the “top-down” mode and the strategy of global chemome, the component group compatibility research of the five classes of effective composition groups has been carried out, which are saponins (mainly derived from *Renshen* and *Gancao*), volatile oils (mainly derived from *Baizhu*), polysaccharides (mainly derived from *Fuling*, and partly from *Renshen*, *Baizhu*, and *Gancao*), triterpenes (mainly derived from *Fuling* and *Gancao*) and flavonoids (mainly derived from *Gancao*) are the most effective. With the pharmacodynamic model of splenasthenic syndrome, metabonomics, and metagenomics, the substance basis of splenasthenic syndrome was revealed; the changing law of the splenasthenic

syndrome models before and after interference by the material media and component group compatibility were investigated; and compatibility theories for the *Jun* (*Renshen*), *Chen* (*Baizhu*), *Zuo* (*Fuling*), and *Shi* (*Gancao*) herbs in the *Sijunzi* decoction were validated from the constructional relationship of effective substances and pharmacological correlation of integrated systems biology parameters. The essence of the TCM pharmacodynamic evaluation is how to establish the correlation between the syndrome and the drug's therapeutic effect. The global therapeutic effect of TCM formula is difficult to see manifested by a single target or combination of a few targets, whereas systems biology can provide a feasible method for evaluation of the treatment of syndrome from TCM formulas at a global level.

(2) Understanding the *Jun*, *Chen*, *Zuo*, and *Shi* compatibility of raw herbs and component groups is crucial for development of the compatibility theories of TCM formulas. Traditional TCM formulas are composed of raw herbs according to some formula principles, and the *Jun*, *Chen*, *Zuo*, and *Shi* compatibility theories also refer to the raw herb compatibility. Moreover, the compatibility of effective components has been applied to research and development of compound formulas. Our chemomics study of *Qingkailing* injection suggested that, following the anti-cerebral ischemia cascade reaction and using group-effect analysis, four classes of effective component groups that play important roles in the compound are found to be composed of eight raw herbs, and a new TCM formula with compatibility of effective component groups has been successfully developed.^[43] Component TCM formulas, as a new branch of TCM formulas, will definitely strengthen the theory and law investigation of component compatibility of TCM formulas. According to the opinion of chemomics experts, TCM formula is also a global chemome and can be regarded as the compatibility of several related subchemomes. Subchemome has two main styles: raw materials and component groups that represent some characteristics of formulas (also called effective component groups). Therefore, effective component group compatibility is consistent with the raw material compatibility. In the investigation of effective component compatibility for *Qingkailing* injection, the *Jun*, *Chen*, and *Zuo* correlation among the four effective component groups have been validated, which suggests that the compatibility also exists among the effective component groups.

1.4.2.4 Application of Global Systems Biology to the Action Mechanisms of TCM Formulas

1.4.2.4.1 Evaluation of the Pharmacological Model for TCM Formulas The premise of the study of the pharmacological mechanism is to elect the appropriate model of TCM formulas. However, it is difficult to evaluate the accordance between the pharmacological model of TCM theory and the characteristics of TCM formulas. The global systems biology and the theory of TCM share many features. Therefore, it is more reasonable to evaluate the pharmacological models of TCM formulas. The systematic relation and

rationality between selected models and the clinical syndromes can be evaluated under the guidance of TCM theory. The pharmacodynamic models and parameters of TCM formulas can be chosen combining the animal, organ tissues, cells, pharmacological evaluation in molecular level, and the evaluation parameters of systems biology together, to evaluate the correlation and rationality for the chosen model and clinical syndrome. Besides the animal model, high-throughput cell models can be explored, particularly stem cell models.

The past pharmacological studies in TCM utilized many evaluation methods and standards of Western drug pharmacology. Systems biology characterizes various pathologic and physiologic stages using the multilevel network style integrated by genomics, proteomics and metabonomics, to reveal the internal principle of the biological response system. Therefore, the relative parameters of systems biology may possibly be used for pharmacological evaluation. Finally, a comprehensive evaluation system can be established from the evaluation parameters for TCM quantification (semiotic symptomatics behavioristic, and life quality evaluation), pathological biochemistry parameters for Western medicine (clinical biochemistry, imaging, and pathologic dissection) and systems biology (genomics, proteomics, and metabonomics).

1.4.2.4.2 Safety Evaluation of TCM Formulas Based on Systems Biology

Due to the complexity of TCM formulas, the chemical composition is not clear, and neither are the mechanisms of toxicity and toxic targets. Thus it is difficult to evaluate the safety. The development of systems biology, in particular metabonomics and biological chip technologies, does not depend much on previous experience. By combining the evaluation of acute toxicity and long-term toxicity, the safety evaluation for *Liushen* pills using chemomics, metabonomics, and toxicokinetics was carried out, in order to reveal the major acute toxic injuries of *Venenum Bufonis* for the heart, as well as the long-term toxicity of realgar for liver. Our results have demonstrated that *Liushen* pills' compound compatibility has an effect to reduce toxicity and enhance the drug efficacy. The "dosage–effect–time–toxicity" relationship of *Liushen* pills was studied, and the safety and effective dosages were identified.

1.5 RESEARCH STRATEGY AND PROSPECTIVE OF SYSTEMS BIOLOGY IN TCM

1.5.1 Background of Systems Biology in TCM

In systems biology, systematic analysis is performed by integrating various omics information on genes, cells, tissues, and the body at different levels, which is a great tool for studying complex biological systems. TCM is also a complex system. For traditional TCM theories, the most idiomatical characteristics are "global," "dynamic," and "dialectical," which are all consistent with systems biology. New ideas and methods from systems biology are also utilized

in TCM, which facilitates modernization of TCM.^[4] The development of TCM systems biology is important for life sciences. The famous scientist Xueseng Qian pointed out that “after we really understand and summarize the theories and practice of TCM, the whole modern scientific technologies will be influenced, and scientific revolution will be evoked.”

1.5.2 Basic Strategy for TCM Systems Biology

Systems biology developed along with modern life sciences, which has been used in Western modern medicine. As forementioned previously, given the similarities between methods for systems biology and TCM, we can assimilate these two. However, due to the huge differences between Chinese and Western medicine systems, research of systems biology in TCM cannot be “borrow and used.” We should follow the laws of TCM when using systems biology methods. Based on our previous study, the following two basic strategies or principles of systems biology can be applied in TCM.

1.5.2.1 “System to System” Research Strategy of Formula and Syndrome Correspondence

“Formula and syndrome correspondence” is the basic principle of prescription research and clinic application of TCM and is crucial in establishing a pharmacological evaluation system of TCM. Thus, there is the saying in TCM that “drugs and medicines can’t live apart.” The interactions between the two complex systems, TCM and the human body, and the formation of a higher system entity are based on TCM globalization. The clinical TCM experts should participate in the research of TCM systems biology, which is based on the “system to system” (S2S) research strategy of formula and syndrome correspondence, and requires a combination of two aspects: one is the global delineation of system characteristics in the process of a biological body (response system) being interfered with by TCM; the other one is system disclosure of the internal relationship in the chemical substance system of TCM formula. The interactions can be disclosed in the global level with combination of the two systems. The current network pharmacology investigates the biological response system (“point to system,” P2S), taking medicine as a disturbance factor. The P2S mode is different from the P2P mode (“point to point,” single compound acts to single target). However, when a TCM formula as a complex substance system interferes with the body, being taken as “point,” the interactions between the TCM formula’s interfering system and the biological response system cannot be constructed. It is difficult to identify the effective component groups in a TCM formula and to further carry out the compatibility optimization of compounds. Thus, we developed chemomics, a method integrating chemomics of TCM formulas and systems biology (Fig. 1.6), to investigate the interactions between TCM complex system and human biological system, providing new insights into the mechanism of TCM formulas as well as evaluation of the compatibility of TCM formulas.^[4, 5]

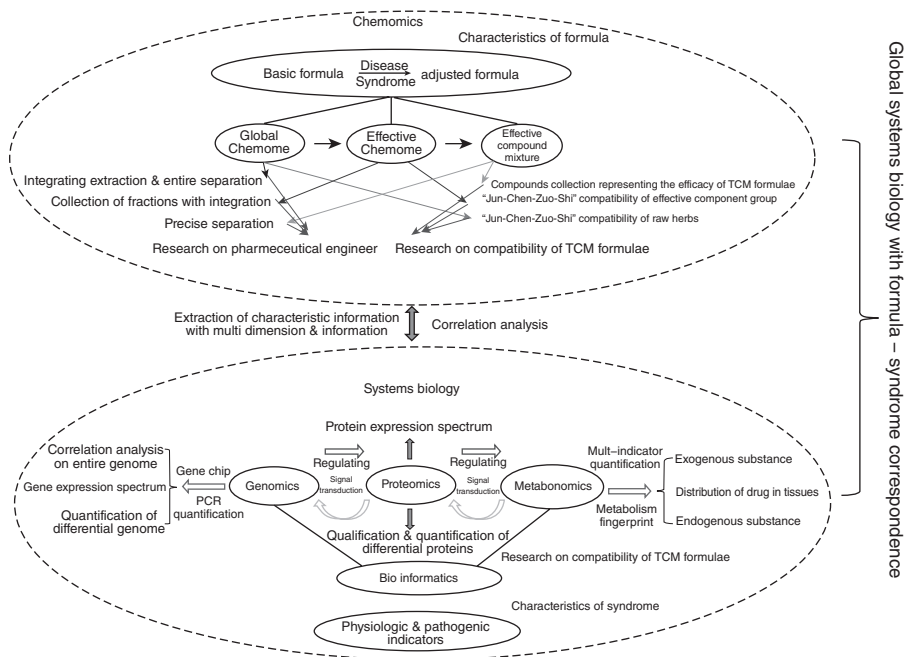


Fig.1.6 “System-to-system” research strategy of TCM formula-syndrome correspondence.

1.5.2.2 Research Strategy of Combination of Macroscopic and Microcosmic Representation TCM emphasizes globalization (macroscopic) but pays less attention to microcosmic characteristics. Western medicine is characterized by anatomy and molecular organism, focusing on the diseased organs, targets of the disease. Systems biology attempts to overcome the disadvantage of “missing the forest for the trees” of traditional biology, explaining the macroscopic characteristics of the system from a points of view that integrates various omics. The omics high-throughput screening models in current systems biology can be used for quantitative/semi-quantitative analysis of the macroscopic characteristics of genomics, proteomics and metabonomics. However, due to the technical limitation, the microcosmic characteristics cannot be quantitatively analyzed. Therefore, the macroscopic representation methods and accurate analysis of microcosmic characteristics should be combined in TCM systems biology, to delineate the characteristics of a complex system. For example, we have combined omics research and the confocal research on special signals to explore the levels of system biology, the gene expression profiling and special gene quantitative real time polymerase chain reaction (RT-PCR) in the level of genomics, and metabolic fingerprint profiling and multimetabolites qualification of special pathways in the level of metabonomics to develop metabonomics platform technologies.^[6, 7]

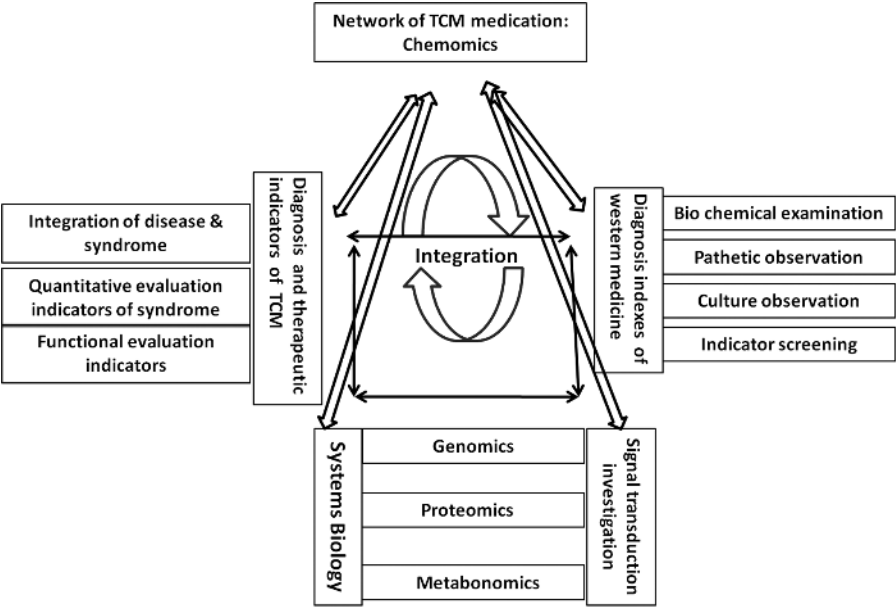


Fig. 1.7 Comprehensive systems for TCM disease–syndrome treatment and efficacy evaluation.

According to the strategy of combining macroscopic representation and microcosmic characteristics, integration of multitechnologies is used to establish the comprehensive systems for TCM disease-syndrome treatment and effect evaluation (Fig. 1.7).

1.5.3 Key Scientific Problems That Need to be Solved by the Systems Biology Approach of TCM

Based on the crucial scientific problems and its potential modernization of TCM, systems biology study of TCM should address the following important issues.

1.5.3.1 Basic Research on Systems Biology Regarding Individual Diagnosis and Treatment of TCM Individual diagnosis and treatment is the essence of differential treatment of TCM, which takes into account conditions in terms of locality, time and people involved. Individual treatment program can be used in accordance with the different objects (physique, cause, and pathogenesis) and different environment. Thus, the circumstance is that the same symptoms receive different treatment and different diseases with the same therapy during the cause of TCM diagnosis and treatment. In recent years, the development of systems biology indicates that many diseases (more complex genetic diseases) are caused by the interaction of hereditary factors

and environmental factors. Various pathogenic factors (mechanism) are likely to exist. The internal basis of individualized treatment principles of TCM may obtain support from studies of systems biology.

For example, to analyze the large amount of patients identified with TCM by genome-wide association studies (GWAs),^[8] gene polymorphism and complexity can be used to understand why people with different physiques, genetic backgrounds, and pathogens (environment) suffer from the same diseases and have different responses to the same medication (Chinese herbal medicine), and why different drugs may cause similar treatment effects. It is also possible to find an answer for the material foundation of same disease with different treatment, and different diseases with the same therapy of TCM from genes–protein–metabolism regulatory and control level.

1.5.3.2 Basic Systems Biology Research of TCM Syndromes Syndrome differentiation is crucial for treatment. Characterization and evaluation of TCM syndrome is critical to TCM systems biology research. Modern research shows that TCM syndrome is complex. It is difficult to express with single factors as physiological and biochemical indicators. However, the overall regulatory and control network can be used to investigate the material base of TCM syndromes based on the net of “genes–protein–metabolism.”^[44–56]

Considering the lack of animal models with TCM syndromes, TCM syndrome systems biology should be combined with TCM clinical investigation. Clinical experts make clinical research programs, criteria of syndrome differentiation and suitable patients with enough cases, since mixed syndromes are widely prevalent.

Fig. 1.3 shows an evaluation system combined with quantitative diagnostic indicator (standardized scale) for different syndrome differentiation in TCM, pathological biochemical indicator of Western medicine, and system evaluation indicator. Based on the relationship among various indicators, the specific indicator can be found which is most helpful for illustration of the syndrome present. The difference found from functional genes, proteins, and metabolic control can be used to know the material basis and science in TCM syndrome differentiation and to improve the clinical diagnosis and treatment ability.

1.5.3.3 Overall Screening and Evaluation Research on TCM Formulas with Systems Biology According to the TCM principle (prescriptions corresponding to syndrome), the formula with exact clinical efficacy should be selected corresponding to syndrome.^[4, 5] The efficacy evaluation models of disease and syndrome can be established (multiple combination of animals, organs and tissues, cells and subcells, molecular targets). Using chemomics to investigate the chemical basis of the formula and different compatibility, we can reveal the relationship between component groups and their efficacy, as well as compatibility. Modern TCM preparations have further been developed that not only have independent intellectual properties and keep the efficacy

of traditional TCM formula, but also have simpler and clearer components with basically clear mechanisms, great stability and safety.

The variation of both chemical component groups and biological efficacy caused by compatibility can be revealed by systems biology tools. The efficacy mechanism of TCM formulas can be explained from the relationship of “system to system.” Through the relationship of chemical information of the change of efficacy and biological information of targets, processes, and controlling net, we can integrate the overall and special parameters to compare the effect of compounds with single or compatible use, to reveal the interaction among different compounds, targets, processes, and controlling net. Based on the net model controlled by multiple factors, the active material basis and the mechanism of activity can be further studied.

1.5.4 Main Development Direction and Key Technology of TCM Systems Biology

The major branches of TCM systems biology include TCM chemomics, TCM genomics, TCM proteomics, TCM metabonomics, and TCM bioinformatics. The combination of overall representation and individual characteristics is not only used in the study of TCM systems biology, but also in strategies and methods for future studies in each branch.

1.5.4.1 TCM Chemomics TCM chemomics is the study of the interrelationship between composition and changes of chemome and the active response of biological systems. Chemome is the assemblage of all chemical or/and chemical groups, and acts on the external disturbance systems (such as TCM formulas) of biological systems under certain circumstances. TCM chemomics usually adopt fingerprints as overall characterization means and multi-indicator components quantitative as precise expression mode of local features. For example, during the chemome study of the *Qingkailing* injection, we respectively set up three kinds of fingerprints as overall characterization, including organic components, inorganic ions, and components of biological macromolecules, and simultaneously separated more than 40 kinds of organic components and 10 kinds of inorganic ions. The content identification and separation were then conducted. By adopting the combination of multidimensional fingerprint chromatogram database and multi-indicator component quantification to the *Qingkailing* injection, we tracked the research on the whole formula, raw herbs, herb couples, herb teams and effective component groups. All these steps provided guidance to the research and development of *Qingkailing* injection.^[42, 43]

1.5.4.2 TCM Genomics TCM genomics combines theory, means of genomics and TCM theory from the level of genes and gene–environment interaction to recognize the scientific connotation of TCM individual diagnosis and treatment systems. We attempted to establish a relation of TCM property,

functions, indications and influence to the regulation and control of related genes expression of specific diseases (TCM syndrome). At the molecular level, we gave an explanation to the scientific content of TCM syndrome differentiation and treatment, and the mechanism of TCM through genomics theory. TCM genomics includes research at the DNA level (such as the genome-wide association analysis and DNA methylation analysis) and RNA level (such as MicroRNA and mRNA), among which gene expression profile (mRNA) has been used in TCM research. Gene expression profile study of correlation between prescription and TCM syndrome finds the gene expression profile database of different TCM syndromes and discovers groups of distinctively expressed genes that have relationships with the specific TCM syndromes. This has significance for the objectivity of TCM syndrome differentiation and knowledge in the syndrome development discipline of TCM. We can further choose TCM with obvious curative effect and relative clear research on material basis and effect mechanism. Using various high-throughput technology (microarray, etc.) and quantitative analysis technique (real-time PCR, etc.), we can study the differences of gene expression during the process of TCM intervention, draw up gene expression profile by comparison and analysis of bioinformatics and statistics and establish gene expression profile database of TCM therapeutic processes. The link with the chemical characteristics database of TCM (chemomics study) and TCM theory offers the study of functional gene expression profile of the TCM role, and reveals signal pathway network of TCM. Based on the above theory and the influence of gene expression with different active component groups (compatibility) to TCM, we can further illustrate our traditional medical theory and its organic function. Meanwhile, we can interpret TCM theory and mechanism for functional gene networks. For example, using an integration screening chip containing 6,000 genes, we studied the overall level of animals and cells for pharmacodynamic evaluation of the *Shuanglong* formula to treat myocardial infarction. We established a gene expression profile database about normal group, model group, sham surgery control group, *Shuanglong* formula group (high, medium, and low dose), and positive medicine control group. Through the bioinformatics process, we found 180 genes differentially expressed and potentially related to myocardial infarction and effects of the *Shuanglong* formula. We further used the fluorescent quantitative PCR method to precisely analyze the ten functional genes of expression differences more than eight times. Through the complete feature of gene chips and by precisely analyzing important genes, we finally obtained the functional gene network of the *Shuanglong* formula established in the process of myocardial infarction treatment.

1.5.4.3 TCM Proteomics TCM proteomics combines theory, means of proteomics and TCM theory to determine the relation of its properties, functions, indications, and influence for the participating regulatory and control process of related protein expression of specific diseases (TCM syndromes). On the molecular level, an explanation of the scientific content of TCM

syndrome differentiation and treatment as well as the mechanism of TCM through the proteomics theory was given. The main content of the study is about the different protein mass spectra related with correlation between prescription and TCM syndrome. We can choose TCM formulas or herbal medicine materials with obvious curative effects and relatively clear research on material basis and effect mechanism, using high-throughput proteomics technical platforms, such as biological mass spectrometry protein chips (SELDI/TOF), two-dimensional electrophoresis (2D-Gel-MALDI/TOF) and multidimensional liquid chromatography/mass spectrometry (MDLC-ESI/MS/MS), and comparing the different protein mass spectra during the process of TCM intervention. By conducting bioinformatics and statistics analysis, we can establish a database for different protein mass spectra of TCM therapy linked with chemical characteristics database of TCM (TCM chemomics research) and TCM theory. The target protein of TCM can be further studied. At this point, combing with bioinformatics, we can reveal the influence of TCM effective substances on protein expression and regulation and interpret TCM theory and its role on protein regulation network. We adopted the comparative proteomics method to ensure the role mechanism of the *Shuanglong* formula promoting differentiation of stem cells into myocardial cells and interpreted the relevant protein signal pathway.

1.5.4.4 TCM Metabonomics TCM proteomics combines quantitative metabonomics methods and techniques as well as TCM theory to study the body's complete metabolism state, disease-related metabolic pathway, and changing features of metabolism during the process that TCM interposes specific diseases (TCM syndromes), clarifies pharmacokinetics, coordinates laws about the TCM effective component groups and their compatibility, and interprets TCM theory and its mechanisms on the metabonomics level. Quantitative metabonomics is a technical system that combines panorama pattern of the entire metabolic fingerprint with a focalization model of more specific metabolic pathways and multi-indicator components quantitatively.^[7, 8] The main features are the combination of overall characterization and local characteristics, integration of qualitative and quantitative, and integration of multiple analysis methods as LC, gas chromatography (GC), MS, nuclear magnetic resonance (NMR), and so on. The primary research contents include the application of TCM quantitative metabonomics in TCM syndrome evaluation and disease diagnosis, application in effective components screening of TCM and overall efficacy evaluation of TCM, and correlation research between pharmacodynamics and pharmacokinetics of TCM. For example, in nephropathy disease research (see Chapter 7), we obtained the metabolic fingerprint of metabolites using UPLC-Q/TOF,^[13] and adopted the PCA method to analyze more than 3,000 species of metabolites by cluster analysis. We obtained samples of diabetic nephropathy in different pathological stages and identified TCM syndromes and types. Combining this with the PLS-DA method, we further discovered more than ten species of new metabolism markers.

Simultaneously, according to prior knowledge, we conducted quantitative analysis accurately on several important substances in metabolic cycle, including fatty acids, phospholipids, amino acid, purine/pyrimidine, and nucleoside. By processing these analysis data, we achieved potential biomarkers of different angles and levels. Our results indicated that only a part of the biomarkers could be identified no matter what mode or circle was used. Even by obtaining overall fingerprints with the capacity of testing thousands of metabolites at the same time with UPLC-Q/TOF, we still lost information on some important metabolites. For example, nucleoside and amino acids-class metabolism markers' information did not reflect enough in the overall fingerprint, and it could be supplemented by using quantitative research of special pathway. Therefore, with the integration of different information, quantitative metabolomics platform technology can achieve more comprehensive, accurate, and specific explanation and characterization for whole-body metabolism states and specific metabolism pathways. The better prediction and judgment of the occurrence and development of disease can reveal the pathogenesis mechanism and provide guidance for clinical treatment.

1.5.4.5 TCM Bioinformatics TCM bioinformatics combines bioinformatics with TCM theory and develops bioinformatics methods of multiple omics information mining related to TCM syndrome, chemomics, genomics, proteomics and metabonomics, and so on. With the integration of gene–protein–metabolism, and multiple levels of network information, TCM theory and mechanisms could be explained comprehensively and systematically on level of overall systems biology from chemomics, genomics, proteomics, and metabonomics. Main research contents include information processing technology of TCM fingerprints, distinction and identification technology of key effective medicine components, distinction and identification theory, and method study of TCM composition effective relationships. It offers how to build a relationship between genes or protein network regulating and controlling model of multiple factors, multiple links, multiple levels, and the integrative adjustment mechanism of multiple targets, multiple links, and multiple levels of TCM.

1.5.5 Research Prospective of TCM Systems Biology

In recent years, much has been achieved by studying TCM formulas, using the pharmacological experiments and chemical analysis methods. However, the curative effect or mechanism of TCM formulas has mostly been explained through modern medical indicators (such as molecules, cells, organs, and overall levels). With the integration of TCM theory and the latest technology of systems biology, we have proposed “system to system” integrative systems biology methods of TCM, which is an innovative method system in accordance with the characteristics and patterns of TCM. With the development and improvement of technical platforms including chemomics, genomic, proteomics, metabonomics, and bioinformatics of TCM, the information obtaining

ability and processing capability of TCM integrative systems biology will be greatly enhanced. It is a combination of “TCM syndrome–design method–compound prescription–curative effect” and research of integration chemomics of the integrative systems biology. Therefore, it will reveal therapeutic material basis and mechanisms of TCM formulas, clarify the compatibility laws of TCM formulas, and guide new drug development. It also provides a communication platform for the integration of TCM with modern sciences, and further inheritance and development of TCM theory. The rapid development of TCM integrated systems biology will greatly expand the modernization research on TCM at the next stage.

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