

# Proteomics and syndrome of Chinese medicine

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- Introduction
- Proteomics and its adaptive multi-disciplinary
- Relationship between proteomics and syndrome of Chinese medicine
- Applications of proteomics in syndrome
- Problems and prospects

## Abstract

Syndrome of Chinese medicine is an understanding of the regularity of disease occurrence and development and its performance of symptoms. Syndrome is the key to recognize diseases and the foundation to treat them. However, because of the complexity of the concept and the limitation of present investigations, the research of syndrome is hard to go further. Proteomics has been received extensive attention in the area of medical diagnosis and drug development. In the holistic and systemic context, proteomics have a convergence with traditional Chinese medicine (TCM) syndrome, which could overcome the one-sidedness and singleness of TCM and avoid the complication and tedious processes. Chinese medicine has a wealth of experience and proteomics has a substantial research potential, the integration of the two aspects will bring a great enhancement of our knowledge of disease.

**Keywords:** traditional • Chinese medicine • proteomics • syndrome

## Introduction

In the theories of traditional Chinese medicine (TCM), health of human body is considered as a particular state of dynamic equilibrium that could be maintained within a certain range by bodies themselves and be disturbed by the influences of multiple factors. When the disequilibrium goes up to a certain extent, the bodies would develop morbidity. There is always a vague state existing between health and illness [1].

Syndrome also called 'Zheng Hou' in Chinese, a summary of pathogeny, location and pathology of one stage in the development of disease, is a comprehensive response to the internal and external action of body and environment, and also changes with the development of diseases [2]. 'Zheng' refers to the law of the development and changes of diseases, and 'Hou' is defined as the symptom developed by the body [1]. TCM grasps the pathogeny, location and pathology of the disease through making a comprehensive analysis of the patient's symptoms and physical characteristics. The TCM syndrome is built on the bases of long-term and substantial clinical practice. During the process of recognition the diseases, doctors of TCM obtain the information about physical characteristics and symptoms of patients firstly by diagnostic means such as 'observing, smelling, consulting and pulse-taking',

and then extrapolate the pathogeny, location, characteristic, the possible trend of conversion, etc. (Table 1).

Syndrome has an important position in the TCM system, which is the key to recognize diseases and the foundation to treat them. Because of the complexity of the concept and non-professional description of it, the research is hard to go further. One single symptom involves multiple anatomical organizations or systems. However, there was not yet a breakthrough in realization of the hypostasis of TCM syndrome, as the previous investigations limited to a local tissues or organs.

## Proteomics and its adaptive multi-disciplinary

Proteomics is the systematic study of the expressed proteins of a tissue or cell type in a parallel manner to provide detailed properties, such as the structure and function, of biological systems in health and disease [3, 4]. The three most important tasks of

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**Table 1** Common TCM aetiology and diagnostic criteria

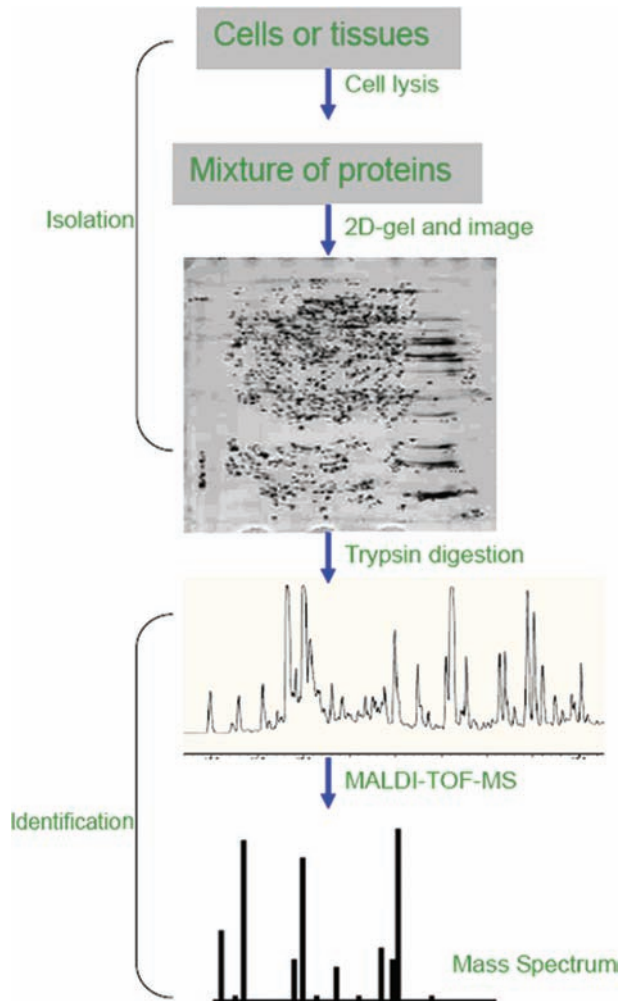
TCM disease name	Aetiology	Cardinal symptom	Similar diseases in western medicine*
Spleen and Stomach of yin-cold <sup>†</sup>	The typical weak yang of middle-jiao affected by the external coldness and uncooked cold foods induce the encroachment of the middle-jiao by the coldness, leading to the lost of the normal 'Sheng Jiang' function of spleen and stomach	Numb limbs, spontaneous perspiration, lassitude, thick white tongue coat, greasy black fat coat, deep and weak pulse-like floating	Acute gastroenteritis
Insufficiency of lung-Qi <sup>‡</sup>	Aeipathia and cough with asthma exhaust the Qi of lung, or insufficiency of the spleen cannot offer inadequate Qi of lung	Weak cough, shortness of breath worsened by action, probably accompanied by clear and thin expectoration, unwillingness to speak, spontaneous perspiration, anemophobia, liable to cold, lassitude, pallid complexion and other symptoms, pale tongue and white tongue coating, weak pulse	Chronic bronchitis
Affection of exogenous wind-heat with dampness syndrome <sup>§</sup>	It is caused by the interaction between infection and climate dampness	Fever, aversion to cold, sore throat, body aches, dry mouth, red tongue, thin yellow or greasy tongue coat, floating or smooth pulse	Acute upper respiratory tract infection fever like SARS
Blood-heat syndrome <sup>‡</sup>	It is caused by the intake of excessive bitter foods and wine, distraction, internal injury caused by excess of seven emotions and other factors	Dry mouth, upset and irritability, dry stool and yellow urine, red tongue, greasy yellow tongue coat, smooth and rapid pulse	Psoriasis
Febrile disease and blood stasis <sup>‡</sup>	The function of internal organs damaged by pathogenic heat resulted in Qi stagnation, such that there is inability to promote the blood run	Fever, abnormal consciousness, efflorescence, deep red tongue, blue purple or petechiae tongue, tongue with rash points, facies labialis of modena, white-eye hyperaemia, the cardinal pulse being thready the fast accompanied by heavy, tight, astringent features	Disseminated intravascular coagulation and its prophase category
Liver depression syndrome	The abnormal evacuating function of liver leads to Qi stagnation	Mental depression or emotional restlessness, inflation or pain of chest and hypochondrium, irritability or depression, bitter taste, sense of suppression in the chest, frequent sighing, wiry pulse, possible concomitant manifestations including insomnia and Amnesia, headache or dizziness, palpitation, exhaustion, anorexia, reduced sexuality, etc.	Ulcerative colitis

\*TCM syndromes and similar diseases in western medicine refer to the different understanding and description of a specific disease respectively according to the diagnostic criteria of TCM and western medicine. It is not a one-to-one correspondence between them because of the close association with a co-existing difference. One certain TCM syndrome may correspond to different diseases in western medicine; a kind of disease in western medicine may also have a variety of relevant TCM syndrome.

<sup>†</sup>Yin and yang is a couple of antithetic aspects. The actual meaning of the yin and yang is 'opposites', such as the negative and positive, declines and rises. Yin and yang emphasizes the balance of energy.

<sup>‡</sup>Qi and blood is a set of most important concepts. Qi is the energy produced by the original material of the life, the function of which is to promote body growth, maintain organs function, metabolism and excretion. When the energy channels were blocked, the illness is caused. The concept of blood in TCM is very different from in modern medicine, the former means all the nutrients from digested food. Qi and blood are closely linked, Qi is evolved from the blood, meanwhile, Qi can promote blood production.

<sup>§</sup>In TCM, wind, cold, wet, dry and fire are five major factors to cause illness. Different diseases caused by different pathogenic factors show different symptoms. Diseases caused by fire usually show symptoms of thirsty and upset, but wet patients often show poor appetite and feeling as if being wrapped around the head. Wind is the most common causes, is changeable and in everywhere. What's more, it can also lead other factors to cause disease. Such as wind-cold illness is caused by the combined effect of wind and cold. Wind is not only come from the external environment, but also can be caused by the disorder of the body functions. For example, when the lung-yang was too exuberant, the wind form lung would blows eyes and cause the increase of red blood in eyes.



**Fig. 1** The important steps in the proteomics work flow. Two-dimensional gel electrophoresis is the central tool for displaying the proteome. Proteins are separated on the basis of charge in the first dimension and molecular mass in the second. The emerging technology of mass spectrometry-based quantitative proteomics provides a powerful tool to systematically and quantitatively assess quantitative differences in protein profiles of different samples and is become an important component of biomedical and clinical research.

proteomics are protein separation, protein identification and characterization and protein bioinformatics (Fig. 1).

Two-dimensional gel electrophoresis is the central tool for displaying the proteome [5]. Proteins are separated on the basis of charge in the first dimension and molecular mass in the second. With the help of specialized software, analysis of gel images allows comparisons of multiple gels to comprehensive proteome databases on the internet, and the difference between healthy and diseased samples can be revealed by a process of subtraction. Being digested with trypsin, the proteins were cleaved at specific amino acid sequences and were broken into mixture of peptides.

The masses of the peptides were measured by mass spectrometry to produce a peptide mass fingerprint. By compared with the peptide masses predicated from theoretical digestion of protein sequences currently contained within databases, the protein can be identified. Ultimately, protein bioinformatics were employed to find the relevant biochemical pathways or disease implications highlighted which were displayed from the complex experimental details [6–8].

With the two-dimensional gel electrophoresis and mass spectrometric detection technology matured and widely used, it becomes much easy to rapid separate and identify of multiple proteins. Thus, it provided the new perspectives for biology studies. Since being proposed in 1995, proteomic has gained rapid developments. In 1997, just two articles could be searched on the topic of proteomic through the Web of Science database in the ISI Web of Knowledge. Five years later, in 2002, the number of published articles themed by proteomics has raise to about 1000. Over the last 5 years, the number maintains at a high level (approximately, 3000 per year). As the new perspectives it provided, since its birth, proteomic has been introduced into almost all domains of biology (Fig. 2) and been placed great expectation on, specially received extensive attention in the area of medical diagnosis and drug development [9–15]. As can be seen from Fig. 2, proteomics technology is closely associated with many fields of medicine, such as oncology, haematology, pathology, immunology, neurosciences, etc. Today proteomics was applied to foster an improved understanding of the pathogenesis, develop new biomarkers for diagnosis and search potential targets for development of drugs [16].

## Relationship between proteomics and syndrome of Chinese medicine

The essence of TCM syndrome is a comprehension of the regularity of disease occurrence and development and its performance of symptoms. Because disease is a regular performance of the body functions, there must be materials, based on which to keep our bodies working. Perhaps, these materials would be reflected at molecular level. Proteins are the embodiment and implementation of life activities, influenced and decided by genetic regulation and environmental factors. Furthermore, proteins are not isolated but interrelated with each other. And clinical studies have shown that most diseases are related to changes in proteins, including protein abundance, structure, function and interaction.

Tongue diagnosis plays an important role in TCM. There will be a corresponding performance in the coated tongue for almost all of the diseases (Table 1). Zhang *et al.* [17] separated the proteome of fur organization in four groups by two-dimensional gel electrophoresis, scanned them into the computer after silver staining, then analysed and compared the electrophoretogram by Image Master 2D Platinum software. In their experiments, there

Field: Subject Area	Record Count	% of 21111	Bar Chart
BIOCHEMICAL RESEARCH METHODS	6944	32.8928 %	██████████
BIOCHEMISTRY & MOLECULAR BIOLOGY	5009	23.7270 %	████████
CHEMISTRY, ANALYTICAL	2431	11.5153 %	████
BIOTECHNOLOGY & APPLIED MICROBIOLOGY	1603	7.5932 %	███
CELL BIOLOGY	1168	5.5327 %	██
ONCOLOGY	861	4.0784 %	█
BIOPHYSICS	812	3.8463 %	█
PHARMACOLOGY & PHARMACY	800	3.7895 %	█
GENETICS & HEREDITY	665	3.1500 %	█
SPECTROSCOPY	634	3.0032 %	█
CHEMISTRY, MULTIDISCIPLINARY	615	2.9132 %	█
NEUROSCIENCES	601	2.8469 %	█
PLANT SCIENCES	526	2.4916 %	█
MULTIDISCIPLINARY SCIENCES	520	2.4632 %	█
MEDICINE, RESEARCH & EXPERIMENTAL	507	2.4016 %	█
MATHEMATICAL & COMPUTATIONAL BIOLOGY	465	2.2026 %	█
MICROBIOLOGY	451	2.1363 %	█
IMMUNOLOGY	389	1.8426 %	█
COMPUTER SCIENCE, INTERDISCIPLINARY APPLICATIONS	352	1.6674 %	█
TOXICOLOGY	336	1.5916 %	█
BIOLOGY	318	1.5063 %	█

**Fig. 2** The relevant disciplines involved in the articles themed by proteomics published during 1997–2009. A total of 21,111 articles were retrieved on 22 June 2010 by searching the theme of Proteomics through the Web of Science database in the ISI Web of Knowledge, then analysed by subject area and sorted by the number of articles. The top 21 names of disciplines and the number of related articles are listed here.

**Table 2** The differences of coated tongue at protein level

Group	Examined protein plots	Differentially expressed proteins
Normal group	(1082 ± 105)	
Thin coating group	(1052 ± 85)	Six protein spots were up-regulated and eight were down-regulated.
Thick coating group	(1129 ± 98)	Six protein spots were up-regulated and seven were down-regulated.
Stripping coating group	(1143 ± 140)	Eight protein spots were up-regulated and seven were down-regulated.

were some differential protein spots found between different tongue groups (Table 2). Therefore, proteomics technology can be utilized in TCM syndromes research and bring its essence to light, providing a scientific basis for diagnosis, prevention and treatment.

In addition, in the holistic and systemic context, proteomics and TCM have a convergence not only taking care of individual genes and proteins, but also paying more attention to the relationship between them [18]. The overcoming of one-sidedness and singleness of TCM (study) and the avoidance of complication and tedious processes resulting from that would enhance the accuracy and efficiency during the study. With the further development, mature of proteomics and its increasing cross-penetration between various disciplines, it will serve as a strong research platform for the study of TCM syndromes and help promoting the disclosure of the nature of the discipline.

## Applications of proteomics in syndrome

At present, there have been researchers applying proteomics in the determination of TCM syndrome to discover the relevant proteins to provide evidences for TCM by comparing the differences (expression, structure, function and interaction) of proteins between healthy persons and patients, eventually to disclose the material foundation and mechanism of diseases (Table 3). As an example, Tan QX, *et al.* exerted serum proteomic techniques to prepare protein map, combing disease differentiation and dialectic, taking as samples the serum of healthy persons and patients with liver depression diseases, and separating proteins by two-dimensional gel electrophoresis. After the comparison between the normal groups and liver depression groups, the differential protein group

**Table 3** Application of proteomics in the studies of TCM syndrome

Disorder *	Analytes and methods	Results	Conclusions	Reference
Unstable angina patients with blood stasis syndrome	Plasma proteins Two-dimensional gel electrophoresis and mass spectrometry	Five proteins (Fibrinogen $\beta$ chain, fibrinogen $\gamma$ chain, $\alpha$ 1-antitrypsin, haptoglobin $\beta$ chain and haptoglobin $\alpha$ 2 chain) were significantly highly expressed in the plasma of these patients, whereas four proteins (ApoA-IV, ApoA-I, transthyretin and ApoJ) were decreased in their plasma. The proteins expressed higher could be divided into three categories according to their functions: acute phase reactive protein, apolipoprotein and blood coagulation protein.	It was speculated that unstable angina patients with blood stasis syndrome may be related to inflammation, lipid metabolism, and coagulation abnormalities.	20
Kidney-Yang deficiency syndrome	Serum proteins with immunoglobulins and albumin removal Two-dimensional gel electrophoresis and mass spectrometry	Twenty six proteins were found twofold up-regulated levels whereas 33 proteins were down-regulated at least twofold. Among these identified proteins, 33 proteins might be related to human Kidney-Yang deficiency syndromes.	Protein function analysis found that 33 kinds of differential expression of protein were related to Kidney-Yang deficiency syndrome.	21
Ganyang Huafeng syndrome and Xuexu Shengfeng syndrome	Proteins of peripheral blood mononuclear cells Two-dimensional gel electrophoresis, Coomassie Brilliant Blue staining, PDQuest analysis software, and MALDI-TOF-MS	The good 2-DE pattern including resolution and reproducibility was obtained. Fifteen spots were incised and analysed by MALDI-TOF-MS. All proteins preliminarily identified, were related to cytoskeleton, anti-oxidative stress, protein degradation, signal transduction and cell cycle, etc.	Preliminary identification showed differentially expressed proteins related with the cytoskeleton, anti-oxidative stress, protein degradation, signal transduction, cell cycle regulation, etc.	22
Stagnation of blood type of coronary heart disease	Plasma proteins Two-dimensional gel electrophoresis, PDQuest analysis software, and MALDI-TOF-M	Compared with normal plasma, three proteins decreased and six proteins increased in blood plasma of stagnation of blood type of coronary heart disease, the increased proteins included fibrinogen, granzyme, immunoglobulin.	Fibrinogen and granulocyte enzymes are expected to be diagnostic markers for stagnation of blood type of coronary heart disease.	23
Splenic asthenia syndrome	Proteins of small intestinal epithelia Splenic asthenia syndrome animal models, two-dimensional gel electrophoresis technology, improved technique of Coomassie brilliant blue staining, PDQuest software, MALDI-TOF-MS	Thirty-three proteins were identified, of which the expression of 27 proteins decreased whereas six proteins increased. After treated with traditional dry Radix Ginseng decoction, the increasing expressions of the proteins mostly were down-regulated and the decreasing expressions of the proteins mostly were up-regulated. The functions of identified proteins were almost related to the main functions of small intestinal cell.	The main protein features of intestinal epithelial cells of Spleen-type mice showed two-way changes in protein expression, and the down-regulation was the main mode. Differential expression of proteins involved various parts of cells. The regulation on cell proteome by White Ginseng Decoction may be one of the important mechanisms of white ginseng invigorating the spleen.	24

Continued



**Table 3** Continued

Disorder*	Analytes and methods	Results	Conclusions	Reference
Noxious heat blood stasis syndrome (NH-BS)	Serum proteins NH-BS animal models, two-dimensional gel electrophoresis technology, improved technique of Coomassie brilliant blue staining	Thirteen proteins, in the serum of rats with NH-BS significantly changes compared with the normal control group, of which 11 proteins were up-regulated and two proteins were down-regulated. After being treated with RPR, five of the increased proteins significantly decreased whereas one of the decreased significantly increased.	Paeonia may regulate certain proteins points such as xPr1, xPr2, xPr3, xPr4, xPr9 and xPr16 to treat noxious heat blood stasis syndrome.	25
Liver stagnation syndrome	Serum proteins Two-dimensional gel electrophoresis, improved technique of Coomassie brilliant blue staining	Compared with the normal control group, 12 proteins were differentially expressed in the serum of patients with liver stagnation syndrome.	The replicable and stable serum protein techniques were established; the serum of patients with the same symptom but different diseases were made preliminary screening and 12 different protein spots were found.	19

\*The corresponding concepts of Chinese medicine please refer to footnote indicators in Table 1.

was found and 12 differential protein spots were identified. The further analysis demonstrated these differential proteins were mainly related to immunization, neuroendocrine and nutrient metabolism [19], consistent with the existing research findings. Certain achievements in the field have been acquired (Table 3).

Meanwhile, proteomics was also applied to study the pharmacology of TCM (Table 4). The most popular pathway is the combined use of 2D gel electrophoresis, biological mass spectrometry and other proteomics technology to isolate, identify and characterize proteins, the expressions of which were changed [26–30]. Furthermore, the further characterization ranging from the basic physicochemical properties to the prediction of potential post-translational modifications, from three-dimensional structures to potential physiological function, was performed to find the relationship between the changes of physiological and proteome [16]. These differentially expressed proteins (group) provide clues to search for biomarkers and drug targets and are advantageous to standardizing TCM diagnosis and improving the scientificity of disease treatment.

## Problems and prospects

Being constrained by research tools, levels of recognition, analytical methods and other factors, the endeavour to introduce proteomics into the studies of TCM symptoms is still confronted with many difficulties. Currently, researchers mainly apply proteomics technology to detect and recognize differential proteins. However, the investigation of protein function is still not gone far enough. Although abundant proteomics responsible for TCM syndrome have been discovered, the problem what the role these proteomics play in the generation and development has not yet been resolved.

Despite of the rapid advancements acquired by proteomics in the past years, there are also some disadvantages: low reproducibility, difficult separation of proteins of big or small isoelectric point. Moreover, some hydrophobic proteins, insoluble membrane proteins and large molecular weight, low-abundance proteins may be ignored in the process of examination.

The notion of TCM syndrome possesses the obvious macroscopic. The scope of TCM symptoms commonly covers multiple organs, organization or functional system throughout the body, whereas proteomics explore and recognize the regularity of vital movement from the perspective of proteins. How to resolve the discrepancies between micro and macro cognition is one pending problem demanding the continuous exploration, the other one is how to appropriately process and analyse the thousands of data derived from related experiments.

Nevertheless, the combination of proteomic studies with the TCM symptoms gives us a great temptation. The history of development of modern biology takes on the features that the direction has changed from part to whole and from linear thinking to complexity. The overall dynamic development of proteomics more reflects its growing unity with the thinking methodology of TCM. Both proteomics and TCM answer the same question what is the nature of disease from two different angles. Proceeding with the microcosmic, the former reveals the essence of the generation, development and healing of diseases by investigating proteins, whereas the latter grasp the disease regularity through awareness of symptoms on the base of the long-term clinical practice from a macrocosmic perspective. Chinese medicine has a wealth of experience, meanwhile proteomics has a substantial research potential. The organic integration of the two aspects will bring a great enhancement of our knowledge of disease, not at the microscopic, but also at macroscopic. Besides that, the integration is mutually beneficial to each side. On one hand, proteomics introduce new thoughts and give a big impetus to TCM; on the other hand, the unique theory and perspective of TCM offer flesh consideration for the development of proteomics.

**Table 4** Application of proteomics in the studies of TCM

Chinese crude drug/active ingredients	Analytes and methods	Results	Reference
Puerarin	Total protein from lung tissue Two-dimensional gel electrophoresis, MALDI-TOF-TOF-MS	The expression of calreticulin precursor was almost at zero level in the normal group and puerarin group, but a great quantity in 3-week-monocrotaline group. It could be concluded that the puerarin could inhibit the form of pulmonary hypertension by interfering with calreticulin precursor.	26
Gui-zhi Decoction*	Proteins in hypothalamus Two-dimensional gel electrophoresis	There were obvious differences in expression of some proteins between model group and treated group. In treated groups, the expression of eight proteins ( <i>Mr/pl</i> : 28.9 kD/4.47, 24.4 kD/6.28, 25.4 kD/6.39, 25.9 kD/6.39, 17.6 kD/7.38, 17.2 kD/7.43, 24.9 kD/7.39, 26.9 kD/7.59) was increased, whereas six proteins ( <i>Mr/pl</i> : 14.3 kD/4.83, 28.5 kD/4.39, 16.2 kD/4.11, 15.3 kD/6.70, 30.5 kD/7.09, 30.5 kD/7.13) were decreased and one changed in isoelectric point. The changes in expression level and modification of some proteins may contribute to the antipyretic action of Gui-zhi Tang.	27
Dry <i>Radix Ginseng</i>	Proteins of small intestinal epithelia of the splenic asthenia syndrome mice Two-dimensional gel electrophoresis, proved technique of Coomassie brilliant blue staining, PDQuest software and MALDI-TOF-TOF-MS	Among 33 identified protein spots, the expression of 27 protein spots decreased and 6 increased. After treated with traditional dry <i>Radix Ginseng</i> decoction, the expression of those proteins was adjusted to normal levels. The adjustive action of dry <i>Radix Ginseng</i> of Chinese traditional medicine on proteomics of small intestinal epithelia is one of the important mechanism of invigorating spleen of dry <i>Radix Ginseng</i> .	28
Tianma Gouteng Decoction†	Two-dimensional gel electrophoresis, MALDI-TOF-MS and database analysis	About 20 protein spots were expressed at different levels between normal and disease groups. Among these spots, five were up-regulated twofold and 10 were down-regulated at least twofold in disease group. After being treated Tianmai Gouteng Decoction, the expression of those proteins was adjusted to the normal levels.	29
Total flavones of <i>hippophae rhamnoides L.</i> and <i>choerospondias axillaries fructus</i>	Proteins from myocardial ischaemia Surface enhanced laser desorption/ionization mass spectrometry, protein chip (IMAC3, SAX2 and NP20)	Six differentially expressed proteins were identified in total flavones of <i>hippophae rhamnoides L.</i> (TFH) treated myocardium, in which five down-regulated and one up-regulated. Seven differentially expressed proteins were identified in total flavones of <i>choerospondias axillaries fructus</i> (TFC) treated myocardium, among these proteins, five down-regulated and two up-regulated. The myocardial protection of TFH and TFC may be mediated by the differential expression of these proteins which could be the key protein candidates for different kinds and amounts.	30
Qi-Boosting Toxin-Resolving Granule	Proteins from HNEI cells Two-dimensional gel electrophoresis, silver staining and PDQuest 2-de microsoft	The mean number of protein spots was $1120 \pm 89$ for treated HNEI cells with Qi-Boosting Toxin-Resolving Granule and that was $1281 \pm 102$ for untreated cells, the differentially expressed protein spots were 673, among which 218 were up-regulated whereas 455 down-regulated in treated HNEI cells.	31

\*Guizhi Decoction is one of the most famous prescription, first recorded in the 'Treatise on Cold-induced Febrile Diseases'. It was containing ramulus cinnamomi, paeonia lactiflora, radix glycyrrhiza, rhizome zingiberis recens and ziziphus jujube Mill.

†Tianma Gouteng Decoction contains gastrodia elata Blume, gardenia jasminoides Ellis, scutellaria baicalensis Georgi, eucommia ulmoides Oliver, leonurus japonicus Houtt, Chinese taxillus Twing, caulis polygoni Multiflori, cyathula officinalis Kuan and ramulus uncariae cumuncis.

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## Conflict of interest

The authors confirm that there are no conflicts of interest.

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