Differential Proteomics Reveals the Pharmacological Mechanisms of Traditional Chinese Medicines

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ABSTRACT: Traditional Chinese medicines (TCMs) have been used for more than 3,000 years. However, most TCMs are used based on previous experience, and their exact mechanisms remain unclear. Proteomics, a major component of systems biology, is a powerful tool that could produce breakthroughs in the study of the pharmacological mechanisms of TCMs. Researchers have made substantial progress investigating the mechanisms of TCMs using proteomics approaches. In this review, we summarize the current applications of proteomics in the mechanistic study of TCMs. Proteomics technologies and strategies that might be used in the future to enhance the study of TCMs have also been discussed. This review demonstrates the wide applicability of TCMs in disease treatment and highlights a number of examples in which proteomic analysis lead to increased understanding of the mechanisms of action of various TCMs. It will lay the foundation for further application of differential proteomics in TCMs studies.

INTRODUCTION

Traditional Chinese medicines (TCMs) have been developed and used for more than 3,000 years in China and other Asian countries, but their clinical applications are limited because the pharmacological mechanisms of most are not well understood. It is therefore vital to use modern technology to determine the cellular mechanisms of TCMs. The mainstream strategy to develop TCMs as pharmacological agents is to separate, extract, and identify the effective components from herbs or animals, observe their biological and medical effects in cellular and animal models, and then explore the signaling pathways affected by the compounds at the molecular level. The antimalarial medicine 'artemisinins' is a prime example. By isolating the compound from the TCMs qinghao, Prof. Youyou Tu led a team that transformed an ancient Chinese healing method into the most powerful antimalarial medicine in use **(1)**. As_2O_3, another compound isolated from a TCM, is used to treat leukemia **(2)**. These two drugs are comparable in effectiveness to Western medicines. However, the complexity of TCMs are have generally precluded their use in Western medicine. Therefore, to promote TCM-based drug development and enable their use worldwide, it is necessary to elucidate the molecular mechanisms of TCM components in living systems. The variety of chemical components contained in TCMs and the complexity of the interactions between TCMs and the human body, makes isolating and identifying these mechanisms quite difficult.

Proteomics, a primary component of systems biology, has gained much attention in the fields of medical diagnosis, drug development, and mechanism studies **(3, 4)**. Differential proteomics, also called comparative proteomics, is used to study protein changes between two or more samples in different physiological or pathological states **(5)**. It employs both labeling and label-free technology to provide mechanistic insight for complex samples. For example, cell lines or tissues with and without TCM treatments can be either labeled with various tags or grown label-free (Figure 1). The lysates are then digested with trypsin, followed by analysis by mass spectrometry (MS). Proteins with altered levels can be further analyzed by bioinformatics and additional benchwork to uncover pathways regulated by TCMs.

The classic experimental procedure for differential proteomics in studying TCMs involved separation, comparison, and identification. First, proteins are extracted from cells or animal models with and without TCM treatment. Two-dimensional electrophoresis (2-DE), for example, is used to separate the proteins.

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Protein spots on paired gels are compared and selected for identification by MS. To overcome the limitations in protein separation inherent to 2-DE, the technique can be replaced by liquid chromatography. Moreover, various labeling technologies, such as isobaric tags for relative and absolute quantification (iTRAQ), tandem mass tags (TMTs), and stable isotope coded with amino acids in cell culture (SILAC) can be used, and label-free quantification is also an option. Proteins with differences among samples are identified by their mass and represent, potential drug targets. This global analysis of protein alterations can provide much insight into a drug's cellular mechanisms.

APPLICATIONS OF TCM PROTEOMICS

Type 2 Diabetes Mellitus

Type 2 Diabetes Mellitus (T2DM) is a primary chronic disease characterized by insulin resistance and the failure of pancreatic cells to compensate via adequate insulin secretion **(3)**. Studies using traditional 2-DE coupled with matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF)-MS identified seven serum proteins that were differentially expressed in a rat model of T2DM. These proteins included the down-regulated apolipoprotein E, apolipoprotein A-I, Ig gamma-2A chain C region, and up-regulated transthyretin, haptoglobin, serum amyloid P-component, and prothrombin. After administration of Tian-qi-jiang-tang capsules, which include *Astragalus membranaceus* (Fisch.) Bge (huang-qi in Chinese), *Panax ginseng* C.A. Mey. (ren-shen in Chinese), *Coptis chinensis* Franch (huang-lian in Chinese), *Ligustrum lucidum* Ait. (nv-zhen-zi in Chinese), *Trichosanthes kirilowii* Maxim. (tian-hua-fen in Chinese), *Dendrobium nobile* Lindl. (shi-hu in Chinese), *Lycium chinense* Mill (di-gu-pi in Chinese), *Cornus officinalis* Sieb.et Zucc. (shan-zhu-yu in Chinese), *Rhus chinensis* Mill. (wu-bei-zi in Chinese), *Eclipta prostrata* (L.) L. (han-lian-cao in Chinese), the majority of these proteins were restored to levels observed in healthy rats **(6)**.

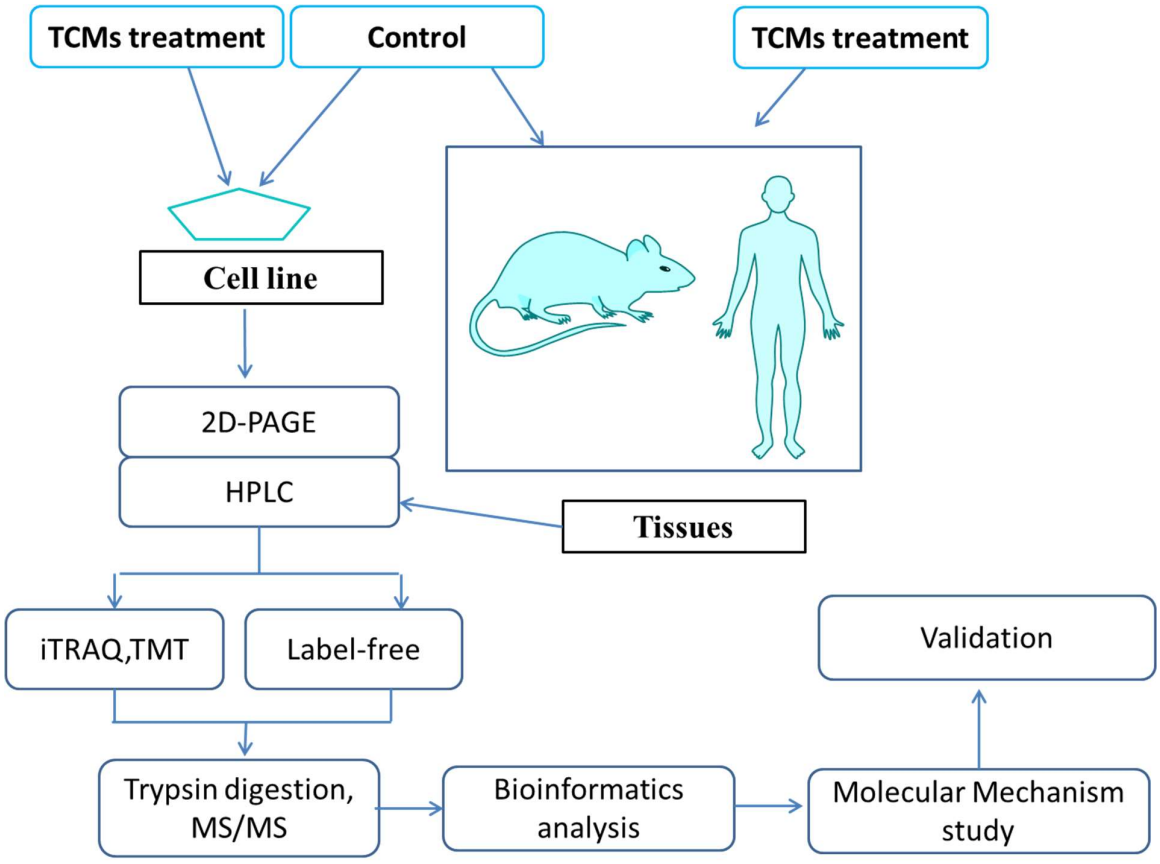

Figure 1. Scheme of general approaches for the application of proteomics in the mechanistic study of TCMs This review summarizes the recent applications of proteomics in TCM research and development, focusing on how proteomics can explain the pharmacological effects of TCMs on various diseases including diabetes, cardiovascular disease, neurological disease, liver diseases, inflammatory responses, and cancer (Table 1).

Another study using surface-enhanced laser desorption/ionization (SELDI)-TOF-MS and bioinformatics analysis reported that the ginsenoside Re, extracted from *Panax ginseng* C.A. Mey, may alter the expression of c-reactive protein, improving diabetes**(7)**. Furthermore, in a diabetes-associated model of cognitive decline, a fluorescence-based differential gel electrophoresis method identified nine candidate proteins involved in diabetes development, such as pyruvate dehydrogenase and dystrophin related protein 2, and defined potential therapeutic targets for zi-bu-pi-yin (composed of the *Panax ginseng* C.A. Mey (ren-shen in Chinese), *Paeonia lactiflora* Pall. (bai-shao in Chinese), *Salvia miltiorrhiza* Bunge (dan-shen in Chinese), *Poria cocos* (Schw.) Wolf (fu ling in Chinese), *Lablab purpureus* (Linn.), *Citrus reticulata* Blanco (chen-pi in Chinese), *Dolichos lablab (bai-bian-dou in Chinese)*, *Glycyrrhiza uralensis* Fisch. (gan-cao in Chinese), *Nelumbo nucifera* Gaertn (lian-zi in Chinese), *Polygala tenuifolia* Willd (yuan-zhi in Chinese), *Santalum album* Linn. (tan-xiang in Chinese), *Dioscorea opposita* Thunb. (shan-yao in Chinese) in the hippocampus **(8)**. Lower limb macroangiopathy is a major complication in T2DM, which can cause the development of ulcers. The Mai Tong formula (MTF) is a classical Chinese herbal formula used to treat lower limb macroangiopathy in T2DM patients. It contains *Radix Astragali* (Huangqi), *Fructus Mori* (Sangshen), *Angelica sinensis* (Danggui), *Salvia miltiorrhiza* Bge (dan-shen), *Alisma plantago-aquatica* Linn (Zexie), and *Lonicera japonica* Thunb (Jinyinhua). Gong et al. used iTRAQ quantitative proteomic assays to investigate the differential proteins and key pathways affected by MTF treatment in a spontaneous diabetic rat model **(9)**. MTF treatment regulated the expression of fatty acid synthase, guanine nucleotide binding protein, recombinant human protein kinase CAMP-dependent regulatory type II alpha, myosin heavy chain 11, and myosin heavy chain 6.

Cardiovascular Disease

Tongxinluo (TXL) is a TCM that is used to treat cardiovascular diseases in China. Li et al. applied a TMT quantitative proteomics approach to detect TXL target proteins in a model of cardiac microvascular endothelial cell ischemia-reperfusion (I/R) injury**(10)**. TXL treatment increased the expression of six proteins (acyl-coenzyme A synthetase medium chain family member 2B, cyclin-dependent kinase inhibitor 1B, heme oxygenase 1, transcription factor SOX-17, sequestosome-1 isoform 1, and TBC1 domain family member 10B). Moreover, TXL decreased the expression of five proteins (angiopoietin-2 isoform c precursor, cytochrome c oxidase assembly factor 5, connective tissue growth factor precursor, cathepsin L1 isoform 2, and eukaryotic elongation factor 2 kinase in this model.

Hyperin (quercetin-3-O-galactoside), a flavonoid component of *Apocynum venetum* L (Luo-bu-ma in Chinese) is generally used to treat endothelium dysfunction. However, its pharmacodynamic mechanism remains unclear. Liu et al. applied iTRAQ-based quantitative proteomic analysis to investigate the protective effects of hyperin against H_2O_2-induced injury in human endothelium-derived EA.hy926 cells **(11)**. They found that hyperin effectively protected H_2O_2-induced cells against injury by regulating apoptosis through the anti-apoptotic BCL-family proteins MCL1 apoptosis regulator and BH3-interacting domain death agonist. The results showed that hyperin is a promising candidate drug for the treatment of thrombotic diseases.

Tian-ma is a TCM commonly used to treat hypertension and heart disease. Feng et al. found that tian-ma decreased not only the expression of contractile proteins (e.g., actin alpha 2, smooth muscle) but also other related structural proteins (e.g., desmin) **(12)**. It also increased the expression of extracellular matrix glycoproteins (e.g., fibulin 5) and anti-thrombotic proteins (e.g., annexin A2) in aortic tissue. Tian-ma effectively prevents hypertension by suppressing vascular smooth muscle contraction, improving blood vessel elasticity, stabilizing the arterial structure.

Salvia miltiorrhiza (dan-shen in Chinese) and *Panax notoginseng* (san-qi in Chinese) are well-known TCMs for the treatment of cardiovascular diseases and are often used in combination. However, the mechanisms of their cardio-protective effects remain unclear. Studies using 2-DE coupled with MALDI-TOF explored the cardioprotective effects of salvianolic acids (SA) from dan-shen, notoginsengnosides (NG) from san-qi and a combination of SA and NG (CSN) in an IR rat model. The CSN treatment exhibited better effects than SA or NG alone**(13)**. SA and NG have differing protein targets in the treatment of cardiovascular diseases, and the ability of CSN to adjust both sets of targets may be the basis for its superior cardioprotective effects. Another study reported that NG inhibits ADP-induced platelet aggregation by up-regulating the expression of growth factor receptor-bound protein 2, thrombospondin 1, and tubulin alpha 6, and down-regulating the expression of DJ-1 protein, thioredoxin, peroxiredoxin3, Cu-Zn superoxide

dismutase, thioredoxin-like protein 2, ribonuclease inhibitor, myosin regulatory light chain 9, potassium channel subfamily V member 2, and laminin receptor 1 **(14)**. Salvianolic acid B (SB) is also extracted from dan-shen, and is believed to prevent IR injury. SB interacts with epidermal growth factor receptor to activate a downstream signaling cascade involving heat shock protein (HSP) 27 and mitofilin **(15)**. In addition, SB suppresses the adhesion and aggregation of platelets by regulating intracellular Ca^{2+} levels and the binding of cytoskeletal proteins to integrin a2β1 **(16)**. Ding-xin recipe (DXR), has been used to treat arrhythmias for over 20 years. To better understand its underlying mechanism of action, 2-DE coupled with MALDI-TOF-MS were used to identify differentially expressed proteins in a rat model of IR-induced arrhythmia with or without DXR pre-treatment. DXR could weaken IR-induced arrhythmias, possibly through increased expression of prohibitin and glutathione, reduced expression of interleukin-6, and neutrophil infiltration **(17)**.

Apigenin is a flavonoid that may attenuate atherogenesis in apolipoprotein E(-/-) mice by inducing macrophage apoptosis through the suppression of Akt Ser 473 phosphorylation and downregulation of plasminogen activator inhibitor-2 **(18)**. Shen Song Yang Xin (SSYX) is a TCM, that has been used to treat arrhythmia, which includes Panax ginseng, dwarf lilyturf tuber, and Nardostachys root. Liu et al. **(19)** established a rabbit model of bradycardia and explored protein changes in the heart after SSYX treatment by iTRAQ-based quantitative proteomic analysis. SSYX upregulated the expression of ryanodine receptor 2, ATPase sarcoplasmic/endoplasmic reticulum Ca^{2+} transporting 2, and voltage-dependent anion channel 1, while down-regulating the expression of proteins related to glycometabolism and lipid metabolism. Shuanglong formula (SLF) is a classic TCM used for the treatment of heart disease. Fax et al.**(20)** used comparative proteomics analysis to determine that SLF might induce mesenchymal stem cells into cardiomyocyte-like cells by regulating the expression of cytoskeletal, energy metabolism, and signal transduction proteins. Qi Shen YiQi (QSYQ) is used to treat cardiac dysfunction. It consists of Astragalus membranaceus (Huangqi), *Salvia miltiorrhiza* (dan-shen), *Panax notoginseng* (san-qi), and *Dalbergia odorifera* (Jiang-xiang, DO). Chen et al. **(21)** used proteomics analysis to explore the mechanism behind QSYQ suppression of cardiac hypertrophy in a rat model of ascending aortic stenosis (AAS) model. They found that QSYQ markedly attenuated cardiac hypertrophy by regulating the levels of protein involved in energy metabolism and oxidative stress.

Neurological Disorders

Gastrodia elata Blume (tian-ma in Chinese) is often used for the treatment of convulsions, pain, headache, dizziness, vertigo, and neurodegenerative diseases. Previous study using iTRAQ proteomics investigated tian-ma's effects on neuronal signaling pathways in human neuronal SH-SY5Y cells **(22)**. The results showed that tian-ma promotes neuroregenerative signaling cascades by regulating chaperones and proteasomal degradation pathways (e.g. calreticulin, FKBP proly1 isomerases 3 and 4, HSPs 70 and 90) and modulating other proteins (reticulons 1 and 4, neural cell adhesion molecule 1, protein kinase C and casein kinase substrate in neurons 2, and PDZ and LIM domain 1 and 5) with various regenerative capacities related to neuro-synaptic plasticity. Furthermore, tian-ma promotes neuro-regenerative processes by decreasing stress-related proteins and increasing neuroprotective genes such as nucleoredoxin; drebrin-like; MOB family member 4, phocein; chloride intracellular channel 4 (mitochondrial); antigen identified by monoclonal antibody Ki 67; and BCl-2 associated X protein, which have various effects on neuro-synaptic plasticity in mouse neuronal N2a cells **(23)**. The TCM *Uncaria rhynchophylla* (Miq) Miq. ex Havil. (gou-teng in Chinese), is used for the treatment of convulsive disorders, including epilepsy. Proteomics analysis using 2-DE and MALDI/TOF/TOF revealed that macrophage migration inhibitory factor (MIF) and cyclophilin A were downregulated in the frontal cortex and hippocampus of kainic acid-induced epileptic rats. However, gou-teng treatment overcame this effect, suggesting that MIF and cyclophilin A participate in its anticonvulsive effects **(24)**. Huperzine A, extracted from the TCM *Huperzia serrata* (she-zu-shi-shan in Chinese), is used to treat neurodegenerative diseases such as Alzheimer's disease. A recent study implemented label-free quantitative proteomics to investigate the effects of huperzine A on neuronal cells, and demonstrated that hupreazine A protects N2a cells from amyloid β oligomer-induced cell death by downregulating tumor protein p53 expression **(25)**. EGb 761, extracted from *Ginkgo biloba* (yin-xing in Chinese), is used to treat neurodegenerative diseases. A study using 2DE showed that EGb 761 targets protein phosphatase 2A subunit B, peroxiredoxin- 2, and collapsing response mediator protein 2 (CRMP2) in a rat model of middle cerebral artery occlusion. Another study showed that baicalin, extracted from *Scutellaria baicalensis* Georgi (Huang-qin in

Chinese), effectively regulated the expression of proteins involved in energy metabolism, in a rat model of middle cerebral artery occlusion **(26)**. Stroke is a leading cause of death worldwide, and tissue plasminogen activator is approved by the United States Food and Drug Administration for the treatment of stroke in the acute phase. Bu-yang-huan-wu decoction (BHD), a TCM prescription, has long been used to ameliorate neurological functional recovery after stroke. An iTRAQ-based proteomics approach was used to identify the differentially expressed proteins after BHD treatment in a mouse model of cerebral IR injury. BHD treatment maintained the integrity of the blood-brain barrier, suppressed excitotoxicity, enhanced energy metabolism, increased the expression of the neurogenesis marker doublc cortin, and inhibited the expression of glycogen synthase kinase 3 and microtubule associated protein tau **(27)**. Another study reported that BHD attenuates ventricular remodeling caused by left anterior descending artery ligation in rats **(28)**.

Inflammatory Responses

Inflammation is a complicated biological response of vascular tissues to harmful stimuli. In Western medicine, it is inhibited by steroids or antibiotics. TCM has used a variety of natural formulations to reduce inflammatory responses for thousands of years. However, the mechanism of action of TCMs that reduce inflammation are yet to be explored. The TCM Xiao-qing-long-tang in Chinese, named Shoseiyutoin Japan, is used to treat allergic bronchial asthma clinically. A study using 2-DE showed that Xiao-qing-long-tang reduced spectrin 2 expression in the lung tissue of a mouse model of ovalbumin-sensitized allergic airway inflammation **(29)**.

Liver Diseases

Hepatic fibrosis is induced by a wound-repair response to liver injury arising from viral hepatitis and metabolic and alcoholic liver diseases **(30)**. The TCMs *Scutellariae radix* (Huang-qin) and *Rheum palmatum* L. (Da-huang) are used to treat liver diseases, including fibrosis. Pan et al. applied 2-DE coupled with MALDI-TOF-MS analysis to discover the protein targets and molecular mechanisms by which Huang-qin and Da-huang improved dimethylnitrosamine-induced liver fibrosis in a rat model **(31)**. They found that these TCMs inhibit hepatic fibrosis mainly by modulating redox status, and regulating the modification of intracellular molecules. Similarly, saffron (Zang-hong-hua) has been reported to alleviate hepatic IR injury by modulating protein oxidation **(32)**. Yin-Chen-Hao-Tang (YCHT) is a well-known TCM formula used for to treat various liver injuries. However, the underlying mechanisms and drug targets of YCHT are largely unclear. Sun et al. **(33)** used 2-DE-MALDI-TOF/TOF-MS to detect YCHT protein targets. YCHT treatment reduced the levels of zinc finger protein 407, haptoglobin, macroglobulin, and alpha-1-antitrypsin and increased the levels of transthyretin, vitamin D-binding protein, and prothrombin. In addition, Lee et al. **(34)** found that YCHT affected liver fibrosis by down-regulating the expression of monocyte chem.-oattractant protein-1 and tissue inhibitor of metalloproteinase- 1 in bile duct-ligated rats. Gypenoside has also been reported to treat liver fibrosis. Song et al. **(35)** found that gypenoside may protect against fibrosis via increasing the expression of ALDH in a CCl_4-induced rat model of liver fibrosis. In another study using 2-DE coupled with MALDI-TOF-MS, the authors showed that *Salvia miltiorrhiza* polysaccharide inhibited nuclear factor-kB activation by increasing the expression of peroxiredoxin 6. This resulted in decreased lipid peroxidation, inducible NO synthase expression, and inflammation in a Bacille Calmette-Guerin (BCG)-primed mice model of lipopolysaccharide-induced immunological liver injury **(36)**. Fu-zheng-hua-yu (FZHY) is an effective Chinese herbal product that can cure liver fibrosis. A study using 2-DE coupled with MALDI-TOF-MS showed that FZHY regulated the expression of aldehyde dehydrogenase, gamma-actin, fructose-bisphosphate aldolase B, aldo-keto reductase, S-adenosylhomocysteine hydrolase isoform, and HSP 90 in a dimethyl nitrosamine-induced rat model of liver fibrosis **(37)**. The results indicate that the molecular mechanism by which FZHY affects liver fibrosis may be through the modulation of proteins involved in metabolism and stress responses, as well as myofibroblast activation.

Cordyceps sinensis (Dong-chong-xia-cao) is also used in the treatment of liver diseases. Wang et.al evaluated the protective effects of Dong-chong-xia-cao against hepatocellular carcinoma (HCC) in a diethylnitrosamine -induced rat model through proteomic analysis **(38)**. They found that Dong-chong-xia-cao relieved HCC by regulating redox imbalance, protein ubiquitination, and tumor growth-associated transcription factors. Lariciresinol is another TCM used to treat HCC. Ma et al. found that lariciresinol regulated the expression of glyceraldehyde-3-phosphate, UDP-glucose 4-epimerase, annexin A1, heat shock protein 27, haptoglobin, tropomodulin-2, tubulin alpha-1A chain, and brain acid soluble protein in HepG2 cells,

suppressing proliferation and causing cell cycle arrests in S phase, resulting in apoptosis **(39)**.

Gamboge (teng-huang) is a TCM used to treat various types of cancer. Its active component, 1,3,6,7-tetrahydroxyxanthone (TTA), can inhibit HCC cell growth. To identify TTA targets in HCC cells, Fu et al. used 2-DE coupled with MALDI-TOF-MS/MS, to demonstrate that TTA up-regulated cyclin dependent kinase inhibitor 2A and 14-3-3σ to inhibit cell growth **(40)**. Another 2-DE study in HCC cells reported that 1,3,5-trihydroxy-13,13-dimethyl-2H-pyran [7,6-b] xanthone (TDP), also extracted from teng-huang, suppressed the expression of HSP27 **(41)**. As_2O_3 has been reported to treat HCC as well as acute promyelocytic leukemia. Yoo et al. found that As_2O_3 induced DNA damage and oxidative stress in human HCC SK-Hep-1 cells **(42)**.

Yiguanjian decoction is a TCM used to treat cirrhosis. Through proteomics analysis, Shen et al. found that Yiguanjian could regulate the expression of superoxide dismutase 1, glutathione synthetase, DJ-1, glutathione S-transferase Yb-1 subunit, and aldo-keto reductase family 7, A2 in a CCl_4-induced rat model of cirrhosis **(43)**.

Cancer

TCMs are used extensively as anticancer drugs. Curcumin, an active component isolated from the TCM *Curcuma longa* L (Jiang-Huang), exhibits cytotoxic effects on multiple cancer cell lines. In MCF-7 breast cancer cells, 2-DE analysis revealed that curcumin treatment upregulated the expression of phosphoglycerate dehydrogenase, endoplasmic reticulum protein 29, and platelet activating factor acetylhydrolase 1b catalytic subunit 2, and downregulated the expression of TAR DNA binding protein, serine and arginine rich splicing factor 1, and eukaryotic translation initiation factor 3 subunit. In human neuroblastoma cells, curcumin elicits different responses in cisplatin-sensitive and resistant cells. Curcumin treatment caused cell cycle arrest in G2/M and increased polyubiquitination in sensitive cells. In resistant cells, it impaired the ubiquitin-proteasome system and increased reactive oxygen species (ROS) production **(44)**.

Berberine is a bioactive compound isolated from the TCM *Coptis chinensis* Franch (Huang-Lian) that exhibits cytotoxicity in various cancer cell lines. Comprehensive proteomics approaches were employed to discover the cytotoxic mechanism of berberine in HepG2 and MCF-7 cells. In HepG2 cells, berberine caused G0 cell cycle arrest or apoptosis. Its target proteins were involved in mitogen-activated protein kinase phosphorylation, metabolism, cell cycle regulation, and DNA damage responses **(45)**. In MCF-7 cells, berberine induced ROS generation and apoptosis and its target proteins were involved in protein folding, proteolysis, redox regulation, trafficking, cell signaling, electron transport, metabolism, and centrosome structure **(46)**.

Ganoderic acid D (GAD) is a triterpene isolated from the TCM *Ganoderma lucidum* (Ling-zhi) that inhibits proliferation in HeLa human cervical carcinoma cells. Yue's laboratory employed 2-DE-MALDI-TOF-MS/MS-based proteomics to analyze the cytotoxic mechanism of GAD in HeLa cells and identified 21 differentially expressed proteins, which were then analyzed *in silico* for possible binding to GAD. The results showed that GAD could bind six isoforms of the tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein family, annexin A5, and aminopeptidase B **(47)**. Tanshinone IIA, an active component isolated from the TCM *Salvia miltiorrhiza* Bunge (dan-shen), exhibits cytotoxic activity against multiple human carcinoma cell lines. Comprehensive proteomics was employed to determine its molecular targets in HeLa cells. Identified targets were related to apoptotic processes, spindle assembly, and p53 activation, and included serpin family B member 5. The results indicated that tanshinone IIA may inhibit cervical cancer cell growth by disturbing microtubule assembly **(48)**.

Aloe-emodin (1,8-dihydroxy-3-(hydroxymethyl)-anthraquinone) is a bioactive compound isolated from the TCM *Rheum palmatum* (Dahuang) that induces apoptosis in lung cancer cells. Lee and his colleagues found that aloe-emodin could increase the expression of certain chaperone proteins, such as HSP70 and protein disulfide isomerase, and decrease the expression of nucleophosmin 1 in the H460 lung cancer cell line **(49)**. Baicalein is an active flavonoid isolated from the TCM *Scutellaria baicalensis* (Huang-qin) that exhibits cytotoxicity in multiple human cancer cell lines. A 2-DE based proteomics approach was applied to discover baicalein targets in colorectal cancer cells. Baicalein treatment increased the expression of peroxiredoxin-6, which decreases ROS production and suppresses the growth of colorectal cancer cells **(50)**.

Triptolide, the main active compound isolated from the TCM *Tripterygium wilfordii* Hook. F (Lei-gong-teng), is an epoxide diterpenoid that has pro-apoptotic and antiproliferative effects on various cancer cell lines **(51)**. Differentail proteomics was employed to detect triptolide-regulated proteins, in colon cancer cells. Treatment could evoke division and perinuclear translocation of 14-3-3ε **(52)**.

Celastrol is another quinone triterpene compound isolated from Lei-Gong-Teng. Hansen and his colleagues found that celastrol suppressed the proliferation of human lymphoblastoid cells by increasing the expression of proteins related to antioxidant defense and inhibition of apoptosis **(53)**.

Rhizoma Paridis total saponin (RPTS), the major bioactive component isolated from the TCM Rhizoma Paridis (Chou-Lou), and demonstrates anti-tumor effects. Cheng and his colleagues used 2-DE-MALDI-TOF-MS to determine that RPTS decreased the expression of dUTP diphosphatase, heterogeneous nuclear ribonucleprotein K, and guanine monophosphate synthase, and increased the expression of deoxyribonuclease gamma, NME/NM 23 nucleoside diphosphate kinase 1, and centrin-2 in HepG2 cells **(54)**.

In addition, paclitaxel, camptothecin, and vinblastine and vincristine, extracted from *Taxus* (Zi-Shan), *Camptoteca acuminate* Decne (Xi-Shu), and *Vinca rosea* L (Chang-chun-hua), respectively, are widely used to treat many kinds of cancers.

CONCLUSION AND PERSPECTIVE

Differential proteomics is a powerful tool for investigate the molecular mechanisms of TCMs. It can provide global views of molecular pathways and identify protein–drug interactions. However, limitations in protein identification, such as low identification rates for low-abundance and poorly soluble proteins, can hinder its application.

In addition, where many studies have identified differentially expressed proteins after TCMs treatments, few have delved into the mechanistic details. Most proteins are activated or inactivated by posttranslational modifications, including phosphorylation, acetylation, methylation, ubiquitination, and glycosylation. Proteomic techniques cataloging these changes after TCM treatment would be highly instructive in elucidating the mechanisms of these drugs.

Moreover, TCMs are complex and unstable in composition, which may affect the consistency of proteomics results.

Other omics technologies, such as genomics, metabolomics, and transcriptomics, may also play crucial roles in TCM research. We believe that integrating omics technologies with bioinformatics, will enable systematic screening that will bring TCM research into a new era, where more comprehensive views of TCM-induced biological alterations can be obtained, advancing the study and exploitation of these valuable compounds.

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Table 1. Summary of the applications of proteomics to explore the pharmacological mechanism Of TCMs for various diseases

Disease	TCM	Specie or cells	Therapeutic targets or pathways	Ref
type 2 diabetes mellitus	Mai Tong Formulae	Rat	AMPK signaling pathway	**(9)**
Type 2 diabetes mellitus	Zi-Bu-Pi-Yin recipe	Rat	dystrophin related protein 2, pyruvate dehydrogenase complex subunit PDH-E1Alpha	**(8)**
Type 2 diabetes mellitus	Tian-Qi-Jiang-Tang Capsule	Rat	Haptoglobin, transthyretin, prothrombin	**(6)**
Type 2 diabetes mellitus	Yi-Qi-Yang-Yin-Hua-Tan-Qu-Yu-Recipe	Rat	Cell division control protein 42 homolog, ras homolog gene family member A	**(55)**
cardiovascular diseases	Tongxinluo	Cardiac microvascular endothelial cells	acyl-CoA synthetase medium chain family member 2B, cyclin-dependent kinase inhibitor 1B, heme oxygenase 1, SRY box 17, sequestosome 1, TBC1 domain family member 10B	**(10)**
bradycardia	Shenxianshengmai	Rabbit	acetylcholinesterase, nicotinic receptor, proteins involved in TCA cycle and oxidation-respiratory chain	**(56)**
Chronic obstructive pulmonary disease	Bu-fei-yi-shen formula	Rat	Oxidative stress and focal adhesion pathway	**(57)**
cardiovascular disease	Salvia miltiorrhizae and Ligustium wallichii Franch	Rat	protein disulfide isomerase family A, member 3	**(58)**
thrombotic diseases	quercetin-3-O-galactoside	human endothelium-derived EA.hy926 cells	MCL1 apoptosis regulator, BCL2 family member, BH3 interacting domain death agonist	**(11)**
cardiovascular diseases	ShenSongYangXin	Rabbit	Aspartate carbamoyltransferase, ATPase sarcoplasmic/endoplasmic reticulum Ca^{2+} transporting 2,voltage dependent anion channel 2	**(57)**
stroke	Buyanghuanwu decoction	Mouse	doublecortin, glycogen synthase kinase 3 and Tau	**(27)**

Depression	Shen-Zhi-Ling tablet	Human	Von Willebrand factor, protein Z-dependent protease inhibitor, alpha2-macroglobulin, apolipoprotein C-III	**(58)**
cardiac hypertrophy	QiShenYiQi Pills	Rat	energy metabolism	**(19)**
early atherosclerotic lesions	Apigenin	Transgenic Mice	serprin family B member 2	**(59)**
Ischemic myocardial injury	Buyang Huanwu decotion	Rat	artial natriuretic factor, HSPβ-6 and peroxiredoxin-6	**(21)**
cardiovascular diseases	Dingxin Recipe	Rat	prohibitin 、 fatty acid binding protein 3、interleukin6	**(18)**
cardiovascular diseases	Tianma	Rat	actin alpha 2, smooth muscle 、 desmin、fibulin 5、annexin2	**(12)**
Cardivoscular disease	Salvianolic acid B	H9C2 cells	heat shock protein27 and mitofilin	**(28)**
acute myocardial infarction	Shuanglong Formula	autologous mesenchymal stem cells	cell differentiation	**(17)**
cerebral artery occlusion	Baicalin	Mice	energy metabolism	**(26)**
blood deficiency	Si-Wu tang	Human	haptoglobin, clusterin, component C4B, GTP binding protein 2, transthyretin, heamoglobin beta	**(15)**
blood deficiency	Si-Wu tang	Mice	Peroiredoxin-V, annexin 1, cofilin, carbonic anhydrase I	**(20)**
blood deficiency	Si-Wu tang	Mice	lymphocyte specific protein 1, proteasome 26S ATPase subunit 4, hematopoietic cell protein-tyrosine phosphatase, glyceraldehyde-3-phosphate dehydrogenase, growth factor receptor binding protein 14 , galectin 12	**(60)**
platelets	Salvianolic acid B	Rat	integrin α2β1	**(61)**
platelets	Notoginsengnosides	Rat	growth factor receptor bound protein 2, thrombospondin 1,Cu-Zn superoxide dismutase, parkinsonism associated deglycase	**(38)**
Alzheimer's disease	Hupreazine A (shi-shan jian A in Chinese)	neuroblastoma N2a cells	p53	**(25)**
Alzheimer's disease	Gastrodia elata Bl.(Tian-ma in Chinese)	mouse neuronal N2a cells	nucleoredoxin, drebrin-like, MOB family member 4, phocein, marker of proliferation Ki-67, BCL2 associated X, apoptosis regulator, heat shock protein 70/90, and FKBP prolyl isomerase 3/4	**(23)**
neurodegenerative diseases	Ginkgo biloba L.(Yin Xing in Chinese) extracts	Rat	PPAP subunit B, dihydropyrimidinase-like 2	**(62)**
Alzheimer's disease	Yizhi Jiannao Granule	Mice	NADH dehydrogenase iron-sulfur protein 6, aconitate hydratase, voltage-dependent anion channel 1, Rho GDP dissociation inhibitor (GDI) alpha, glutathione S-transferase mu1, beta 2-globin and ubiquinol-cytochrome c reductase	**(63)**
Epileptic seizures	Uncaria rhynchophlla (Miq.) Jacks.(Gou-teng in Chinese)	Rat	macrophage migration inhibitory factor, cyclophilin A	**(24)**

Allergic airway inflammation	Xiao-Qing-Long-Tang	Mice	Spectrina 2	**(29)**
Lymphoblastoid cells	Celastrol	Lymphoblastoid cells	heme oxygenase 1	**(53)**
Promyelocytic leukemia	Arsenic trioxide and retinoic acid	acute promyelocytic leukemia NB4cells	calcium signaling and IFN pathway	**(64)**
essential hypertension	Ping-gan-qian-yang formula	Rat	heat shock protein 27,mitofusin-2, Rho GDP-dissociation inhibitor 2, annexin-A1	**(65)**
liver fibrosis	gypenoside	Rat	aldehyde dehydrogenase 1 family, member B1, aldehyde dehydrogenase 2 family member, aldehyde dehydrogenase 7 family, member A1	**(35)**
liver fibrosis	Fu-zheng-hua-yu recipe	Rat	Uridine diphosphate-glucuronosyltransferase 2A3, cytochrome P450 2B1 and cytochrome P450 3A18 in retinol metabolism pathway	**(66)**
liver injury	Salvia miltiorrhiza polysaccharide	Mice	peroxiredoxin 6	**(36)**
hepatocellular carcinoma	Cordyceps sinensis (C. sinensis)	Rat	redox imbalance, protein ubiquitination tumor growth-associated transcription factors	**(67)**
hepatocellular carcinoma	lariciresinol	HepG2 cells	ubiquitin-proteasome pathway	**(39)**
liver diseases	Scutellariae radix and Rhei rhizoma	Rat	S-transferase Mu, glutathione S-transferase Mu 2	**(31)**
liver fibrosis	Fu-Zheng-Hua-Yu	Rat	Vimetin,S-adenosylhomocysteine hydrolase isoform , HSP 90	**(37)**
liver fibrosis	*Bupleurum marginatum* Wall.ex DC	Rat	Uridine diphosphate-glucuronosyltransferase, adenylate kinase isoenzyme 1, thioredoxin 1, acyl-CoA oxidase 2, glycogenin 1, alpha serine/threonine kinase, acyl-CoA synthetase medium-chain family member 1, carbonyl reductase family member 4	**(68)**
hepatic ischemia-reperfusion (IR) injury	saffron	Rat	protein oxidation	**(32)**
hepatic injury	Yin-Chen-Hao-Tang	Rat	zinc finger protein 407,haptoglobin, macroglobulin, alpha-1-antitrypsin; significant up-regulation of transthyretin, vitamin D-binding protein, prothrombin	**(33)**
hepatocellular carcinoma	1,3,6,7-tetrahydroxyxanthone from Garcinia oblongifolia Champ.et Benth	HepG2 cells	cyclin dependent kinase inhibitor 2A, 3-monooxygenase/tryptophan 5-monooxygenase activation protein, epsilon	**(40)**
hepatocellular carcinoma	1,3,5-trihydroxy-13-13-dimethyl-2H-pyran[7,6-b] xanthone from Garcinia oblongifolia Champ.et Benth	HepG2 cells	heat shock protein 27	**(41)**

liver injury	Yin-Chen-Hao-Tang	Rat	Zinc finger protein 407, haptoglobin, macroglobuin,a-1-antitrypsin, transthyretin,vitamin-D-binging protein and prothrombin	**(69)**
liver cirrhosis formation	Yiguanjian Decoction	Rat	superoxide dismutase 1, parkinsonism associated deglycase, glutathione S-transferase, Yb-1 subunit, aldo-keto reductase family 7, A2	**(43)**
hepatocellular carcinoma	Arsenic trioxide	human hepatocellular carcinoma (HCC) SK-Hep-1 cells	DNA damage and oxidative stress	**(42)**
liver fibrosis	Yin-Chen-Hao-Tang	Rat	monocyte chemoattractant protein-1, tissue inhibitor of metalloproteinase-1	**(34)**
kidney-yang deficiency	Curculigo orchioides, Epimedium brevicornum Maxim, Cistanche deserticola, Aconitum carmichaelii, Rhizoma zingiberis(Xian-Mao,Yin-Yang-Huo,Rou-Cong-Rong,Fu-Zi,Gan-Jiang,respectively in Chinese),	Rat	heat shock protein 60, catalase, glutathione peroxidase, carbamoylphosphate synthetase I, ATP synthase, lactotransferrin, H(+)-transporting two-sector ATPase, electron transfer flavoprotein subunit alpha, calpain 12	**(70)**
'Yin-deficiency-heat syndrome	Zhi-bai-di-huang granule	Rat	Zinc-alpha-2-glycoprotein, C-reactive protein, complement C1q sub-component, mannose-binding protein C, l-selectin, plasminogen and kininogen-1	**(71)**
kidney injuries	*Desmodium styracifolium*	Rat	Cathepsin D, mitogen activated protein kinase 14 , cyclin dependent kinase 2	**(72)**
liver-yang hyperactivity type of hypertension	Ping-gan-qian-yang formula	Rat	isocitrate dehydrogenase,steroidogenic acute regulatory protein ,ferritin light chain,elongation factor Tu, Rho GDP disassociation inhibitor 1, flavin reductase, basic transcription factor 3	**(73)**
hepatocellular carcinoma	Rhizoma paridis total saponin	HepG2 cells	deoxyribonuclease 1-like 3, heterogeneous nuclear ribonucleoprotein K [Homo sapiens	**(54)**
hepatocellular carcinoma	Berberine	HepG2 cells	mitogen-activated protein kinase 1	**(45)**
human gastric cancer	Ginsenoside F2 (F2)	SGC7901 human gastric cancer cells	p53 signaling pathway, Bcl-xl/Beclin-1 pathway	**(74)**
acute Lung Injury	Jie-Geng-Tang	Mice	PI3K/Akt signal pathway	**(75)**
nasopharyngeal Carcinoma	Radix Ophiopogonis	Human	vascular endothelial growth factor A, tumor protein p53, heat shock	**(76)**

			protein family A (Hsp70) member 8	
breast cancer	Arsenic trioxide	MCF7 cells	p53	**(77)**
breast cancer	Berberine	MCF7 cells	ROS generation	**(78)**
breast cancer	Curcumin	MCF7 cells	TAR DNA binding protein serine and arginine rich splicing factor 1, eukaryotic translation initiation factor 3 subunit A, phosphoglycerate dehydrogenase „ endoplasmic reticulum protein 29	**(79)**
thyroid cancer	Honokiol	Thyroid cancer cells	glyceraldehyde-3-phosphate dehydrogenase, tubulin alapha-1A chain, alpha-enolase, 78 kDa glucose-glucose -regulated protein, proliferating cell nuclear antigen	**(80)**
non-small cell lung cancer	gamabufotalin	A549 cells	heat shock protein 90	**(81)**
non small lung cancer	Aloe-emodin	H460Cell	heat shock protein 70 150kDa oxygen-regulated protein, protein disulfide isomerase	**(82)**
neuroblastoma	Curcumin	SH-SY5Y cells	Polyubiquitinated proteins	**(44)**
colon cancer	Triptolide	SW480cells	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, epsilon	**(83)**
colorectal cancer	Baicalein	Colorectal cancer cells	peroxiredoxin-6	**(50)**
cervical carcinoma	Tanshione IIA	HeLa human cervical carcinoma cells	heat shock protein 27	**(48)**
cervical carcinoma	Ganoderic acid D	HeLa human cervical carcinoma cells	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, epsilon	**(47)**
influenza virus	Lonicera japonica	Mice	apolipoprotein AI precursor, transthyretin, haptoglobin, major urinary protein	**(84)**
implantation dysfunction	DS-1-47	Mice	collagen α-1 (VI) chain, keratin 7, keratin 14, myosin regulatory light chain 12B, myosin light polypeptide 9, heat shock protein β-7, C-U-editing enzyme APOBEC-2	**(85)**
type I osteoporosis	Qianggu decoction	Human	Angiotensinogen, stromelysin-1, heparanase, glyceraldehyde-3-phosphate dehydrogenase	**(86)**
psoriasis	Yin-Xie-Ling	Human	peptidase inhibitor 3, C-C motif chemokine ligand 22, interleukin 12B	**(87)**