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Review

Application of proteomics to determine the mechanism of action of traditional Chinese medicine remedies

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ABSTRACT

Ethnopharmacological relevance: The rationale for using traditional Chinese medicine (TCM) is based on the experience that has been gained from its wide use over thousands of years. However, the mechanisms of action of many TCM are still unclear. Proteomics, which mainly characterizes protein functions, protein–protein interactions, and protein modification in tissues or animals, can be used to investigate signaling pathway perturbations in cells or the whole body. Proteomics has improved the discovery process of effective TCM compounds, and has helped to elucidate their possible mechanisms of action. Therefore, a systematic review of the application of proteomics on TCM research is of great importance and necessity. This review strives to describe the literature on the application of proteomics to elucidate the mechanism of action of TCM on various diseases, and provide the essential discussion on the further utilization of proteomics data to accelerate TCM research.

Materials and methods: Literature survey was performed via electronic search on Pubmed with keywords 'Proteomics' and 'Traditional Chinese Medicine'. The papers written in English were acquired and analyzed in this review.

Results: This review mainly summarizes the application of proteomics to investigate TCM remedies for neuronal disease, cancer, cardiovascular disease, diabetes, and immunology-related disease.

Conclusions: Researchers have applied proteomics to study the mechanism of action of TCM and made substantial progresses. Further studies are required to determine the protein targets of the active compounds, analyze the mechanism of actions in patients, compare the clinical effects with western medicine.

Contents

Abbreviations: TCM, traditional Chinese medicine; 2DE, 2-dimensional electrophoresis; LC–MS/MS, liquid chromatography coupled with tandem mass spectrometry; SILAC, stable isotope labeling by amino acids in cell culture

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1. Introduction

Traditional Chinese medicine (TCM) has been used and developed for more than several thousand years in China, but its clinical application is limited because the pharmacology of most remedies is unclear. Conventional medicine does not accept the rationale of TCM because it is based on original prescription, which is incompatible with modern pharmacology (Bauer and Chan, 2010). For TCM to be modernized, it is critical to use modern technology to determine the mechanism of action of TCM remedies. One way to develop TCM is to separate, extract, and identify the effective compounds. A description from a 1900 year-old book led researchers to identify artemisinin as the effective constituent of the malaria treatment with the acrial part of *Artemisia annua* L. (青蒿). (Tu, 2011). Chen's group studied a formula that contained multiple constituents, which was used to treat leukemia. They discovered that the effective component of the TCM decoction was arsenic trioxide (As_2O_3). Their later study determined that the mechanisms of action of As_2O_3 mainly involved the induction of the apoptotic pathway. Consequently, As_2O_3 is now widely used to treat acute promyelocyte leukemia (Zhu et al., 2002; Wang et al., 2008). Translational medicine is the conventional strategy that is used to overcome the difficulty of investigating the efficacy of TCM remedies, which are based on the decoction of multiple herbs. PHY906 consists of 4 herbs and is derived from the formulation of Huang Qin Tang that was initially described in Chinese canonical medicine approximately 1800 years ago. Lam et al. (2010) using a gastrointestinal cancer model to study its efficacy to reduce chemotherapeutic toxicity, found that, in mice, PHY906 restored normal intestinal structure, increased proliferating progenitor cells, blocked inflammatory cell migration to the gut, and inhibited inflammatory factors (Lam et al., 2010). The only 2 herbal drugs that are approved by the Food and Drug Administration are Veregen, a polyphenon from tea extract that is used for genital warts, and Fulyzaq, which contains extracts from the resin of *Croton lechleri* Muell. Arg. (秘鲁巴豆) that are used for diarrhea. The main obstacle preventing more TCM remedies from meeting the stringent requirements of conventional medicine is efficacy and economic utilization of modern pharmacological tools to investigate their mechanism of action.

Systems biology, which consists of genomics, epigenomics, proteomics, and metabolomics, seeks to describe the complex interactions between biological system components and to predict biological system behavior (Oberg et al., 2011). Genomics is a discipline in genetics that applies recombinant DNA, DNA sequencing, and bioinformatics to sequence, assemble, and analyze the function and structure of genomes (Freyhult et al., 2008). Epigenetic regulation involves heritable alteration of gene expression by modifying chromatin structure without changing primary DNA sequences (Boonsanay et al., 2012). Metabolomics is one of the key approaches of systems biology that consists of studying biochemical networks having a set of metabolites, enzymes, reactions and their interactions (Tagore et al., 2014). Proteomics has emerged as a powerful tool to investigate physiological conditions, mutations, changes in response to external factors, and adaptation. Due to rapid developments in proteomic analytical tools such as 1-dimensional polyacrylamide gel electrophoresis and 2-dimensional electrophoresis (2-DE) coupled with tandem mass spectrometry-based isobaric tags (one of the most effective methods for analyzing the complete proteome of cells, organs, and tissues), proteomics now allows the systematic quantitative and qualitative mapping of the whole proteome during disease (Hussain and Huygens, 2012). Proteomics can identify altered proteins as potential drug targets, and the global analysis of protein alterations can help understand a drug's mechanism of action. Proteomics can also identify post-translational protein modifications such as phosphorylation, glycosylation, acetylation, and proteolysis, and sequence variants such as mutants, alternatively spliced isoforms, and amino acid polymorphisms (Mann and Jensen, 2003; Zhang and Ge, 2011). Post-translational modification, which can occur after disease progression or drug treatment, substantially affects protein function and protein-protein interactions *in vitro* and *in vivo*. By analyzing these protein changes in tissue and cultured cells before and after TCM treatment, proteomics is a powerful tool to study the mechanism of action of TCM remedies (Cho, 2007). Published papers related to the topic of the present review were searched in Pubmed using the key words 'proteomics' and 'traditional Chinese medicine'. A total of 148 papers, including 28 reviews, were found (up to 10 February 2014). 53 papers written in Chinese character were not involved in this review. Among 120 original research papers, some were focused on studying syndromes of TCM and clinical application of TCM, and some were focused on application of Chinese medicine resources. Those papers which described the pharmacological effects, mechanism of action, and targeted proteins in specific diseases were selected to be discussed here.

This review summarizes the recent application of proteomics in TCM research and development, focusing on how proteomics can determine the mechanism of action of TCM remedies for various diseases including neurological disease, cancer, diabetes and the immunological response (Tables 1 and 2). Our own findings on the application of proteomics to determine the anticancer mechanisms of natural compounds are also discussed. Finally, the review

Table 1
Summary of the application of proteomics to determine the mechanism of action of TCM remedies for various diseases.

Disease name	TCM or compound	Targets or signaling pathways	References
Alzheimer's disease	Hupreazine A	p53	Tao et al. (2013)
Alzheimer's disease	*Gastrodia elata* Bl. (天麻)	Nxn, Dbnl, Mobkl3, Mki67 and Bax; HSP 70/90 and FKBP3/4	Manavalan et al. (2012)
Epileptic seizures	*Uncaria rhynchophylla* (Miq.) Jacks. (钩藤)	MIF and cyclophilinA	Lo et al. (2010)
Cerebral artery occlusion	Baicalin	Energy metabolism	Zhang et al. (2009)
Neurodegenerative diseases	*Ginkgo biloba* L. (银杏) extracts	PPAP subunit B and CRMP2	Koh (2011)
Cardivouscular disease	Salvianolic acid B	HSP27 and mitofilin	Feng et al. (2011)
Arrhythmias	Dingxin recipe	Prohibitin	Jia et al. (2012)
Ischemic myocardial injury	Buyang Huanwu decoction	Atrial natriuretic factor, HSP β-6 and peroxiredoxin-6	Zhou et al. (2012)
Type 2 diabetes mellitus	Tian-Qi-Jiang-Tang Capsule	Haptoglobin, transthyretin and prothrombin	Zhang et al. (2010)
Type 2 diabetes mellitus	Zi-Bu-Pi-Yin recipe	DRP-2 and PDHE1α,	Shi et al. (2011)
Allergic airway inflammation	Xiao-Qing-Long-Tang	Spectrinα2	Nagai et al. (2011)
Immunological liver injury	*Salvia miltiorrhiza* Bge. (丹参) polydacchride	PRDX6	Sun et al. (2011)
Liver fibrosis	Fuzheng Huayu	Vimentin, S-adenosylhomocysteine hydrolase isoform and HSP90	Xie et al. (2013)
Liver injury	Yin-Chen-Hao-Tang	Zinc finger protein 407, haptoglobin, macroglobuin, α-1-antitrypsin, transthyretin, vitamin D-binging protein and prothrombin	Zhang et al. (2011)

Table 2
Summary of the application of proteomics to determine the mechanism of action of compounds and plants for cancer diseases.

Type of cancer	Plant	Compound	Cell lines	Mechanism of action	References
Promyelocytic leukemia	–	Arsenic trioxide and retinoic acid	NB4	Calcium signaling and IFN pathway	Zheng et al. (2005)
Breast cancer	*Curcuma longa* L.	Curcumin	MCF-7	TDP-43,SF2/ASF, eIF3i, 3-PGDH and ERP29	Fang et al. (2011)
Neuroblastoma	*Curcuma longa* L.	Curcumin	SH-SY5Y	Polyubiquitinated proteins	D'Aguanno et al. (2012)
Cervical carcinoma	*Ganoderma Lucidum* (Leyss. ex Fr.) Karst.	Ganoderic acid D	HeLa	14-3-3ξ	Yue et al. (2008)
Hepatocellular carcinoma	*Coptis chinensis* Franch.	Berberine	HepG2	MAPK	Tan et al. (2006)
Breast cancer	*Coptis chinensis* Franch.	Berberine	MCF-7	ROS generation	Chou et al. (2012)
Non small lung cancer	*Rheum palmatum* L.	Aloe-emodin	H460	HSP70, 150 kDa oxygen-regulated protein and protein disulfide isomerase	Lai et al. (2007), Lee et al. (2005)
Colorectal cancer	*Scutellaria baicalensis* Georgi	Baicalein	DLD-1	peroxiredoxin-6	Huang et al. (2012)
Colon cancer	*Tripterygium wilfordii* Hook. f.	Triptolide	SW480	14-3-3ξ	Liu et al. (2012)
Lymphoblastoid cells	*Tripterygium wilfordii* Hook. f.	Celastrol	Lymphoblastoid cells	Heme oxygenase 1	Hansen et al. (2011)
Hepatocellular carcinoma	*Garcinia oblongifolia* Champ. et Benth.	1,3,6,7-tetrahydroxyxanthone	HepG2	P16 and 14-3-3σ	Fu et al. (2012a)
Hepatocellular carcinoma	*Garcinia oblongifolia* Champ. et Benth.	1,3,5-trihydroxy-13,13-dimethyl-2H-pyran [7,6-b] xanthone	HepG2	HSP27	Fu et al. (2012b)
Cervical cancer	*Salvia miltiorrhiza* Bge.	Tanshione IIA	HeLa	HSP27	Pan et al. (2010)
Hepatocellular carcinoma	*Paris polyphylla* Smith var. *chinensis* (Franch.) Hara	*Rhizomaparidis* total saponin	HepG2	Dnase gamma and hnRNPK	Cheng et al. (2008)

points out the challenges and perspectives for the application of proteomics in TCM research.

2. Application of proteomics to traditional Chinese medicine of neurological disorders

Some of the TCM remedies that are used to treat neurological disorders such as dementia, neurodegenerative diseases, and neuronal injury have been shown to have neuroprotection effects, which suggests that they may be potential sources of anti-dementia drugs (Wang et al., 2010). The whole plants of *Huperzia serrata* (Thunb. Ex Murray) Trev. (蛇足石杉) has been widely used in China for hundreds of years to relieve pain, dizziness, nausea, and vomiting. The main active ingredient, huperzine A, is an acetylcholinesterase inhibitor. A recent proteomics study using a centrifugal reactor and nano-LC–MS/MS to investigate the effects of huperzine A on neuronal cells showed that hupreazine A protects N2a cells from amyloid β-induced cell death by decreasing the p53 concentration (Tao et al., 2013). The TCM remedy *Gastrodia elata* Bl. (Tianma in Chinese, 天麻) is often used to treat headaches, hypertension, and neurodegenerative diseases. A recent proteomics study used 2-DE-LC–MS/MS-based isobaric tagging to investigate Tianma's potential to treat neurodegenerative diseases such as Alzheimer's disease. The results showed that Tianma's mechanism of action may promote neuro-regenerative processes by inhibiting stress-related proteins and mobilizing neuroprotective genes such as Nucleoredoxin (Nxn), Drebrin-like protein (Dbnl), Mps one binder kinase activator-like 3 (Mobkl3), Ki67 protein (Mki67) and Bax with various regenerative modalities and capacities that are related to neuro-synaptic plasticity in mouse N2a cells (Manavalan et al., 2012). Furthermore, Tianma modulated neuro-regenerative signaling cascades by controlling chaperone/proteasomal degradation pathways such as heat shock protein (HSP) 70/90 and FKBP3/4 in a human neural SH-SY5Y cell model (Ramachandran et al., 2012). Notably, the authors used two softwares to analyze the proteomic data. First, they used STRING (Search Tool for the Retrieval of Interacting Genes) to analyze the physical and functional interaction of the proteins and they revealed the link among chaperone proteins and other metabolically modulated proteins. Second, they used IPA (Ingenuity Pathways Analysis) to show the complex interactive link to the identified proteins within their networks. These data demonstrated the network proteins including PKA, PKC, PLC, and RHOA *etc.* may also involve in the protection effect of Tianma on neuronal cells.

The TCM remedy *Uncaria rhynchophylla* (Miq.) Miq. Ex Havil. (钩藤) is also used to treat neurological disorders such as epilepsy. MIF and cyclophilin A down-regulation has been observed in the frontal cortex and hippocampus of rat brain tissue using 2-DE. However, *Uncaria rhynchophylla* treatment overcame this effect, which suggests that MIF and cyclophilin A partly participate in its anticonvulsive effect (Lo et al., 2010). Furthermore, using an ischemia/reperfusion mouse brain model, baicalin, a major polyphenolic compound that is derived from the dried roots of *Scutellaria baicalensis* Georgi (黄芩), has been shown to be a possible treatment for stroke. The 2-DE analysis results showed that baicalin effectively regulated protein expression in energy metabolism, including cellular processes, development, and biological regulation (Zhang et al., 2009). The TCM *Ginkgo biloba* L. (银杏) extract is used for various health disorders such as cardiovascular disease, cerebrovascular diseases, and dementia. Using a rat cerebral ischemia model, it has been shown that EGb761, the standard extract from the leaves, has neuroprotective effects against neurodegenerative diseases, with evidence that it targets various proteins such as protein phosphatase 2A (PP2A) subunit B and Collapsing response mediator protein 2 (CRMP2) (Koh, 2011). These studies suggested that applying proteomics within a translational medicine model could permit the determination of the mechanisms of action of TCM remedies.

3. Application of proteomics on traditional Chinese medicine in Cancer

More than 60% of anticancer drugs are naturally occurring, modified natural compounds, or synthetic compounds that are based on the structure of naturally occurring molecules (Gordaliza, 2007). Camptothecin, which is derived from *Camptoteca acuminata* Decne (喜树), and its derivatives can inhibit DNA topoisomerases by stabilizing certain intermediate complexes during DNA synthesis.

Vinblastine and vincristine, which is derived from *Vinca rosea* L. (长春花), can arrest the cell cycle by blocking the formation of microtubules and binding to the α and β subunits of tubulin in the S phase. Paclitaxel, which is derived from *Taxus*, inhibits mitosis by acting as a microtubule-stabilizing agent.

Many bioactive natural compounds have been screened and studied in the last few decades (Li-Weber, 2013). Arsenic trioxide (As_2O_3), curcumin and berberine are under clinical trials on various tumors and their mechanisms of action are still under hot investigation. Chen and colleagues first found that As_2O_3 was the effective compound within a TCM decoction to treat acute promyelocytic leukemia (APL) (Zhu et al., 2002). An integrated cDNA microarray, proteomics, and computational biology approach was used to determine that the administration of arsenic trioxide and retinoic acid had the following effects: (1) activated transcription factors and cofactors, calcium signaling, and the proteasome system; (2) stimulated the IFN pathway; (3) degraded the PML-RARα oncoprotein; (4) restored the nuclear body; (5) arrested the cell cycle; and (6) caused the gain of apoptotic potential (Zheng et al., 2005). Curcumin, which is derived from the rhizomes of *Curcuma longa* L. (姜黄), and its derivatives have gained interest due to their anti-oxidant, anti-proliferative, anti-angiogenic, and anti-tumorigenic properties. Several studies used proteomics to analyze the effects of curcumin on different cancer cell lines. In the MCF-7 breast cancer cell line, proteomic analysis identified 12 differentially expressed proteins that contributed to multiple functional activities such as DNA transcription, mRNA splicing and translation, amino acid synthesis, protein synthesis, folding and degradation, lipid metabolism, glycolysis, and cell motility (Fang et al., 2011). In neuroblastoma cell lines, curcumin causes different responses in cisplatin-sensitive cells and resistant cells. Shotgun analysis demonstrated that 66 proteins were differentially expressed in response to curcumin treatment in sensitive cells, and 32 proteins were significantly modulated in the treatment-resistant cells (D'Aguanno et al., 2012). Interestingly, the cisplatin resistant cells exhibited impaired ubiquitin-proteasome system (UPS) function and ROS production after curcumin treatment, which suggests that curcumin could act as a proteasome inhibitor. Recently, it is reported that curcumin could regulate cancer-linked inflammation proteins (such as COX-2 and iNOS), transcription factors (NF-κB, STAT3, Sp, AP-1, GADD153/CHOP, HIF-1α), growth factors (VEGF, HER2), apoptotic proteins (p53, Bcl-2, survive, DNA topoisomerase II, DHAC2, p300, hTERT) and cell cycle proteins (cyclin D1, cyclin E, cyclin B, p21, p27) associated with the prevention and therapy of cancer (Hasima and Aggarwal, 2013). The ideal way to elucidate the mechanism of action of an effective compound is to determine the targeting proteins. Berberine is a natural product isolated from the roots of *Coptis chinensis* Franch. (黄连) which has been shown to have anti-neoplastic properties. The proteomics analysis was conducted in HepG2 liver cancer cells and MCF-7 breast cancer cells. In HepG2 cells, berberine caused G0 cell cycle arrest and apoptosis. The mechanism included a network of proteins involved in mitogen-activated protein kinase (MAPK) phosphorylation, metabolism, cell cycle regulation and DNA damage response (Tan et al., 2006). In MCF-7 cells, the proteomic and redox-proteomic analysis revealed that the complex working system of berberine involved reactive oxygen species (ROS) generation and apoptotic processes. This study demonstrated that the protein expression and thiol reactivity of 96 and 22 protein features, respectively, were significantly changed, and revealed that berberine-induced cytotoxicity in breast cancer cells involves the dysregulation of protein folding, proteolysis, redox regulation, protein trafficking, cell signaling, electron transport, metabolism and centrosomal structure (Chou et al., 2012).

The ideal way to elucidate the mechanism of action of an effective compound is to determine the targeting proteins. By combining proteomics, an *in silico* docking program, and surface plasmon resonance (SPR), Guo and his colleagues determined that ganoderic acid D, a main component of *Ganoderma triterpenes*, induces cytotoxicity through interacting with protein 14-3-3ξ (Yue et al., 2008). Aloe-emodin (1,8-dihydroxy-3-(hydroxymethyl)-anthraquinone) is one of the active constituents from the root and rhizome of *Rheum palmatum* L. (大黄). In non-small lung cancer cell line H460, the increase in the relative abundance of certain chaperone proteins such as HSP70, 150 kDa oxygen-regulated protein and protein disulfide isomerase, and the degradation of nucleophosmin were associated with aloe-emodin-induced cell death (Lee et al., 2005; Lai et al., 2007). Natural flavones such as Apigenin, Chrysin, Wogonin, and Baicalein have anticancer activities. Their mechanisms of action are still under investigation. A proteomics study demonstrated that Baicalein caused the up-regulation of peroxiredoxin-6, which attenuates the generation of ROS and inhibits the growth of colorectal cancer cells (Huang et al., 2012).

The *Tripterygium wilfordii* Hook. f. (雷公藤) is a representative TCM that has been widely and successfully used for centuries to treat many diseases. Its anticancer activity and underlying mechanism of action have been investigated for the last 4 decades. The diterpenoid epoxide triptolide and the quinone triterpene celastrol are two important bioactive ingredients that exhibit a divergent therapeutic profile and can perturb multiple signaling pathways (Liu et al., 2011). In colon cancer, a proteomics study revealed that triptolide treatment could induce cleavage and the perinuclear translocation of 14-3-3ξ, which is the main protein of cell cycle arrest and cell death (Liu et al., 2012). To investigate the effect mechanism of celastrol in cultured human lymphoblastoid cells, the proteomic study on celastrol was performed using SILAC (stable isotope labeling by amino acids in cell culture), a quantitative proteomics technique. Hansen found that celastrol substantially modified the proteome composition, and 158 of 1800 proteins with robust quantitation showed at least a 1.5-fold change in relative abundance. Up-regulated proteins play key roles in cytoprotection with a prominent group involved in quality control and the processing of proteins that traverse the endoplasmic reticulum. Increased levels of proteins that are essential for cellular protection against oxidative stress including heme oxygenase 1, several peroxiredoxins and thioredoxins as well as proteins that are involved in the control of iron homeostasis were also observed. Specific analysis of the mitochondrial proteome strongly indicated that the mitochondrial association of certain antioxidant defense and apoptosis-regulating proteins increased in cells that were exposed to celastrol (Hansen et al., 2011).

Gambogic acid, a xanthonoid derived from the brownish or orange resin of *Garcinia hanburyi* Hook. f. (藤黄), was found to inhibit the growth of various cancer cells through multiple signaling pathways such cell cycle arrest and apoptosis (Guo et al., 2004; Zhao et al., 2004). This natural chemical was intensely investigated in the last decade and has shown promising anti-tumor activity in clinical trials (Chantarasriwong et al., 2010; Anantachoke et al., 2012; Chen et al., 2012). However, the toxicity of gambogic acid to animal's kidney and liver has limited its application in cancer therapy (Guo et al., 2006; Qi et al., 2008). In the last few years, we conducted a series of studies to investigate the mechanism of action of gambogic acid and other novel compounds that are derived from the *Garcinia* species (Han and Xu, 2009). First, we investigated the stability and toxicity of gambogic acid that was stored in different solvents (Han et al., 2005). We then applied proteomics to demonstrate that stathmin could be a potential target of gambogic acid in hepatocellular carcinoma (Wang et al., 2009). Later, we used bioassay guided fractionation to identify more than 80 compounds with anticancer potential from *Garcinia* species such as *Garcinia lancilimba* C.Y. Wu ex

Y.H. Li (长裂藤黄), *Garcinia xishuangbannaensis* Y. H. Li. (版纳藤黄), *Garcinia oblongifolia* Champ. et Benth. (岭南山竹子) and *Garcinia nujiangensis* C.Y. Wu ex Y.H. Li (怒江藤黄) (Han et al., 2008a; Han et al., 2008b; Huang et al., 2009; Xia et al., 2012). We systematically studied the possible mechanisms of action of these active compounds. Our systematic proteomic analysis of these active compounds showed that 1,3,6,7-tetrahydroxyxanthone, an active compound from *G. oblongifolia*, suppressed cell growth by the up-regulation of p16 and 14-3-3σ in hepatocellular carcinoma cells (Fu et al., 2012a). Our proteomics data also showed that 1,3,5-trihydroxy-13,13-dimethyl-2H-pyran [7,6-b] xanthone, which is also derived from *G. oblongifolia*, can induce cancer cell death by suppressing HSP27 (Fu et al., 2012b). It is interesting that 2-DE analysis has shown that tanshione IIA, a phenanthrene quinine extracted from the root of *Salvia miltiorrhiza* Bge. (丹参), has also been shown to down-regulate HSP27 expression in cervical cancer cells, indicating that HSP27 plays an important role in the cancer cells (Pan et al., 2010). Interestingly, we managed to use the protein docking simulation to find out the protein-small molecules interaction from the proteomics data, which suggested that proteomics was an essential tool to predict the protein targets of the active compounds.

Proteomics also made great contribution to the mechanistic study of TCM mixtures or decoctions. Cheng et al. (2008) reported that total saponin of the rhizomes of *Paris polyphylla* Smith var. *chinensis* (Franch.) Hara (重楼) induced apoptosis in HepG2 cells by up-regulating dUTPase, hnRNP K, GMP synthase and down-regulating DNase gamma, nucleoside diphosphate kinase A, and centrin-2-as determined by proteomics.

4. Proteomics in cardiovascular disease

Salvia miltiorrhiza Bge. and *Panax notoginseng* (Burk.) F.H. Chen (三七) are well known Chinese herbs for the treatment of cardiovascular disease, but their underlying mechanisms of action are unclear. Proteomic analysis using a rat cardiac ischemia-reperfusion model has been used to study the mechanisms of action of salvianolic acid and notoginsengnoside, which are derived from *Salvia miltiorrhiza* and *Panaxnotoginseng*, respectively. This study showed that salvianolic acid and notoginsengnoside inhibit the eukaryotic translation elongation factor 2 that is involved in cell proliferation. In addition, salvianolic acid also inhibited the activity of protein disulfide isomerase and prohibitin, indicating that the 2 compounds could have different targets for producing their cardiovascular protective effects (Yue et al., 2012). In a rat myocardial cell ischemia-reperfusion injury model, salvianolic acid B, another compound that is derived from *Salvia miltiorrhiza*, has been shown to interact with epidermal growth factor receptor (EGFR) to activate the downstream signaling cascade involving HSP27 and mitofilin (Feng et al., 2011). Dingxin recipe, which is composed of the seeds of *Ziziphus jujuba* Mill. var. *spinosa* (Bunge) Hu ex H. F. Chow (酸枣仁), the roots of *Sophora flavescens* Alt. (苦参), the roots of *Coptis chinensis* Franch. (黄连), the sclerotium of *Poria cocos* (Schw.) Wolf (茯苓), the roots of *Codonopsis pilosula* (Franch.) Nannf. (党参), the roots of *Salvia miltiorrhiza* Bunge (丹参), the roots of *Paeoniae lactiflora* Pall. (赤芍), the fruits of *Trichosanthes kirilowii* Maxim. (瓜蒌), the sporocarp of *Ganoderma lucidum* (Leyss.ex Fr.) Karst. (灵芝), and the roots and rhizomes of *Panax notoginseng* (Burk.) F.H. Chen (三七), is a TCM remedy that has been used to treat arrhythmia for several years. To better understand its underlying mechanism of action, 2-DE gels were used to identify the differentially expressed proteins in an arrhythmia model in rats with or without dingxin recipe pre-treatment. The alleviation of ischemia/reperfusion-induced arrhythmias by the dingxin recipe was shown to be related to the prohibitin increase, glutathione depletion, and interleukin-6 and neutrophil infiltration (Jia et al., 2012). Buyang Huanwu decoction (BYHWD), which is composed of the roots of *Astragalus membranaceus* (Fisch.) Bge. (黄芪), the roots of *Angelica sinensis* (Oliv.) Diels (当归), the roots of *Paeoniae lactiflora* Pall. (赤芍), the roots of *Ligusticum chuanxiong* Hort. (川芎), the seeds of *Prunus persica* (L.) Batsch (桃仁), the flowers of *Carthamus tinctorius* L. (红花), and the bodies of *Pheretima aspergillum* (E. Perrier) (地龙), has been used to improve the neurological functional recovery of stroke-induced disabilities or inhibit ischemic myocardial injury in China. A proteomics-based approach was used to identify the response of differentially expressed proteins to BYHWD treatment in the ischemia-induced ventricular remodeling of rats. This study showed that atrial natriuretic factor was down-regulated; heat shock protein beta-6 and peroxiredoxin-6 were up-regulated in the BYHWD-treated group, and they were among the successfully identified proteins. The results suggested that BYHWD could alleviate ventricular remodeling that is induced by left anterior descending artery ligation (Zhou et al., 2012).

5. Proteomics in type 2 diabetes mellitus

Type 2 diabetes mellitus (T2DM) is one of the most prevalent chronic diseases in the world, and it is characterized by insulin resistance coupled with the failure of pancreatic cells to compensate by adequate insulin secretion. Comparison of 2-DE mapping in T2DM rats has shown that Tian-Qi-Jiang-Tang capsule, composed of the roots of *Astragalus membranaceus* (Fisch.) Bge. (黄芪), the roots and rhizomes of *Panax ginseng* C.A. Mey. (人参), the roots of *Coptis chinensis* Franch. (黄连), the fruits of *Ligustrum lucidum* Ait. (女贞子), the roots of *Trichosanthes kirilowii* Maxim. (天花粉), the stems of *Dendrobium nobile* Lindl. (石斛), the velamen of *Lycium chinense* Mill. (地骨皮), the sarcocarp of *Cornus officinalis* Sieb. et Zucc. (山茱萸), the whole plants of *Eclipta prostrata* (L.) L. (旱莲草), and the galls of *Rhus chinensis* Mill. (五倍子), which was used as anti-T2DM prescription in China inhibits haptoglobin, transthyretin, and prothrombin (Zhang et al., 2010). Furthermore, in a diabetes-induced cognitive decline model, a fluorescence-based differential gel electrophoresis proteomic method identified novel candidate proteins that are involved in diabetes development, such as DRP-2 and PDHE1, and also defined potential intervention targets for Zi-Bu-Pi-Yin recipe in the hippocampus (Shi et al., 2011), which is composed of the roots and rhizomes of *Panax ginseng* C. A. Mey. (人参), the rhizomes of *Dioscorea opposita* Thunb. (山药), the sclerotium of *Poria cocos* (Schw.) Wolf (茯苓), the roots of *Paeoniae lactiflora* Pall. (白芍), the roots of *Salvia miltiorrhiza* Bunge (丹参), the seeds of *Lablab purpureus* (Linn.) Sweet (白扁豆), the seeds of *Nelumbo nucifera* Gaertn. (莲子), the rhizomes of *Acorus tatarinowii* Schott. (石菖蒲), the roots of *Polygala tenuifolia* Willd. (远志), the woods of *Santalum album* Linn. (檀香), the peels of *Citrus reticulate* Blanco(橘红), and the roots of *Glycyrrhiza uralensis* Fisch.(甘草).

6. Proteomics in inflammatory responses

Inflammation is complex biological response of vascular tissues to harmful stimuli, which is inhibited by steroid or antibiotic in Western medicine. TCM has a variety of natural formulations to reduce inflammation responses for thousands of years. However, the mechanisms of action of TCM remedies that reduce inflammation are unclear. Xiao-Qing-Long-Tang decoction composed of the herbaceous stems of *Ephedra sinica* Stapf (麻黄), the roots of *Paeonia lactiflora* Pall. (芍药), the roots of *Asarum sieboldii* Miq. (细辛), the roots of *Zingiber officinale* Rosc. (干姜), the roots of *Glycyrrhiza uralensis* Fisch.(甘草), the twigs of *Cinnamomum cassia*

Presl (桂枝), the fruits of *Schisandra chinensis* (Turcz.) Baill. (五味子), and the tubers of *Pinellia ternata* (Thunb.) Breit.(半夏), which is Chinese herb prescription, named Shoseiyuto in Japan, is used to treat allergic rhinitis, bronchitis, bronchial asthma, and cold symptoms. Proteomic analysis, using an ovalbumin-sensitized allergic airway inflammation model showed that Xiao-Qing-Long-Tang reversely reduced spectrin 2 expression in lung tissue (Nagai et al., 2011). Analysis using 2-DE, in a lipopolysaccharide-induced immunological liver injury model, showed that *Salvia miltiorrhiza* polysaccharide protected against immunological liver injury by inhibiting NF-κB activation via the up-regulation of PRDX6 and the subsequent attenuation of lipid peroxidation, iNOS expression, and inflammation (Sun et al., 2011). Some TCM remedies were reported to have protective effects on certain organs so that they could be used as adjunctive therapy to reduce toxicity and side effects. Cheng et al. (2008) devoted many efforts to elucidate the mechanisms of PHY906, a four herbs decoction for cancer therapy (Wang et al., 2011; Liu and Cheng, 2012). Fuzheng Huayu (FZHY), which contains six traditional Chinese drugs, the roots of *Salvia miltiorrhiza* Bunge (丹参), the fungus of *Cordyceps sinensis* (Berk.) Sacc. (冬虫夏草), the seeds of *Prunus persica* (L.) Batsch (桃仁), the whohe plants of *Gynostemma pentaphyllum* (Thunb.) Makino (绞股蓝), the pollen of *Pinus massoniana* Lamb. (松花粉), and the fruits of *Schisandra chinensis* (Turcz.) Baill. (五味子), is an effective Chinese herbal product that is used to treat liver fibrosis. The complicated liver protective mechanism of FZHY was revealed by a proteomics study that was conducted by Xie et al. (2013). Using a dimethylnitrosamine-induced liver injury rat model, they determined that 12 proteins were responsible for fibrogenesis induced by dimethylnitrosamine in rats, and among them, 8 proteins in fibrotic liver were regulated by FZHY, including aldehyde dehydrogenase, vimentin isoform CRA_b, gamma-actin, vimentin, fructose-bisphosphate aldolase B, aldo-keto reductase, S-adenosylhomocysteine hydrolase isoform, and HSP90. These results indicate that the mechanism of action of FZHY anti-liver fibrosis could be associated with the modulation of proteins that are associated with metabolism and the stress response, as well as myofibroblast activation (Xie et al., 2013). Yin-Chen-Hao-Tang (YCHT, Inchin-ko-to or TJ-135 in Japan), consisting of the acrial part of *Artemisia annua* L. (青蒿), the fruits of *Gardenia jasminoides* Ellis (栀子), and the roots and rhizomes of *Rheum Palmatum* L. (大黄), is a widely used TCM in clinical practice for jaundice and the treatment of liver disorders (Zhang et al., 2011). Wang and his team performed proteomics to identify the possible target proteins of YCHT in a CCl_4-induced liver injury mouse model. As a result, 15 modulated proteins were identified in the CCl_4-treatment group, out of which 7 were found to be significantly altered by YCHT. YCHT treatment caused a statistically significant down-regulation of zinc finger protein 407, haptoglobin, macroglobulin, and alpha-1-antitrypsin; the significant up-regulation of transthyretin, vitamin D-binding protein, and prothrombin, appear to be involved in metabolism, energy generation, chaperone, antioxidation, signal transduction, protein folding and apoptosis (Sun et al., 2013).

The proteomics studies on inflammatory diseases are complex and difficult to analyze. In the FZHY study, the researchers induced liver fibrosis models using intraperitoneal injection of dimethylnitrosamine, followed with FZHY treatment. To obtain the proteomics data, the liver from 3 animals were selected from each group and homogenized for 2-D electrophoresis and image analysis. To better understand the mechanism of FZHY recipe, the data analysis was performed by comparing the normal group and model group, model group and FZHY group. The complexity caused some contradictory results in the proteomics data. For instance, vimentin isoform CRA_b protein showed both down and up-regulation between FZHY and model group. It might due to the different cleavage or modification of this protein in three different groups. To better understand the function of this protein, the authors analyzed the changes among three groups and found out that vimentin isoform CRA_b was down-regulated by dimethylnitrosamine and rescued by FZHY. However, the authors did not provide the evidence by using other analytical method such as western blotting or RT-PCR. It will be interesting to further study this protein to understand the detailed mechanisms of FZHY on liver fibrosis animals.

7. Conclusion and perspectives

It is expected that applying modern technology to TCM research and development will accelerate the integration of TCM into Western medicine. Proteomics can identify therapeutic targets and determine the mechanisms of action of TCM remedies. It can also be used to study the effect of a single compound, active fractions and herbal prescriptions. Other omic technologies such as genomics, metabolomics and metabonomics also play important roles on TCM research (Buriani et al., 2012). Genomics, including DNA microarray, cDNA microarrays, microRNomics analyze the changes of genes and miRNAs expression pattern under TCM treatment. Combined with bioinformatics and statistical analysis, it can provide the possible molecular mechanisms, downstream effects, and targeted pathways. However, the changes in DNA, mRNA and miRNA expression may poorly correlate with the changes in protein expression. Moreover, the post-translational modification (PTM) of proteins, such as acetylation, ubiquitination, phosphorylation, and methylation, cannot be detected by genomic techniques. Metabonomics measures the metabolic profiling (*e.g.* low molecular weight metabolite) of the systems (cells, animals or patients) and provides the holistic picture on the effects of drugs (Lao et al., 2009). It also can analyze the urine or plasma samples which is easy to perform in large scale research. A recent review summarized the usage of metabonomics to analyze the TCM syndrome research (Zhang et al., 2013). Since the metabonomics and proteomics analyze different molecules during drug treatment, they complement each other when apply to study the mechanism of TCM. The combination of these two omic techniques is a powerful tool to elucidate the pharmacological response, biological pathways and novel biomarkers (Wang et al., 2013).

Fig. 1 shows the general proteomics procedure to apply when studying the mechanism of action of single compound or TCM extracts. The whole procedures involve sample preparation, 2D gel electrophoresis, spots extraction, MS/MS analysis, bioinformatics analysis, and candidate's validation *in vitro* and *in vivo*. SILAC technique provides a conventional way to avoid 2D electrophoresis and incorporates isotopically labeled amino acid into the proteome of the cells. Then the whole labeled proteome can be mixed with the one from unlabeled cells and the differences in protein expression can be read out by comparing the abundance between these two samples. Bioinformatics analysis is one of the most challenge steps when apply proteomics on TCM. Since the active compounds or TCM mix always affects multiple signaling pathways and protein targets, the essential data mining will increase the efficiency of target evaluation. Currently, there are many software platforms for the network and pathway analysis (*e.g.* STRING, KEGG); researchers are encouraged to test the proteomics data in various databases to visualize the functional contexts of TCM. Recently, the national institutes of health (NIH) of the U.S. launched a program called library of integrated network-based cellular signatures (LINCS), which creates a network-based understanding of biological changes in gene expression and other cellular process when cells are exposed to perturbing agents (http://www.lincsproject.org/). It will be interesting to compare

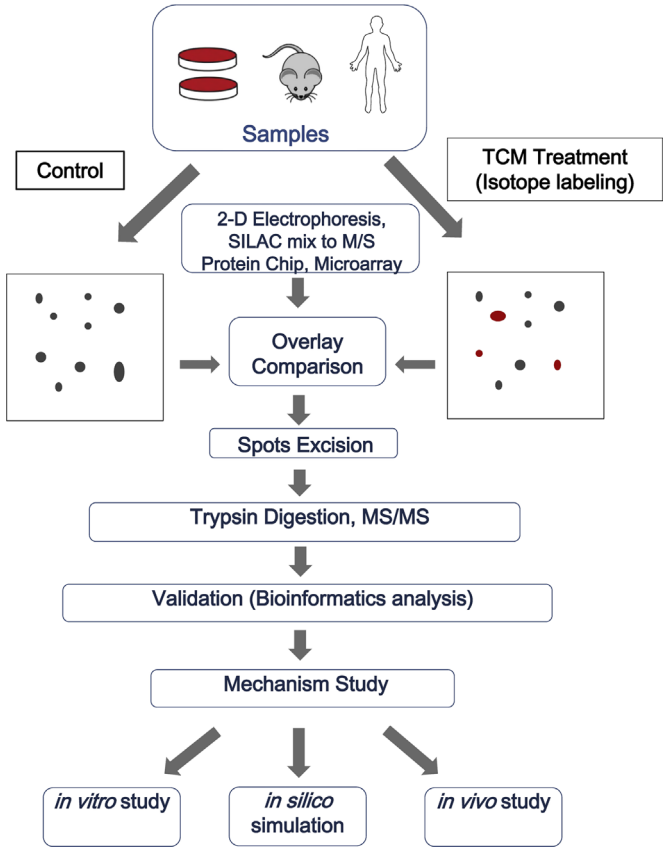

Fig. 1. Scheme of general approaches for the application of proteomics in the mechanistic study of TCM. Samples (cultured cells, animals or patients) are treated with TCM (single compounds or decoction), followed by 2D gel electrophoresis and staining. After comparison, the differentially expressed proteins are selected for digestion and mass spectrometry. The candidates are chosen for further mechanistic studies including *in vitro* study, *in vivo* study, and *in silico* simulation. Alternatively, samples can be labeled with isotope in TCM treated samples (SILAC, stable isotope labeling by amino acid in cell culture). The samples then mix with control samples and go through MS/MS analysis without 2D gel electrophoresis. The SILAC method is more efficient and reproducible compared to 2D electrophoresis. Other methods such as protein chips, protein microarray are be used to analyze the proteomics of various samples.

the pharmacological effect of TCM with the agents to predict the mechanism of action by using this database.

Proteomics could expand the new field of TCM research and promote the internationalization of TCM. Advances in proteomics and biotechnology will be combined with other technologies to address many of the aims of TCM research. Combining proteomics and bioinformatics (functional proteomics) to study protein signaling and protein-drug interactions could help to develop molecular evidence-based TCM research (Ventura, 2005). High speed and high throughput proteomics will help process the large number of TCM remedies and, with other related technologies, could also allow TCM remedy effects to be studied for the whole body and not just at the molecular and cellular level. It may also facilitate scientific dialog between TCM and conventional Western medicine (Dai et al., 2012). Post-translational modifications (PTMs) are the key regulators of protein activity and involve the modifications of proteins by small compounds, lipids, or even a group of chemicals. It would be interesting to apply proteomics to detect PTMs and other parameters such as protein–protein interactions after TCM treatment (Altelaar et al., 2013). The rapid development of proteomics will allow scientists to perform in depth analyses of samples including homogenous cell populations and microdissected tissue. The current increase in the size of the TCM market provides an unprecedented incentive to apply the latest proteomics techniques to their mechanisms of actions.

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