Transcriptomics: a sword to cut the Gordian knot of traditional Chinese medicine

The systemic effects of traditional Chinese medicine (TCM) seem to be a Gordian knot, impossible to untie for decades. With the advent of transcriptomics, a useful sword is provided to cut the knot and shed some light on complex bioprocesses and intrinsic connections among them. Here, we revisit studies on TCM ZHENGs using this approach, highlight its applications on elucidating the potential scientific basis of ZHENG and investigating mechanisms of action for the TCM formula, and demonstrating its unique role in novel TCM drug design and discovery through active ingredient detection from TCM and compatibility theory study of TCM. The limitations and future perspectives of transcriptomics approaches to TCM study are also discussed.

Keywords: compatibility • formula • mechanism • traditional Chinese medicine • transcriptomics • ZHENG

Background

Rooted in a well-developed and complete ancient Chinese philosophy, traditional Chinese medicine (TCM) is a comprehensive medical system that has been practiced in China and other Asian countries for over 3000 years. TCM theory views diseases as exterior expressions of an unbalanced internal world (Yin-Yang and five elements) of the human body. This abstract interpretation of diseases makes it difficult to perceive underlying molecular and cellular mechanisms for the beneficial outcomes of TCM, even with current state-of-the-art analytical tools and methods. The ambiguity on scientific essence of TCM often raises questions about its clinical practice under current standards of modern medicines, which significantly limits recognition of the full potential of TCM, thus it is not surprising that TCM only serves as an alternative/complementary treatment option in the western world. Corresponding with the increasing trend in the use of system biology and the shift of the drug discovery paradigm from 'one target, one drug' to 'multi-target, multidrug' [1,2], increasing popularity of TCM has recently been observed world-

wide. This increased popularity is in part due to converging perspectives on diseases and drug treatment by TCM and this movement of modern pharmacology. Furthermore, the developments of several -omics technologies, including transcriptomics, have been promoted accordingly and have accelerated the discovery of 'magic shotguns' for complex diseases. At the same time, these novel approaches offer exciting opportunities to investigate the holistic effects of TCM from a systemic point of view [3].

Transcriptomics is a potent tool that offers convenient and reliable access to changes in the expression of genes, which could shed light into multiplexed mechanisms of drugs [4–6]. Compared with widely used traditional transcriptional methods, such as northern blotting and quantitative real-time polymerase chain reaction, transcriptomics overcomes limitations in the measurable number of genes and allows simultaneous determination of coexpression of thousands of genes at various levels, for example, organ, tissue and cell. In the early stages of this technology, its great potential in TCM study was well recognized, with a number

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of studies focusing on the effects of individual TCM medicinal herbs on gene expression profiles of various cell lines [7,8] or tissue samples [9,10]. These studies significantly enhanced our understanding of various biological processes induced by the treatment of medicinal herbs and corroborated with implications of many previously described pathways [11,12]. However, single TCM normally is the building block of a TCM formula that is a combination of multiple TCMs to treat diverse TCM syndromes (ZHENG). As the foundation of TCM clinical pharmacotherapy, important challenges in the field concern the understanding of this intriguing cell biology induced by TCM formula and the molecular basis for ZHENG differentiation. To modernize TCM in order to meet stringent standards for medical drugs, it is also critical to uncover the rationale underlying the compatibility rules of TCM formula and identify active ingredients responsible for therapeutic and/or toxicological effects of the formulae. During the past decade, transcriptomics has represented a promising approach to addressing these demanding questions, as shown in Figure 1.

The objective of this review is to focus on transcriptomic studies of TCM formulae. Such studies have provided important insight into the potential scientific basis of TCM syndromes (ZHENG) and mechanisms of action for TCM formula. We also showed promising

results for active ingredient detection from TCM and compatibility theory of TCM, which could be used in novel TCM drug design and discovery, a critical area of modern TCM study. In addition, the application of transcriptomics to understand unique TCM physical therapies for diseases was presented. Finally, we discussed limitations and potential future development of this approach.

Transcriptomics for the analysis of ZHENG in TCM

ZHENG, Chinese medicine syndrome, is the illustration of one or several clinical phenotypes that are gathered from four diagnostic procedures of Chinese Medicine (observation, listening, questioning and pulse analysis), which reflects the particular imbalance of human homeostasis [13]. Based on guidelines from the Chinese Diagnostics Branch of the Chinese Association of Chinese Medicine, there are 124 common ZHENGs in the clinic [14,15], which provide essential medical descriptions on diseases, such as pathology, development and features, and serves as the foundation for the prescriptions of TCM therapy. ZHENG can be viewed as a classification system for patients on TCM; however, it is difficult to establish a linear relationship between ZHENG and modern pathophysiological descriptions. Therefore, the differentiation

Figure 1. Overview of study of transcriptomics in traditional Chinese medicine. Treatment with traditional Chinese medicine, including pharmacotherapy and physical therapy, on the biological system (cells, model animals or patients) would induce multifaceted alterations in the organism at different levels. Transcriptomics is one powerful sword available to cut this Gordian knot and uncover one facet of this complex problem from a gene profiling point of view.

Table 1. Transcriptomics studies of ZHENG in traditional Chinese medicine.			
ZHENG	Disease	Tissue/model	Related bioprocess
Kidney deficiency/kidney Yang deficiency/control	NS*	Organs/rat	Oxidative phosphorylation of energy metabolism [17]
Kidney Yang deficiency/control	NS	Peripheral blood mononuclear cell/human	Immune, cell cycle, signal transduction [18] Energy metabolism, signal transduction, immune [19]
Kidney Yang deficiency/control	NS	Brain/mouse	Hormone secretion, inflammation, immune, apoptosis [20]
Kidney deficiency/control	NS	Organs/rat	Neuroendocrine immune [21]
Pulmonary Qi deficiency/control	Chronic obstructive pulmonary disease	Peripheral lymphocytes/human	Immune [22]
Pulmonary Yin deficiency/control			
Cold/hot	Rheumatoid arthritis	Peripheral CD4 ⁺ lymphocytes/human	Nucleotide metabolism [23] Proliferation, differentiation, immune [24]
Cold/hot/control	Rheumatoid arthritis	Peripheral CD4 ⁺ lymphocytes/human	Fatty acid metabolism, immune [25,26]
Cold/control	NS	Peripheral blood mononuclear cell/human	Energy metabolism [27,28]
Cold/control	End-stage renal disease	Peripheral blood mononuclear cell/human	Inflammation, immune, metabolism, proliferation [29]
Blood stasis/control	Coronary heart disease	Peripheral blood mononuclear cell/human	Inflammation, immune response [30,31]
Liver and kidney Yin deficiency/control	Chronic hepatitis B	Peripheral blood mononuclear cell/human	Inflammation, peroxide activity, immune, energy metabolism [32]
Dampness heat/control			
Damp obstruction/control	NS	Organs/rat	Energy metabolism, signal transduction, oncogenesis [33,34]
Damp-heat accumulation/control	Chronic hepatitis B	Peripheral leukocyte/human	Sensory perception, neurological system process, cognition [35]
Liver depression and spleen deficiency			
Deficiency/cold/hot/control	Rheumatoid arthritis	Peripheral CD4 ⁺ lymphocytes/human	Protein transcription, protein ubiquitination, apoptosis, nucleotide metabolism, immune [36]
Spleen deficiency/control	Chronic superficial gastritis	Gastric mucosae/human	Protein ubiquitination, post-translational modification, DNA replication and repair, metabolism [37]
Spleen–stomach damp-heat syndrome			
*Nonspecific disease.			

of ZHENG is critical for phenotype-oriented diagnosis and TCM therapy. A major obstacle to applying

ZHENG differentiation in current clinical practice is its subjective nature and unrepeatability since a TCM

practitioner's experience and personal interpretation of clinical observations of a patient play a significant role in this process. An important scientific question about ZHENG is often asked; what is the molecular basis of these ZHENGs? The answer, however, remains obscure despite of years of efforts using traditional pharmacological methods. From a systemic biology point of view, the comprehensive approach of transcriptomics converges with the holistic concept and practices of TCM, which also advances our understanding of ZHENG at the genomic level. A review [16] published in 2013 has provided a nice summary on -omics science in TCM ZHENG study, including transcriptomics. Here, we supplement some of the most recent applications of this platform in TCM ZHENG differentiation. **Table 1** lists published works that rely on gene expression profiles to study ZHENG and discriminate multiple ZHENG. Clearly, only a small subset of ZHENG was studied. The majority of works were performed on human samples rather than using animal models. As shown in **Table 1**, pathways related to metabolisms and immune responses were frequently connected to ZHENGs, which suggested that ZHENGs of TCM partially reflected the alterations in cellular processes of patients. However, the specificity of ZHENGs remains to be further illustrated.

Chronic hepatitis B (CHB), the major cause of cirrhosis and hepatocellular carcinoma, is a worldwide health problem, particularly in China. In TCM therapy for CHB, the dampness-heat accumulation syndrome (DHAS), liver depression and spleen deficiency syndrome (LSDS) are identified as two major ZHENGs. However, a biological basis for TCM classification remains elusive. Lu *et al.* [35] were interested in classification of these two ZHENGs from CHB patients using their transcriptional expression profiles. Leukocyte samples were collected from three hepatitis B-caused cirrhosis patients with DHAS or LSDS and then subjected to microarray analysis. Compared with the control group, more than 2000 differentially expressed genes (DEGs) were mapped and the co-expression gene network was constructed accordingly. Topological analysis on this network identified three important genes, *PNP*, *AQP7* and *PSMD2*, from both ZHENG groups and RT-PCR experiments further validated this results. Using another cohort of 40 hepatitis B-caused cirrhosis patients (20 patients with DHAS and 20 patients with LSDS), the expression level of *AQP7* and *PSMD2* in these patients was proven to correlate with their ZHENG. Moreover, further gene function analysis revealed that DEGs in DHAS group were mainly related to cellular process, while there were more genes involved in stimulus response for LSDS.

The protocol described in the article above is a standard workflow to study TCM ZHENG differentiation using transcriptomics. Based on network analysis on DEG, the molecular mechanism of ZHENGs was investigated to correlate with corresponding clinical symptoms. However, two unavoidable problems were faced by these studies on ZHENGs. First, ZHENGs are derived from manual observation and interpretation of phenotypic conditions of patients. It is rather difficult to reproduce ZHENG in animal models since some symptoms are not attainable in animals, such as the color of tongue. Human samples would be recommended for ZHENG-related studies. Another problem is related to unclear pathophysiology of ZHENG, thus it is unknown if the anatomical location of biological samples would influence the understanding of ZHENG due to varied expression profiles of genes. It is interesting to perform multiple transcriptomics studies on different tissues from the same ZHENG and compare (dis)similarity of DEG among samples, which would offer more comprehensive knowledge on ZHENG.

Transcriptomics for TCM formula-related study

In this review, we focused on the applications of transcriptomics to study TCM formula, one of the major utilized forms of TCM in the clinic. Under the guidance of TCM theory, TCM formula is established by a rational combination of multiple TCMs according to a series of phenotypic syndromes of the patient (ZHENG). In response to differential ZHENG among patients, the selection of TCMs and their relative content ratio in a formula are flexible and adjustable. In this sense, TCM formula can be viewed as an ancient practice of personalized medicine. Therefore, to uncover the scientific essence of TCM, formula is a good starting point that bridges ancient theory and clinical therapy of TCM and it is of utmost importance to investigate them from a holistic aspect. With the introduction of the transcriptomic approach, our insights on the molecular basis for TCM formula have been greatly accelerated at the genome-wide RNA expression level. Here, we gazed on studies addressing three critical questions around TCM formula, for example, complex mechanisms of action, active ingredient identification and compatibility rules of formula.

Mechanism study

From various perspectives, it is important to understand how a drug works *in vivo* to exert its therapeutic effects, for example, mechanisms of action, since this information is deeply related to drug development, its safety profile and so on. Even for modern medicine

with a single active ingredient, polypharmacology has been a known issue in mechanistic study. Complexity for this question is exponentially increased when we look into a system-like TCM formula where hundreds of chemicals are mixed together to interact with an unknown number of cellular targets and produce profound systemic effects. Transcriptomics, as a unique and powerful tool to investigate these potential mechanisms through global gene expression profiling, has been integrated with mechanistic study of TCM formula since early stages of this technology and significant progress have been made to elucidate mechanisms of action of multiple TCM formula using transcriptomics, as shown in **Table 2**.

Through years of work on this topic, a general workflow has emerged that is routinely applied in mechanistic study of TCM formula. First, one formula or several components of a formula are prepared, and it is important to document this process accurately since it is subject to human interventions [38–40]. Subsequently, suitable cell lines, proper animal models or human samples are selected to study pathological conditions of interest. A technology platform, normally microarray or RNA-seq, is subsequently decided on to run transcription tests on drug-treated samples [41,42]. In the last, but most important, step of this workflow, transcriptional data analysis is performed by various system biology methods [43,44] and our group has published an extensive review on this issue [45]. These works have accelerated our understanding on molecular mechanisms of TCM formula at the gene expression level. Interestingly, compared with ZHENG-related works, most mechanistic studies of TCM formula were performed on cell lines or experimental animals rather than human samples. To improve correlation with clinical efficacy, future works should focus on transcriptional alterations of biological samples of patients treated by TCM formula. In addition, results from transcriptomics studies usually only revealed multiple pathways induced by the treatment formula, however, relative contribution and importance of these pathways to their beneficial effects remain to be further illustrated by other pharmacological methods.

Active ingredient detection

It seems contradictory to isolate active ingredients from TCM formula since we have been emphasizing their holistic effect as a whole entity. However, this area of research is at the center of the product manufacturing process. To ensure safe and effective TCM products for clinical use, it is critical to monitor the content of active ingredients (a group of chemicals exerting desired therapeutic effects under certain conditions) while removing those pharmacologically

nonrelevant compounds to prevent the occurrence of adverse events. Classic practice to address this question solely relies on chemical characteristics of TCM product, such as concentration measurement of those compounds with the highest relative contents or fingerprint methods based on chromatograms [60]. These methods established important criteria to evaluate quality of TCM products. However, the limitation of these chemical-based approaches is obvious, such as disconnection between overall *in vivo* efficacy of a TCM formula and abundance of several chemicals. The use of transcriptomics as a means to determine holistic effect induced by TCM formula presents a promising approach to bridge the gap between its cell biology and chemical properties, which also offer an opportunity to detect active ingredients based on their influence on overall gene expression profile.

Inspired by this idea, Wang *et al.* designed an experiment to find out the active ingredients in Xue-Sai-Tong Injectable (a clinical TCM preparation for treating cardiovascular diseases). Samples collected from risk region of Xue-Sai-Tong-treated myocardial infarction rat heart were submitted for transcriptomics experiment. More than 500 DEGs were extracted based on the microarray data and rat genome database. They further filtered the list of DEGs with literature mining and target prediction. Finally, a target compound network was constructed with 335 relationships. In order to assess the holistic effects, Wang *et al.* [61] presented the content-weighted network-based efficacy, a measurement based on ingredients' target degree in the network and their relative content within Xue-Sai-Tong Injectable. Via this method, they concluded a five-compound mixture (ginsenoside Rg1, ginsenoside Rb1, notoginsenoside R1, ginsenoside Re and ginsenoside Rd) representing 95% efficacy of whole formula and this new combination was experimentally proved to demonstrate similar effects of original TCM preparation in an *in vivo* study.

San-Huang-Xie-Xin-Tang is a TCM formula for treating gastritis, gastric bleeding and peptic ulcers. Clinical reports have demonstrated this formula to produce beneficial effects on hepatocellular carcinoma. Cheng *et al.* [62] tested the antihepatocellular carcinoma effects of San-Huang-Xie-Xin-Tang and individual TCMs of this formula by microarray technology. Hierarchical clustering analysis on expression profile on HepG2 cells indicated that a very similar expression pattern was shared by San-Huang-Xie-Xin-Tang and one TCM (*Rhizoma Coptidis*) treated cells. Expression data also reveal that *Rhizoma Coptidis* displayed better effect than the primary formula on several important signaling pathways for anticancer activities, such as p53 signaling, p53 activation and DNA dam-

Table 2. Transcriptomics studies on traditional Chinese medicine formula.

Formula name	Composition	Disease	Model	Description	Ref.
Danggui Buxue Tang	<i>Radix Astragali</i> , <i>Radix Angelicae Sinensis</i>	Osteoporosis	Cell	Genes in cell proliferation and differentiation were upregulated and the expression of <i>CCL-2</i> , <i>CCL-7</i> , <i>CCL-8</i> and <i>Gal-9</i> were regulated by Danggui buxue tang which point to a potential antiosteoporosis effect	[46]
Zilongjin	<i>Radix Astragali</i> , <i>Radix Angelicae Sinensis</i> , <i>Solanum lyratum</i> , <i>Solanum nigrum</i>	Tumor	Cell	483 DEGs were identified in four different lung cancer cell lines. These genes were mapped to three anticancer properties-related pathways including apoptosis, cell cycle regulation and mitogen-activated protein kinase cascade	[47]
Si-Wu-Tang	<i>Radix Rehmanniae praeparata</i> , <i>Radix Angelicae Sinensis</i> , <i>Rhizoma Ligustici Chuanxiong</i> , <i>Radix Paeoniae Alba</i>	Tumor	Cell	Similar expression profiles were induced by estradiol or Si-Wu-Tang-treated MCF-7 cells, suggesting phytoestrogenic effect of this formula, which is confirmed by estrogen-responsive element luciferase reporter assay	[48,49]
QiShenYiQi	<i>Astragalus membranaceus</i> , <i>Salvia miltiorrhiza</i> , <i>Panax notoginseng</i> , <i>Dalbergia odorifera</i>	Cardiovascular disease	Rat	55 potential targets of QiShenYiQi were identified, which mainly involved in apoptosis signaling, immune, inflammation pathways	[50]
Xue-Sai-Tong	<i>Panax notoginseng</i>	Cardiovascular disease	Rat	Via database and literature filter, 70 DEGs were consider to be the potential targets of Xue-Sai-Tong, most of which were related to cell death and inflammatory response	[51]
Bufei Yishen	<i>Radix Astragali</i>	Chronic obstructive pulmonary	Rat	Multiple cellular targets, such as interleukins, myosin filament assembly components and mitochondrial electron transport-related molecules, were altered for beneficial activities of this formula	[52]
Youguiyin	<i>Radix Rehmanniae praeparata</i> , <i>Rhizoma Dioscoreae</i> , <i>Fructus Corn</i> , <i>Fructus Lycii</i> , <i>Radix Glycyrrhizae</i> , <i>Cortex Eucommiae</i>	Femoral head necrosis	Rat	The treatment of this formula induced expression changes of genes relevant to cell apoptosis, signal transduction and cell proliferation, etc	[53]
Huo Luo Xiao Ling	<i>Salviae miltiorrhizae</i> , <i>Angelica sinensis</i> , <i>Gummi olibanum</i> , <i>Commiphora myrrha</i>	Rheumatoid arthritis	Rat	Huo Luo Xiao Ling treatment-effected pathways directly related to arthritis pathogenesis, included immune response, inflammation, cellular proliferation and apoptosis, and metabolic processes	[54]
Xueshuan Xinmaining	<i>Rhizoma Chuanxiong</i> , <i>Radix et Rhizoma Salviae Miltiorrhizae</i> , <i>Hirudo</i> , <i>Bovis Calculus</i> , <i>Moschus</i> , <i>Pubescent Holly Root</i> , <i>Sophorae Flos</i> , <i>ginseng</i> , <i>Borneolum syntheticum</i> , <i>Bufonis venenum</i>	Cerebral thrombosis; coronary heart disease	Rat	This formula yields the therapeutic effect on blood circulation and blood circulation by affecting <i>F13a1</i> , <i>Car1</i> and <i>Tbxa2r</i>	[55]
PHY906	<i>Glycyrrhiza uralensis</i> , <i>Paeonia lactiflora</i> , <i>Scutellaria baicalensis</i> and <i>Ziziphus jujuba</i>	Tumor	Mouse	Microarray data showed that PHY906 induced the repression of transcription and immune-suppression in tumors	[56]

Table 2. Transcriptomics studies on traditional Chinese medicine formula (cont.).

Formula name	Composition	Disease	Model	Description	Ref.
Tangshen	<i>Radix Astragali</i> , <i>Radix Rehmanniae</i> , <i>Radix Notoginseng</i> , <i>Radix et Rhizoma Rhei</i> , <i>Fructus Corni</i>	Diabetic nephropathy	Mouse	Beneficial effect was achieved via JAK/STAT/SOCS signaling pathway	[57]
Shensong-yangxin	<i>Ginseng</i> , <i>Ophiopogonis</i> , <i>Cornus officinalis</i> , <i>Salvia miltiorrhiza</i> , <i>Ziziphi spinosae semen</i> , <i>Taxilli herba</i> , <i>Paeoniae Radix Rubra</i> , <i>Eupolyphaga seu steleophaga</i> , <i>Nardostachyos</i> , <i>Coptis chinensis</i> , <i>Schisandrae sphenantherae fructus</i> , <i>Os draconis</i>	Bradycardia	Rabbit	Genes related to pacemaker current, calcium ion homeostasis and signaling were affected by Shensongyangxin treatment	[58]
NF3	<i>Radix Astragali</i> , <i>Radix Rehmanniae</i>	Foot ulcers	Clinical trial	NF3 changed the genes involved in fibroblast regeneration, angiogenesis and anti-inflammation	[59]

age signal ing pathways. These findings suggested that *Rhizoma Coptis* might contribute significantly to the antihepatocellular carcinoma effect of San-Huang-Xie-Xin-Tang.

Compatibility rules

TCM compatibility rules/principles are the foundation for formula construction. Among these rules, ‘Jun-Chen-Zuo-Shi’ (also known as ‘sovereign-minister-assistant-courier’) rule guides the basic structure of four major TCMs in the formula, which correspond to their ranked contributions to overall clinical effects [63,64]. However, the rationality of this rule is not fully understood at molecular level. In order to answer this question, Wu *et al.* [65] selected the Qi-Shen-Yi-Qi formula, known for its efficacy in cardiovascular disease and applied transcriptomics approach to study the role of four medicinal herbs of this formula. Authors investigated the influence on gene expression profiles on acute myocardial ischemic rat model by the formula and four separate TCMs. A network-based algorithm was proposed to calculate a recovery index by integrating a series of network topology-based parameters with fold changes of gene expression levels. Using this index, the capability of TCM treatment to recalibrate the network balance from diseased condition back to normal was indicated for the whole formula and each TCM. By mapping the expression profile to an acute myocardial ischemia-related gene network, Wu *et al.* found that *Radix Astragalus membranaceus*, *Radix Salvia miltiorrhiza*, *Panax notoginseng* and *Dalbergia odorifera* T. Chen corresponded to Jun, Chen, Zuo and Shi in Qi-Shen-Yi-Qi, respectively. The influences of four herbs on the gene expression network were found to decrease in the sequence of Jun, Chen, Zuo, Shi, which is consistent with their roles in TCM compatibility

rules. Compared with the complete formula, network analysis demonstrated that these four herbs displayed distinctive but weaker regulatory effect than combined, which underscores the holistic property of TCM formula from a gene expression network point of view.

Compatibility rules dissection is one of the most complicated areas in TCM study. Works on compatibility are relatively rare [66–68]. The explorations on formula-composed principles in transcriptomics are even more scarce [69,70] and existing studies are still lacking further explanation.

Transcriptomics for other TCM treatments

In addition to pharmacotherapy, another significant part of TCM treatment involves external physical interventions on pathological conditions, such as acupuncture and massage. There are many unsolved questions concerning their potential mechanisms. Moxibustion belongs to acupuncture clinical practice and has been demonstrated to be useful for pain relief in certain acupoints. Yin *et al.* [71] applied the transcriptomics technology to investigate the analgesic effects of moxibustion. Freund’s Complete Adjuvant was injected into the plantar surface of the left hind paw of the rat to model pathological condition of pain. After two consecutive weeks of persisted injection, 15 min of moxibustion were applied on Zusanli acupoint of rat for 1 week. Expression levels of about 500 genes were differentially altered after treatment. Pathway-enrichment analysis suggested most of these genes are mapped to metabolism in physiological condition and immune response in pathological condition. These results indicated an effect of moxibustion in pain relief.

To increase controllability and provide extra effect on the human body, electroacupuncture is a modern form of acupuncture which introduces electrical

stimulators into traditional acupuncture. Frequency of electroacupuncture has previously been proved to be related with the release of central neural chemical mediators. To reveal the mechanism of curative effect, Wang *et al.* [72] designed a transcriptomics study on rats treated with electroacupuncture. Spinal dorsal horn was separated from rats stimulated by 2 and 100 Hz electroacupuncture at two acupoints (Zusanli and Sanyinjiao) bilaterally. Gene expression in dorsal horn suggested that both low- and high-frequency electroacupuncture could regulate over expression in 1000 significant genes. A total number of 516 genes with the same regulation trend by two frequencies were found to be involved in the release of central neural chemical mediators. In contrast, other DEGs induced in the 2 Hz group were mostly enriched in the neural plasticity process, while 100 Hz stimulation would affect genes in stress and immune regulation.

Massage or manipulation is normally regarded as a form of recreation, which could produce significant therapeutic effect in some cases. Tai *et al.* [73] performed a study on 30 children with cerebral palsy and 10 healthy age- and sex-matched children. After 3 month of selected spinal manipulative therapy, physical performance, including muscle and joint activity, was improved for children with illness. Under the scale of cerebral palsy therapeutic effective evaluation criteria, 90% of the cases were effective. Besides, the gene expression profile of peripheral blood mononuclear cells (collected before and after the treatment) in study objects showed that the manipulative therapy successfully reversed the change of various inflammatory cytokines and receptors, such as *IL-10*, *IL-13*, *IL-9R*, *IL-15*, *CCL2*, *CCL3*. These findings demonstrated that massage therapy can increase the expression of anti-inflammatory factors and downregulate proinflammatory factors.

Limitations & conclusions

For decades, systemic effect by TCM system has seemed to be a Gordian knot impossible to untie. Transcriptomics provides us with a sword to cut it and sheds some light on the complex processes triggered in the cell and their intrinsic connections. Through this approach, significant progress has been made in various related fields of TCM, as we have discussed. However, transcriptomics in TCM study still faces a number of problems, which are summarized below:

Embracement of a more advanced platform

As a mature approach that has been widely used and well assessed [74–78], microarray platforms occupy a central place in the majority of work we reviewed herein. With rapidly evolving next-generation sequenc-

ing technology, RNA-seq makes it possible to assess whole genome-wide expression with high-throughput and reliable accuracy and is becoming a common tool for transcriptomics study [79]. However, the application of RNA-seq in TCM-related work is rather limited [80–82].

Gap between gene expression & translated protein

Transcriptomics measures changes in mRNA levels of genes that do not necessarily correspond to the amount of protein expressed in the cells. Classically, a number of experimental approaches, such as Western blot, are required to validate findings from gene profiling studies. However, information gathered from transcriptomics is still incomplete, which may limit our conclusions, as suggested by a recent study [83]. Taking the holism basis of TCM into consideration, it is reasonable to believe that integration of transcriptomics with proteomics and/or metabolomics would enrich our knowledge of the systemic effects of TCM at multiple levels and improve our interpretation of results. Few studies have incorporated multiomics data to analyze complex diseases [84,85]. Despite several technical issues related to data fusion, this is a promising novel direction for TCM research.

Accumulation of TCM data

This is a follow-up issue related to the large amount of information around TCM. Information aggregation has been the key-driving force in many areas including social network, epidemiology and so on [86,87]. The fierce development of system biology is dependent on the construction of comprehensive and structuralized databases, such as OMIM [88] and DrugBank [89]. In TCM study, existing databases show notable insufficiencies in term of size, completeness or originality [90–94]. For example, the number of high-throughput gene expression data of TCM in GEO repository [95] is much lower compared with those for conventional drugs and many groups that have published TCM gene profiling studies did not deposit their data into public database, hampering further correlation analysis or in-depth data mining. These facts suggest that it is necessary to build an integrative platform that connects the preexisting knowledge on TCM and encourages more data sharing for future studies.

Another relevant question about data is its heterogeneity in term of quality, which is originated from multiple sources from experimental design, execution and analysis. Data quality from transcriptomics experiments should be seriously considered, as reviewed in [96]. Since TCM itself is a complex system, more attention should be paid to avoid confounding factors

in transcriptomics experimentations, such as sample pooling and statistical analysis of data, which would improve data quality and allow more biologically relevant interpretation.

Future perspective

Despite a number of limitations, it is concluded that transcriptomics remains a powerful tool to investigate whole gene expression influenced by TCM formula treatment, which would continuously expand our knowledge on TCM theory and practice at the genetic level. We anticipate that the coanalysis of integrative studies [97,98] with the combination of multiple -omics approaches at multiple levels, including genes (mRNA and miRNA), proteins and metabolites, will become

common practice in TCM studies within the next few years.

Financial & competing interests disclosure

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Executive summary

Traditional Chinese medicine & transcriptomics

- Traditional Chinese medicine (TCM) is a comprehensive ancient medical system with standalone theory and practice.
- Theory of TCM have not been well understood via modern science, limiting the development of TCM practice;
- The holistic idea within TCM requires systemic biology to reveal its mechanisms and corresponding tools for research.
- Transcriptomics shows advantages in global expression detection and has become a powerful tool in TCM studies, mostly in ZHENGs and formula.

ZHENGs, the building block of TCM theory

- ZHENGs are TCM terms in the classification system for patients based on clinical symptoms.
- Over 100 ZHENGs are commonly observed in the clinic but few have been studied by transcriptomics.
- ZHENGs are difficult to study in animal models and human samples are recommended for transcriptomics study.

Formula, the major utility forms of TCM in the clinic

- TCM formula is a rational combination of TCMs according to TCM theory and ZHENGs.
- Mechanistic study of TCM formula follows a classic work flow of transcriptomics application in pharmacology.
- Transcriptomics helps to recognize the active ingredients of TCM formula.
- Compatibility rules of TCMs combination have not been widely studied by transcriptomics.

Other practice of TCM

- Acupuncture and massage, two types of physical therapies in TCM, demonstrate noticeable effects on certain medical conditions, however, their mechanisms have not been well investigated before. Transcriptomics is a useful approach for this.

Limitations in current studies

- New transcriptomic platforms, such as RNA-seq and single cell sequencing technology, should be applied in TCM transcriptomics studies.
- Expression profile alone cannot fully represent the response of the organism and integration with other omics approaches would enrich our understanding on TCM.
- High-throughput data of TCM study are lacking in quantity and quality. Database construction and information sharing are important for future studies.

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