Published By Guangdong Pharmaceutical University, Guangzhou
Journal of Holistic Integrative Pharmacy
2020,1(2): 102-108

REVIEWS

A study of toxicogenomics and its current applications in the safety of Traditional Chinese Medicine

LIAN Xueping, AI Ni, LU Xiaoyan, FAN Xiaohui*
Department of Chinese Medicine Science and Engineering, College of Pharmaceutical Sciences, Zhejiang University, Hangzhou 310058, China

[Abstract] Continuous advances in "omics" technologies have brought toxicogenomics into sharp focus in the medical community. Toxicogenomics has been increasingly utilized in studies of the safety of traditional Chinese medicine (TCM). Toxicogenomic technology, which assesses the systematic changes of the body upon exposure to toxins at the genome-wide level, has been extensively adopted in studies that are concerned with identifying toxicity biomarkers and investigating mechanisms and predictions of toxicity. This study concisely summarizes the present status of toxicogenomics and highlights its current applications in TCM safety by discussing the mechanism of TCM toxicity, the identification of potential biomarkers studies on of toxicity, and the examination of toxicity dynamics pertinent to drug compatibility.

[Key words] Toxicogenomics; Traditional Chinese medicine (TCM); Gene chip; Database; Safety assessment; Toxicology

1 Introduction

Safety has been a major concern throughout the medical community in recent years and the area of traditional Chinese medicine (TCM) modernization is no exception[1-3]. The technology of toxicogenomics has been increasingly utilized by toxicologists[4] and more frequently used in TCM safety studies[5] in the context of ever-evolving high-throughput omics, such as genomics, transcriptomics, proteomics, and metabolomics. TCM toxicology publications primarily focus on acute, subacute, and long-term toxicity experiments in laboratory animals. The detection criteria of these studies often only involve toxicity endpoints (e.g., pathological section), with insufficient consideration of toxicity mechanisms, particularly at molecular levels. Overall changes after body/toxin interactions may be detected with the use of toxicogenomics at the genome-wide level. This would be a great help in identifying biomarkers of toxicity and thus to study the mechanisms and predictions of toxicity. With wide applications in drug screening, new drug development, and rational use of drugs in clinical practice[6-7], the

This article has been published in China Journal of Chinese Materia Medica (Page 2690-2695, Volume 40, Issue 14, 2015).
[Reasearch funding] This work was supported by National Natural Science Foundation of China (No.81173465); New Century Excellent Talents in University (No.NCET-12-0488).
[*Corresponding Author] E-mail: fanxh@ zju.edu.cn.
These authors have no conflict of interest to declare.

use of toxicogenomics would also help produce novel study conceptions and technical tools for TCM safety studies (Fig.1). Our group is a team that undertook practical studies on toxicogenomics early in China. In close collaboration with the U.S. Food and Drug Administration (FDA)'s National Center for Toxicological Research and the group led by Professor Yue Gao from the Academy of Military Medical Sciences (AMMS), we have developed new methods of assessing TCM toxicity with such technologies as gene chip, including those that are cross-organizational, cross-platform, and early evaluations. This study briefly describes the general picture of toxicogenomic studies and the current applications of toxicogenomics in TCM safety in order to elucidate its use in the studies of these investigations.

2 Platform for Toxicogenomic studies

The rapid progress of toxicogenomics is attributed to the ever-advancing methods in gene chip technology. Gene chip (also known as DNA microarray) technology has evolved into the most important technical platform of transcriptomic studies. In toxicogenomic studies, cDNAs are usually reverse-transcribed from the mRNAs that are extracted from tissue or cells with fluorescently labeled nucleotides and are then hybridized on a gene chip. Expression level is then obtained based on the fluorescence intensity. Subsequently, the gene expression profile of specimens from different groups is compared to identify the differentially expressed genes for further analysis[8]. Apart from mRNA, miRNA (microRNA) is also a major concern in toxicogenomic studies. MiRNAs, which are 19–24 nucleotides-long, non-coding RNAs, regulate gene expression by binding to mRNAs with complementary sequences and controlling their translation[9]. Studies published in recent years have shown that miRNA plays a significant role in genome-wide expression regulation, as evidenced by a role in fundamental activities, such as early development, cell proliferation and differentiation, apoptosis, and lipid metabolism; it is thus carefully considered by toxicogenomic researchers[10-11]. Additionally, analysis of single

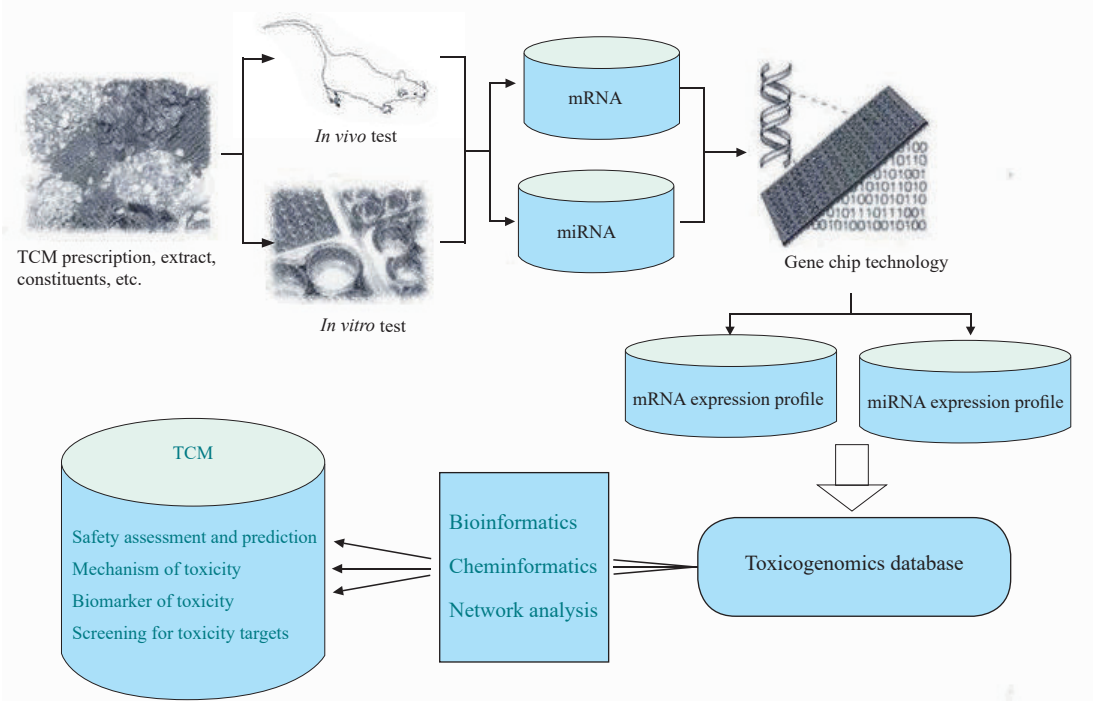

Fig. 1 Toxicogenomics-based strategy for safety studies of traditional Chinese medicine

nucleotide polymorphisms (SNPs) is an important method for toxicogenomic studies. The use of microarray technology in SNPs analysis may help identify differences in individual responses to toxins. For example, if differences in individual susceptibility to chemical poisoning can be determined by analyzing changes in a nucleotide of DNA sequence , it may provide a foundation for individualized therapy[12]. It is particularly notable that rapid advances in next generation sequencing (NGS) has led to its use in toxicogenomic studies by some groups in recent years[13-14]. The FDA recently disclosed the results from the microarray quality control (MAQC-III) project which aimed to test NGS platforms for data reproducibility; these findings have established technical standards for the application of NGS in toxicogenomic studies[15-16].

3 Present status of Toxicogenomic studies

The importance of toxicogenomics has been realized by many health regulatory authorities. The United States, European Union, and Japan are aware of its potential and have developed relevant regulatory policies or guidelines[17-18]. The FDA has included toxicogenomics in opportunity lists and conducted regulatory science studies[15,19-20], while the U.S. Environmental Protection Agency specified in a policy report the substantial impact that toxicogenomics will have on risk assessment and toxicogenomic study programs, such as Toxicology in the 21st Century (Tox21)[21].

The academic and industrial communities attach even greater importance to toxicogenomics. For drug development, it is crucial to determine the appropriate selection of candidate drugs in the early phase of development and the proper evaluation of the potential toxicity of candidate drugs[22]. With high-throughput toxicogenomic technology, more comprehensive toxicologic information regarding the effects of compounds on potential target organs of animals, such as the liver[23], kidney[24], and heart[25] is made available. Toxicogenomic tools have been used in each phase of drug development, including compound screening[26], investigation into the mechanism of toxicity[27], identification of toxicity targets or biomarkers[28], and prediction of potential toxicity[29]. The tools are also applied to numerous studies concerning clinical or preclinical general toxicity[30], developmental toxicity[31], carcinogenicity[32], and screening for sensitive individuals[33]. Toxicogenomics is expected to promote the rational use of drugs in clinical practice as an effective tool for risk warning and reassessment in the post-marketing phase[34]. Besides, toxicogenomic approaches, which have been taken in research regarding species differences and interspecies extrapolation, establish a linkage between basic and clinical toxicology studies and are anticipated to deliver an effective solution to species differences when applied to potential toxicity analyses[18,35].

As gene chip technology advances, dry lab, in the place of the wet lab, pose a technical bottleneck to toxicogenomics. How to efficiently derive, manage, and analyze the high-throughput data represents the greatest challenge faced by toxicogenomic researchers at present. The Microarray Gene Expression Data Society (MGED) proposed a paradigm whose purpose is to collect all published genome data and developed an instruction, the Minimum Information about a Microarray Experiment (MIAME). A majority of scientific journals consider MIAME as an essential part of publication criteria. With the advent of NGS, MGED sets out new guidelines, such as the Minimum Information About a High-throughput SEQuencing Experiment (MINSEQE)[36]. A complete toxicogenomics database encompasses not only genome data but also information on compound property, pathological section, and experiment design factor (e.g., dosage, timing, and species). Much of toxicogenomic data is deposited in multiple databases, such as

ArrayTrack[37], ArrayExpress[38], Gene Expression Omni-bus[39], Open TG-GATEs[40], DrugMatrix[41], Pred-Tox[42], and Comparative Toxicogenomics Database (CTD)[43]. Analytical tools for these databases have been developed and improved, such as KEGG (http://genome.jp/kegg/)[44] and Cmap (http://www. broadinstitute.org/cmap/)[45]. Our group established a TCM toxicity knowledge database TTKB in cooperation with the study team led by Professor Gao Yue from AMMS. We also established an online platform for drug hepatotoxicity prediction named LTMap (http://tcm.zju.edu.cn/ltmap/). This was established by integrating data of over 20,000 chips from the Open TG-GATEs from the Japanese Toxicogenomics Project. LTMap is available for the assessment of potential TCM hepatotoxicity and mechanisms of action[46].

4 Typical Applications of Toxicogenomics in safety assessment of Traditional Chinese Medicine

4.1 Investigation into the mechanisms of adverse reactions and prediction of toxicity

It is difficult to copy general study methods for the toxicology of chemical drugs. Reasons for this difficulty include the fact that TCMs are characterized by "multiple components, multiple targets, multiple effects"; asymptotic analysis results, from overall to cellular and molecular levels, make the process unsatisfactory[47-48]. However, with a toxicogenomic tool, a researcher may fully examine the safety of TCM at the gene level and perform a statistical analysis in accordance with existing gene expression databases by comparing characteristic gene expression profiles before and after the administration of TCM. The mechanism of action of TCM at the gene level can thus be understood and then made it possible to predict toxicity. With the use of toxicogenomic technologies such as gene expression profile and miRNA chips, our group determined the mechanisms of TCMs (e.g., *fructus meliae toosendan*) and a common TCM biotoxin aflatoxin B1-induced hepatotoxicity[28]. In addition, Chen et al.[49] evaluated how the administration of *Rhizoma Dioscoreae Bulbiferae* (RDB) affected the gene expression profile of mouse liver. The analysis of differentially expressed genes revealed a disturbance in the synthesis of hepatic-cytoskeleton-associated protein and a compromise to the cytoskeleton structure after using the drug. This finding indicated RDB's possible inhibition of hepatocellular repair and regeneration. Meanwhile, the hepatotoxicity of RDB was also noted to be cumulative and closely associated with the duration of administration. Kiel et al.[50] found that enteritis did not improve with administration of a high dose of frankincense extract, and the high dose caused dysregulation of gene expression in a large number of genes associated with lipid metabolism as demonstrated in the analysis of the hepatic gene expression profile. These findings are suggestive of suspected hepatotoxicity when a high dosage of frankincense extract is administered. Using toxicogenomic methods, Pan et al.[51] assessed the potential risk of *Valeriana jatamansi* Jones (VJJ) by comparing the behavioral indicators between the diazepam-induced physical dependence mice model and the group receiving VJJ. The drug was found to show limited effects on genes closely associated with physical dependence, and a low potential risk is therefore expected.

Results of other means of detection cannot be compared to the efficiency and coverage of toxicogenomics. For example, toxicogenomics techniques reveal overall changes of the body exposed to toxins at the genome-wide level and investigate the mechanisms of drug toxicity at the molecular level. These techniques can also have broad use in studies regarding the mechanism of TCM toxicity. In addition, treatment with TCMs

is often characterized by high dosage, prolonged course, and rough preparation as compared to that of chemical drugs. The toxicity of TCMs, as a result of accumulation, is relatively lower and should not be assessed only on single or several known components due to the presence of multiple targets. Therefore, it is difficult to examine TCM toxicity using traditional toxicologic methods[52]. A toxicogenomic approach can assist in predicting toxic phenotypes of TCM in animals that require more time to present and in identifying gene expression alterations induced by drug toxicity before pathological changes. It provides an effective solution to toxicity detection and allows for a more reliable assessment of TCM toxicity.

4.2 Targets and potential biomarkers of TCM toxicity

Drug targets are usually the key molecules that are directly linked with a specific illness or pathological state in metabolic or signaling pathways[53]. In contrast, biomarkers are measurable indicators of changes in organs, cells or biomolecules caused by various endogenous and exogenous factors that are active in organisms[54]. Drug toxic effects cannot be identified or reduced without screening and studying for both targets and biomarkers. Both of these goals can be achieved with the analysis of extensive toxicogenomic data. With the use of gene chips,Yan et al.[55] examined gene expression alterations in renal tissue of rats on oral administration of total rhubarb anthraquinones,aiming to screen the targets that are involved in nephrotoxicity. In the drug group, 143 upregulated and 101 downregulated genes were found. The team also investigated the toxic mechanism of rhubarb anthraquinones from the aspect of the functional genome and were able to draw logical inferences about the nephrotoxicity targets and toxicity mechanism of the drug. Su et al.[56] carried out a parallel study on the miRNA expression changes in liver and serum in the acetaminophen (APAP) or an herb (*dioscorea bulbifera*)-induced liver injury in Sprague Dawley (SD) rat. Serum biochemical and histopathological tests and quantitative real-time polymerase-chain-reaction (qRT-PCR) assay discovered miR-122, miR-192, and miR-193 as potential diagnostic biomarkers of drug-induced liver damage.

A large quantity of differentially expressed genes associated with toxicity can be detected rapidly with the use of high-throughput gene chip technologies. These genes most likely represent direct targets of toxins or biomarkers of toxicity. In particular, the alterations of these genes appear much earlier than the pathological endpoints currently in use[57]. Besides, numerous toxicogenomics databases allow researchers to rapidly identify potential toxicity targets or biomarkers using gene chip data and to conduct studies on the mechanisms in the human body at a safe dose level of TCM. However, because of technical complexity and diversified technical platforms, findings may vary with chips or platforms for analysis. Changes in gene expression can also differ according to sampling timing and dosage. As a result, more experiments are required to verify whether the genes that are differentially expressed in response to toxins have a role as biomarkers in the prediction or diagnosis of TCM toxicity.

4.3 Study of the principles of TCM prescription compatibility

Syndrome differentiation is an essential part of TCM prescription, and "compatibility" is the theoretical underpinning of prescription[58]. With a prescription, general status is modulated by multiple drugs which are synergetic and complementary to one another and produce more detoxifying effects, which has irreplaceable advantages in the treatment of complicated illness[59]. By far, the majority of studies emphasize chemical levels in their prescription compatibility

principles. This is illustrated by the investigation into changes in prescription chemical composition before and after compatibility, as well as attempts to clarify the basic toxic substance of TCM. Nevertheless, these studies do not focus on the overall effects of the toxic substance on the body over the course of treatment[60]. The application of toxicogenomics will assist in the transformation of this traditional approach with regards to TCM toxicology studies. In holding a holistic view of TCM and adopting toxicogenomic methods, we may consider the molecular mechanism of TCM compound compatibility, principles of compatibility, and connotation of formulae. These goals can be achieved by comparing the gene expression profiles between single herb and compound prescription. Yang et al.[61] examined the acute toxicity of toad venom on the rat heart using a toxicogenomic method. In combination with qRT-PCR, low-dose toad venom was found to adversely affect cardiac contraction by disturbing ionic homeostasis and destroying actins structure, resulting in stress reactions, such as antiapoptosis of cardiac cells and abnormal lipid metabolism. Additionally, more ferric ions accumulated at a high dose of toad venom, thereby stimulating apoptosis. This finding showed the dose-dependent impact of the drug on the heart. However, significant reactions were not observed when toad venom was prescribed as a component of Shexiang Baoxin Pills (heart-protecting musk pills). This fact indicates toxicity reduction by TCM compatibility. Li et al.[62] evaluated the impact of the compatibility of *Gastrodiae Rhizoma* with *Uncariae Ramulus cum Uncis* on renal gene expression in spontaneously hypertensive rats (SHRs). The results suggested a reversal in the expression of the genes related to G-protein coupled receptor mediated signaling pathway, lipid metabolism, and insulin resistance. This finding suggested that the kidney toxicity of *Gastrodiae Rhizoma* was reduced after compatibility.

5 Challenges and outlook

Toxicogenomics provides an objective and comprehensive assessment of TCM safety at the gene expression level and produces scientific data for TCM risk assessment. However, the wide application of toxicogenomics in TCM safety studies is considered exceptionally challenging. Theoretically, toxic responses to every substance can be found in the genome-wide expression profile, and gene expression variation is an important contributor to adverse drug reactions[4]. Nevertheless, altered expression of some genes is a normal result of a cell or animal's exposure to an external stimulus, rather than a toxic effect produced by a drug. Furthermore, changes in gene expression do not necessarily cause alterations in protein expression or function. Hence, whether the genes identified in the microarray analysis are reliably involved in toxic response requires more consideration. Similarly, how to best characterize changes in gene expression with regards to protein expression and spatiotemporal correlation regarding the dose-effect relationship are also problems to be addressed[63-64]. Besides, as one of TCM safety study methods, toxicogenomics still requires popularization, whereas it is currently often used to examine TCM toxicity in liver and kidney and less frequently adopted in other organ systems.

As chip technology advances, consistency in gene chip quality can be achieved. Toxicogenomics data will be standardized and accordingly, become highly reproducible. Development and improvement of various databases will open up the possibility of establishing a standard gene atlas for toxicogenomics. As a result, the following issues are expected to be handled: spatiotemporal correlation regarding endpoints and gene expression profile of TCM toxicity, drug dose-effect relationships, the association between genes and severity/reversibility of lesions, and interspecies extrapolation. A toxicogenomic approach can also be used to examine the mechanisms of

TCM toxicity and to identify toxicity targets and biomarkers. In addition, it may have applications in screening TCM toxic components, optimizing TCM compound preparations, and investigating individual susceptibility. Intracellular components (e.g., DNA, RNA, protein, and metabolites) are analyzed using a holistic approach that involves bioinformatics and network pharmacology in the combination of toxicogenomics, proteomics, and metabolomics. In this process, a variety of interactions and changes in metabolic and regulatory pathways observed at separate levels (e.g., gene and protein) are integrated. This is an example of the methodology of systems biology, which displays a tendency toward a modern TCM safety assessment. This integrated toxicogenomics-based strategy can be used to elaborate upon the complicated toxic effects of TCM in a comprehensive and systematic way, increase the reliability of risk assessment and provide powerful tools for TCM safety research. It will undoubtedly boost the development of TCM toxicologic studies and speed up the modernization of TCM.

References

[1] Mei QX, Zeng CY. A Tentative discussion on the modernization and safety of Chinese Traditional Medicine[J]. *Clini Med J*, 2009(2):23 (in Chinese).

[2] Li S, Li M, Wei YF, et al. Review on research of safety evaluation for Traditional Chinese Medicine[J]. *Mod Chin Med*, 2014(2):172 (in Chinese).

[3] Cheng KF, Leung PC. Safety in Chinese medicine research[J]. *Open J Safety Sci Technol*, 2012, 2(1):32.

[4] Nuwaysir EF, Bittner M, Trent J, et al. Microarrays and toxicol- ogy: the advent of toxicogenomics[J]. *Mol Carcinogen*, 1999, 24(3):153.

[5] Youns M, Hoheisel JD, Efferth T. Toxicogenomics for the pre- diction of toxicity related to herbs from traditional Chinese medi-cine[J]. *Planta Med*, 2010, 76(17):2019.

[6] Suter L, Babiss L E, Wheeldon EB. Toxicogenomics in predic-tive toxicology in drug development[J]. *Chem Biol*, 2004, 11(2):161.

[7] Goetz AK, Singh BP, Battalora M, et al. Current and future use of genomics data in toxicology: opportunities and challenges for regulatory applications[J]. *Regul Toxicol Pharm*, 2011, 61(2) : 141.

[8] Ju ZL, Wells MC, Walter RB. DNA microarray technology in toxicogenomics of aquatic models: methods and applications[J]. *Comp Biochem Phys C*, 2007, 145(1):5.

[9] Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function[J]. *Cell,* 2004, 116(2):281.

[10] Lockhart DJ, Dong H, Byrne MC, et al. Expression monitoring by hybridization to high-density oligonucleotide arrays[J]. *Nat Biotechnol*, 1996, 14(13):1675.

[11] Gundert-Remy U, Oberemmm A, Onyon L. How can toxicog- enomics inform risk assessment[J]. *Toxicol Appl Pharm*, 2004, 197(3):152.

[12] Deeken JF, Figg WD, Bates SE, et al. Toward individualized treatment: prediction of anticancer drug disposition and toxicity with pharmacogenetics[J]. *Anti-Cancer Drug*, 2007, 18 (2):111.

[13] Su Z, Ning B, Fang H, et al. Next-generation sequencing: a rev-olutionary tool for toxicogenomics[J]. *Gen Appl Syst Toxicol*, 2011, DOI: 10. 1002 /9780470744307. gat232.

[14] Ning B, Su Z, Mei N, et al. Toxicogenomics and cancer suscep-tibility: advances with next-generation sequencing[J]. *J Environ Sci Health C*, 2014, 32(2) : 121.

[15] Consortium SM. A comprehensive assessment of RNA-seq accu-racy, reproducibility and information content by the sequencing quality control consortium[J]. *Nat Biotechnol*, 2014, 32(9):903.

[16] Wang C, Gong BS, Bushel P, et al. The concordance between RNA-seq and microarray data depends on chemical treatment and transcript abundance[J]. *Nat Biotechnol*, 2014, 32(9):926.