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TITOLO

La tecnologia del DNA
microarray e la Medicina
Tradizionale Cinese

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Summary

Functional genomics, a new screening strategy and methodology, so called “genomic screening”, aims at placing all of the genes in the genome within a functional framework. The advantage of this approach is that it takes a more holistic perspective and uses the information provided by the human genome sequences to measure how what we absorb interacts with our body, or more specifically our genes, proteins and metabolism. DNA microarrays have proven to be a powerful technique to analyze gene expression profiling. Traditional Chinese Medicine (TCM) formulas usually contain several major ingredients, each playing a unique role while working together synergistically, to achieve optimum therapeutic results. The DNA microarray technology can be used in combination with other conventional chemical and biological/clinical techniques to screen the target molecules of the action of Traditional Chinese Medicines, to identify the new effective components from Traditional Chinese Medicines, and to explore the mechanisms of the effects of Traditional Chinese Medicines. In this review, the use of DNA microarray technology to study Traditional Chinese Medicine will be introduced; additionally, the application of microarray-based expression profiling in unveiling the molecular modes and synergistic effects of principal ingredients in TCM will be discussed, hoping that this will facilitate the integration of Traditional Chinese Medicine within modern biological and medical sciences.

Riassunto

La genomica funzionale è una nuova metodologia di screening, il cosiddetto “screening genomico”, che aspira a collocare tutti i geni del genoma su un framework funzionale. Questo approccio segue una prospettiva più olistica e utilizza le informazioni fornite dalle sequenze del genoma umano per misurare come ciò che assorbiamo interagisce con il nostro corpo, o più precisamente con i nostri geni, le proteine e il metabolismo. I microarrays hanno dimostrato di essere una potente tecnica per analizzare il profilo dell'espressione genica. Le formule della Medicina Tradizionale Cinese (MTC) di solito contengono diversi ingredienti importanti, ognuno con caratteristiche e ruoli unici ma che insieme lavorano si-

nergicamente per garantire i migliori risultati terapeutici. La tecnologia del DNA microarray può essere utilizzata in combinazione con altre tecniche convenzionali, chimiche e biologico/cliniche, per lo screening delle molecole target della MTC per identificarne i nuovi componenti e per esplorare i meccanismi dei loro effetti. In questa review verrà introdotto l'utilizzo della tecnologia del DNA microarray per lo studio della MTC e verrà discusso come l'applicazione del profilo di espressione basato sui microarray possa facilitare la comprensione dei suoi principali ingredienti, a livello molecolare e relativamente agli effetti sinergici, sperando che questo possa facilitare l'integrazione della MTC all'interno della moderna scienza biologica e medica.

Introduction

Traditional Chinese Medicine (TCM) is thousands of years old and has historically been a rich source of lead molecules in the study of human health, disease and prevention. It is popular in Hong Kong and much of Asia, and has won wide recognition in the West in recent decades, as alternative treatment, especially in chronic conditions (1, 2). During the SARS outbreak in 2003, China used a combination approach, and the World Health Organization (WHO) later recognized China's efforts for its efficacy, safety and quality. However, the value of TCM has not been fully recognized worldwide due to the lack of definitive information on active ingredients, and scientific evidence of effectiveness of TCM remains still largely unknown.

In recent years, many herbal me-

dicines have successfully been used to treat various symptoms or diseases, and have demonstrated a variety of effects through direct or indirect interactions with different genes or proteins (3). However, the mammalian genome contains huge number of genes, of which typically more than 10,000 are transcribed in a given cell. Utilizing the complete DNA sequences of the genomes of many organisms and expanding gene databases, researchers seek evidence on whether and how TCM works by RNA expression analysis, hunting for candidate disease genes or characterization of tissue-specific genes.

On strategy of functional genomics is to place all the genes of the genome within a functional framework. Fundamentally it is based on the gene expression profiles that are used to analyze gene regulation in biomedical research as

well as for transcriptional therapy as long as the transcriptional effects are well characterized. Combined with other techniques, functional genomics can be employed for biological characterizations of natural products, for high throughput screening of effective compounds found in TCM formulas, identification of qualified herb targets, and determination of efficacy and side-effects of active compounds; all these should facilitate the integration of Traditional Chinese Medicine within modern biological and medical sciences.

Functional genomics technologies

Functional genomics refers to the development and application of global (genome-wide or system-wide) experimental approaches to assess gene function by making use of the information and rea-

gents provided by the mapping and sequencing of genomes. It is characterized by high throughput or large-scale experimental methodologies combined with statistical and computational analysis of the results; all of which perform a critical and expanding role for mining the data sets for particularly valuable information, and to yield new insights into the behavior of biological systems (4).

Serials of traditional approaches have been applied to different research areas, for example, subtractive hybridization, differential display, representational difference analysis (RDA), suppression subtraction hybridization (SSH), etc. Besides, several advanced approaches have been established for monitoring gene expression on a genome-wide scale, including DNA microarray, oligonucleotide chips and serial analysis of gene expression (SAGE). SAGE is a sequence-based genomics tool that features comprehensive gene discovery and quantitative analysis of overall gene expression patterns with digital analysis (5,6).

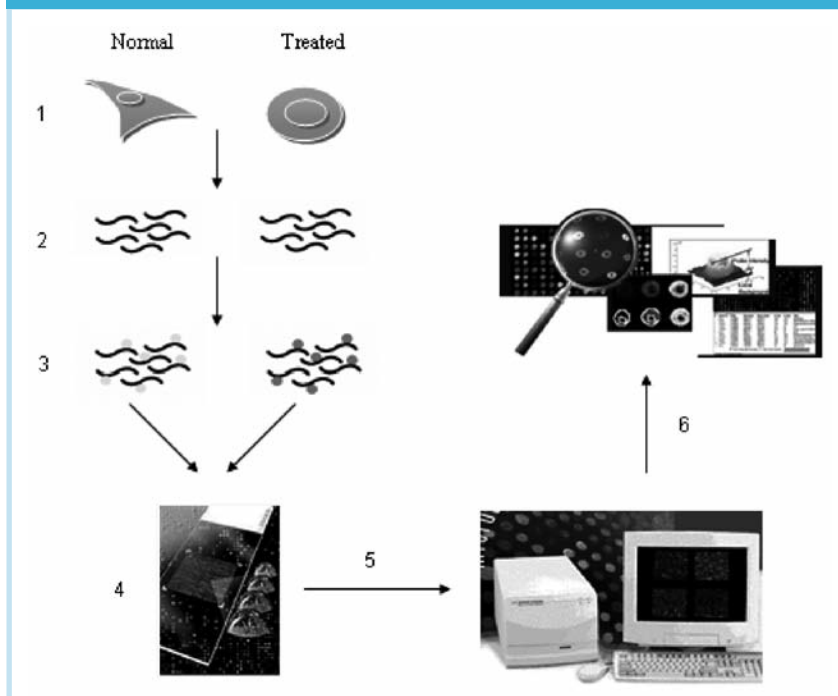
cDNA microarray technique is a hybridization-based analytical tool aiming at monitoring expression levels of thousands of genes simultaneously (7, 8). It allows researchers to obtain gene expression profiles with speed, efficiency and sensitivity. In general, a cDNA microarray experiment platform is

composed of three steps, including construction of cDNA microarray, labeling and hybridization of cDNA probes to targets on microarray, and image acquisition and data analysis. Figure 1 summarizes the basic procedures of cDNA microarray technique. Thousands of cDNAs or synthetic oligonucleotides are fixed on small membranes, silica wafers, or glass slides in high density, with parallel hybridization of two different mRNA samples and subsequent analysis by means of two-color

fluorescence; the changes of gene activity can be detected by measuring the intensity of hybridization signals.

With the successful bid for €1 Million EU funding together with a consortium consists of 29 beneficiary partner institutions and small-and-medium-sized enterprises as well as more than 20 additional non-beneficiary institutions from the EU and China, King's College London [UK] is now leading a project entitled 'Good Practice in Traditional Chi-

Figure 1 - Diagram shows the main steps of DNA microarray technology. 1: Drug treatment; 2: total RNA or/and mRNA extraction; 3: reverse transcription and fluorescence labeling; 4: hybridization to the cDNA microarray; 5: scanning the hybridized array; 6: interpreting the scanned image



nese Medicine Research in the Post-genomic Era' (GP-TCM). This project aims at reviewing the current status of Traditional Chinese Medicine (TCM) research, identify problems and propose solutions by applying modern methods of investigation, especially to discuss fora that explore the role of functional genomics methodology in researching the safety, efficacy and mechanisms of action of Chinese herbal medicines; all these should play an important role in the unification of Western and Chinese approaches to medicine, as well as a forum for the exchange of opinions, experience and expertise among scientists in the EU and China (9).

DNA microarray technology and the development of TCM

Several innovative technology platforms based on microarray technology have been well established and are used to study and develop TCM, including genotyping biochip for TCM herbal authentication and quality control; microfluidic biochip for high-throughput screening of effective compounds from TCM; and cDNA microarray for quality control, drug screening and target discovery (10-12). Rong et al. described a genome-wide biological response fingerprinting (BioReF) approach on the basis of

regulated expression levels and the involvement in the cellular signaling pathways to define a set of marker genes for signature pattern of a specific botanical formulation (13). This set of marker genes represents the biological responses of human cells to the chemical composition of botanical drugs it also served as potential quality control in terms of the consistency of biological activities. Yan et al. used high-density oligonucleotide microarray to study nephrotoxicity of total rhubarb anthraquinones on Sprague Dawley rats, and identified mitogen-activated protein kinase (MAPK) 6 to be the target gene which causes cell cycle arrest and proliferation inhibition, and contributes to nephrotoxicity on S.D. rats (14).

Genomics and combinational chemistry dramatically increase the range of biological targets and compounds available for high throughput screening. Many groups have used cDNA microarray technique to study gene expression profiles in combination with a series of cellular and animal disease models to develop the lead compounds and new products for disease prevention and treatment against major human diseases. For example, Li *et al.* analyzed gene expression profile of inferior colliculus affected by Qingyangshenylycosides, and found that they can prevent many of the audioge-

nic seizure-induced gene expression changes, while revealing the action mechanism of the anticonvulsant effect; further analysis of the function of these genes may help to identify therapeutic targets for epilepsy (15). In one review article about the pharmacogenomics and the Yin/Yang actions of ginseng (16), mechanistic studies based on cDNA microarray analysis were introduced; a group of genes related to cell adhesion, migration and cytoskeleton were found to be up-regulated, which might be concomitant to the observed phenomena in angiogenesis effects of ginsenosides; this finding may lead to the development of more efficacious ginseng-derived therapeutics for angiogenesis-related diseases, including tumor progression and cardiovascular dysfunctions, etc. Table 1 also lists more biological and gene expression analysis of TCM studies.

With the DNA microarray application in TCM, many herbal medicines were found to exhibit a variety of effects through regulating a wide range of gene expressions or protein activities. The term "Herbogenomics" was coined recently to describe the approach to understand mechanisms of action of TCM, and to identify effective molecular targets for the discovery and development of novel therapeutics (17). Combined with modern technologies, i.e. fingerprint

Table 1 - Biological function study and gene expression profile analysis of selected natural products

Natural product	Biological function	Gene expression analysis	References
<i>Radix Paeoniae</i>	Inhibits hepatoma cells growth	The differential gene expression of water-extract of <i>Radix Paeoniae</i> -treated HepG2 was examined. Gene expression of BNIP3 was up-regulated while ZK1, RAD23B, and HSPD1 were down-regulated.	Lee et al. Life Sciences 2002; 71: 2267-2277
<i>Scutellaria barbata</i>	Growth inhibitory effects on a number of human cancers	Ethanol extracts of <i>S. barbata</i> treated A549 cell. 16 genes, involved in DNA damage, cell cycle control, nucleic acid binding and protein phosphorylation, underwent more than 5-fold change; CD209, related to dendritic cell (DC) function, was dramatically down-regulated by 102-fold	Yin, et al. Life Sciences 2004; 75: 2233-2244.
Genistein	Inhibitory actions in human breast cancer (MCF-7) cells	Genistein up-regulated heat shock protein 105 (HSP) and down-regulated serum response factor (SRF), estrogen receptor (ER) α , disabled homolog 2 (DOC 2) and recombination activation gene 1 (RAG-1) as well as their pathway specific genes. The inhibitory action of genistein on human breast cancer cells appears to be complex and is only partially mediated by the alteration of estrogen receptor-dependent pathways.	Chen, et al. Biochim. Biophys. Acta, Mol. Basis Dis. 2003; 1638: 187-196
Protodioscin	Anti-proliferative effect of methyl protodioscin on the HepG2 cells	Down-regulation of cyclin B1 and the signaling pathways leading to up-regulation of Bax and down-regulation of BCL2, suggesting that methyl protodioscin may be a novel anti-mitotic agent.	Wang et al. Cancer Lett. 2006; 241: 102-109
<i>Tripterygium hypoglaucom</i> Hutch	Apoptosis of HL-60 cells	The herb induced HL-60 cells apoptosis via NF-kappa B and c-myc signaling pathways	Zhuang et al. Phytomedicine 2004, 11(4): 295-302.

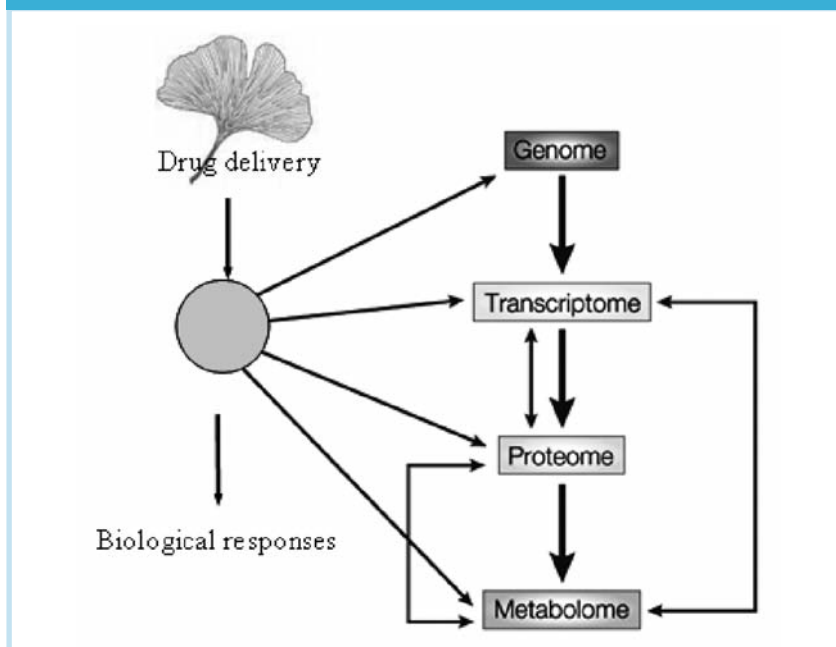
profiling, LC-mass spectrometry to establish relationships between genomic DNA fragment patterns and ratios of active components,

functional genomics can be employed for identification of biologically active compounds from complex mixtures.

As data on TCM rapidly increase, there is an urgent need to explore these resources effectively by mining the huge volume of literature.

In 2008, a database, TCMGeneDIT, was developed, and provides association information about TCM, genes and diseases, relationships among effectors, TCM effects and effect receivers, and correlations between TCM and ingredients from a vast amount of biomedical literature and information about protein-protein interactions and biological pathways from public databases. This database integrating TCM with life sciences and biomedical studies should facilitate clinical research and the understanding of therapeutic mechanisms involved by TCM, that seems to induce or suppress gene activities (18, 19).

Figure 2 - Commonly performed-omics technologies to explain the mechanisms behind the biological changes



Application of omics technologies in TCM research

An omics technology has come to mean an approach capable of generating a comprehensive data set of whatever is being measured, as shown in Figure 2, there are four major types of omics technologies that are commonly performed: genomic, transcriptomic, proteomic as well as metabolomics. Each of them is distinct; the combined data can explain the mechanisms behind the biological changes.

Besides of our previous introduction about “genomic” which measures genotypes for hundred of thousand single nucleotide poly-

morphisms spread throughout the genome, and “transcriptomic” study based on cDNA microarray or oligonucleotide arrays techniques, “proteomic” and “metabolomics” have also been newly developed and applied for biomarker research. Proteins are vital parts of living organisms, they are the main components of the physiological metabolic pathways of cells, proteomic technique is used to identify all proteins in an organism, basically, after protein extraction from samples, they are separated using chromatography, one dimensional protein gels which separate based on size alone

or two dimensional protein gels which can separate proteins based on charge and then size, and digested with trypsin and run through mass spectroscopy to identify the peptides and proteins by comparing the database about size of the peptides with the theoretical digests of known proteins. On the other hand, metabolome refers to the complete set of small molecule metabolites, such as metabolic intermediates, hormones and other signaling molecules, and secondary metabolites, etc. Metabolomics is a newborn cousin to genomics and proteomics, it characterizes all of the small molecule meta-

bolites profiles in an organism with the rapid, high throughput technique, and MS and NMR are by far the two leading technologies for metabolomics. However, a key limitation to metabolomics is the fact that the human metabolome hasn't been all well characterized. Although omics technologies are powerful for systems biology and functional genomics research, one of the challenges is to integrate transcriptomic, proteomic and metabolomic information to give a more complete picture of living organisms.

Conclusions

Traditional Chinese Medicine is characterized by complexity and holism in both diagnostic and therapeutic principles. DNA microarray technology has been demonstrated to be a powerful tool to study functional genomics of TCM; it is able to identify and characterize the active components of the complex, to provide significant information for understanding the efficacy of certain TCM from the genomic point of view in a systematic way; and to facilitate the functional screening of numbers of TCM at both *in vitro* and *in vivo* levels. Together with rapid growth of proteomics and metabolomics, disease pathogenesis, pharmacology and toxicology,

they should play an integrative role in the global development of Traditional Chinese medicine.

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