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Genomic approach for Traditional Chinese Medicine reappraisal

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L'approccio genomico per la rivalutazione della Medicina Tradizionale Cinese

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PAROLE CHIAVE

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Summary

Genomic tools and knowledge have changed the way for new drug discovery. Applying modern genomic technologies to reappraise Traditional Chinese Medicines (TCM) may give this old medicine a new life and may even help find out novel leading compounds for new drug development. In this paper, I present three examples to demonstrate the usefulness of genomic technologies: Example I shows that cDNA microarray can be used as a genome fingerprint for TCM biological quality control (QC). The current method for TCM QC is a chemical profile based on the high performance liquid chromatography (HPLC). However, a gene expression profile offers distinct advantages because such data can be quantitative and objective, and may be more biologically relevant than the chemical profile. From the analysis of transcripts in the microarray, few marker genes can be identified and their gene expression levels serve as a biological index. In the second example, we show that microRNA can be a novel mechanism to explain how a TCM works, and we may be able to discover new diagnostic biomarker(s) through TCM studies. In the third example, we show that green tea can reduce the methylation in several genes which causes down regulation of gene expression in cancer cells, thus resulting in anti-proliferation effect. The green tea studies can also provide a paradigm for TCM studies because of the complexity of the components in green tea, and that various tea preparations are similar to many TCMs. In this post-genome era, advanced genomic tools and techniques offer an exciting and promising avenue to reappraise TCM.

Riassunto

Gli strumenti e la conoscenza della genomica stanno cambiando la ricerca farmacologica. Applicando le moderne tecnologie genomiche per rivalutare la Medicina Tradizionale Cinese (MTC), si può dare a questa antica medicina una nuova vita e si può addirittura contribuire a scoprire nuovi composti guida per lo sviluppo di nuovi farmaci. In questo articolo vengono presentati tre esempi per dimostrare l'utilità delle tecnologie genomiche. Il primo esempio mostra come il cDNA microarray può essere utilizzato come un fingerprint genetico per il controllo di qualità biologi-

ca (CQ) della MTC. Il metodo corrente per il controllo di qualità della MTC è un profilo chimico basato sulla cromatografia liquida ad alte prestazioni (HPLC). Tuttavia, un profilo di espressione genica offre notevoli vantaggi in quanto tali dati possono essere quantitativi e oggettivi, e può essere biologicamente più rilevante rispetto al profilo chimico. Dall'analisi dei trascritti nei microarray, possono essere identificati pochi geni marker e i loro livelli di espressione genica possono servire come un indice biologico. Nel secondo esempio si discute come il microRNA possa essere un nuovo meccanismo per spiegare come funziona la MTC, e come attraverso il suo studio sia possibile scoprire nuovi biomarker diagnostici. Nel terzo esempio si dimostra come il tè verde possa ridurre la metilazione in diversi geni che causano la down-regolazione dell'espressione genica nelle cellule tumorali, con conseguente effetto anti-proliferazione. Gli studi sul tè verde possono anche fornire un paradigma per gli studi relativi alla MTC a causa della complessità dei suoi componenti, e al fatto che diverse preparazioni di tè sono molto simili a molte delle preparazioni della medicina tradizionale. In questa era post-genoma, gli avanzati strumenti di genomica e le nuove tecniche offrono un'interessante e promettente strada per rivalutare la MTC.

Introduction

The philosophy between Chinese and western medicines is significantly different. The ancient Chinese considered the human body a whole unit, and the human body is also considered a reflection of the universe. While observing the diurnal and seasonal changes, the ancient Chinese proposed the Yin-Yang theory as the fundamental mechanism for Chinese medicine. In general, Yin represents dark, cold, inhibitory or rest; and Yang represents bright, hot, excitatory or movement. Therefore, the philo-

sophy of Chinese medicine is macrocosmic. On the other hand, the philosophy of western medicine has been on microcosmic basis. By investigating a small molecule and its function, the western scientists hope to find the solution to cure diseases. The microcosmic approach has been successful for less complex disorders such as infectious diseases that used to be the most prevalent and important diseases in human history. Since a few decades, scientists and physicians started to realize that using either microcosmic or macrocosmic approach would not be

sufficient to understand human body and disease mechanisms. This is particularly true for complex and chronic diseases that are caused by multiple factors and pathways, and have become the most prevalent diseases in the modern societies. The consensus to study multiple molecules simultaneously and interpret the results using a more integrated way has been reached. This consensus actually is a merge of the philosophy in the Chinese medicine and western medicine. However, the tools and methods were not available to conduct a study using both micro-

and macrocosmic approaches until the Human Genome Project, which brought scientists from different backgrounds to create the modern biotechnologies. One of the milestone developments is the invention of the microarray which allows us to investigate hundreds of thousands of molecules simultaneously. Using the massive data from microarray experiments, we begin to understand not only the change of each molecule, but also the interactions between thousands of molecules. The dream of combination of micro- and macrocosmic investigation is now within reach.

Genome medicine and technologies have revolutionarily changed drug development and medical practice. Considerable progress has been made in exploiting the tremendous amount of genomic data to identify the new and potential drug targets, and to reappraise the mechanisms of action of existing drugs. Applying modern genomic technologies to reappraise traditional Chinese medicine (TCM) may give this old medicine a new life, and may also discover novel leading compounds for new drug development.

Unlike western drugs which are “one compound, one drug”, the TCM often uses one or several natural products to treat diseases. Indeed, the “multiple components vs. multiple targets” mechanism is

important for treating chronic and complex diseases because several factors are involved in the pathogenesis of these diseases. Actually, many complex diseases such as hypertension and cancer are currently treated by multiple drugs that target different pathogenic pathways and molecules to reach a more satisfactory therapeutic goal. Therefore TCM treatment could be an ideal therapeutic strategy for complex diseases.

However, TCM has faced several problems including the variation of ingredients due to seasonal changes, different cultivations, or non-standardized preparations. In addition, the active/therapeutic components and mechanisms are often unclear. Most of TCM do not have well-conducted clinical trials to support their safety and effect. Using the modern genomic technologies may help us to solve some of these problems. To show the usefulness of genomic tools for TCM reappraisal, three examples are given in these Proceedings.

Example 1. cDNA microarray as a genome fingerprint for TCM biological quality control (QC).

QC plays a critical step for TCM because the TCM uses natural products, especially botanicals, whose ingredients are subject to seasonal and cultivating factors. The current method for TCM QC is chemical profile (i.e. finger-

print) based on the high performance liquid chromatography (HPLC). Gene expression studies using either the candidate gene or genome-wide approach have been widely used in biomedical research. A gene expression profile offers distinct advantages because such data can be quantitative and objective, and may be more biologically relevant than chemical profile. Rong et al. (1) compared the gene expression levels between the cells treated and untreated with a TCM using cDNA microarray. From the analysis of more than 47,000 transcripts in the microarray, they found that 145 genes had significant differences of expression levels between cells with and without treatment. They then selected 9 marker genes which were considered the surrogates of these 145 differentially expressed genes. The authors repeated the same cell studies with the same TCM whose botanicals were harvested at the same place but at different seasons. In this repeated experiment, they only checked the expression levels of these 9 marker genes for cost-effective purpose. They found that in general the gene expression from these 9 marker genes showed no difference between the first and second batch of the TCM. Accordingly, the selected 9 marker genes may serve as a biological index. In addition, the HPLC fingerprints also showed

consistent patterns between the first and second batch of the TCM. Their study indicates that the gene expression profile or genome fingerprint may provide additional information to assure the QC of a TCM. Although this study did not further perform *in vitro* or *in vivo* tests to show that biological QC can be more relevant to the TCM therapeutic effect, this study suggests that the modern genome technologies can be employed in TCM QC. Since the HPLC chemical fingerprint may not show the real therapeutic components, genetic fingerprint based on few selected marker genes may provide a cost-effective tool and may offer more therapeutic and biological relevant information than chemical profiles.

Example 2. A novel mechanism for TCM

MicroRNAs (miRNAs) are a recently recognized class of highly conserved, noncoding short RNA molecules (about 22 nucleotides) that regulate gene expression on the post-transcriptional level. MiRNAs suppress protein synthesis by inhibiting the translation of protein from mRNA or by promoting the degradation of messenger RNA (mRNA), thereby silencing gene expression.

MiRNAs are important regulators for cell growth, differentiation and apoptosis (2, 3). Consequently,

dysregulation of miRNA function may lead to human diseases. Interestingly, each miRNA can have a potential to interact and fine tunes hundreds of target genes. From this point of view, miRNAs bear the characteristic of fine tuning and multiple targets with TCM. Our laboratory has been interested in the role of miRNAs in relation to atherosclerosis. Recently, some studies already reported that some miRNAs are involved in the development of atherosclerosis (4, 5). We used Sanqi (a dry root of *Panax notoginseng*) to treat the vascular smooth muscle cells (VSMC) and performed the microRNA microarray experiments. Sanqi is a widely used TCM to treat heart disease. By comparing the changes of miRNAs between the treated and untreated cells, we identified several miRNAs that may be involved in atherosclerosis. We currently focus on one miRNA that was predicted to interact with the CCNE1 and CXCL3 genes. Previous studies have implied that the CCNE1 gene is involved in cell proliferation (<http://www.ncbi.nlm.nih.gov/omim>) and CXCL3 in atherosclerosis (6). We first proved that our interested miRNA can directly interact with the 3' UTR binding sites of these two genes, and then we did show a dose-dependent inhibitory effect of this miRNA on CCNE1 mRNA levels. Further studies show that

transfecting the miRNA precursor into the VSMC cells can inhibit ox-LDL induced cell proliferation (Fig. 1) and migration. Our study also indicates that this miRNA can significantly reduce monocyte chemotactic protein-1 (MCP-1) in the ox-LDL treated VSMC. Moreover, our preliminary results also show that the serum miRNA levels in the patients with coronary stenosis are higher than the controls. The more severe the degree of stenosis, the higher the level of this miRNA is detected. The results from our study have a couple of implications: (1) miRNA can be a novel mechanism to explain how a TCM works, (2) using novel genome technologies, we identified an atherosclerosis-related miRNA and its target genes that can be drug targets, and (3) we may be able to discover new diagnostic biomarker[s] through TCM studies.

Example 3. Green tea and epigenetics

Green tea has been shown to have many beneficial effects via numerous mechanisms (Fig. 2). The anti-cancer effect has been one of the major health effects from green tea. Epigenetic modification is basically an interaction between protein and DNA structure without modifying the actual DNA sequence, and the two major epigenetic mechanisms are DNA

Figure 2 - Dose-dependent inhibitory effect of miRNA on cell proliferation

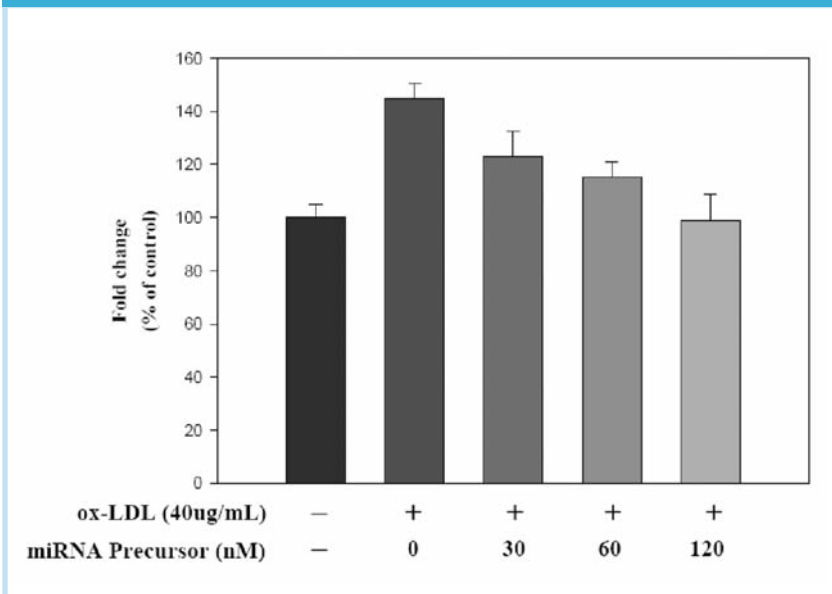
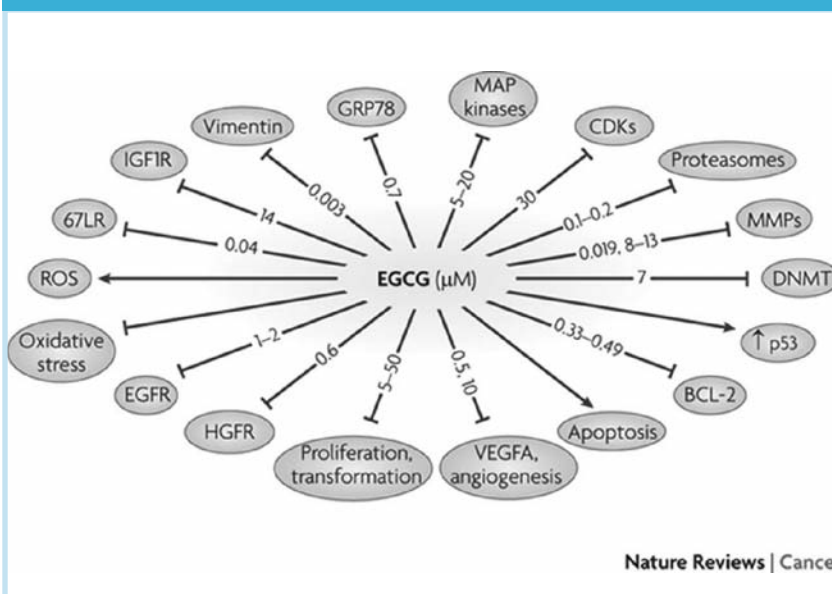


Figure 2 - Figure 2. The possible targets for the cancer prevention effect of EGCG in green tea. The numbers are the reported effective concentrations in IC50 or Kd. (from Yang *et al.* Nat Rev Cancer. 2009 ;9(6):429-39)



methylation and histone modification. Epigenetic processes can cause alteration of gene expression, repair, and cell-cycle, etc. and have been implicated in several diseases including cancer and cardiovascular disease. Pharmacological intervention to influence the epigenetic process can result in a therapeutic effect. Therefore, a disease might be cured by altering the epigenetic regulation of gene expression, either directly by modifying the control of the misregulated genes, or indirectly by ‘reprogramming’ cells toward a ‘normal’ gene expression pattern (7).

(-)-epigallocatechin-3-gallate (EGCG), the major polyphenol in green tea, has many interesting activities and is believed to be a key active ingredient. Fang and colleagues⁸ conducted a series of experiments to demonstrate the EGCG effect on reducing the methylation enzyme activity, reactivating methylation-silenced genes in cancer cells, and finally on anti-proliferation of the cancer cell line. Their study clearly shows that EGCG can prevent or reverse cancer related gene-silencing via an anti-epigenetic mechanism. In addition, their study can also provide a paradigm for TCM studies because the complexity of the components in green tea and various tea preparations are similar to many TCM.

In conclusion, the genomic technologies provide a great opportunity to let us reappraise TCM. With the modern biotechnologies, we may solve some of the long lasting problems to let more scientific data and better support the manufacturing of TCM. If so, scientists can conduct more meaningful clinical trials to finally determine the safety and efficacy of TCM.

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