Application of microarray assay to nephrology

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Introduction

The Human Genome Project (HGP) has now completed the determination of the 3 billion human genetic codes [1]. The goal of the HGP is at least 5-fold: (i) providing the complete human DNA sequence; (ii) identification of sequence variation of the human genome (single nucleotide polymorphism; SNPs) [2]; (iii) development of functional genomics; (iv) comparative genomics among species; and (v) development of bioinformatics and computational biology. The HGP has a profound impact on the scientific research of biological science. The cDNA cloning is done by computer and the analysis of gene expression can be done by gene tips. Even positional cloning could be done by computer in the very near future. Individual medicine will develop and the patient will be treated by tailor-made therapy because the human genome variation such as SNPs will tell us the susceptibility to the disease and will result in the subclassification of the disease. In the last decade, we have been focusing on the structural analysis of the human genome, and our current question is what the physical structure of the genome. However, our main research interest is moving to the function of the genome as a post-genome project. The function of the gene will become a central issue in a few years, and one will ask what the genome does in the cell or in the body. A promising strategy to image the change in large-scale of gene expression is the microarray or DNA chip and computational analysis. This review looks at the state of microarray and also raises the problems we have to face today.

Status of microarray assay and its limitations

Comprehensive knowledge of the whole genome sequence will make feasible the analysis of the expression of all genes in response to various physiological or pathological conditions. In mammals, the genome encodes ~100,000 genes. Analysis of the expression of all genes cannot be achieved by the methods available at present. Recent DNA technology allows us to handle hundreds of thousands of genes simultaneously by microarrays [3,4]. Microarrays could be easy to recognize by imaging a large scale (over thousands) northern or southern blot analysis. Thousands of cDNAs or synthetic oligonucleotides are fixed on small membranes, silica wafers or glass slides at high density for hybridization to either fluorescent or radiolabelled probes. The changes in gene activity can be detected by the intensity of the hybridization signals.

When using microarray to compare the expression of genes in several conditions, a considerable numbers of genes would be shown up as potential targets for individual medicine will develop and the patient will be treated by tailor-made therapy because the human genome variation such as SNPs will tell us the susceptibility to the disease and will result in the subclassification of the disease. In the last decade, we have been focusing on the structural analysis of the human genome, and our current question is what the physical structure of the genome. However, our main research interest is moving to the function of the genome as a post-genome project. The function of the gene will become a central issue in a few years, and one will ask what the genome does in the cell or in the body. A promising strategy to image the change in large-scale of gene expression is the microarray or DNA chip and computational analysis. This review looks at the state of microarray and also raises the problems we have to face today.
**Application of microarray to the study of nephrology**

The information concerning large-scale gene expression could be used not only to determine those genes that may play important roles in renal function, but also to identify genes related to the initial insult or the progression of renal diseases. New methods described above may be useful to monitor differential patterns of the expression of thousands of genes in the normal kidney vs various renal disease conditions, or developmental stages. When those techniques are used to analyse experimental models of genetically modified animals such as a mouse strain, genes would be...
organized according to their possible functions and expression patterns, without considering the genetic diversity found in the human. Accumulation of gene expression profiling data in various pathological conditions may illuminate the key transcripts for the diseases. Because most mouse genes are considered to have human counterparts, it may be easy to apply the results obtained from experimental model to human diseases. The mechanisms of the progression of kidney diseases have remained unresolved as a central issue in nephrology.

The massive proteinuria, which results in an intrinsic renal toxicity, provokes renal fibrosis, finally leading to the deterioration of kidney function [11]. To study the mechanisms of renal proteinuria, we investigated a mouse model of protein overload proteinuria by microarray assay. A schematic diagram of the microarray assay is shown in Figure 1. A total of 18,000 kinds of duplicated genes were analysed using mRNA isolated from kidneys on days 0, 7 and 21 after protein overload. More than 1600 genes were confirmed in the control kidney. The expression of 92 genes, including osteoponin, was increased >2-fold on day 7. The expression of >60 genes, e.g. NF-κB activator and fibroblast-inducible secreted protein, was increased on day 21. On the other hand, the expression of 35 genes, including KAP mRNA, was decreased to less than half of the control level. In conclusion, >10% of the transcripts identified in the kidney were changed under the condition of proteinuria. Microarray assay seems to be feasible to analyse large-scale gene expression in the kidney.

If there were unique transcripts or even clusters of gene expression related to kidney disease, the information might be utilized to understand the mechanisms of human kidney diseases, to design new drugs, to assess the possibility of genetic disorders and to predict the prognosis for each case. It may help to provide patients with the most suitable treatments. The therapeutic strategies could be worked out in a customized manner at a molecular level. To achieve these final goals, more comprehensive information should be collected systematically, e.g. the construction of an array database [12] concerning renal diseases.

Conclusions

The progress of genome projects and the rapid expansion of databases for genetic bioinformatics will open up a new research field, along with innovative new technologies, which will allow us to analyse large-scale gene expression. We may see a new fundamental movement of cells by analysis of dynamic changes of clusters of genes, which may not be recognized by the observation of change of expression of a single gene.

References