Review

Application of DNA Microarrays in Occupational Health Research

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Abstract: Application of DNA Microarrays in Occupational Health Research: Shinji Koizumi, Department of Hazard Assessment, National Institute of Industrial Health—The profiling of gene expression patterns with DNA microarrays is recently being widely used not only in basic molecular biological studies but also in the practical fields. In clinical application, for example, this technique is expected to be quite useful in making a correct diagnosis. In the pharmacological area, the microarray analysis can be applied to drug discovery and individualized drug treatment. Although not so popular as these examples, DNA microarrays could also be a powerful tool in studies relevant to occupational health. This review will describe the outline of gene expression profiling with DNA microarrays and prospects in occupational health research.

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During the 50 yr since the discovery of the DNA double helix, human beings have made an enormous effort to develop biotechnology. This contributed much to the elucidation of a great number of new biological processes useful for explaining the mystery of life, and also to the welfare of people, in particular, through its practical use in medicine. On the other hand, the use of biotechnology appears to be still limited in research relevant to occupational health. It might be significant to consider how this great intellectual property can be applied in this area. The DNA microarray analysis described hereafter is one of the most promising tools in current biotechnology. This review is intended to introduce the outline of gene expression profiling with DNA microarrays and prospects in occupational health research.

Outline of DNA microarray analysis

In 2000, the draft of the human genome was presented. However, it remained to be solved when and where in the body the respective gene products are synthesized, and how they function. In this sense, the word “post-genome research” has become frequently heard these days. DNA microarray analysis is one of the most powerful tools to support studies of this kind. “Gene expression” means that messenger RNA (mRNA) molecules that are the complementary copies of DNA containing the genetic codes are synthesized, followed by the synthesis of functional protein molecules with the mRNAs as templates. The expression of genes is precisely controlled temporally and spatially in an organism. For example, when a human body is exposed to a toxic chemical, a number of genes are activated to produce proteins necessary to reduce its toxicity or recover from the damage caused by it, but once the toxicant is removed from the environment, gene expression no longer required is immediately switched off. In order to study the functions and regulatory mechanisms of genes, several methods to estimate the levels of primary gene products, mRNAs, have been developed. Northern blotting is a representative method that determines the level of a specific mRNA the nucleotide sequence of which is known. However, it has been considered almost impossible to study the expression of all the genes, the number of which is estimated to be 40,000 to 50,000, by this kind of method. As compared with such a conventional technique, the DNA microarray analysis is an epoch-making one that can analyze the expression of tens of thousands of genes in a single experiment.

The term “microarray” originally stood for an array of a number of cloned DNA molecules fixed on a slide glass. A similar term “DNA chip” was used to mean an array of short DNA oligomers directly synthesized on a slide by the photolithographic technology. Recently, however, the microarray also is often referred to by this name. The high density of the fixed DNA spots made economical and high-throughput screening possible.
DNA microarrays carrying more than 20,000 spots of DNA probes are now commercially available, and the expression profile of such a number of genes can be monitored in a single analysis. For example, by analyzing a pair of tumor and corresponding normal cell species, thousands of genes can be screened simultaneously for tumor-specific changes in gene expression. Although there are variations in the method of microarray analysis, a typical procedure (for the comparison of gene expression profiles of two biological samples by dual fluorescent labeling) is shown in Figure. The major steps are as follows.

(i) Extraction of mRNA from a pair of biological samples of interest (cultured cells, experimental animals, human samples, etc.)

(ii) Enzymatic synthesis of two sets of complementary DNAs (cDNAs) in vitro and simultaneous labeling with two different fluorescent dyes, respectively

(iii) Competitive hybridization of the mixture of the two sets of labeled cDNAs with the DNA probes fixed on a microarray

(iv) Measurement of the fluorescence on each DNA spot for the two dyes

(v) Estimation of changes in respective mRNA levels based on the ratio of the two fluorescence dyes on each DNA spot

To prepare spotting-type microarrays, an automatic spotter\(^{10}\) is required. By introducing this apparatus, custom-made microarrays can be produced in a laboratory, but it usually costs much. It is also required to prepare a number of DNA samples to be spotted, which needs a work force. This step can be skipped by utilizing commercially available microarrays (e.g. from Affymetrix, Inc., Agilent, Clonetech, Superarray, Inc. etc.\(^{11}\)). Those ready-made arrays are being improved in variety and quality, and are quite useful only if they match the purpose of research. After hybridization, fluorescence associated with each spot is measured by a fluorescence image analyzer. The analysis step can also be carried out by contract with one of the companies dealing with the microarray products. For further information about the DNA microarray technique, refer to recent reviews\(^{7,10-12}\) and the homepages of the manufacturers.

Studies with DNA microarrays

According to a literature search by PubMed, about 3,700 papers were found to meet the keyword “microarray” in June, 2003. The Table shows the numbers of corresponding papers published each year since 1996. It is obvious that the use of microarrays has rapidly increased year by year. In 2003, more than 1,000 articles were published within half a year. With regard to basic research, a variety of studies were carried out, for example, on the mechanisms of diseases including cancers and on the gene expression associated with biological events such as inflammation.

**Figure.** A typical procedure of gene expression profiling by DNA microarray. In this example, the gene expression profiles are compared in two cultured cell samples (unexposed control cells and cells exposed to a chemical), using corresponding complementary DNAs (cDNAs) labeled with red and green fluorescent dyes, respectively.

**Table.** The number of reports on microarray

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*From January 1 to June 30
as differentiation and aging. In addition to these, practical researches are also increasing. In the clinical area, DNA microarrays are useful in identifying gene expression patterns for disease type classification, appropriate prognosis markers, and genes relevant to disease susceptibility. These studies would provide valuable information for correct diagnosis and therapy\textsuperscript{13-15}. In the pharmacological area, the DNA microarray is a promising tool for individualized drug administration with maximal effects and minimal side effects\textsuperscript{16}.

On the other hand, DNA microarray analysis has not been so popular in the area of occupational or environmental health. For example, out of the 1,020 papers on microarray published in the first half of 2003, the effects of chemicals were taken up only in 19 articles (for reference, the number of reports relevant to cancer was about 250). Some of these papers concerned the biological effects of environmental pollutants and occupational hazardous materials, including bisphenol A\textsuperscript{17}, arsenic\textsuperscript{17, 18}, nickel\textsuperscript{19} and dichloroacetic acid\textsuperscript{20}, providing several suggestive findings. At present, the use of DNA microarrays in this area is still limited, and we should wait for a while before this technology proves its merit.

Application of DNA microarrays in research relevant to occupational health

DNA microarrays could be applied in the following aspects relevant to the occupational health.

\textit{(a) Understanding the mechanistic background of health effects}

In order to take the most appropriate measures against occupational hazardous factors including chemicals and physical agents, it is essential to understand the mechanistic background of their health effects. Comprehensive searches of the changes in gene expression after exposure to these factors would provide keys to the molecular mechanisms of their biological action. For example, an analysis of a pair of organs derived from mice unexposed and exposed to a chemical would identify its target genes, which help us understand the mechanisms of toxicity expression and protective responses to it. Such information also could lead to correct risk assessment, for example, through the development of appropriate effect indicators as described below.

\textit{(b) Toxicity testing}

The development of toxicity tests with low cost and easy handling is required to improve efficiency in the management of industrial chemicals. Target gene search of chemicals by DNA microarray analysis can contribute to this purpose. By identifying an appropriate gene(s) indicative of a specific health effect, more convenient and less expensive tests replaceable with conventional animal testing could be established. If sufficient such data are accumulated, even unknown health effects of a new chemical will be correctly predicted from its effect on the gene expression profile. DNA microarrays might also be useful in the extrapolation from laboratory animals to humans: the inter-species difference in susceptibility to a certain chemical could possibly be predicted from the difference in gene expression patterns.

\textit{(c) Search of indicators for hazard prevention and health management}

DNA microarrays are also useful in searching for the novel biological indicators of exposure. An appropriate change (or a set of changes) in human gene expression caused by exposure to a hazardous factor might serve as an indicator for monitoring the effect of exposure. Such indicators reflect the physiological state of the human body as a consequence of toxicant exposure, and could be used in biological monitoring for hazard prevention and health management. In practical use, however, it should be considered that the collection of biological samples does not impose a severe burden on the subjects. That is, it is favorable that the sample is easy to collect and the required amount is small. It has been reported that the expression of the metallothionein gene in peripheral blood lymphocytes could serve as an effect indicator of heavy metal exposure\textsuperscript{21}. Comprehensive screening with DNA microarrays would provide more useful indicators. The accuracy of the bioassay would be increased by using a set of multiple genes as the indicator: even unknown sources of exposure may possibly be specified from the gene expression patterns.

\textit{(d) Managing high-risk populations}

Individualized health management will be an important subject of occupational health in future, since different genetic backgrounds generate variation in susceptibility to occupational hazardous factors. DNA microarray analysis also helps in the search for susceptibility indicators. For example, resistance to chemicals is expected to depend on the genes relevant to their absorption, distribution, metabolism and excretion, and recovery from damage caused by them. The polymorphism in these genes could possibly result in differences in susceptibility. Since many such genes are inducible, microarray screening of gene expression induced by exposure would facilitate identifying the susceptibility indicators. Once the polymorphism of a specific gene is correlated with susceptibility to a chemical, microarrays with synthetic oligonucleotide probes could serve as a powerful genotyping tool to detect a high-risk population.

It should be noted that the outcome of DNA microarray studies can make the mechanism-based control of health
hazards possible. Once a new relationship between a chemical and its target gene has been noticed, direct analysis of the signaling pathway lying between them becomes possible, which leads to an understanding of the regulatory mechanism. Accumulation of such data will develop into a picture of the regulatory network among a number of hazardous factors and their target genes. In addition, the identification of target genes also provides clues for inquiring how gene expression is connected with terminal health effects. These data will logically predict a variety of practically useful biological factors, for example, co-existing factors stimulating or inhibiting health effects, targets for efficient blocking of toxicity with minimal side effects, targets for enhancing protective functions, and so on. Such mechanistic information would give important suggestions in the application of microarray results described in (b)–(d) above, as well as in diagnosis and therapy.

Considerations

Although one tends to expect much of this state-of-the-art technology, a few points should be noted for practical use.

(a) Limitation to the genes to be analyzed

It is often stated that the DNA microarray makes a comprehensive screening possible, but it should be noted that even an array with a maximal number of probes cannot cover all the mRNA population, since the collection of gene probes has not yet been completed. This represents a characteristic of methods that uses probes with specified sequences, essentially distinct from the techniques that can detect unspecified changes in gene expression such as differential display and subtraction cloning. This problem is being relieved as the library of probe DNAs is improved.

(b) Cost of analysis

It seems ideal if one can prepare DNA microarrays containing only the genes of interest, but it usually requires financial resources and/or a work force. A more practical choice is to look for ready-made arrays that match the purpose of the research. Even in this case, cost may still be a problem. For example, in screening thousands of genes to detect the target genes of a certain chemical, detailed dose response and time course experiments are often not allowed due to high cost. Also, repeated experiments for confirming reproducibility are often restricted to a minimum for the same reason. To obtain the best results under such limitations, experiments should be carefully designed, and the quality of the biological materials must be strictly controlled.

(c) Reliability

In DNA microarray experiments, false positives are often generated. One of the reasons for this might be the cross hybridization due to sequence similarities. Unequal melting temperatures also affect signal intensity and cross reactions. It is therefore risky to draw conclusions from a single microarray analysis. It is desirable that the genes of interest, obtained by the primary microarray screening, are inspected for their altered expression by more quantitative methods such as northern blotting and real time polymerase chain reaction.

(d) Practical use

It is impatient to expect that an altered gene expression detected by microarray screening can directly be applied for a practical use, for example, as an effect indicator of exposure. Further careful inspection is essential before concluding the suitability for applications. Specificity of the altered expression and factors affecting the response are important points to be considered. The information about the mechanism of induction or repression is of great help in this process, and improves the quality of applications.

Although the use of DNA microarrays in the field of occupational health is still limited, it is hoped that this technology will come into wider use, which may be facilitated by the understanding of its potential by researchers in this area and improvement of the cost problem. The DNA microarray technology and related biotechnology are becoming more and more popular these days, and it might be of significance to consider further how they can be introduced into, and efficiently applied in occupational health research.

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References


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