Analysis Of Gene Expression Pattern In Human Cancer Using DNA Microarrays

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The development and progression of cancer and the experimental reversal of tumorigenicity are companied by complex changes in the pattern of gene expression.[1]

DNA micro-array technology is used to profile complex disease and discover novel disease–related genes. This technique has been successfully used to investigate gene expression in processes as complex as inflammatory disease, tumor suppression and to identify heat shock in human T cell.[2] DNA micro-arrays or DNA-chip technology allows expression monitoring of hundreds and thousands of genes simultaneously and provide a format for identifying genes as well as changes in their activity. The DNA micro-array helps us study genome-wide expression patterns in complex biological systems. These tools have shown great promise in finding the meaning of complex diseases such as cancer. [3]

There is some interest in the potential application of DNA micro-array analysis for gene expression profiling in human cancers. Micro-arrays of DNA provide a powerful tool for studying the development and progression of cancer phenomena. It might be useful for tumor classification, for the elucidation of regulatory networks that are disturbed in tumor cells and for the identification of genes that might be of use for diagnostic purposes or as therapeutic targets. [4]

HYBRIDIZATION TO DNA MICRO-ARRAYS

The Southern blot was the first array. It was only a small step to filter-based screening of clone libraries, which introduces a one correspondence between clones and hybridization signals. In Southern blotting, DNA fragments are produced by digestion with restriction endonucleases. The fragments produced by the cleavage are separated by gel electrophoresis. The DNA is then denatured in order to separate the strands and transferred onto a nitrocellulose filter. The DNA is fixed to the membrane and is ready for hybridization. Specific DNA sequences can be identified with radio-labeled sequences (probes), either DNA or RNA. [5]

The next advantage was the use of libraries, stored in micro-titer plates and stamped onto filters in a fixed position, and each clone could be uniquely identified and information about it accumulated.

One high throughput method by which to gain information about gene function is the griddled DNA microarray, in which microscope slides containing hundreds to thousands of immobilized DNA samples are hybridized in a manner very similar to the southern blot. [6]

DNA micro-arrays provide a simple and natural vehicle to explore the genome in a way that is both systematic and comprehensive. The power and universality of DNA micro-arrays as experimental tools are derived from the exquisite specificity and affinity of complementary base pairing. [7] The method used in Brown's labs is simple to describe. Briefly, arrays of thousands of discrete DNA sequences are printed on glass microscope slides using a robotic arrayer. To compare the relative abundance of each of these gene sequences in two DNA or RNA samples, the two samples are first labeled using different fluorescent dyes. They are then mixed and hybridized with the arrayed DNA spots. After hybridization, fluorescence measurements are made with a microscope that illuminates each DNA spot and measures fluorescence for each dye separately: these measurements are used to determine the ratio and in turn the relative abundance sequence of each specific gene in the two mRNA or DNA samples.

The explosion of interest in array technologies was sparked by two key innovations. The first was the use of non-porous solid supports, such as glass, which facilitated miniaturization and fluorescence-based detection. The second was the development of methods for high-density spatial synthesis of oligonucleotides. [8] The advantage of glass slides is that DNA samples can be co-

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valently attached onto a treated glass surface. Secondly, glass is durable material that sustains high temperatures and washes of high ionic strength. Thirdly, it is non-porous, so the hybridization volume can be kept to a minimum, thus enhancing the kinetics of annealing probes to targets. Fourth, as consequence of its low fluorescence, it does not significantly contribute to background noise. [6]

MOLECULAR BASIS OF CANCER

Cancer is not one disease but many. It has also become clear in the past several decades that any particular cancer arises as a result of the gradual accumulation of many different types of genetic changes in a single cell. Whereas no single genetic change may be sufficient, the number of accumulated changes required to alter the behavior of a normal cell to a cancer cell is still not clear. [9] Naturally, occurring cancer is most often a consequence of multiple factors that interact over long periods of time.

To become a cancer cell, a normal cell undergoes many significant changes. It continuously multiplies when normal cells would be quiescent; it invades surrounding tissues, often breaking through the basal laminas that define the boundaries of the tissue. Cancer cells are usually closer in their properties to immature normal cells than to more mature cell types. A normal cell can be transformed into a malignant cell by the expression of one or a few oncogenes. Most oncogenes are derived from growth-controlling genes that encode one of five types of protein: growth factors, receptors, intracellular signal transducers, nuclear transcription factors and cell cycle control proteins.

Oncogenes often act collaboratively, no one oncogene being sufficient to induce malignant growth. Oncogenes are formed in cells or carried into cells by transforming agents, viruses, chemical carcinogens, and radiation. All of this evidence support the concept that carcinogenesis is multi-causal and multi-step. The search for cancer genes defines the central challenge in cancer biology.

MICROARRAYS AND HUMAN CANCER

Gene expression micro-arrays hold great promise for studies of human cancer. The high-throughput mRNA analysis platforms such as micro-arrays are capable of producing large gene expression data sets with the potential to provide novel insight into fundamental cancer biology at the molecular level.[10] The quantitative measurement of gene expression profiles from specific cell populations offer viewing of in *vivo* molecular anatomy cells and their diseased counterparts. There is the specific collection of genetic changes and the pattern of altered gene product expression and function that provides the actual definition of any cancer in the context of, but not strictly dependent upon, its site of origin. Mitelman and colleagues studied cytogenetic data leading to the logical conclusion that a large number of genes contribute to the multi-stage process of cancer development, many of which have not yet been discovered. [11]

The applications of these microchips generally fall into one of two classes: diagnostics and genomics. More specific diagnostic applications are already in production by Affymerix, a biotechnology company. Using this chip, Hacia et al designed high-density arrays consisting of over 96.600 oligonucleotides 20-nucleotides (nt) in length to screen for a wide range of heterozygous mutations in the 3.45-kilobases (kb) exon 11 of the hereditary breast and ovarian cancer gene BRCA1.[12] This study showed that heterozygous carriers for a mutation can still be detected despite the presence of the wild-type gene.

DeRisi et al used DNA chips for studying gene expression in cancer. They spotted DNAs, rather than oligonucleotides, onto chips to analyze expression changes in human melanoma cell line following suppression of the tumorigenic phenotype.[13] In this experiment, they used a high density microarray of 1.161 DNA elements to search for the difference in gene expression associated with tumor suppressions in this system. Fluorescent probes for hybridization were derived from two sources of cellular mRNA, which were labeled with different fluors to provide a direct and internally-controlled comparison of the mRNAs corresponding to each gene.[13] This experiment produced a wide of the diverse systems that altered in this model system of tumorigenicity and focused attention on specific gene products and pathway that may be of particular importance in this tumor type.

Beside for diagnostic purposes, micro-arrays have also been used to identify gene expression patterns that predict response to therapy. Rosenwald et al have developed to predict the likelihood of survival after chemotherapy for diffuse large B-cell lymphoma that is based on patterns of gene expression in the biopsy specimen of lymphoma. [14] The outcome of their research may help identify patients with diffuse large B cell who are unlikely to be cured by conventional therapy.

From information above, we know that gene expression micro-arrays hold great promise for studies of human cancer. DNA micro-arrays have allowed investi-

gators to develop expression-based classifications for many types of cancer, including breast, brain, ovary, lung, colon, kidney, prostate, gastric, leukemia, and lymphoma.

CONCLUSION

There is a great deal of work to be done to keep cancer biologist busy. Knowledge gained from microarray studies of human neoplasia will be invaluable in furthering understanding of the molecular events that underlie tumor development. All of it will provide new diagnostic, prognostic, and therapeutic targets for the benefit of patients.

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ERRATA

In the review article of our previous edition (Askandar Tjokroprawiro, Obesity and Insulin Resistance: Molecular Basis for Clinical Appraisal, Acta Medica Indonesiana 2002; XXXIV: 147), there was several mistakes in the Institution name.

Division of Metabolic-Endocrine, Department of Internal Medicine, Faculty of Medicine of The University of North Sumatera, Dr. Adam Malik Hospital, Medan, North Sumatera, Indonesia

The corrected word was supposed to be

Diabetes and Nutrition Center, Department of Internal Medicine, Faculty of Medicine of The University of Airlangga, Dr. Soetomo, Surabaya, Indonesia

The other mistake was sentences in the review article (Ika P Wijaya, Djumhana Atmakusuma, Karmel LT. Protein C, Protein S, and Anti-Thrombine III as Natural Anticoagulant, Acta Medica Indonesiana 2002; XXXIV: 155).

Inner vein thrombosis

The corrected word was supposed to be

Deep vein thrombosis