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APPLICATION OF DNA MICROARRAY TECHNOLOGY FOR ANALYSIS OF GENE EXPRESSION IN ANIMALS: AN OVERVIEW

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Abstract

DNA microarray technology is a powerful tool for quantifying gene expression in animals. It works by allowing researchers to simultaneously measure the expression levels of thousands of genes in a single experiment. This technology is based on the hybridization of labelled cDNA or RNA to a microarray, which is a small glass slide or a chip that contains thousands of DNA probes. Some applications of DNA microarray technology for the quantification of gene expression in animals are: gene expression profiling - DNA microarrays allow researchers to profile the expression levels of thousands of genes simultaneously, this technology being widely used to study gene expression in a wide range of organisms, including humans, mice, rats, and other animals; disease diagnosis - DNA microarrays can be used to diagnose diseases based on changes in gene expression patterns; drug discovery, etc. This paper present newest and the most important studies of specific literature regard on DNA microarray technique. The aim of this review is to show that the DNA microarray technology is a powerful tool for quantifying gene expression in animals.

Introduction

Microarray technology has allowed the identification of over- and under-expressed gene sets in various pathologies: breast cancer, prostate cancer, lung cancer, as well as in the deregulation of certain physiological processes: induction of apoptosis and response to therapy. The integrated analyzes of several studies have highlighted generalities and particularities of gene expression in certain pathologies.

The use of microarrays, in biomedical research, is not only limited to determining the gene expression profile, being also used to detect CNV-type changes (DNA or chromosomal copy number variation) at the level of the entire genome, with a high resolution, up to at a level of 5-10 kilobases and even up to 200 bp resolution in the case of high-resolution array CGH (HR-CGH) variants. The DNA microarray technique is a multiplex technology used in molecular biology and medicine studies. It developed from the ***Southern Blotting*** method of analysis – DNA fragments are attached to a substrate and hybridized with labeled probes representing gene fragments or whole genes. Recently discovered DNA "microarrays" allow researchers to analyze the expression of several genes, quickly and efficiently in a single experiment. They represent a major step in DNA analysis methodology and illustrate how new technologies provide "powerful tools" for research.

Material and method

This study was carried out by consulting a number of 20 bibliographic sources from the specialized literature, both from the country and abroad (scientific works, books and book chapters from national and international databases - *Web of Science, Science Direct, Scopus , Google Scholar, Enformation, CabiDirect*, etc. Statistical databases, national statistical institutes in the milk industry - Food and Agriculture Organization, National Institute of Statistics, Europe-EU, etc., were also consulted.

Results and discussions

There are three types of samples that can be used to make chips: two are genomic and the third is "transcriptomic" (measures mRNA levels). They differ in the type of DNA fixed in the spots.

Chips for the detection of changes in the level of gene expression, also called gene expression analysis chips (microarray expression analysis chip or simply expression chip). The spots of these chips contain cDNA obtained by mRNA reverse transcription of some known normal or mutant genes. The cDNA will hybridize at that point compared to the control, with fluorescence directed toward the red. Gene expression chips can be used in: *diagnosis of genetic diseases; identification of mutations of some genes involved in multifactorial diseases (especially the cell cycle control genes involved in the proliferation of neoplastic cells); the development of new drugs (Nguyen D. V., 2002).*

Comparative genomic hybridization chips to identify gene amplifications and deletions in the genome or to observe changes in the number of copies of a gene involved in the genesis of a certain disease (especially cancers). These chips target large portions of genomic DNA; for each target DNA spot of the chip, the chromosomal location must be known. The hybridization mixture will contain fluorescently labeled genomic DNA samples collected both from the normal tissue and from the one to be researched. As a result, if the number of copies of the investigated gene has increased, a larger amount of DNA extracted from the tissue to be investigated will hybridize with the target spots compared to a smaller amount of control ***DNA and mutation/polymorphism analysis chips*** to detect single gene mutations or single nucleotide polymorphisms (SNP) *(FloaresA. et. al., 2010)* (Figure 1).

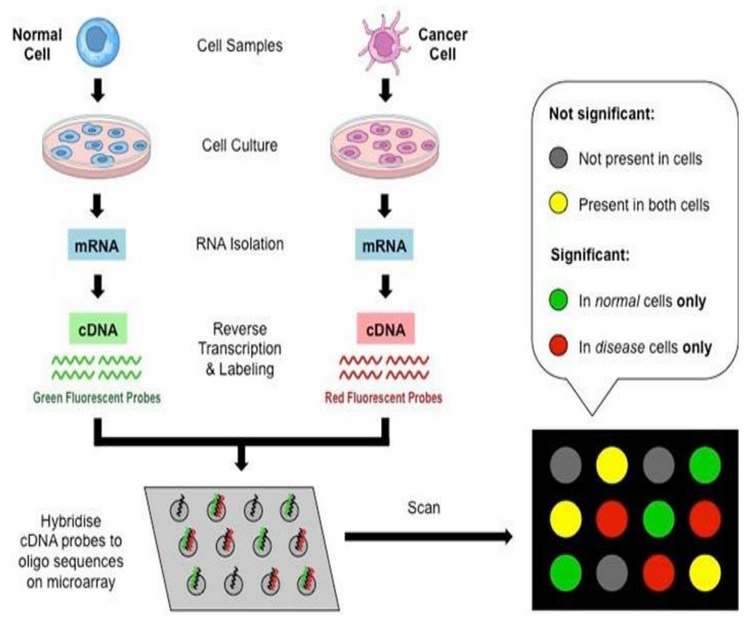

Figure 1. Experimental protocol for DNA analysis using the microarray technique *(https://www.onlinebiologynotes.com/dna-microarray-principle-types-and-steps-involved-in-cdna-microarrays/)*

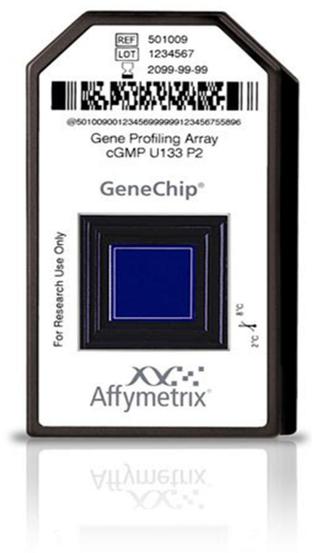

Figure 2. Affymetrix GeneChip *(https://www.thermofisher.com)*

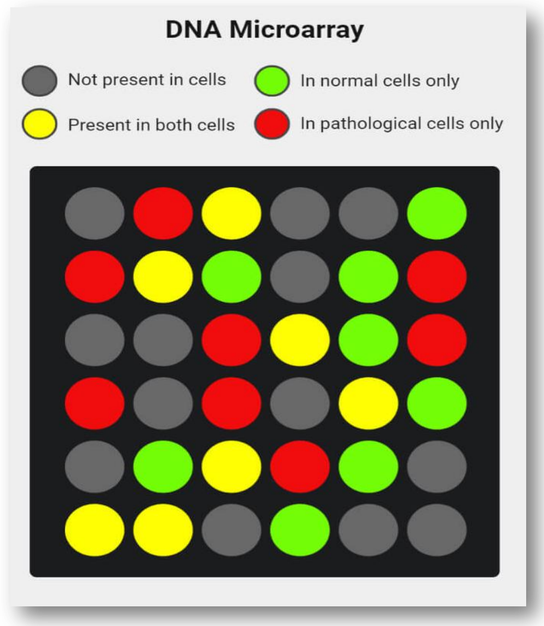

Figure 3. DNA Microarray *(https://www.onlinebiologynotes.com/dna-microarray-principle-types-and-steps-involved-in-cdna-microarrays/)*

Scheme of a microarray experiment involves hybridization step with 2 cDNA samples to be compared (diseased tissue/healthy tissue samples; treated cells/untreated cells) labeled with two different fluorophores. Fluorescent markers: Cy3, with emission wavelength 570 nm (corresponding to the green light spectrum) and Cy5 with emission wavelength 670 nm (corresponding to the red light spectrum). The two types of samples - Cy-labelled cDNA are mixed and hybridized on the same microarray chip that will be scanned to analyze the fluorescent signal intensity of the two fluorochromes. The relative intensities of the fluorescent signal of the two markers can be analyzed as a ratio in order to identify genes with stimulated or inhibited expression - up-regulated or down-regulated *(Plous C.V., 2007).*

Affymetrix is one of the first companies to produce microarrays, developing technology and synthesis based on combinatorial chemistry. These methods have been applied to construct high-density arrays of oligonucleotides on glass or silicon substrates (chips) (Figure 1, Figure 2). Each spot on the chip represents a specific gene; each color represents the DNA extracted from the healthy tissue (the control) or the DNA sample extracted from the tissue to be investigated (the sample). Depending on the type of chip used, the location and intensity of each color specifies the expression level (presence/absence) of a gene (or its mutant) in the DNA samples (Wiltgen M., 2007). (Figure 3).

Conclusions

Currently, microarrays are considered very powerful tools in the medical field. This new technology enables disease diagnosis and a better understanding of how gene expression is altered in various medical conditions.

Moreover, it allows the comparison of a control tissue and a tissue treated with a certain drug, to study the effects of a possible medical treatment. To do this, the normal state and the diseased state are compared before and after the administration of the drug.

It can also determine why certain drugs lead to unwanted side effects.