DNA MICROARRAY TECHNOLOGY IN DERMATOLOGY

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Rabab Abdel Hamid

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LIST OF ABBREVIATIONS

AA	:	Alopecia areata
AAP	:	Alopecia areata persistant type
AAT	:	Alopecia areata Totalis
ACD	:	Allergic contact dermatitis
AD	:	Atopic dermatitis
AK	:	Actinic keratosis
AU	:	Alopecia areata universalis
BCC	:	Basal cell carcnioma
BM	:	Betamethasone valeratec
cDNA	:	Complementary Deoxyribonucleic acid
Chd	:	Coronary heart disease
COMP	:	Cartilage oligomeric matrix protein
cRNA	:	Complementary RNA
cSCC	:	Cutaneous squamous cell carcinoma
CSD	:	Common significantly dysregulated
Cy3	:	Cyanine 3
Cy5	:	Cyanine 5
DEGs	:	Differentially expressed genes
DMR	:	Differentially methylated regions
DNA	:	Deoxyribonucleic acid
ECM	:	Extracellular matrix
eSNP	:	Single-nucleotide polymorphisms
EVMM	:	Extravascular migratory metastasis
FLG	:	Filaggrin
GEO	:	Gene Expression Omnibus
GO	:	Gene ontology
GWAS	:	Genome-wide association studies
HLA	:	Human leukocyte antigen
HTS	:	Hypertrophic scars
ICD	:	Irritant contact dermatitis
ID	:	Identity document
IL	:	Interleukin

IV	:	Ichthyosis vulgaris
KD	:	Keloid
KGF	:	Keratinocyte growth factor
KGFR	:	Keratinocyte growth factor receptor
MeDIP-Seq	:	Methylated DNA immunoprecipitation sequencing
MetS	:	Metabolic syndrome
miRNA	:	Micro Ribonucleic acid
MM	:	Multiple myeloma
mRNA	:	Messenger Ribonucleic acid
NFs	:	Normal Fibroblasts
NK	:	Natural Killer
NKG2D	:	Natural killer cell receptor
NKGD	:	Natural killer cell receptor
NM	:	Nodular melanoma
PCR	:	Polymerase chain reaction
RA	:	Rhuamtoid arthritis
RNA	:	Ribonucleic acid
SCC	:	Squamous cell carcnioma
SLE	:	Systemic lupus erythematosis
SNP	:	Single nucleotide polymorphism
SSc	:	Systemic sclerosis
SSM	:	Superficial spreading melanoma
TNF	:	Tumour necrosis factor
UV	:	Ultraviolet

INTRODUCTION

Microarray analysis allows scientists to understand the molecular mechanisms underlying normal and dysfunctional biological processes. It has provided scientists with a tool to investigate the structure and activity of genes on a wide scale. Microarray technology could speed up the screening of thousands of DNA samples simultaneously. DNA microarrays have been used for clinical diagnosis and for studying complex phenomena of gene expression patterns. ***(Singh and Kumar 2013).*** Present review article focus on history, technique and applications of DNA microarray technology.

DNA microarray is a technology that has revolutionized the functional genomics with a wide array of applications. It is an arrangement of a large number of known genes or corresponding cDNA probes, for a given physiological condition of any living being; that are placed precisely as dots on a glass slide or chip or coated on beads. It works on the principle of Southern hybridization wherein DNA is hybridized with DNA to confirm the expression of a gene. The only difference is, in microarrays probesare placed on solid surface and test DNA is in the hybridization solution, which is just opposite to Southern hybridization where DNA to be diagnosed is placed on nylon or nitrocellulose membrane and probe is in the hybridization solution. During hybridization, fluorescent dyes attached to probes produce emissions of specific color based on complete, partial and no binding of DNA to the probes. After hybridization these emissions can be observed and recorded under a confocal laser microscope and further analyzed with the help of image analysis software to understand set of genes up or down regulated in the test DNA and to determine fluorescence intensities that allow the quantitative comparison between the two test DNAs for all genes on the array. This technique is useful in gene expression profiling, comparative genomic hybridization, checking Gene ID, Single Nucleotide Polymorphism (SNP) detection, alternative splicing detection, fusion gene detection and genome tilling to empirically detect expression of transcripts, or alternative splice forms. It has been widely applied in studies related to cancer biology, microbiology, plant science, environmental science, etc. ***(Ravi et al. 2014)***

AIM OF THE ESSAY

The aim of this essay is to review the updates on the microarray techniques and its application in dermatological diseases and conditions.

History

The idea of performing chemical or biological reactions with one reagent spatially immobilized is not new. Southern's 1975 paper described a technique that needs no introduction, and forever altered molecular biology. ***(Southern ,1975).*** DNA arrays are logical extension of the method described by Gillespie and Spiegelman in 1965, in which DNA immobilized on a membrane can bind a complementary RNA or DNA strand through specific hybridization and the methods described for applying DNA to a treated cellulose surface ***(Southern and Mitchell ,1975)*** and DNA blotting hybridization ***(Southern, 1975).*** Several publications from the 1980s describe the use of such arrays in DNA mapping and sequencing ***(Bains and Smith , 1988),(Khrapko et al.,1989).*** Scientists at the California-based biotech company Affymax produce the first DNA chips ***(Fodor , 1991).*** Miniaturized microarrays for gene expression profiling was used in 1995 ***(Schena et al., 1995)*** A complete eukaryotic genome (Saccharomyces cerevisiae) ona microarray was published in 1997 ***(Lashkari et al., 1997).*** A DNA microarray which is used to follow changes in gene expression as Deinococcus radiodurans recovers from a sub-lethal dose (3000Gy) of ionizing radiation was constructed in 2002 ***(Battista , 2002).***

Principle

DNA Microarrays are based on the principle of Southern blotting where fragmented DNA is attached to a solid substrate like nylon or nitrocellulose membrane and then hybridized with the chemiluminiscence or radioactively labelled probe prepared from a known gene or DNA fragment under stringent conditions. The hybridization between two DNA strands takes place by the property of complementary nucleic acid sequences to specifically pair with each other by forming hydrogen bonds between complementary nucleotide base pairs. A high number of complementary base pairs in a nucleotide sequence results in tighter non covalent bonds between the two strands. After washing-off of nonspecific bonding sequences only strongly paired strands remain hybridized. In DNA microarrays, this situation is slightly altered wherein, thousands of non-labelled probes are blotted on the solid surface and these chips are subjected to hybridization with the fluorescently labelled target sequence, i.e., sample DNA (Fig.1). On binding of fluorescently labelled target sequence to a probe sequence, a signal is generated that depends on the strength of the hybridization determined by the number of paired bases, the hybridization conditions (such as temperature), and washing after hybridization. Total strength of the signal, from a spot (called as feature), depends upon the amount of target sample binding to the probes present on that spot. Microarrays use relative quantification in which the intensity of a feature is compared to the intensity of the same feature under a different condition, and the identity of the feature is known by its position. ***(Singh and Kumar 2013)***

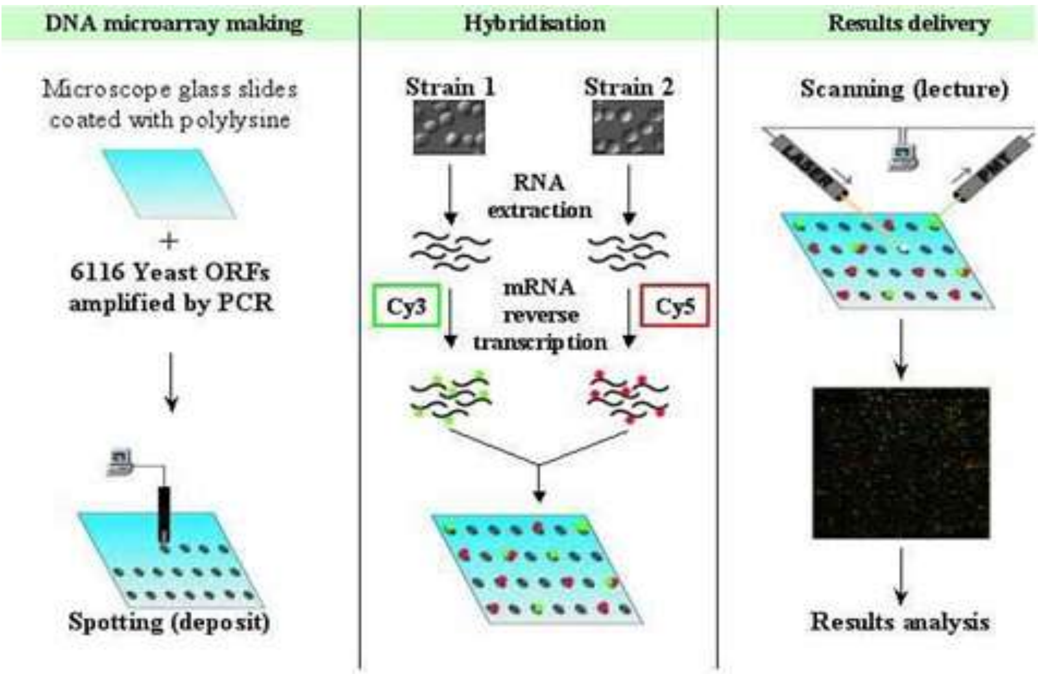

Fig. (1): Microarray Principle

(http://www.transcriptome.ens.fr/sgdb/presentation/principle.php)

Dna Microarray Technique

The major steps of a microarray technology are:

- Preparation of microarray
- Preparation of probes (biological sample)
- Hybridization of the probes with the array
- and finally, scanning, imaging and data analysis.

(Esteve-Nunez et al., 2001), (Labana et al., 2005)

1 .Preparation of Microarray:

DNA Microarray, is a collection of DNA probes that are arrayed on a solid support and are used to assay, through hybridization in the presence of complementary DNA that is present in a sample ***(Marmur and Doty , 1961).*** DNA Microarray is a chip of size of fingernail having 96 or more tiny wells and each well has thousands of DNA probes or oligonucleotides arranged in a grid pattern on the chip ***(Sundberg et al., 2001),(Afshari , 2002).*** Thousands of different genes are immobilized at fixed locations on chip and it means that a single DNA chip can provide information about thousands of genes simultaneously by base pairing and hybridization. There are two types of DNA microarray: cDNA microarrays and oligonucleotide arrays. ***(Ponder , 2001) (Druker***

et al., 2001)

cDNA arrays are produced by printing a double stranded cDNA ona solid support (glass or nylon) using robotic pins.Oligonucleotide arrays are made by synthesizing specific oligonucleotides in a specific alignment on a solid surface using photolithography. ***(Gray and Collins, 2000)***

2. Preparation of Probes (Biological Sample):

Two types of probes are currently available for DNA microarrays as suggested in Figure 2.

Complementary DNA microarrays: in which one cDNA microarrays labels *two RNA samples*, e.g.; healthy vs. diseased, with fluorescent dyes of different colors, usually red and green. The two labeled RNAs are mixed and hybridized to the microarray; the microarray washed and scanned using red and green wavelengths separately. This allows one to calculate the ratios of the two RNAs hybridized to a given spot, i.e.; relative ratios of RNA amounts in the healthy vs. diseased sample. ***(Schena et al., 1996)*** A skin-specific cDNA array, named DermArray, containing >4000 gene probes is commercially available. ***(Curto et al., 2002)***

Oligonucleotides microarrays: In which *a single RNA sample* is hybridized to each oligonucleotide microarray, which means 2 oligonucleotide microarrays are necessary for a