DNA microarrays and their use in dermatology

V. Mlakar and D. Glavač

SUMMARY

Multiple different DNA microarray technologies are available on the market today. They can be used for studying either DNA or RNA with the purpose of identifying and explaining the role of genes involved in different processes. This paper reviews different DNA microarray platforms available for such studies and their usage in cases of malignant melanomas, psoriasis, and exposure of keratinocytes and melanocytes to UV illumination.

KEY WORDS

DNA microarrays, malignant melanoma, psoriasis, keratinocytes, melanocytes

Introduction

The latest microarray technology is a result of rapid development in the 1990s and early 2000s. Although the foundation for microarray technology was established much earlier in 1975, when the first hybridization between nucleic acids was performed (1), it took researchers another 20 years to develop microarrays, due to underdeveloped computer, robotic and DNA technologies.

Several platforms are used for microarray experiments. All platforms use hybridization between probe nucleic acids bound to solid support (microarray) and labeled target nucleic acids in sample (Figure 1).

Platforms can be grouped according to the type of fabrication and type of probe (Figures 1 and 2) (2).

Microarrays can be fabricated either by spotting pre-existing DNA (oligonucleotide, cDNA microarrays) (3) or by direct synthesis of oligonucleotides on the slide surface (Figure 1) (4). The first group of technologies use standard PCR and oligonucleotide synthesis for development of cDNA and oligonucleotides, respectively, which are spotted on slides afterwards. Synthesis of oligonucleotides directly on slides is done using two strategies. Steve Fodor and colleagues developed the first strategy in 1991, when they used photolabile groups on nucleotides and photolithographic masks for synthesis. Alan Blanchard developed the second, which adopted standard oligonucleotide synthesis on-chip format by using ink-jet synthesis, which allowed application of picoliter volumes (5).

Two types of probes are currently available for DNA microarrays as suggested above (Figure 1). The first is complementary DNA (6), which requires the creation of a cDNA library (usually in *E. coli*), amplification by PCR, and spotting on microarrays. The other type is oligonucleotides, which are typically 25 nucleotides long when photolithographic synthesis is used and 60–80 nucleotides long when ink-jet synthesis or standard oligonucleotide synthesis is used.

Various support materials (solid surfaces) have been used to date (Figure 1). Probes were first spotted and immobilized onto nylon membranes (7). However, glass supports are used today because of four main advantages. Glass is not porous; therefore, target nucleic acids do not have to diffuse to the probe. This feature also reduces volume and improves kinetics. Second, the rigid and nonabsorptive surface of glass requires spotting of smaller amounts of DNA. This increases the number of spots on an array and better defines their locations. Third, glass has low autofluorescence and therefore does not contribute to background noise. The final advantage is that glass allows for multiple targets to be labeled with different fluorophores and hybridized at the same time, which was not possible with nylon membranes, where radioactive labeling was primarily used (8).

DNA microarrays are used in two distinct types of analysis, both of which exploit the principle of complementary nucleic acid hybridization. Researchers can investigate either RNA or DNA (Figure 1). Both molecules are usually labeled with fluorescent dyes, usually Cy 3 or Cy5, which facilitate signals after hybridization. The first approach is expression profiling, in which the specific type and amount of RNA can be determined in the cells (9). The second approach is genotyping, in which DNA is interrogated for possible changes in sequence (SNPs, insertions, deletions, etc.) (10–13).

Most microarray studies done in dermatology have dealt with malignant melanoma. Researchers have been particularly interested in differences between the expression profiles of normal and cancerous tissue in order to identify genes involved in tumor development and progression. In addition to expression profiles, there was also interest in characterizing the changes in a tumor's genetic material using microarrays, which might explain tumor development. Microarrays were also used to study response of keratinocytes and melanocytes to UV illumination, and differences between normal and psoriatic skin.

Malignant melanoma and microarrays

Bittner et al. proposed that discrete and previously unrecognizable cancer taxonomy could be identified by reviewing the systematized data from gene expression experiments. Using 31 melanoma specimens and a microarray with 6,971 unique genes, they managed to separate melanomas into two distinct groups. They found no correlation between the groups and sex, age, biopsy site, Breslow thickness, Clark's level, and survival. They also did not manage to correlate mutation status in p16 or β-catenin mutation status, *in vitro* pigmentation, and cell passage number with these specific clusters. Using gel invasion assay and detection of capacity to induce vascular structures, it was shown that melanomas in the major cluster have reduced motility, invasive ability, and vasculogenic mimicry. Genes with reduced expression in the major cluster compared to more invasive melanomas included integrin β1, integrin β 3, integrin α1, syndecan 4, and vinculin. Samples outside this major cluster showed increased expression of fibronectin (14). Even though no correlation was found between expression profile and other characteristics of melanoma, molecular data might be of significant importance for diagnosis and prognosis in the future.

Table 1. Use of different platforms by citations.

Platform	Reference	Usage
cDNA (spotted)	14, 17, 20, 21, 22	Expression profiling
Oligonucleotide	15, 18, 19, 23, 24	Expression profiling

One of the recent studies on metastatic processes used poorly metastatic melanoma cells to select for highly metastatic melanoma cells with the help of *in vivo* selection. The study suggests that tumorigenesis and metastasizing are two distinct processes. The researchers also identified fibronectin, RhoC, thymosin β4, and several other proteins of extracellular matrix – collagen a2(I) and a1(III), matrix Gla protein, fibromodulin and biglycan – as potential genes involved in the metastasizing , by comparing poorly- and highly-metastatic cells. The role of RhoC in modulation of the cytoskeleton and metastasizing was confirmed by function assay. It has been shown that enhanced metastasizing could be induced by transfection of non-metastatic melanoma cells with wild-type RhoC. Metastasizing was reverted in cells that had overexpression of the dominant negative mutant of RhoC (15).

Another study done by Weeraratna et al. also demonstrated the effect of cell adhesion and motility on metastasizing through Wnt5a signaling. Incensement of Wnt5a is likely to be mediated through specific protein kinase C pathways, which are thought to be associated with cytoskeletal organization. This in turn should lead to the invasive phenotype of melanoma cell line. Additional support that the Wnt5a/Frizzled-5 pathway is in-

volved in generation of invasion melanoma phenotype is the desensitization of Frizzeled-5 (a Wnt5a receptor) by an antibody, which resulted in a decrease in the activation of the presumptive PKC pathway and the inhibition of *in vitro* motility and invasion phenotype of melanoma cells (16).

Comparison of the vertical and radial growth phases is accompanied only by loss of expression of a set of genes. These genes are involved in cell adhesion and extracellular matrix molecules such as CDH3, MMP10, integrin α2, and laminin γ2. CDH3 and MMP10 were also shown to be more strongly expressed in radial than vertical growth phases, which showed stronger expression in 12 of 19 and 11 of 22 cases, respectively, using immunohistochemistry. In no case was the immunostaining stronger in the vertical phase than in the radial growth phase. Like Bittner and colleagues, Haqq et al. also distinguished two different subtypes of metastatic melanomas. They also found no statistically significant correlation between subtypes and age, site of biopsy, Breslow thickness, and V599E mutation in B-RAF gene. It was also shown that both significant gains and losses of gene expression are present in transition from nevus to melanoma. Up-regulated genes were SPP1 (osteopontin), CXCL1 (melanoma growth-stimulating activity), and RAB32, which are all known to play a role in melanoma progression. Down-regulated genes with potential tumor suppressor activity were WIF1, ECM2, and SLIT3. Interestingly, Haqq et al. also showed that melanocytic linage markers S100B and MLANA were unable to distinguish between any of the multiclass sample groups. They also showed over-expression of a reverse transcriptase homologue of an endogenous retrovirus, nuclear receptor co-activator receptor protein 3, and PHIP (pleckstrin homology domain-containing protein), and loss of genes implicated in maintenance of normal melanocyte differentiation (ZNFN1A5 and HPS1) in metastatic lesions (17).

Arguing that chromosome changes could have an effect on differential expression of genes, Okamoto et al. used M-FISH and CGH with CDD banding for analysis of chromosomal aberrations of metastatic melanomas. Comparing the results of chromosomes aberrations with expression profiling, which showed higher mRNA expression levels of CTNNB1, E2F1, GPNMB, GPRK7, KBRAS2, LDB2, LIMK1, MAPK1, MEL, MP1, MUC18, NARCM, PBX3, and RAB22A genes at breakpoints, suggested that chromosomal aberration might lead to gene over-expression. Higher expression of CDK6, LDB2, MP1, and PBX3 genes was also confirmed by RT-PCR. The functional role of CDK6 was also shown by down-regulation with siRNA, which reduced cell growth up to 70% within 72 hours (18).

UV irradiation and gene expression in keratinocytes and melanocytes

Changes in gene expression of keratinocytes can be grouped into 3 waves: the early wave from 0.5 to 2h, intermediate changes from 4 to 8h, and late changes from 16 to 24h. Early changes in gene expression occur in genes that presumably protect cells from the harmful effects of UV. UV activates several transcription factors – junB, junD, c-fos, ETR101, EGR1, and URY – and suppresses c-Myc. Over-expression of c-Myc causes extensive premalignant epidermal proliferation by deregulating cell growth, promoting genomic instability, and inhibiting DNA damage-induced growth arrest proteins. It is of note that UV regulates a high number of RNA processing enzymes. Up-regulation of several mitochondrial proteins appears to be associated with the cells' additional need for energy and possibly the removal of reactive oxygen species. Accordingly, the cells also shut down gluconeogenesis and lipogenesis. The intermediate phase shows differential expression of genes whose products are secreted: chemokines, cytokines, and growth factors (IL8, Gro-α, Gro-β, MDNCF, and MIP2-β). They are chemotactic and presumably invite inflammatory cells and activate melanocytes that initiate tanning. In the late phase, the most strongly induced genes are keratinocyte differential markers (components of the cornified envelope). Desmosomal proteins are suppressed, which may facilitate movement of keratinocytes. UV also regulates actin binding protein expression, which affects shape, motility, and polarity. Interestingly, genes not found to be changed were p53, most cell cycle proteins, and proteins of extracellular matrix (19). In contrast, Murakami et al. showed enhancement of p53 and p21WAF1 expression. They also showed induction of ERK3, mitogen-activated protein kinase 3 (MAP kinase p38), growth arrest, and DNA-damage-induced protein (GADD45) (20).

In melanocytes, expression of 198 genes was changed 1.9 times or more; 159 of these were previously known. These differentially expressed genes encoded proteins involved in binding, synthesis, or modification of nucleic acids, ribosomal proteins, solute carriers, porins, ionic channels, cell receptors, and transcription factors. When comparing these results to a study using a similar platform done by Bittner et al., Valery and colleagues identified 36 genes modulated the same way as in melanomas (21). UVA has a similar effect to UVB, as shown by Jean et al. Differentially expressed genes were involved in DNA repair, cell survival, proliferation, and irradiation. Interestingly, they observed high up-regulation of stress response proteins HSP 70,

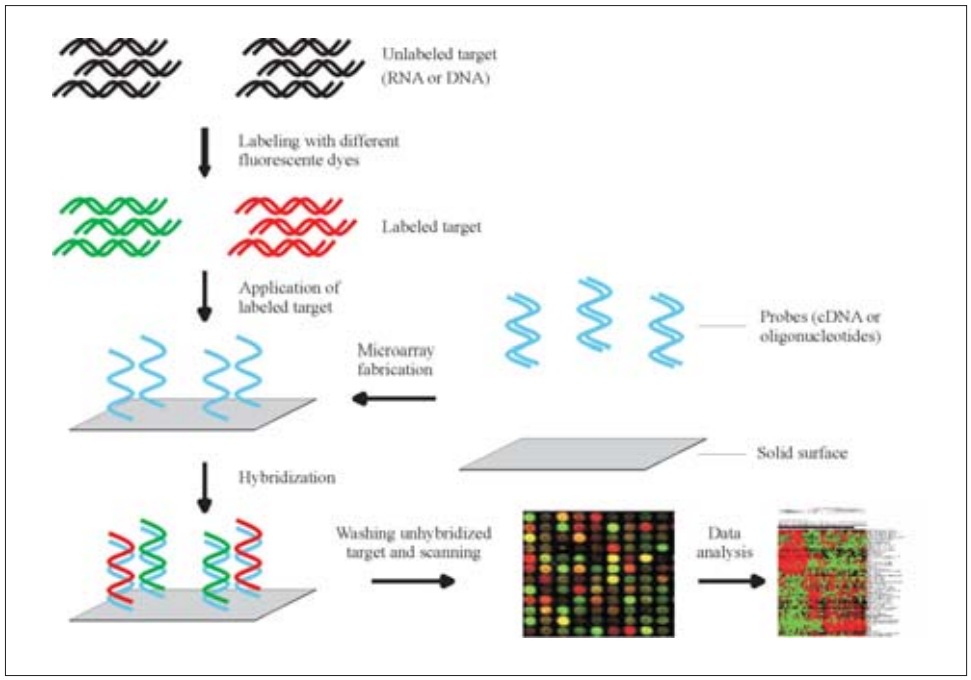

Figure 1. DNA microarray experiment work-flow.

HSP40, and HSP86, which could also affect caspase-dependent or independent apoptotic pathways (22).

Psoriasis and other autoimmune skin diseases

In a study by Bowcock et al., 177 genes with differential expression were recognized when comparing involved skin versus normal skin from 15 non-related psoriatic patients. Among these 177 genes, S100 calcium binding proteins showed the largest expression difference between involved and uninvolved skin in comparison to normal skin. Other transcripts up-regulated in both involved and uninvolved skin were transcobalamin I (vitamin B12 binding protein), CD47, IL8, ECGF1, SPRR2C, and STAF50 (23). The same group used a U95A microarray, with which they generated a list of 1,338 genes that are potentially psoriasis related, and 60% of these encode newly discovered proteins. Further attention was turned toward immune signaling cascades, and 131 genes with differential expression were found. Involvement of IL1H1 and IL1HY1 in psoriasis was also confirmed with RT-PCR. Nineteen chemokines were determined to be differentially expressed, and 11 of these were described in relation to psoriasis for the first time. This finding may be important because chemokines are known to regulate T cell or dendritic cell trafficking. It was proposed that expression of CCR7 leads to the entry of T cells into psoriasis lesions (24).

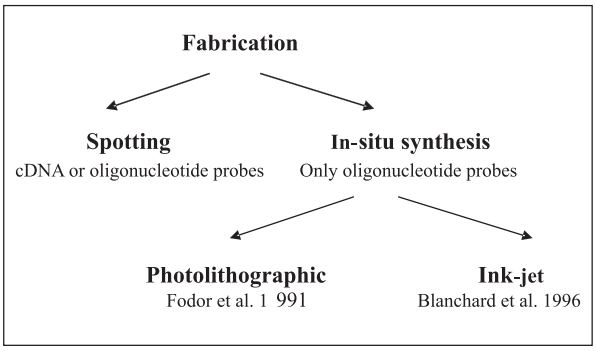

Figure 2. Relation between fabrication and type of probe on microarray.

Conclusion

The multiple DNA microarray platforms available on the market are all products of development in the 1990s and early 2000s. DNA microarrays can be used to study either RNA or DNA. The first approach is called expression profiling, and enables identification and quantification of a cell's RNAs. This approach has been used in studies of malignant melanomas, psoriasis, and UV irradiation of cells in attempt to identify and better understand the role of genes in the development of diseases or responses to environmental stress. DNA microarray technology is used also for genotyping. This approach yields interesting results in studies of malignant melanomas, in

which changes in chromosomes were compared with mRNA expression levels. Microarrays have been used to identify many genes implicated in tumor development and progression. Subsequently, they may serve as potential targets for new drugs and markers for diagnostic procedures.

Abbreviations

CCR7 – C-C chemokine receptor type 7
CDD – cytidine deaminase
CDH3 – cadhenin 3
CDK6 – cyclin-dependent kinase 6
CGH – Comparative genomic hybridization
CTNNB1 – catenin (cadherin-associated protein), beta 1
CXCL1 – chemokine (C-X-C motif) ligand 1
ECGF1 – endothelial cell growth factor 1
ECM2 – extracellular matrix protein 2
EGR1 – Early growth response protein 1
ERK3 – mitogen-activated protein kinase 4
ETR101 – immediate early response 2
E2F1 – E2F transcription factor 1
GADD45 – growth arrest and DNA-damage-inducible
GPNMB – glycoprotein (transmembrane) nmb
GPRK7 – MAP kinase interacting serine/threonine kinase 2
HSP – Heat shock protein
HSP1 – protamin 1
IL8 – interleukin 8
IL1HY1 – Interleukin-1 family member 5
KBRAS2 – NFKB inhibitor interacting Ras-like 2
LDB2 – LIM domain binding 2
LIMK1 – LIM domain kinase 1
MAPK1 – mitogen-activated protein kinase 1
MDNCF – Monocyte-derived neutrophil chemotactic factor – IL8
M-FISH – Multiplex fluorescence in situ hybridization
MIP2-β – Macrophage inflammatory protein 2-beta
MLANA – Melan A
MMP10 – Matrix metalloproteinase 10
MP1 – Mitogen-activated protein kinase kinase 1-interacting protein 1
MUC18 – melanoma cell adhesion molecule
PBX3 – Pre-B-cell leukemia transcription factor 3
PHIP – pleckstrin homology domain interacting protein
RAB32 – member RAS oncogene family
RAB22A – member RAS oncogene family
RT-PCR – real time PCR
SLIT3 – slit homolog 3
SPP1 – secreted phosphoprotein 1
SPRR2C – small proline-rich protein 2C
STAF50 – tripartite motif-containing 22
UV – ultraviolet
WAF1 – cyclin-dependent kinase inhibitor 1A
WIF1 – Wnt inhibitory factor 1
ZNFN1A5 – zinc finger protein, subfamily 1A, 5, isoform CRA_a

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AUTHORS' ADDRESSES

Vid Mlakar, Microbiologist, Department for molecular genetics, Institute of Pathology, Faculty of Medicine, University of Ljubljana, Zaloska 4, 1000 Ljubljana, Slovenia

Damjan Glavac, PhD, Professor, Head of the department, Department for molecular genetics, Institute of Pathology, Faculty of Medicine, University of Ljubljana, Zaloska 4, 1000 Ljubljana, Slovenia