Microarray applications: emerging technologies and perspectives

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Analysis of gene expression in RNA samples using DNA microarrays has made considerable progress in the past few years. In addition, microarray applications were extended to proteins and have more recently been used for compound classification in the pharmaceutical industry. These were some of the topics addressed at the *EuroBiochips* conference [organized by IBC Life Sciences (http://www.ibc-lifesci.com)], which was held in Hamburg, Germany, on 22–24 June 2004.

In his keynote presentation, Samir Hanash (University of Michigan Medical School; http://www.med.umich.edu/medschool) highlighted that none of the array techniques currently available for the profiling of protein abundance is equivalent to DNA microarray technology. However, over the past three years, the Human Proteome Organisation (HUPO; http://www.hupo.org) initiative has collected antibodies that are specific for 5000 proteins with a view to constructing antibody arrays. One key initiative of the plasma proteome project is the quantification of human plasma proteins: analysis of human plasma could lead to the identification of protein markers that correspond to common diseases. To this end, plasma proteins are labeled with fluorescent Cy-dyes, separated in 2D gels and analyzed using mass spectrometry. Another objective of the HUPO initiative is the identification of posttranslational modifications of tumor antigens and drug-binding proteins in human tumors and cell lines. Furthermore, the spotting of antibodies of several protein markers that occur in different types of cancer on microarrays facilitated the investigation of proteins that are important for diagnosis of distinct cancer types.

Emerging technologies

Ulf Landegren (University of Uppsala, Sweden; http://www.uu.se) presented an interesting universal approach for the analysis of transcripts and proteins using padlock probes [1]. Padlock probes are linear oligonucleotide probes that, after hybridization with specific target nucleic acid sequences (DNA or RNA), are converted to intramolecular DNA circles. The circles can be amplified via rolling circle replication reactions and identified by, for example, hybridization to universal tag microarrays. In proximity ligation, pairs of reagents, such as antibodies or aptamers that can bind a target protein, are equipped with DNA strands that can be ligated when the reagents have bound the protein. After amplification, target proteins can also be detected by hybridization on tag arrays. Therefore, padlock probes could be used in conjunction with a universal tag microarray to quantify DNA, RNA and proteins.

Karin Schütze (PALM Microlaser Technologies, Germany; http://www.palm-microlaser.com) described a technique of non-contact laser microdissection developed by PALM that can be used to extract single cells from their cellular environment. PALM's microdissection technology uses a pulsed nitrogen laser, which is directed into the path of an inverted research microscope and focused to a diameter of <1 μm. The resulting photofragmentation generates a microplasma under the single cell thereby catapulting it into a collection device. Because the microplasma collapses rapidly, it is claimed that the cell is not damaged and biomolecules such as DNA, RNA or proteins can be extracted without undergoing degradation. According to PALM, the microlaser can be used for cellular microsurgeries and pure sample processing, which is necessary for the analysis of single cells taken from, for example, solid tumors using proteomics or genomics approaches. Another interesting application of the PALM microlaser technology is the preparation of single chromosomes or distinct parts of chromosomes; this technology is already used for genetic fingerprinting in the area of forensics.

Hans Peter Lang (IBM Zürich Research Laboratory; http://zurich.ibm.com) presented the use of nanomechanical cantilevers as array sensors: the cantilevers are 500 μm long, 100 μm wide and 0.5 μm thick. The high flexibility of nanomechanical cantilevers renders them sensitive to the binding of molecules on their surface; molecule adsorption triggers a mechanical motion that can be detected optically using microfabricated lasers. In addition, the cantilevers can be coated with oligonucleotides or antibodies, and thus the binding of complementary RNA molecules or antigens results in the bending of the cantilevers. Because of their high sensitivity, cantilever sensors do not

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require fluorescent labeling of biomolecules or amplification procedures. Possible applications for this technology could be the rapid diagnostic analysis of biomarker proteins, for example, after acute myocardial infarction (results could be generated in minutes rather than waiting for time-consuming ELISA assays) or as a 'chemical nose' for the detection of toxic agents in the air.

Current and prospective applications for DNA and RNA analyses using Affymetrix (http://Affymetrix.com) arrays were outlined by Fiona Brew (Affymetrix). In addition to expression profiling of RNA samples, Affymetrix is also focusing on human single nucleotide polymorphisms (SNP) analyses. The 10K-array and the 100K-array set were designed in collaboration with Perlegen (http://www.perlegen.com) for the mapping of 10,000 or 100,000 SNPs, respectively: each assay requires only 250 ng of genomic DNA and a single PCR primer. Using the 100K-array set, costs for SNP detection will be less than one cent per single SNP. In the field of expression profiling arrays, Affymetrix will include more organisms such as grape, soybean, cow, pig and chicken. Furthermore, the design of human arrays for all-exon studies is planned. To circumvent the 3' bias of the existing labeling procedure, Affymetrix is developing a whole-transcript assay using random hexamers to generate a random-primed cDNA. Affymetrix claims that every predicted human exon will be represented on the all-exon arrays. Another interesting topic was the development of tiling arrays for the detection of transcriptional activity in non-coding regions of the human genome, which could be relevant for gene regulation [2]. In the area of automization of target preparation, Affymetrix and Caliper (http://www.calipertech.com) collaborated to develop a robotic station that can prepare 30 μg of cRNA using 2–5 μg total RNA as starting material.

Microarray applications for patient profiling

Mathias Gebauer (Aventis; http://www.aventis.com) reported results on the expression profiling of tissue samples from osteoarthritis (OA) patients and cellular model systems using the SensiChip™ technology developed by Zeptosens (http://www.zeptosens.com). The SensiChip™ two-color platform is based on planar waveguide technology, which uses an evanescent excitation for microarray detection. Therefore, the background of slides is significantly reduced and sensitivity is increased (detection limit is approximately 1–3 mRNA copies per cell without amplification steps). Fluorescence labeling of RNA samples with amounts >1 μg can be performed via reverse transcription, or, if less RNA material is available, by T7 RNA polymerase-mediated *in vitro* transcription (up to 20 ng of total RNA can be used). In the experiments performed within the 'Leitprojekt Osteoarthrose', RNA samples from ten patients in the late stages of OA were profiled against a pool of normal donors. The use of a customized SensiChip™ microarray spotted with 70mer oligonucleotides that represented 350 OA-relevant genes led to the identification of approximately 200 genes that were significantly regulated during OA. Furthermore, time-course experiments with interleukin-1β-stimulated primary human-chondrocytes were used to demonstrate the involvement of two selected genes in catabolic activities during progression of OA.

Anders Lönneberg from DiaGenic (http://www.diagenic.no) presented an attempted array-based diagnosis of breast cancer using blood cells as the sample. In a pilot study, DiaGenic analyzed the expression patterns of 1435 genes in peripheral blood cells of 24 women diagnosed with breast cancer and 32 women with no sign of this disease. A distinct set of genes has been identified that correctly predicted the diagnostic class in 93% of the samples. The majority of the genes used were expressed at a lower level in breast cancer samples when compared with normal controls and predominantly encoded proteins involved in protein synthesis. DiaGenic claims that the expression pattern of the identified genes can be used to discriminate and predict the class of breast cancer with significant accuracy.

Microarray applications for toxicology and compound classification

An approach to predicting the toxicity of compounds was discussed by Alex Nie from Johnson & Johnson (http://www.jnj.com). A major goal of the Toxicogenomics program established at the company is to predict hepatic toxicity of drug candidates. A reference transcriptional database of 120 paradigm hepatotoxitants was constructed using cDNA microarrays that represent about 3400 rat genes. Three male rats per compound were treated with a single high-dose for 24 hours, and then RNA was prepared from liver. After hybridization of microarrays, distinct gene sets for three different toxicity signatures could be defined – peroxisome proliferators (20 genes), macrophage activators (32 genes) and oxidative stressors (60 genes). The identified sets of marker genes will be used to predict different types of hepatotoxicity to prioritize drug candidates at an early stage of development. However, to increase the statistical significance of compound classification via expression profiling experiments, it might be necessary to include more rats that have been treated with different compound concentrations.

Christoph Freiberg (Bayer HealthCare; http://www.bayerhealthcare.com)

described the use of RNA expression profiling in antibacterial drug discovery to characterize the mechanism of action (MOA) of antibacterial agents. Almost all genes contained in the genome of *Bacillus subtilis* were amplified by PCR and spotted on a microarray. A collection of more than 76 antibacterial reference compounds was generated that covered all classical and emerging antibacterial target areas (e.g. fatty acid, cell wall, nucleotide and DNA synthesis). Bacteria were incubated with these reference compounds at two different time points and concentrations. After two-color labeling, RNAs prepared from compound-treated bacteria and untreated controls were hybridized on the *B. subtilis* genome microarray. The resulting expression profiles were then analyzed by clustering to characterize the MOA of reference compounds. Additionally, expression data were used to map regulatory units and pathways affected by antibacterial agents. Based on the data generated from a novel pyrimidone antibiotic with an unknown MOA, this approach enabled the prediction of the *B. subtilis* peptidyltransferase that is involved in the bacterial translation machinery as a potential molecular target.

Concluding remarks

The *EuroBiochips* conference was well organized and focused on novel developments in microarray technologies, as well as their applications for patient and compound profiling. Discussions on the use of microarrays as a strategy for compound profiling highlighted that compound classification based on expression profiling will be an important perspective for microarray technologies and will have an increasing impact on lead optimization in the future.

References

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