Introduction

Today, researchers can choose from a broad variety of methods for global transcriptional profiling. Among the different technical approaches, microarray technology has gained a premier position. In principle, microarrays can be produced either by robotic printing (“spotting”) of DNA on a chemically modified glass surface [1], or by in situ synthesis of oligonucleotides on a silane-reacted quartz substrate [2]. Spotted arrays usually contain cDNA-specific PCR amplicons (cDNA arrays), ranging from several hundred to a few thousand base pairs in size. Generally, no more than one amplicon is used to probe a given gene. Although they are technically challenging and require both optimized protocols and workflow, CDNA arrays are typically produced by individual research groups or core facilities. Alternatively, they can be purchased from several commercial suppliers. But after the discovery of frequent discrepancies in the annotation of cDNA clones, investigators began to realize potential drawbacks of this highly advocated technology. In situ synthesis of oligonucleotide probes requires sophisticated equipment for photolithography and solid phase chemistry, which is usually too complex and elaborate for an academic environment. A widespread commercial implementation of this technology is the Affymetrix GeneChip platform, which currently uses 11-16 pairs (11 for the arrays used in this study) of perfect-match and single-base-mismatch 25-mer oligonucleotides for each gene. Recently, large collections of longer oligonucleotides (50-80 bases), produced by established suppliers using conventional phosphoramidite chemistry, have become increasingly popular as probes for spotted DNA arrays. Technical advantages of oligonucleotide arrays include a constant DNA concentration across all spots and biophysically optimized sequences, reducing secondary structures, avoiding repetitive sequence motives and providing a fixed range for both Tm and length. All this accounts for more uniform, stable and predictable hybridization conditions. The overall costs for long oligonucleotide arrays will often be lower when labor and other costs associated with cDNA libraries, such as replication, amplification or sequence verification, are regarded.

Considering this diversity of approaches and the resulting technical differences, researchers are highly interested in the potential drawbacks of this highly advocated technology. In situ synthesis of oligonucleotide probes requires sophisticated equipment for photolithography and solid phase chemistry, which is usually too complex and elaborate for an academic environment. A widespread commercial implementation of this technology is the Affymetrix GeneChip platform, which currently uses 11-16 pairs (11 for the arrays used in this study) of perfect-match and single-base-mismatch 25-mer oligonucleotides for each gene. Recently, large collections of longer oligonucleotides (50-80 bases), produced by established suppliers using conventional phosphoramidite chemistry, have become increasingly popular as probes for spotted DNA arrays. Technical advantages of oligonucleotide arrays include a constant DNA concentration across all spots and biophysically optimized sequences, reducing secondary structures, avoiding repetitive sequence motives and providing a fixed range for both Tm and length. All this accounts for more uniform, stable and predictable hybridization conditions. The overall costs for long oligonucleotide arrays will often be lower when labor and other costs associated with cDNA libraries, such as replication, amplification or sequence verification, are regarded.

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We have shown that, overall, expression profiles obtained with either long (Operon) or multiple short (Affymetrix) oligonucleotide microarrays display a reasonable correlation, with variable concordance of individual genes. Based on patient samples, we obtained results which are in good agreement with previous studies that utilized cell line-derived RNA. Projecting these findings to a larger series of array experiments, one could expect to obtain similar albeit not identical results, concerning, e.g., a hierarchical clustering or a gene expression signature, with either of the two investigated platforms. On the level of individual genes and quantitative precision, however, our results reaffirm that microarrays have to be considered a screening technology and that their data should be regarded with caution. This should be kept in mind particularly when comparing data from different array platforms.

![Fig 1: Comparison of relative gene expression for the genes OSF2, GMDS, TMPRSS2 and BGN. Expression ratios were determined for tumour versus control tissue of the indicated patients, using either Affymetrix GeneChip arrays (A-B), Operon long oligonucleotide arrays (C) or real-time quantitative PCR analysis (D). Affymetrix ratios were either normalized by variance stabilization (A) or the MA5 algorithm (B).](image)

Identification of novel tumour-associated genes differentially expressed in the process of squamous cell cancer development

Chemically induced mouse skin carcinogenesis represents the most extensively utilized animal model to unravel the multistage nature of tumour development and to design novel therapeutic concepts of human epithelial neoplasia. We combined this tumour model with comprehensive gene expression analysis (see Figure 2) and could identify a large set of novel tumour-associated genes that have not been associated with epithelial skin cancer development yet [4]. Expression data of selected genes were confirmed by semi-quantitative and quantitative RT-PCR as well as in situ hybridisation and immunofluorescence analysis on mouse tumour sections. Enhanced expression of genes identified in our screen was also demonstrated in mouse keratinocyte cell lines that form tumours in vivo. Self-organizing map clustering was performed to identify different kinetics of gene expression and co-regulation during skin cancer progression. Detailed analysis of differential expressed genes according to their functional annotation confirmed the involvement of several biological processes, such as regulation of cell cycle, apoptosis, extracellular proteolysis and cell adhesion, during skin malignancy. Finally, we detected high transcript levels of ANXA1, LCN2, and S100A8 as well as reduced levels for NDR2 protein in human skin tumour specimens demonstrating that tumour-associated genes identified in the chemically induced tumour model might be of great relevance for the understanding of human epithelial malignancies as well.

![Fig 2: Correlation between expression ratios measured on NIA and ArrayTAG microarrays. The expression ratios for 181 genes, which show significantly altered expression levels (linear ratio greater or smaller than 2.71) in SCC and are present on both chips, were plotted in a 2D graph. The x-axis represents the logarithmic (ln) ratio values of the NIA microarray, whereas the y-axis indicates the logarithmic (ln) ratio values of the ArrayTAG microarray. The square of the correlation coefficient, R², is 0.78, which indicates a high correlation between the expression ratios obtained from both microarrays.](image)

Outlook

We produce high-quality cDNA and 70mer oligonucleotide microarrays using standardized conditions (quality management) and will apply them for comprehensive RNA expression studies. Our focus will be both the analysis of murine model systems of medulloblastoma, squamous cell carcinoma and mantle cell lymphoma but also the analysis of defined human tumor entities, e.g., of the central nervous system.