MicroRNAs

Small Molecules with Big Impact in Cancer

MicroRNAs form one of the major regulatory gene families by targeting mRNAs for cleavage or translational repression within viruses, plants and animals. MiRNA expression patterns were found to classify cancer types with a greater reliability than the respective profiles of protein-coding genes [1]. This outstanding potential as diagnostic biomarkers is furthermore supported by the extraordinary stability of miRNAs in routinely collected formalin-fixed paraffin-embedded (FFPE) tissue samples [2].

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MicroRNAs Play an Important Role in Tumourigenesis

MicroRNAs (miRNAs) are a family of endogenous ~22nt non-coding RNAs that regulate gene expression with a strong sequence specificity. To date, over 8600 miRNAs have been identified and deposited in the online miRBase sequence database [3], including currently more than 690 miRNA sequences for the human genome.

Due to their function as gene regulators, miRNAs have been shown to control cell growth, differentiation and apoptosis. An impaired miRNA expression was found in many different human tumour types. It is therefore evident that changes in miRNA expression levels might also play an important role in tumourigenesis. Though most miRNAs showed lower expression levels in tumours compared with normal tissues independent from the cell type, also overexpressed miRNAs have been found. In this respect, miRNA genes are thought to function as both tumour suppressors and oncogenes [4]. For instance, the expression level of miRNA *let-7* has been shown to be reduced in lung cancer [5]. In animals, the expression of *let-7* is dependent on developmental timing, with low levels in early stages of development and highest expression levels in differentiated adult tissues. Downregulated expression of *let-7* results in loss of differentiation, which is a hallmark of cancerogenesis. Furthermore, it was demonstrated that the *RAS* oncogene is a direct target of miRNA *let-7*, suggesting that *let-7* works as a tumour suppressor gene in lung oncogenesis by its function as regulator of *RAS*. A second example is the downregulation of *miR-15* and *miR-16* in chronic lymphotic leukemias, which target the antiapoptopic factor *BCL2*.

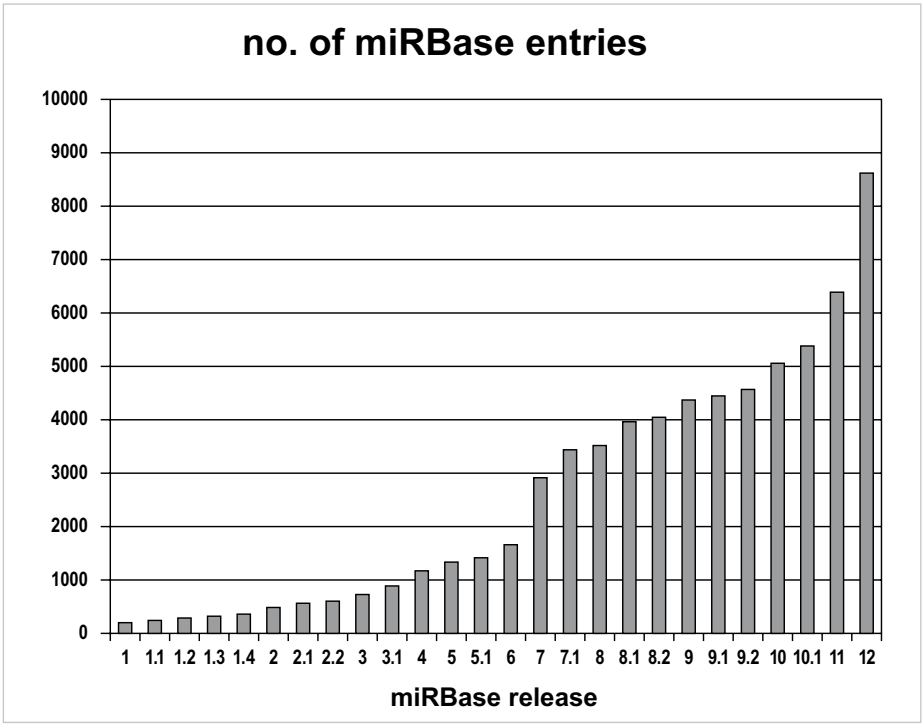

Fig. 1: Number of entries in the miRBase database from the Sanger Institute starting with 218 entries in release 1.0 (Dec. 2002) up to 8619 entries in release 12.0 (Sep. 2008).

On the other hand, oncogenic miRNAs promote cancer development by negatively inhibiting tumour suppressor genes or genes that regulate cell differentiation or apoptosis. An example is the *miR-17-92*, whose expression is significantly increased in several cancers like lung cancer and lymphomas and which emerges to enhance lung cancer cell growth. Another hint for the contribution of miRNAs in tumourigenesis is the fact that more than 50% of miRNA genes known so far are located in cancer-associated genomic regions [6].

Classification of Cancer by Studying miRNA Expression

Based on expression profiles of miRNAs, classification of tumours with respect to developmental origin as well as differentiation state became possible with a much higher accuracy classification based on conventional mRNA expression studies [1]. In a systematic study including multiple human cancers, nearly all analysed

miRNAs exhibited differential expression, enabling the discrimination of tumours of different developmental origin. This classification of human cancers was done by examining expression profiles of a relatively small number of about 200 miRNAs, while the study of expression profiles of about several thousand mRNA genes did not achieve the same result. Other studies showed a high correlation of miRNA expression with specific biopathologic features like tumour stage or proliferation index [7]. In contrast to the evaluation of mRNA expression profiles which often do not have a direct biological consequence due to post-transcriptional modifications on the way from DNA to protein, miRNAs reflect more directly the functional level of the gene.

Another huge benefit of using miRNAs for the classification of cancer is their improved stability in formalin-fixed paraffin-embedded (FFPE) tissues, which are routinely collected and archived in clinical research. While the longer mRNAs most likely undergo degradation and chemical modification, the tiny miRNA outlast the fixation procedure as well as long term storage. An extensive comparison with snap frozen cells showed the reliabilty of using FFPE samples for achieving miRNA expression profiles [2].

Microarray-based Methods for miRNA Expression Profiling

The most common method for high-throughput analysis of expression levels of miRNAs is the use of oligonucleotide-based microarrays [8], enabling the measurement of differential gene expression of hundreds of miRNAs in a large number of samples simultaneously. Another microarray-based method, the so-called Microfluidic Primer Extension Assay (MPEA) based on the febit Geniom microarray technology uses unlabelled miRNAs for hybridisation on highly flexible microfluidic microarrays. In a second step, Klenow fragment of DNA polymerase I is added directly into the channels of the microfluidic chip, where the specific elongation of the bound miRNAs takes place. The method therefore combines in a double-staged way the specificity of a hybridisation assay with the discriminatory power of an enzymatic extension [9].

The MPEA shows several advantages over any other existing microarray-based method. Since miRNA is used directly without preceding treatment like enrichment, PCR-based amplification or labelling, it is ensured that no experimental bias is introduced. Compared to the conventional hybridisation assay, which is most suitable to discriminate mismatches in the central position of the hybridised target, MPEA provides an additional level of specificity due to the fact that the enzymatic elongation only can occur with nearly perfect matches at the 3´-end resulting in significantly less false positive signals coming from cross-hybridisations. The MPEA also shows several key benefits compared to the conventional RNA-primed, array-based Klenow Extension (RAKE) assay [10]. While the RAKE assay uses microarrays consisting of oligonucleotides bound with their 5`-end to the surface, the oligonucleotide capture probes of the MPEA arrays are attached with their 3´-end to the surface. This fact does not only exclude self-elongation of the probes, but especially leads to exposition of the elongation reaction away from the surface to the lumen of the microchannel without any steric hindrance. A significantly lower amount of sample RNA is needed due to the use of microfluidic channels. The sensitivity is even high enough to reliably analyse nanogram amounts of total RNA coming from FFPE tissue samples without a need for amplification.

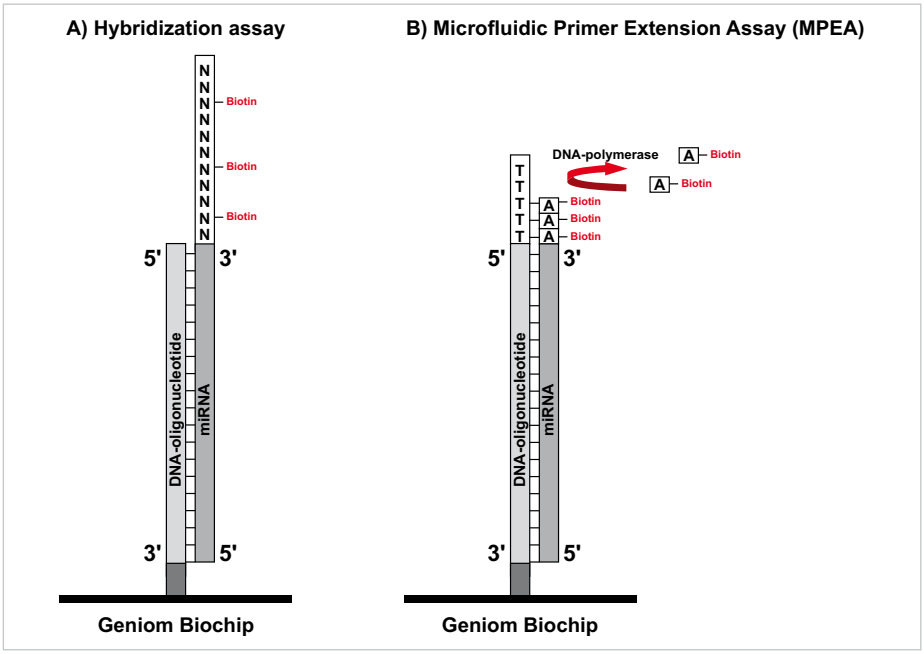

Fig. 2: Comparison of two microarray-based assays used for miRNA profiling. In both cases, labelling was done using biotinylated nucleotides, which enables the subsequent staining with a streptavidin-phycoerythrin-conjugate. A): For the conventional hybridisation assay, miRNAs are labelled first (here exemplarily shown using poly(A) tailing introducing a 20-50 tail of partially amine-modified nucleotides, which are then coupled to Biotin-XX-NHS-ester) and hybridised afterwards to the microarray containing reverse complementary oligonucleotides. B): Principle of the MPEA. The hybridised, unlabelled miRNA functions as a primer for an enzymatic elongation in which biotinylated nucleotides are incorporated.

References

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