A Sensitive Method for MicroRNA Gene Expression Profiling Applied to Prostate Cancer Tissue and Cell Lines LNCaP and PC3


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Introduction

MicroRNAs (miRNAs) are a class of small non-coding RNAs encoded in the genomes of animals and plants that play a role in targeting messages of protein-coding genes for cleavage or translational repression. Many microRNAs are evolutionarily conserved, suggesting they play roles in essential processes. Genetic studies in various species have demonstrated that many miRNAs are regulated in a temporal and spatial manner and have important regulatory functions in development, differentiation, cell proliferation, cell death and morphogenesis. Direct demonstrations of the involvement of miRNAs in human disease have been recently published. Reduced levels of miRNA 141 and 143 are found in colorectal neoplasia (Michael et al, 2003). Two miRNAs correlate with disease progression in chronic lymphocytic leukemia (CLL). Deletions at 13q14 occur in 65% of prostate cancers, and in other cancers. Both miR-15 and miR-16 are located at this locus, and appear to be deleted or down-regulated in the majority of CLL cases (Calin et al, 2002). Croce et al (2004) reported 185 miRNAs and 584 miRNAs located to previous reported noncoding germline loci and found that 32.5% of the miR genes are located in fragile sites and cancer-associated genomic regions.

Methods

MicroRNA labeling

Micronucleus, the damaged genome

C elegans embryogenesis study

Poly (A) tail

Human studies: microRNA role in cancer

miRNA in chronic lymphocytic leukemia

New frontier for gene expression profiling

Results

MicroRNA expression profiling of prostate tissues

Normal tissue and tumor from a radical prostatectomy case were amplified and labeled for microarray analysis. The experiment was done with three replicates. Studies are currently underway to examine differences in miRNA profiles between normal tissue and prostate. Profiles will be examined to identify any changes in miRNA expression that occur during prostate cancer progression.

Conclusions and Future Studies

The methods described here allow for highly sensitive and reproducible miRNA expression analysis using microarray technology. Our results demonstrate highly correlated expression between amplified samples, between amplified and unamplified samples, and between replicates. These methods enable comparison of miRNA expression profiles between cell lines or between normal tissue and tumors. We have begun to apply these methods for identifying miRNAs that may play important roles in prostate cancer, specifically differences in miRNA expression that may occur during the transition from hormone-sensitive to hormone refractory prostate cancer. The ability to amplify miRNAs starting at picogram quantities allows for the ability to study miRNA expression from tissue samples where starting material may be limited, such as needle core biopsies.

Future studies include:

1. Validation studies of microarray results by Taqman RT-PCR quantitation of microRNAs in collaboration with Applied Biosystems and a non-radioactive method being developed by Genisphere.
2. Investigation of miRNA expression profiles during prostate cancer progression.
3. Functional studies of miRNAs in prostate cancer cell lines.