Introduction

- Abnormal expression of microRNAs (miRNAs) in cancer implies that these small molecules play a role in oncogenesis. Therefore miRNAs may comprise a novel class of diagnostic and prognostic signatures.
- Here, we study the global expression profiles of miRNAs in breast cancer and normal adjacent tissue in order to identify possible new biomarkers for breast cancer.
- To study the global miRNA expression profiles in breast cancer, we use the mirCURY™ LNA Array platform based on locked nucleic acid (LNA) modified capture probes that have uniquely high affinities for miRNA.
- For quantitative validation of new miRNA biomarkers, we apply LNA-enhanced real-time PCR detection methods.

Methods

Samples

- Biopsies from primary tumors and from normal adjacent tissue (1 cm and 5 cm) were collected from 11 female patients undergoing surgery for invasive ductal carcinoma (Fig. 1).

microRNA extraction

- Total RNA was isolated by guanidinium isothiocyanate/phenol/chloroform extraction. From 50 mg breast tissue, 0.5-10 µg total RNA was routinely retrieved.

Microarray expression profiling

- 1 µg total RNA was analyzed for miRNA expression on mirCURY™ LNA Arrays containing capture probes for 491 miRNAs. The miRNA labeling and hybridization procedure is outlined (Fig. 2).

Confirmation of expression pattern with real-time PCR

- The expression pattern of a number of selected miRNAs was confirmed with the gene-specific LNA-enhanced real-time PCR assays. The real-time PCR detection was either based on hydrolysis probes or Sybr green intercalating dye (Fig. 3, 7 and 8).

Data analysis

- The miRNA expression data were analyzed with dChip 2006. Unsupervised hierarchical clustering was applied to both samples and genes using the centroid linkage method and (1 – Pearson correlation) distance metric.

Conclusions

mirCURY™ LNA Arrays

- Superior sensitivity, < 50 amol miRNA detected (< 1 µg total RNA)
- Excellent specificity and discrimination of miRNA family members
- High reproducibility, both intra-array, and inter-batch
- Fast and simple RNA labeling
- No need for amplification or miRNA enrichment

LNA-enhanced real-time PCR

- High sensitivity, < 10 ng total-RNA
- Unique specificity, single mismatch discrimination
- Good reproducibility

miRNA profiles

- Known, breast-cancer associated miRNAs were confirmed with the mirCURY™ LNA Arrays and validated by LNA-enhanced real-time PCR.
- A number of novel miRNAs not previously connected with breast cancer were identified with the mirCURY™ LNA approach.
- Some of these miRNAs may represent novel diagnostic signatures.
- We are currently validating the new potential biomarkers with a 454 pyrosequencing approach, LNA-enhanced real-time PCR methods, and on the FlexiMir™ microRNA Luminex platform.

Results – miRNA profiling

Identification of novel breast-cancer associated miRNAs

In addition to the known miRNAs, we identified numerous novel (i.e. not previously reported in humans) breast-cancer associated miRNAs. LNA probes for these new miRNAs are currently being designed.