Supplementary materials and methods.

Microarray printing and data analysis.

The LNA-modified oligonucleotide probe set for all annotated miRNAs from mouse \textit{(Mus musculus)} and human \textit{(Homo sapiens)} in the miRBase Release 7.1, including a set of positive and negative control probes, was purchased from Exiqon (Vedbaek, Denmark) and used to fabricate the LNA microarrays. The list of capture probes in the LNA oligonucleotide probe set version 7.1 for all annotated miRNAs from mouse \textit{(Mus musculus)} and human \textit{(Homo sapiens)} in the miRBase Release 7.1 can be found at www.exiqon.com. The capture probes were diluted to a final concentration of 10 µM in 150 mM sodium phosphate buffer (pH 8.5) and spotted in quadruplicate onto Codelink slides (Amersham Biosciences) using the BioRobotics MicroGrid II arrayer (Genomic Solutions, MI, USA) at 45% humidity and at room temperature. Spotted slides were post-processed as recommended by the manufacturer.

Spot and background intensities were extracted from the scanned images using TIGR spotfinder version 3.0 (1). Data were normalized using the variance stabilizing normalization package version 1.8.0 (2) for R (www.r-project.org) and intensities of replicate spots were averaged. Probes displaying a coefficient of variance > 100% were excluded from further data analysis.

Normal and tumor breast tissue
Total RNA extracted from normal or tumor breast tissue specimens (3.5 µg per sample) was 3´end labeled using T4 RNA ligase and Cy3- or Cy5-conjugated RNA linkers (3;4). Only probes displaying signal intensities of >500 above background were included in the analysis and the intensities from at least two (n=2) replicate hybridizations were averaged.

Breast cell lines
Total RNA extracted from the breast cell lines (5 µg per sample) was 3´end labeled using T4 RNA ligase and Cy3-conjugated RNA linker (3;4). miRNA probes displaying signal intensities 3x above the average of the local background intensity plus 3x SD were considered as expressed miRNAs. Hierarchical clustering was done using the TIGR Multi Experiment Viewer (1).

References


StarFire probes used for Northern blot analysis

First name is the official name of the microRNA deposited at the Sanger Center (http://microrna.sanger.ac.uk/sequences/index.shtml), the name after the equal sign is the name used in the paper.

These sequences are complementary to the microRNA sequence.

> mmu-miR-1 = miR-1
  TGATAGGCCCTGTACAATGCTGCT
> hsa-miR-7 = miR-7
  ACAAACACCAGTGACTCTCAA
> mmu-miR-7 = mmu-miR-7
  TGGCTACCGCGTGCTCTTAA
> hsa-miR-9 = miR-9
  CACAGTTAAAAAGGTCCTCAGGGA
> hsa-miR-9* = mmu-miR-9*
  GCATTATTACCTCAGGTCAGA
> hsa-miR-15b = miR-15
  AGCCAAAGCTCAGGCGATCCGA
> hsa-miR-15 = hsa-miR-15
  GAAAGAGGCTCCTCAGTGCT
> hsa-miR-16 = miR-9
  AACAAAATCACTAGTCTTCCA
> hsa-miR-16 = miR-16
  TGGCTACCGCGTGCTCTTAA
> hsa-miR-17-5p = miR-91
  ACTTTCGGTTATCTAGCTTTA
> hsa-miR-17-5p = hsa-miR-17-5p
  TGGCTACCGCGTGCTCTTAA
> hsa-miR-18 = miR-18
  TATCTGCACTAGATGCACCTTA
> hsa-miR-19 = miR-9
  TCATACAGCTAGATAACCAAAGA
> hsa-miR-19 = hsa-miR-19
  TCATACAGCTAGATAACCAAAGA
> hsa-miR-20 = miR-20
  TGTAAACCATGATGTGCTGCTA
> hsa-miR-20 = hsa-miR-20
  TGTAAACCATGATGTGCTGCTA
> hsa-miR-21 = miR-21
  TCAACATCAGTCTGATAAGCTA
> hsa-miR-22 = miR-22
  ACAGTTCTTCAACTGGCAGCTT
> hsa-miR-23b = miR-23
  GTGGTAATCCCTGGCAATGTGAT
> hsa-miR-24 = miR-24
  CTGTTCCTGCTGAACTGAGCCA
Supplementary Figure 1. miRNA expression was determined by Northern blot analysis in total RNA extracted from breast cell lines: IMECs, MCF10A[myc], SK-BR-3, MDA-MB-231, MDA-MB-468. miRNA levels were quantified from radioactive signals of Northern blots. For each miR, the highest signal obtained among these cell lines was assigned the arbitrary value of 100, and lines showing weaker signals were given correspondingly lower values. Values across samples are represented by a color scale from 0 (black) to 100 (bright yellow). Of these 92 microRNAs examined, 36 were detected in at least one of these 5 breast cell lines. IMECs is the only non-tumorigenic breast cell line in this panel. These studies reveal patterns of miRNA expression that are unique to tumorigenic lines. miR-1 and miR-155 were detected at very low levels in one experiment, but their expression could not be confirmed in subsequent experiments.
Supplementary Figure 2.
The miRNA expression profile of four breast cell lines and HeLa cells. Data shown are averaged, normalized miRNA expression obtained from two (HELA, MCF7, MCF10A, MDA-MB-453) or three (MDA-MB-231) independent microarray hybridizations. Yellow, black and blue denote high, medium and low expression, respectively. Small nuclear RNAs (U3, U6 and U8) were used as reference spots in the arrays.
Supplementary Figure 3. Decreased Expression of let-7a in invasive carcinoma. In situ hybridization (ISH) analyses using 5’ fluorescein-conjugated LNA-modified DNA probes complementary to mature let-7a were performed on 4 μm sections from archived formalin-fixed paraffin-embedded whole tissue blocks of randomly-selected patient cases represented in the tissue microarray (TMA). Concordant ISH results from tissue cores on the TMA and whole N/T tissue were obtained. Selected cases from TMA (A-C: Normal; F-H: Tumor) and whole tissue sections (D-E: Normal; I-J: Tumor) show lower levels of let-7a expression in carcinoma cells. Pictures from an additional case are displayed to illustrate the overall stronger signal in normal epithelia and the consistency of ISH signal across the entire tissue section (K-R): Normal epithelial structures (K), Normal tissue adjacent to invasive carcinoma (L & M), and core of invasive carcinoma (N). Sections were counterstained before mounting using DAPI to reveal nuclei and this is displayed in the lower panels (O-R).
Supplementary Figure 4. Expression of miR-21 in normal and tumor-associated fibroblasts. In situ hybridization (ISH) analyses using 5\' fluorescein-conjugated LNA-modified DNA probes complementary to mature miR-21 were performed on 4 μm sections from archived formalin-fixed paraffin-embedded whole tissue blocks of randomly-selected patient cases represented in the tissue microarray (TMA). Concordant ISH results from tissue cores on the TMA and whole N/T tissue were obtained. Besides expression in epithelial cells as described in the main text, miR-21 was also detected in some cases in normal fibroblast (A) and/or tumor-associated fibroblasts (B-C). In tumor tissue, miR-21 was detected only in carcinoma cells (B-C), in carcinoma cells and tumor-associated fibroblasts (D-E), or less commonly only in tumor-associated fibroblasts (F). Tissue sections were counterstained before mounting using DAPI to reveal nuclei and this is displayed in the lower panels.
Supplementary Figure 5. Expression of miR-145 in human and murine smooth muscle of blood vessels and murine colonic myoepithelia. In situ hybridization (ISH) analyses using 5’ fluorescein-conjugated LNA-modified DNA probes complementary to mature miR-145 were performed on 4 μm sections from archived formalin-fixed paraffin-embedded whole tissue blocks of randomly-selected patient cases represented in our tissue microarray (TMA) or from murine tissues. Concordant ISH results from tissue cores on the TMA and whole N/T tissue were obtained. Besides expression in mammary gland myoepithelial cells as described in the main text, miR-145 was also consistently detected in smooth muscle cells of blood vessels (A-D). In our limited sample of vascular tissue, miR-145 was detected at lower levels in tumor-associated vessels (C and D) than in matched normal vessels (A and B). panels A&C and B&D represent two different patient cases. miR-145 expression was detected in muscularis externa, muscularis mucosae and myoepithelial cells around crypts of murine colon (E) and smooth muscle cells of blood vessels in murine kidney (F). Tissue sections were counterstained with DAPI before mounting to reveal nuclei, which are displayed in the lower panels.
Supplementary Figure 6. Expression of miR-205 in ER^PR^HER2^ patient cases. *In situ* hybridization (ISH) analyses using 5’ fluorescein-conjugated LNA-modified DNA probes complementary to mature miR-205 were performed on 4 μm sections from archived formalin-fixed paraffin-embedded whole tissue blocks of ER-PR-HER2- patient cases represented in our tissue microarray (TMA). Concordant ISH results from tissue cores on the TMA and whole N/T tissue were obtained. Intense signal of miR-205 is detected in myoepithelial cells in normal tissue (A), whereas miR-205 is undetectable in blood vessels (B). Expression of miR-205 varies in intensity in invasive carcinomas from different ER-PR-HER2- cases (C-G), though consistently lower than in normal myoepithelia. Panels C-E represent tumor tissue from breast cancer survivors, whereas F-G represent tissue from two deceased patients. Tissue sections were counterstained with DAPI before mounting to reveal nuclei, which are displayed in the lower panels.
Supplementary Figure 7. Expression of miR-451 in erythrocytes in human mammary gland and murine heart. In situ hybridization (ISH) analyses using 5’ fluorescein-conjugated LNA-modified DNA probes complementary to mature miR-451 were performed on 4 μm sections from archived formalin-fixed paraffin-embedded whole tissue blocks of randomly-selected patient cases represented in our tissue microarray (TMA). ISH experiments for miR-451 were not performed on TMA, because miR-451 expression was detected exclusively in mature enucleated red blood cells within human mammary gland (A-B). miR-451 is also detected in erythrocytes in murine heart tissue. Tissue sections were counterstained with DAPI before mounting to reveal nuclei, which are displayed in the lower panels.