Supplementary Information 1

Materials and Methods

miRNA microarray

Total RNA was labeled with Cy3 and hybridized with a miRNA labeling and hybridization kit (Agilent Technologies, Santa Clara, CA, USA) according to the manufacturer's instructions. The G4470A Human MiRNA Microarray (Agilent Technologies), which consists of 534 probes for 470 human and 64 virus mature miRNAs based on Sanger miRBase release 9.1, was used. The arrays were washed and scanned with a laser confocal scanner (G2565BA, Agilent Technologies) according to the manufacturer's instructions. The intensities of fluorescence were calculated by use of Feature extraction software (Agilent Technologies).

Analysis of miRNA microarray data

Raw data obtained from Feature extraction software were transformed by setting values less than 0.01 to 0.01. Then, the data for a given miRNA were normalized to the median of that miRNA in the 5 non-neoplastic gastric epithelia, followed by filtering based on raw signal intensity of 10 or greater; three miRNAs dead in miRBase release 13.0 were excluded. Of the 470 human miRNAs, 305 were left after the filtering and were used for further statistical analyses. Differentially expressed miRNAs were identified by Mann-Whitney U test with the Benjamini and Hochberg

multiple testing correction to control the false discovery rate. miRNAs with corrected *P*-values of < 0.05 were considered to be differentially expressed.

Gene-expression microarray

Briefly, Cy3-labeled cRNA targets were generated by use of Quick Amp labeling Kit (Agilent Technologies). A human 44 K oligoarray was used for hybridization, in accordance with the manufacturer's recommendations (Agilent Technologies). A laser confocal scanner (Agilent Technologies) was used to measure signal intensities in the expression microarray analysis. Feature Extraction software (Version 9.5, Agilent Technologies) with the manufacturer's recommended settings was applied for the microarray image analysis. Analysis of the raw data obtained from Feature Extraction software was performed with Genespring GX10 Software (Agilent Technologies). For comparison among multiple arrays, probe set data were median-normalized per chip. Then, data were centered across the genes in four control samples, followed by filtering based on signal intensity and flagged values. Differentially expressed genes were identified by using Student's t test with the Benjamini and Hochberg multiple testing correction to control the false discovery rate. Genes with corrected P-values of < 0.05 were considered to be differentially expressed.

Legends for Supplementary Figures

Supplementary Figure 1. miRNA profiles of gastric carcinomas are different from those of normal tissues.

The tree was generated by unsupervised hierarchical cluster analysis of 305 miRNAs expressed in all the 27 cases. Rows, miRNAs; columns, cases. For each miRNA, red means higher expression and blue means lower expression than the average expression of the 5 normal gastric epithelium samples. The lower bars indicate intestinal-type carcinoma, diffuse-type carcinoma and normal control, represented by pink, red and green, respectively.

Supplementary Figure 2. Validation of miRNA expression data from microarray by quantitative RT-PCR.

Expressions of miR-17-5p, miR-93 and miR-29c in 27 cases as assessed by microarray correlated well with those assessed by quantitative RT-PCR. Each dot indicates the expression level of an individual case. The Y axis displays the expression level ($\log_2$). All *p*-values of data associations were less than 0.02 (Mann-Whitney U-test).

Supplementary Figure 3. Quantitative RT-PCR for miR-375 in 12 gastric carcinoma and a islet cell carcinoma cell lines.

The Y axis displays the expression levels of miR-375 (log_2) normalized by the median of 5 normal control tissues.

Supplementary Figure 4. Comparison of the expression level of miR-375 between normal controls (n=12), gastric carcinomas in situ (gastric CIS, n=9) and advanced gastric carcinomas (AGC, n=11).

To analyze the expression level of miR-375 in normal controls, gastric CISs and AGCs, histological sections were prepared from formalin-fixed, paraffin-embedded tissues, and then stained with cresyl violet. Normal and tumor cells were selectively collected from the normal and tumor tissue sections, respectively, by laser-capture microdissection. Total RNA was extracted from the collected cells with a miRNA easy FFPE kit (Qiagen). qRT-PCR was performed as described in Materials and Methods. Comparisons among three groups were done with the Kruskal-Wallis test and the Tukey Honestly Significantly Different (HSD) test. P values of less than 0.05 were considered to be statistically significant. Boxes indicate the distribution of expression values from the 25th to 75th percentile. Horizontal lines in the boxes indicate median values. Whiskers indicate 5th and 95th percentile.

Supplementary Figure 5. Comparison of miR-375 expression levels between cases with (n=14) and without (n=8) lymph node metastasis (A), or intestinal-type (n=14) and diffuse-type (n=8) cases (B).

The expression level of miR-375 was determined by qRT-PCR as described in Materials and Methods. Comparisons among three groups were done with the Kruskal-Wallis test and the Tukey Honestly Significantly Different (HSD) test. P values of less than 0.05 were considered to be statistically significant. Boxes indicate the distribution of expression values from the 25th to 75th percentile. Horizontal lines in the boxes indicate median values. Whiskers indicate the 5th and 95th percentile.

Supplementary Figure 6. Downregulation of miR-375 induces Akt phosphorylation.

An islet cell carcinoma cell line, QGP-1, which expresses a substantial amount of miR-375, was transfected with an anti-miR miRNA inhibitor targeting miR-375 (anti-miR-375, Ambion) or its control (anti-Cont) at concentrations of 30 or 90 nM, and analyzed for the phosphorylation level of Akt. At 72 h after transfection, 5 μg of the cell lysates were subjected to Western blot analysis using antibodies against the phosphorylated form of Akt (Thr308) and alpha-tubulin. Alpha-tubulin was used as an internal control. Experiments were repeated three times and a representative result is shown.