SUPPLEMENTAL METHODS

Illumina oligonucleotide microarray. Transcrip-tional profiling of Con-miR and miR-1248 overexpressing cells was performed using Illumina Sentrix BeadChips. Total RNA (isolated as in the Methods) was used to generate biotin-labeled cRNA using the Illumina TotalPrep RNA Amplification Kit. In brief, 0.5 g of total RNA was first converted into single-stranded cDNA with reverse transcriptase using an oligo-dT primer containing the T7 RNA polymerase promoter site and then copied to produce double-stranded cDNA molecules. The double stranded cDNA was cleaned and concentrated with the supplied columns and used in an overnight in vitro transcription reaction in order to generate single-stranded RNA (cRNA) incorporating biotin-16-UTP. A total of 0.75 g of biotin-labeled cRNA was hybridized at 58°C for 16 hours to Illumina's Sentrix Human HT-12 ver4 Expression BeadChips (Illumina, San Diego, CA). Each BeadChip has approximately 48,000 transcripts with about 15-fold redundancy. Next, the arrays were washed, blocked and the labeled cRNA was detected by staining with streptavidin-Cy3. Hybridized arrays were scanned using an Illumina BeadStation 500X Genetic Analysis Systems scanner and the image data extracted using the Illumina GenomeStudio software, version 1.9.0. For statistical analysis, the expression data were filtered to include only probes with a consistent signal on each chip; the probe original signal filter value was established at detection p value < 0.02. The complete normalized and raw dataset have been submitted to GEO (Accession Number; GSE51309). The resulting dataset was analyzed as described below.

Microarray data analysis. Data from microarray was analyzed using DIANE 6.0.a spreadsheet-based microarray analysis program based on SAS JMP7.0 system. First, raw microarray data were subjected to filtering by the detection p-value and Z normalization and the data are further tested for significant changes as previously described (Cheadle et al. 2003). The sample quality was initially analyzed using scatter plots, principal component analysis, and gene sample Zscores based on hierarchy clustering to exclude possible outliners. ANOVA tests were used to eliminate the genes with larger variances within each comparing group. Genes were determined to be differentially expressed after calculating the Z ratio, which indicates the fold-difference between experimental groups, and false discovery rate (fdr), which controls for the expected proportion of false rejected hypotheses. Significant changes in individual genes were considered if the p value <0.05, absolute value of Z ratio > 1.5 and fdr < 0.3. A total of 3510 differentially expressed genes were identified as significantly changed in miR-1248 overexpressing cells compared to Con-miR. Hierarchy clustering/K-means clustering and Principal Components Analysis (PCA) were performed to identify clustering within the two groups. Array data for each experimental sample was also originally hierarchically clustered in Ilumina Bead Studio version 2.0.

We employed the Parametric Analysis of Gene-set Enrichment (PAGE) algorithm for gene set enrichment analysis by using all of the genes in each sample as input against and the data set supplied by Gene Ontology Institute and MIT Broad Institute (De et al. 2010). For comparisons between Con miRNA and miR-1248 samples, the lists of differentially expressed genes and Z ratios were entered into the PAGE Pathway Analysis software to organize them according to known biological pathways. The Enrichment Zscores for each functional grouping were calculated based on the chance of mRNA abundance changes predicting these interactions and networks by z-test. The P-value was calculated by comparing the number of user-specified genes of interest participating in a given function or pathway relative to the total number of occurrences of these genes in all functional/pathway annotations stored in the knowledge base. All of the Pathways must at least have three genes found in the microarray gene set. The p value <0.05 and fdr <0.3 are the cutoff criteria for the significant pathway selection. The canonical pathway results, sorted by Z ratio, are represented by a heat map in Fig. 6D. The top 25 downregulated pathways are shown in Fig. 6D and the entire heat map of all significant canonical pathways is shown in Supporting Fig. 1.

Gene specific primers. The oligomer pairs (forward and reverse) indicated below were used for real time RT-PCR for each gene: GGCCACAGCTGCCTCTTC and CCAGCAGATTCCATACCAATGA for ACTC1 GGCTAAGAGGAGCTGATTCGTTATC and AGAGATTGGGTTACAGGGACGTAT for C1orf116 CTCCCCTGGATGAAGATGGA and GCTGCCTTGGCCGAAAT for CDK2 GCTCCTCCTGTTCGACAGTCA and ACCTTCCCCATGGTGTCTGA for GAPDH GGCTGCACCTCATTCATCATC and TCATCGCTATCTTTGCGTTCTTC for GAVD1 CCGGGAACGAAAGAGAAGCT and GCGCTTGTGGAGAGGAGTT for IL-6 CTTTCCACCCCAAATTTATCAAAG and CAGACAGAGCTCTCTTCCATCAGA for IL-8 CGCCAGCGATCATGTCTACA and CTCCATCCCGAGTGCAGAAT for LYPD3

GAACTGCTGGAAGGAGACTGGAT TTCCGGTTGAAGATTTTGACAA for TMX1 CGTGAAGGAGTACGTGAATGCT GGCGAATGAGTCCTCAATGC for ZNF185.

and

and

REFERENCES

Cheadle C, Vawter MP, Freed WJ , Becker KG. Analysis of microarray data using Z score transformation. The Journal of molecular diagnostics : JMD. 2003; 5:73-81.

De S, Zhang Y, Garner JR, Wang SA, Becker KG. Disease and phenotype gene set analysis of disease-based gene expression in mouse and human. Physiological genomics. 2010; 42A:162-167.

SUPPLEMENTARY TABLE

Please browse full text version of this manuscript to see the the Supplemental Table S1. Differentially expressed genes that are significantly changed by miR-1248 overexpression.

Figure S1. Significant canonical pathways regulated by miR-1248 expression. miR-1248 was overexpressed in HeLa cells as described in Methods. Total RNA from Con-miR and miR-1248 expressing cells were analyzed using microarray as in Methods and Supplemental Methods. Analysis was performed as in Supplemental Methods and the significant canonical pathways regulated by miR-1248 are shown by heat map. Pathways were sorted by Z ratio.

RNA POLYMERASE I PROMOTER OPENING SYSTEMIC LUPUS ERYTHEMATOSUS NTHI PATHWAY PACKAGING OF TELOMERE ENDS TELOMERE MAINTENANCE GRAFT VERSUS HOST DISEASE LYSOSOME E L'ISUSOME CYTORINE PATHWAY TNFRZ PATHWAY I IMMUNOREGULATORY INTERACTIONS BETWEEN A LYMPHOID AND A NON LYMPHOID CELL P7SNTR SIGNALS VIA NFKB GLYCOSAMINOGLYCAN DEGRADATION
 RIG I LIKE RECEPTOR SIGNALING PATHWAY IL 1R PATHWAY ILLIK PATHWAY
 CO40 PATHWAY
 GLYCOSAMINOGLYCAN BIOSYNTHESIS CHONDROITIN SULFATE
 P75 NTR RECEPTOR MEDIATED SIGNALLING
 SIG CDAPATHWAYMAP SIG CD40PATHWAYMAP P75NTR RECRUITS SIGNALLING COMPLEXES DOWNSTREAM EVENTS IN GPCR SIGNALING DOWNSTREAM EVENTS IN GPCR SIGNALING NAA POLYMERASE I PROMOTER CLEARANCE VIRAL DSRNA TLR3 TRIF COMPLEX ACTIVATES RIP1 NATURAL KILLER CELL MEDIATED CYTOTOXICITY CELL ADHESION MOLECUES CAMS NFKB PATHWAY GPCR LIGAND BINDING ALLOGRAFT REJECTION B CELL ANTIGEN RECEPTOR NFKB IS ACTIVATED AND SIGNALS SURVIVAL CLASS A1 RHODOPSIN LIKE RECEPTORS NRAGE SIGNALS DEATH THROUGH JNK COPI MEDIATED TRANSPORT CHO GTARASE CYCLE RHO GTPASE CYCLE KERATINOCYTE PATHWAY MAPK SIGNALING PATHWAY ACETYLCHOLINE NEUROTRANSMITTER RELEASE CYCLE NOREPINEPHRINE NEUROTRANSMITTER RELEASE CYCLE TYPE I DIABETES MELLITUS CELL JUNCTION ORGANIZATION SIGNALING IN IMMUNE SYSTEM ECM RECEPTOR INTERACTION PATHWAYS IN CANCER AUTOIMMUNE THYROID DISEASE SEMA3A PLEXIN REPULSION SIGNALING BY INHIBITING INTEGRIN ADHESION SEMAGA PLEXIN REPOLSION SIGNALING BY INHIBITING INTEGRIN ADHESION
ENDOCYTOSIS
CYTOSOLIC DNA SENSING PATHWAY
NCAMI INTERACTIONS
OTHER GLYCAN DEGRADATION
DOPAMINE NEUROTRANSMITTER RELEASE CYCLE
MARK PATHWAY
G ALPHA O SIGNALING SEVENTS
COMPLEMENT AND COAGULATION CASCADES
PEPTIDE LIGAND BINDING RECEPTORS
CELLEXTRACELLULAR MATRIX INTERACTIONS
SEROTONN NEUROTRANSMITTER RELEASE CYCLE
NEUROTROPHIN SIGNALING PATHWAY
HUMAN TAKI ACTIVATES NFKB BY PHOSPHORYLATION AND ACTIVATION OF IKKS COMPLEX
HEMATOPOFITIC CELL LIMENGEE
GRIRH SIGNALING PATHWAY
GLUCOSE REGULATION OF INSULIN SECRETION
NEURORACTIVE LIGAND RECEPTORI ENDOCYTOSIS NEUROACTIVE LIGAND RECEPTOR INTERACTION
 DEATH RECEPTOR SIGNALLING
 CALCIUM SIGNALING PATHWAY
 G ALPHA I SIGNALLING EVENTS G ALPHAT ISIGNALLING EVENTS 418B PATHWAY TALLI PATHWAY RNA PATHWAY TIGHT JUNCTION INTERACTIONS CTL PATHWAY THELPER PATHWAY TCYTOTOXIC PATHWAY TCYTOTOXIC PATHWAY TRANSLOCATION OF ZAP70 TO IMMUNOLOGICAL SYNAPSE TRANSLOCATION OF ZAP70 TO IMMUNOLOGICAL SYNAPSE
 PD1 SIGNALING
 BLYMPHOCYTE PATHWAY
 OLFACTORY SIGNALING PATHWAY
 SET PATHWAY
 ATRBRCA PATHWAY
 ATRBRCA PATHWAY
 ATRBRCA PATHWAY
 ASSOCIATION OF TRIC CCT WITH TARGET PROTEINS DURING BIOSYNTHESIS
 RNA POLYMERASE III TRANSCRIPTION TERMINATION
 CARMI PATHWAY
 POGP PATHWAY
 EGF PATHWAY
 N GLYCAN BIOSYNTHESIS
 RNA POLYMERASE
 PHASE II CONJUGATION
 CDT1 ASSOCIATION WITH THE CDC6 ORC ORIGIN COMPLEX
 PROPANOATE METABOLISM PROPANOATE METABOLISM PURINE RIBONUCLEOSIDE MONOPHOSPHATE BIOSYNTHESIS PYRUVATE METABOLISM METABOLISM OF XENOBIOTICS BY CYTOCHROME P450 LATE PHASE OF HIV LIFE CYCLE MRNA 3 END PROCESSING RNA POLYMERASE I PROMOTER ESCAPE INA POLYMERASE I TRANSCRIPTION INITIATION RNA POLYMERASE I TRANSCRIPTION INITIATION HIV1 TRANSCRIPTION INITIATION HIVI TRANSCRIPTION INITATION
 DULAI. INCISION REACTION IN GG NER
 TRANSFORMATION OF LANOSTEROL TO CHOLESTEROL
 PROCESSING OF CAPPED INTRON CONTAINING PRE MRNA
 MRNA SPLICING
 DULAI. INCISION REACTION IN TC NER
 RNA POLYMERASE II TRANSCRIPTION
 ELONGATION AND PROCESSING OF CAPPED TRANSCRIPTS
 GENE EXPRESSION
 STEROID BIOSYNTHESIS

4.51 -3.40

-2.303

-1.196

0.2143

1.6246 3.0349 4.4451

- STEROID BIOSYNTHESIS FORMATION AND MATURATION OF MRNA TRANSCRIPT SPLICEOSOME