SUPPLEMENTAL MATERIAL S11

Microarray analysis

Total RNA (1 µg) was labeled according to the manufacturer's protocols and hybridized to NCode human microarrays (Life Technologies). An Agilent 2 µm high-resolution C scanner (Cat #G2365CA) was used to scan the slides and the data was normalized and analyzed using GeneSpring software (Agilent). Data was clustered according to the hierarchical cluster algorithm from the GeneSpringTM software and R-package (www.r-project.org). For determining differential expression, data was analyzed using the Linear Models for Microarray Data (LIMMA) software package via the R Project for Statistical Computing (www.r-project.org). Data was background-corrected, normalized both within and between arrays[1], and differential expression analysis was performed by fitting a linear model of the data to the experimental design matrix and then calculating Bayesian statistics adjusted for multiple testing using Benjamini-Hochberg analysis[2]. Subsequent data analysis was performed using NRED[3,4]. Significantly differentially expressed genes were defined as those with p values > 0.015 and > 2 fold difference in level of expression in WM1552C relative to melanocytes.

RNA extraction and quantitative real-time PCR

Total RNA was isolated using Trizol (Life Technologies) with subsequent quantification by using an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA). 1 µg of total RNA was reverse transcribed using the High Capacity cDNA kit (Applied Biosystems Inc., Foster City, CA, USA), and qRT-PCR was carried out using TaqMan Assays in the 7500 Real-Time PCR System (Applied Biosystems) according to the manufacturer's protocols. SDS1.2.3 software (Applied Biosystems) was used for comparative Ct analysis with *GAPDH* serving as the endogenous control.

For the human tissue expression analysis, total RNA from 20 different tissues was purchased from Ambion. 1 µg was oligo-dT reverse transcribed using Superscript III (Life Technologies) and qRT-PCR was carried out using the TaqMan Noncoding RNA Assays (*SPRY4-IN1*) and TaqMan Gene Expression Assays (*SPRY4.1* and *SPRY4.2*) in the 7900 Real-Time PCR System (Applied Biosystems) according to the manufacturer's protocols. SDS2.3 software (Applied Biosystems) was used for comparative Ct analysis with *RPLO* serving as the endogenous control.

Northern blot analysis

Total RNA isolated from each sample (20 µg) was separated in 15% TBE-urea polyacrylamide gels by electrophoresis. The RNA was electroblotted onto nylon membranes, cross-linked by ultraviolet light, prehybridized in Ultrahyb-Oligo (Ambion) for 30 min at 42^{o}C, and hybridized at 100 nM with a 5'-biotinylated anti-miRNA DNA oligonucleotide (TCCACTGGGCATATTCTAAAA) at 42^{o}C overnight. The blots were then washed, and the signal was detected by chemiluminiscence (Brightstar Detection kit, Ambion). Anti-U6 probes (10 pM) were used as a reference control.

Phosphatidylserine externalization

Cell death was studied by flow cytometry using Annexin V. Annexin V binds to the negatively charged phospholipids located on the inner surface of the plasma membrane. Annexin V conjugated to fluorescein together with propidium iodide is used to detect non-apoptotic live cells (Annexin V negative, PI negative), early apoptotic cells (Annexin V positive, PI negative) and late apoptotic or necrotic cells (PI positive). Transfected (Stealth siRNA) and untransfected cells were washed twice with PBS, trypsinized and washed again with PBS. Cells were re-suspended in binding buffer (10 mM HEPES + 10 mM NaOH - pH 7.4, 140 mM NaCl, 2.5 mM $CaCl_2$) at a density of 0.5 – 1x 10^6 cell/mL. To the 100 µL of cell suspension, 3 µL of Annexin V FITC (B.D. Pharmingen) and 10 µL of PI (10 µg/mL) was added and gently vortexed. The cells were incubated at room temperature for 15 min in the dark. To each of the samples, 400 µL of binding buffer was added and placed on ice. Flow cytometric measurements were carried using a FACS caliber flow cytometer (Becton and Dickinson, USA). Green fluorescence due to Annexin V-FITC was collected on the FL1 channel and red fluorescence due to PI was collected on the FL2 channel on a log scale. A minimum of 10,000 cells per sample was acquired and analyzed using CellQuest software (Becton and Dickinson).

References

1. Smyth, G.K. & Speed, T. Normalization of cDNA microarray data. *Methods* **31**, 265-73 (2003).
2. Smyth, G.K. Linear models and empirical bayes methods for assessing differential expression in microarray experiments. *Stat Appl Genet Mol Biol* **3**, Article3 (2004).
3. Dinger, M.E. et al. NRED: a database of long noncoding RNA expression. *Nucleic Acids Res* **37**, D122-6 (2009).
4. Dinger, M.E. et al. Long noncoding RNAs in mouse embryonic stem cell pluripotency and differentiation. *Genome Res* **18**, 1433-45 (2008).