Supplemental Material

METHODS

miRNA Microarray

MiRNAs were profiled in both COPD (N=19) and Normal Smokers (N=8) using Exiqon miRNA microarrays as per the manufacturer’s instructions (Exiqon, Woburn, MA). In brief, 15µL of total RNA was labeled using the miRCURY LNA microRNA Power Labeling Kit (Exiqon, Woburn, MA). The RNA was dephosphorylated with calf intestinal alkaline phosphatase and denatured by heating to 95ºC. Hy3 or Hy5 dyes were ligated to the dephosphorylated single-stranded RNA. The labeled miRNA probes were hybridized to the miRCURY LNA arrays v.11.0 for 16 hours at 56º C. After hybridization, the arrays were washed and scanned at 5µm resolution using a ScanArray Express (Perkin Elmer, Waltham, MA).

Messenger RNA Microarray

Samples were prepared for mRNA microarray analysis using Agilent Quick Amp Labeling technologies (Santa Clara, CA), which uses T7 polymerase to amplify targets and cyanine 3- or cyanine 5- to fluorescently label RNA targets. Approximately 500 ng of RNA was used in the labeling and amplification reactions. Following the labeling and amplification of the samples, Qiagen’s RNeasy mini kit (Valencia, CA) was used to purify the labeled cRNA. The purified cRNA was then quantified using the NanoDrop
ND- 1000 UV-Vis Spectrophotometer (Wilmington, DE), and the yield and specific activity of labeled targets were calculated. The labeled cDNA targets were fragmented, mixed with hybridization buffer, and incubated at 65°C overnight. The hybridized slides were then washed and scanned with ScanArray Express (Perkin Elmer, Waltham, MA).

**mRNA and miRNA microarray data processing**

For mRNA data, raw intensities from all samples were merged, normalized using quantile method, and transformed into log2-scale. Present probes with mean intensity over all samples larger than the global mean intensity were chosen and used for further statistical analysis. As for miRNA data, the intensity for each miRNA was computed by taking median value of four replicates since each miRNA on the microarray has four identical replicated probes. MiRNA hybridization intensities were processed as described above for mRNA.

LIMMA and QVALUE, R packages were used to perform statistical testing of differential mRNA and miRNA expression between control smoker and COPD samples and compute positive false discovery rate (pFDR), respectively. We used present probes for statistical testing to increase the ratio of true positives to false positives and defined differentially expressed mRNAs and miRNAs as having pFDR<0.05 with at least ±1.5 fold-change between the groups. For multiple probes mapped to the same Entrez ID or miRNA symbol, the probe with the smallest pFDR value was chosen to represent the differentially expressed mRNA and miRNA.

**In Situ Hybridization and Co-localization**
In brief, the in situ hybridization was done following a protease digestion that utilizes a probe cocktail containing 1 pmol/microL of the 5' digoxigenin labeled LNA probe (Exiqon). Following an overnight hybridization and development in NBT/BCIP, SMAD7 was assessed using a 30 minute antigen retrieval and a dilution of 1:200 and AE 1/3 (Dako) 1:100 with a 1 hour and 30 minute incubation. After development via peroxidase/DAB, the slide was analyzed with the Nuance system (Cambridge Research Institute).

Transfection Studies and Western

Beas2Bs (bronchial epithelial cell line) were cultured in RPMI (Fisher Manassas, Va.) with 10% fetal bovine serum and Penicillin/Streptomycin and maintained at 37°C humidified 5% CO₂ chamber. Transfection with scramble, pre-miR15b or anti-miR15b was performed using Lipofectamine (Invitrogen Carlsbad, Ca.) following manufacturer’s protocol at the specified concentrations. The media was replaced within 24 hours to reduce the toxicity of the transfection complex. Cells were harvested at 24 hours and assessed by QRT-PCR for adequate miR-15b induction. Cells were harvested after 72 hours in RIPA with the addition of phosphatase and protease inhibitors. Protein expression of SMAD7 (Santa Cruz, Ca.), SMURF 2 (Sigma, St. Louis, MO.) and Decorin (Cell Signaling, Danvers, MA.) were then determined by SDS-PAGE.

Migration Assay
Migration rate was assayed using the IBIDI culture insert (München, Germany). The migration insert was set into a 24 well plate. 20K cells were transfected with either scrambled miR or pre-miR15b and suspended in 80ul of media and plated into each well. After 16 hours, the insert was removed and the cells were washed 6 times to remove any non-adherent cells and replaced with full media. Images were taken on an Olympus IX81 immediately (Time 0) and again at 8 and 24 hours. Seven random widths were measured across the wound at Time 0. The number of pixels was converted to microns and the line was then superimposed onto the subsequent time points. The mean and standard deviation of the 7 lines was then calculated using ImageJ software to determine distance of wound closure. Experiments were performed in triplicate.

Results

Study Subjects

Study subjects were grouped according to GOLD classification based on the reported post-bronchodilator FEV1 (forced expiratory volume in one second) percent predicted. All of the COPD subjects by definition had an FEV1/FVC ratio less than the age-predicted reference normal values. The smokers without airflow limitation had a normal FEV1/FVC ratio.