Supplemental File

Increased expression of miR146a dysregulates TLR2 signaling in airway epithelial cells from patients with chronic obstructive pulmonary disease

Hymavathi Reddy-Vari[1], Yerin Kim[1], Charu Rajput[1], and Umadevi S Sajjan[*1,2]

Supplemental Figure 1. HBD2 expression in normal and COPD cell cultures at basal levels and after NTHi infection. (A) Total RNA was isolated from the mucociliary-differentiated COPD and normal bronchial epithelial cell cultures and the expression of DEFB4 and G3PDH was determined by qPCR. The data was normalized to G3PDH and expressed as range with median and the statistical significance was calculated by Mann-Whitney test. (B) The normal and COPD cell cultures were infected with NTHi or treated with PBS (control) and incubated for 3 or 24h. Total RNA was isolated and the expression of *DEFB4* and *GAPDH* was determined by RT-qPCR using gene-specific primers. *DEFB4* expression was normalized to *GAPDH* and expressed as fold increase over PBS-treated cultures. The data represent range with median (n=6) and the statistical significance calculated by ANOVA on ranks with Kruskal-Wallis H.

Supplemental Figure 2. NTHi does not induce the expression of cathelicidin (*CAMP*) in bronchial epithelial cell cultures. Normal and COPD cultures were infected with NTHi and examined for the expression of cathelicidin by RT-qPCR. Cathelicidin mRNA expression was normalized to *GAPDH*. Paired analysis with Wilcoxon signed-rank test was performed to determine the statistical significance.

Supplemental Figure 3. Expression of TLR2 and IRAK-M is not altered in COPD bronchial epithelial cell cultures. (A and C) Total protein isolated from COPD and normal mucociliary-differentiated cell cultures was subjected to electrophoreses, proteins transferred to nitrocellulose membranes, blocked with 5% BSA and the membranes were cut using prestained markers as reference. The membranes were probed with antibodies to TLR2, IRAK-M or GAPDH. (B and D) The band intensities were quantified by ImageJ software, and data were expressed as fold change over GAPDH. Data represent mean ± SD calculated from 4-6 normal and COPD cultures for TLR2 and 4 each normal and COPD cell cultures for IRAK-M.

Supplemental Figure 4. IRAK-1 shRNA does not alter the expression of IRAK-4 and IRAK-M in airway epithelial cell cultures. Normal basal cells transduced with non-targeting or IRAK-1 shRNA were grown as mucociliary-differentiated cultures. Total protein was subjected to electrophoresis, proteins transferred to membranes, blocked with 5% BSA and the membranes were cut using prestained markers as reference and probed with IRAK-4, IRAK-M or GAPDH antibodies (n=3).

Supplemental Figure 5. COPD epithelial cells transduced with miR146a inhibitor does not alter the expression of IRAK-4 and IRAK-M. Total protein from normal epithelial cell cultures (n=2) or COPD epithelial cells cultures transduced with scrambled or miR146a inhibitor (n=3) were subjected to electrophoresis, proteins transferred to membrane and the membrane was cut using prestained markers as reference and probed with antibodies to IRAK-4, IRAK-M or GAPDH.

Supplemental Table 1. Study Population

Subject #	Age (Yr)	Gender	FEV1 (% predicted)	Smoking history (pk years)
COPD				
C6	49	Female	17	25
C10	53	Female	14	37.5
C11	59	Male	22	70
C12	49	Female	13	136
C14	55	Male	17	25
C15	45	Female	17	31.2
C18	67	Male	20	90
C19	64	Male	29	45
C713	73	Male	19	70
C1190	68	Male	42	84
C1209	79	Male	34	50
C1236	59	Male	18	30
Healthy non-smokers				
N17	33	Male		
N18	16	Male		
N19	50	Female		
N20	52	Male		
N22	62	Male		
N24	50	Female		
N27	48	Female		
N31	60	Female		
N53	54	Male		
N396	46	Male		
N1201	70	Male		
N1220	67	Male		
N1259	77	Male		

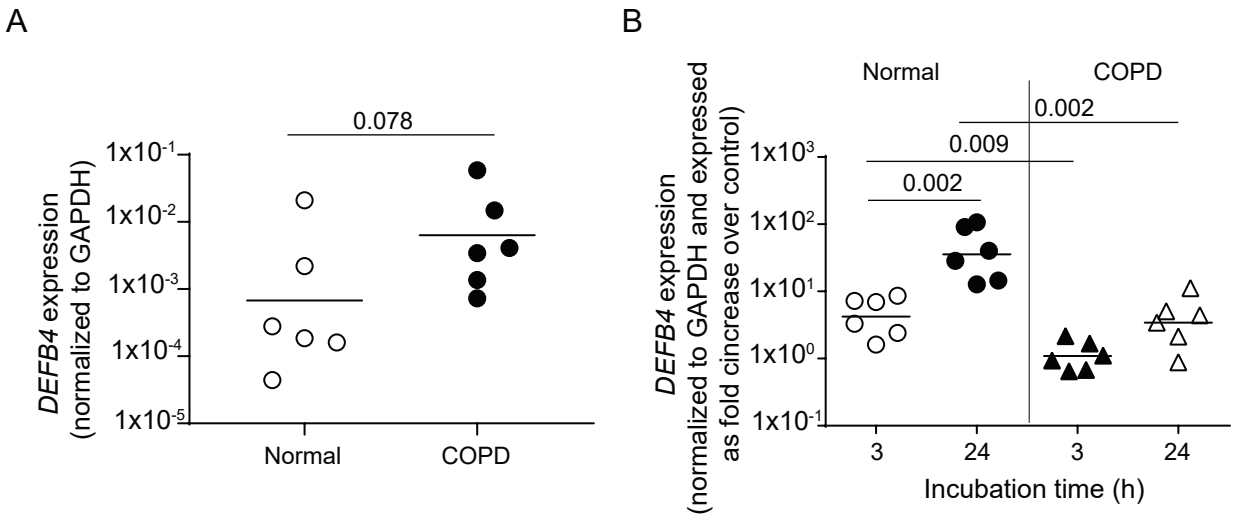
Airway basal cells from C15, C18, C713, C1190, C1209, C1236, N31, N53, N396, N1201, N1220 and N1259 were used throughout the study

For mechanistic studies (gene knockdown studies) airway basal cells from N31, N51, C15, and C17 were used.

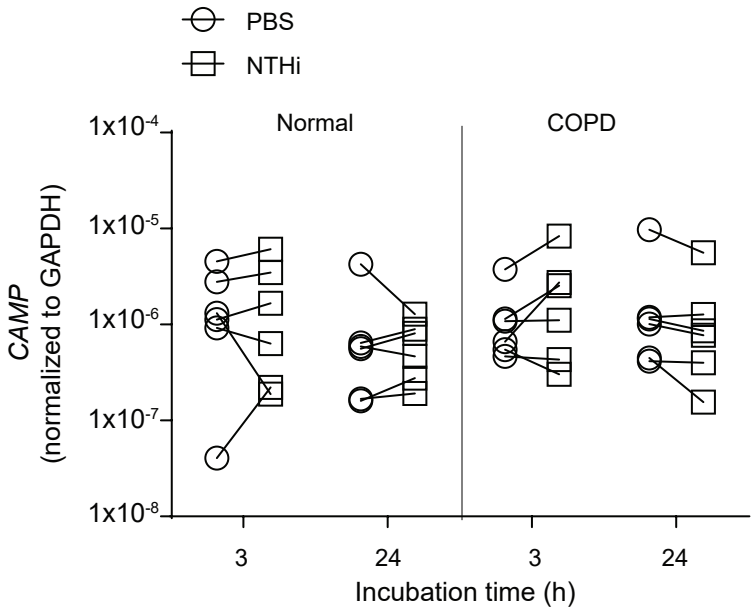
For expression of miR146a, we also used cells from all the donors

Lung tissues from Subject # C1190, C1209, C1236, N1201, N1220, and N1259 were used for immunofluorescence microscopy

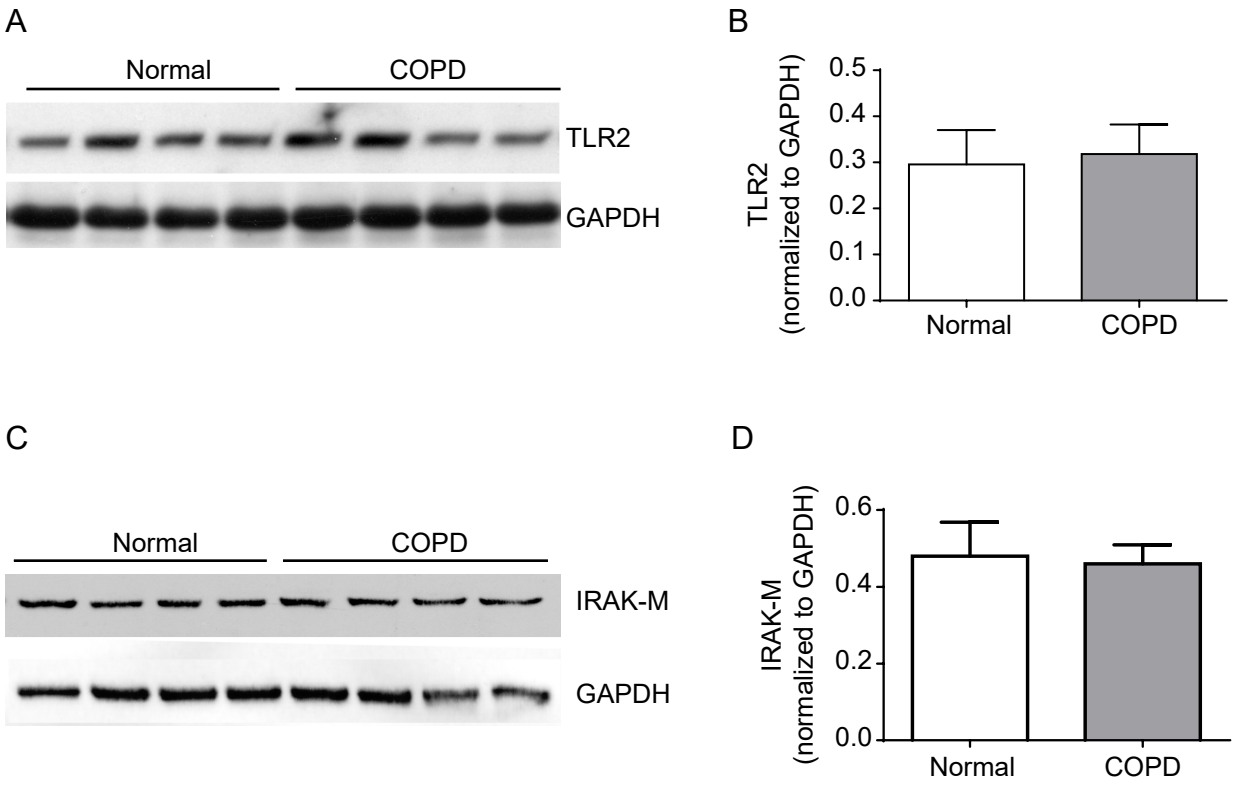
Supplemental Figure 1



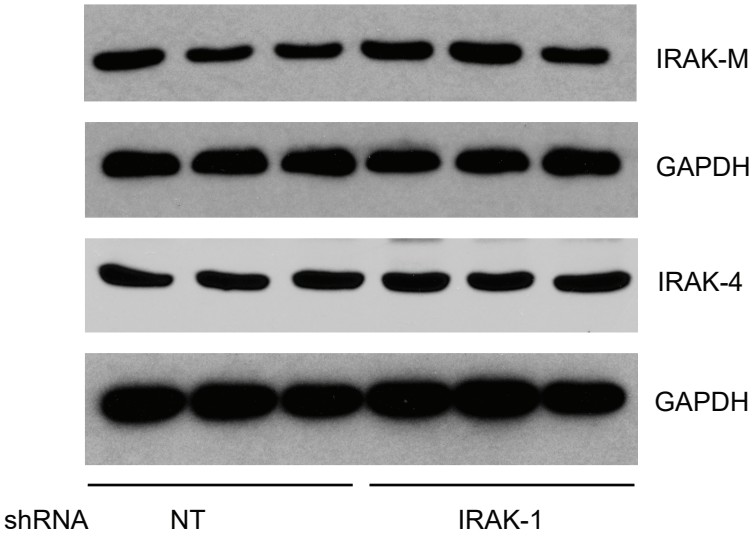
Supplemental Figure 2



Supplemental Figure 3



Supplemental Figure 4



Supplemental Figure 5