Mucin Production During Pre- and Post-Natal Mouse Lung Development

Michelle G. Roy[1], Mahdis Rahmani[1], Jesus R. Hernandez[2], Samantha N. Alexander[1], Camille Ehre[3], Samuel B. Ho[4], Christopher M. Evans[1,2]

[1]Department of Pulmonary Medicine, The University of Texas M.D. Anderson Cancer Center, Houston, Texas. [2]Tecnológico de Monterrey Escuela de Medicina, Monterrey, Nuevo León, México. [3]Cystic Fibrosis/Pulmonary Research and Treatment Center, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina. [4]Department of Medicine, Veterans Affairs San Diego Healthcare System and University of California, San Diego, California

ONLINE DATA SUPPLEMENT

Corresponding Author:
Christopher M. Evans, PhD
Department of Pulmonary Medicine
The University of Texas M.D. Anderson Cancer Center
2121 West Holcombe Boulevard
Room 704
Houston, Texas 77030
(713) 563-7431
(713) 563-0411 [FAX]
cevans@mdanderson.org

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Table E1. Primers used in the current studies.

Transcript	Sequence		Genbank Source
Muc1	Sense	5'-GGT TGC TTT GGC TAT CGT CTA TTT	NM_013605
	Anti	5'-AAA GAT GTC CAG CTG CCC ATA	
	Probe	5'-TGT GCC AGT GCC GCC GAA AGA	
Muc2	Sense	5'-TGA TGA GAT CTG CAA GTC TTG TAC AT	XM_620587
	Anti	5'-TGT TAA GAA TCT TCC CTT CAT CTG G	
	Probe	5'-CAC CAA CAC GTC AAA AAT CGA ATG CCA	
Muc4	Sense	5'-TGC CAG GAC TGT GGA TTT TAA CT	NM_080457
	Anti	5'-CTG ACC ATC TGT AAC GTA GAA GGA	
	Probe	5'-AAG ATC CTG ATT GGC TTT CCC CTC GG	
Muc5ac	Sense	5'-AGA ATA TCT TTC AGG ACC CCT GCT	AJ511870
	Anti	5'-ACA CCA GTG CTG AGC ATA CTT TT	
	Probe	5'-CTC AGC GTG GAG AAT G	
Muc5b	Sense	5'-CAT CCA TCC CAT TTC TAC CAC AA	NM_028801
	Anti	5'-AGG CAA CAT AGA GTT GCT TTT GG	
	Probe	5'-ACA ACC AAG AAC CCT CAA ACA CTA GTC AC AG	
Muc16	Sense	5'-CGT GCC TGA TGT GCT GTT TC	XM_911929
	Anti	5'-GCA TGA CGT TGA ACT TGG TAG TCT	
	Probe	5'-TGG TGA CTG TCT GCA GGA GAA AAA AGG AG	
Muc19	Sense	5'-GCA ACC CCA CAG GCT TAG TG	
	Anti	5'-TTT GAA TCG TAG ATT CTC TCT TCT TCT G	NM_207243
	Probe	5'-TCA GGA CTG CCC AAA GCA AAC ATG G	
CCSP	Sense	5'-CCT TTC AAC CCT GGC TCA GA	X67702
	Anti	5'-AGG GTA TCC ACC AGT CTC TTC AG	
	Probe	5'-CAA AAT GCG GGC ACC CAG	
γ-Actin	Sense	5'-AAT CGC CGC ACT CGT CA	NM_009609
	Anti	5'-CGC CAG CAA AGC CGG	
	Probe	5'- CAA TGG CTC CGG CAT GTG CAA A	

Table E2. AB-PAS positive cells in the conducting airways.

		Cartilagenous bronchi		Axial bronchi		Bronchioles	
Time	*n*	cells/mm ± SEM	*%	cells/mm ± SEM	*%	cells/mm ± SEM	*%
E12.5	*6*	0.0 ±0.0		0.0 ±0.0	0	†N/D	
E14.5	*5*	0.0 ±0.0		0.0 ±0.0	0	†N/D	
E15.5	*5*	‖97.8 ±16.7	46	‖57.2 ±8.6	25	0.0 ±0.0	0
E16.5	*5*	‖35.0 ±10.8	17	‖36.1 ±4.1	13	0.0 ±0.0	0
E17.5	*4*	†,‖95.1 ±10.2	35	‖121.8 ±47.1	32	1.8 ±1.8	1
E18.5	*5*	‖75.1 ±9.5	40	§,‖35.3 ±5.5	21	†5.2 ±1.5	4
PN5	*8*	‖58.6 ±6.9	30	§17.2 ±10.4	9	0.0 ±0.0	0
PN14	*6*	‖74.4 ±11.7	41	†,‖88.3 ±8.4	44	0.5 ±0.5	<1
PN28	*5*	‖43.8 ±12.3	26	‖35.9 ±10.1	15	0.4 ±0.4	<1

* Percentages are calculated against total numbers of cells above the basement membrane in each image used.

† ‘N/D’ - Not determined due to high levels of intracellular glycogen staining (1).

‡ identifies statistically significant differences ($p<0.05$) between AB-PAS positive cell numbers at the indicated timepoints vs. AB-PAS positive cell numbers at PN28 by ANOVA.

§ identifies statistically significant differences between AB-PAS positive cell numbers in axial vs. cartilaginous bronchi at the same timepoint by ANOVA ($p<0.05$).

|| identifies statistically significant differences between AB-PAS positive cell number in cartilaginous and axial bronchi vs. bronchioles at the same timepoint by ANOVA ($p<0.05$).

Table E3. Mean values of individual data points in Figure 2 (fmol per 100 ng input RNA ± SEM and transcript per γ-actin (mol/mol)).

		Muc2		Muc5ac		Muc5b	
Time	*n*	fmol ± SEM	per γ-actin	fmol ± SEM	per γ-actin	fmol ± SEM	per γ-actin
E14.5	*4*	0.185 ±0.104	0.026	0.142 ±0.042	0.019	362 ±75.4	*37.6
E17.5	*8*	0.032 ±0.017	0.007	0.156 ±0.042	†0.030	293 ±27.0	*62.4
E18.5	*4*	0.011 ±0.006	0.002	0.086 ±0.016	†0.014	357 ±59.3	*54.3
PN5	*3*	0.047 ±0.025	0.012	0.111 ±0.042	0.031	369 ±16.3	*,‡101.5
PN14	*6*	0.035 ±0.016	0.005	1.088 ±0.876	0.146	348 ±48.5	*43.8
PN28	*6*	0.007 ±0.004	0.001	0.356 ±0.139	0.062	461 ±36.9	*,‡84.2

		Muc1		Muc4		Muc16	
Time	*n*	fmol ± SEM	per γ-actin	fmol ± SEM	per γ-actin	fmol ± SEM	per γ-actin
E14.5	*4*	4.8 ±2.77	0.42	1.0 ±0.190	0.08	0.013 ±0.003	0.002
E17.5	*8*	6.2 ±2.32	0.94	1.7 ±0.496	0.33	0.020 ±0.008	0.004
E18.5	*4*	8.1 ±4.64	0.93	1.5 ±0.234	0.29	0.005 ±0.001	0.001
PN5	*3*	6.8 ±2.85	1.80	3.5 ±2.038	0.97	0.025 ±0.014	0.007
PN14	*6*	16.6 ±4.49	1.96	2.2 ±0.595	0.27	0.023 ±0.008	0.003
PN28	*6*	10.6 ±3.90	1.74	1.5 ±0.216	0.27	0.020 ±0.006	0.003

		CCSP		γ-actin
Time	*n*	fmol ± SEM	per γ-actin	fmol ± SEM
E14.5	*4*	1.8 ±1.4	0.14	9.88 ±2.74
E17.5	*8*	6.1 ±3.2	0.73	6.07 ±1.71
E18.5	*4*	11.8 ±7.2	1.6	7.44 ±2.33
PN5	*3*	5354 ±4978	1537	3.92 ±0.86
PN14	*6*	7870 ±5000	931	8.47 ±1.44
PN28	*6*	9231 ±6212	1252	5.90 ±0.76

* identifies significance between Muc5b mRNA levels and those of Muc5ac and Muc2.

† identifies significance between Muc5ac and Muc2.

‡ identifies significance between E14.5 and later values.

Differences in Muc5ac and Muc2 levels are not significant over time.

Table E4. Muc5b protein production in developing airways.

Time	*n*	Cartilaginous bronchi nl/mm^2 ± SEM	Axial bronchi nl/mm^2 ± SEM	Bronchioles nl/mm^2 ± SEM
E12.5	*1*	0.0	0.0	0.0
E14.5	*1*	0.9	0.0	0.0
E15.5	*3*	†,§1.0 ± 0.1	1.2 ± 0.8	0.0 ± 0.0
E16.5	*3*	1.1 ± 0.9	*0.4 ± 0.3	0.0 ± 0.0
E17.5	*2*	†,§1.2 ± 0.2	0.7 ± 0.5	0.0 ± 0.0
E18.5	*3*	†,‡3.9 ± 1.1	*0.5 ± 0.3	0.0 ± 0.0
PN5	*3*	1.0 ± 0.1	*0.4 ± 0.1	0.0 ± 0.0
PN14	*3*	§2.5 ± 0.7	§1.4 ± 0.4	0.0 ± 0.0
PN28	*3*	†,§1.5 ± 0.5	†,§1.7 ± 0.2	0.0 ± 0.0

'*' identifies statistically significant differences ($p<0.05$) between Muc5b volume density at the indicated timepoints vs. Muc5b volume density at PN28 by ANOVA.

'†' identifies statistically significant differences ($p<0.05$) between Muc5b and Muc5ac volume densities at the same timepoints and airway levels by ANOVA. See Table E3 for Muc5ac values.

'‡' identifies statistically significant differences ($p<0.05$) between Muc5b volume densities in cartilaginous vs. axial bronchi at the same timepoint by ANOVA.

'§' identifies statistically significant differences between ($p<0.05$) Muc5b volume densities in cartilaginous and axial bronchi vs. bronchioles at the same timepoint by ANOVA.

Table E5. Muc5ac protein production in developing airways.

Time	*n*	Cart. bronchi nl/mm^2 ± SEM	Axial bronchi nl/mm^2 ± SEM	Bronchioles nl/mm^2 ± SEM
E12.5	*1*	*N/D	0.0	0.0
E14.5	*1*	0.0	0.0	0.0
E15.5	*3*	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
E16.5	*2*	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
E17.5	*2*	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
E18.5	*2*	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
PN5	*2*	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
PN14	*3*	†0.6 ± 0.3	†0.5 ± 0.1	0.0 ± 0.0
PN28	*3*	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

* ‘N/D’ - not determined due to sampling.

‘†’ identifies statistically significance differences of changes in Muc5ac volume density over the time course of the study by ANOVA ($p<0.05$).

Figure E1. Quantitative RT-PCR analysis of membrane associated mucin expression in the developing mouse lung. Gene specific probes demonstrate Muc1 (filled circles), Muc4 (open circles), and Muc16 (filled inverted triangles) mucin levels compared to γ-actin. Data are means ± standard errors (n = 3-8 animals/timepoint). Data were analyzed by ANOVA; a p-value < 0.05 was considered a significant difference. ‘ ̇’ identifies significance between Muc1 mRNA levels and those of Muc4 and Muc16. ‘†’ identifies significance between Muc4 and Muc16. ‘‡’ identifies significance between E14.5 and later values for individual mucins. Differences in Muc16 levels were not significant over time.

Reference List

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