Impacts of Cystic Fibrosis on the Pulmonary Tissues of Sheep

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Introduction

Cystic Fibrosis (CF) is a recessive human genetic disease that is caused by mutations in the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) gene. This gene is responsible for transport of Cl^- and HCO_3^- anions in epithelial cells (Fig. 1).

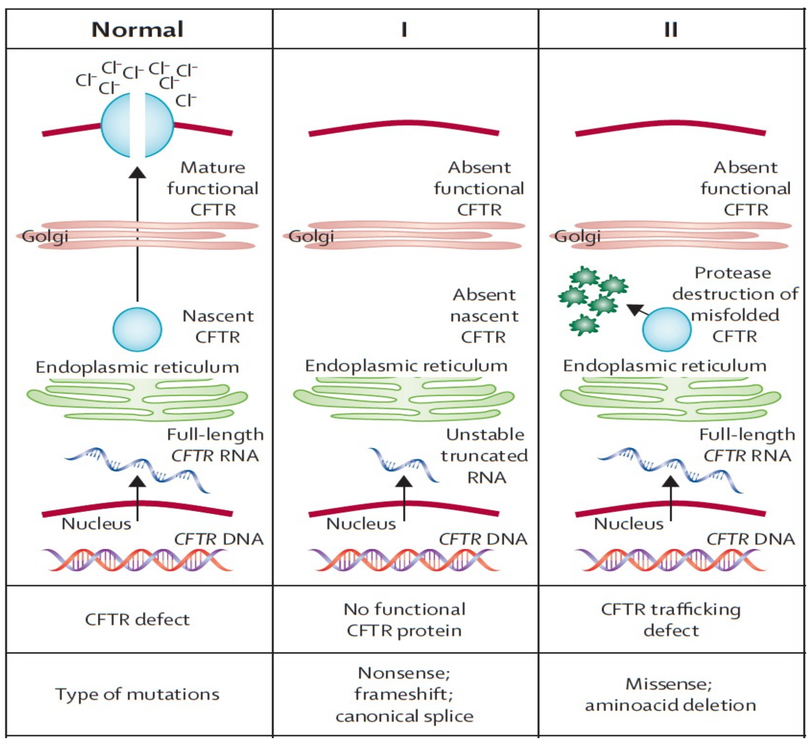

Figure 1. The CFTR expression in normal and mutated cells. Picture adapted from Elborn, 2016.

Among the ~2000 known CF mutations, F508del mutation is found in 84% and G542X mutation in 4.6% of the CF patients in the U.S., respectively. The F508del mutation is a Class II mutation characterized by the deletion of the 'CTT' nucleotides that ultimately deletes the Phenylalanine residue at the position 508 of CFTR. It causes the misfolding of the CFTR protein, which is further degraded by proteases. The G542X is a Class I nonsense mutation associated with nonsense-mediated decay of the mutant transcript causing the absence of protein production (Fig. 1). We previously generated *CFTR*$^{F508del/F508del}$ and *CFTR*$^{G542X/G542X}$ lambs using CRISPR/Cas9 and Somatic Cell Nuclear Transfer (SCNT) techniques (Fig. 2).

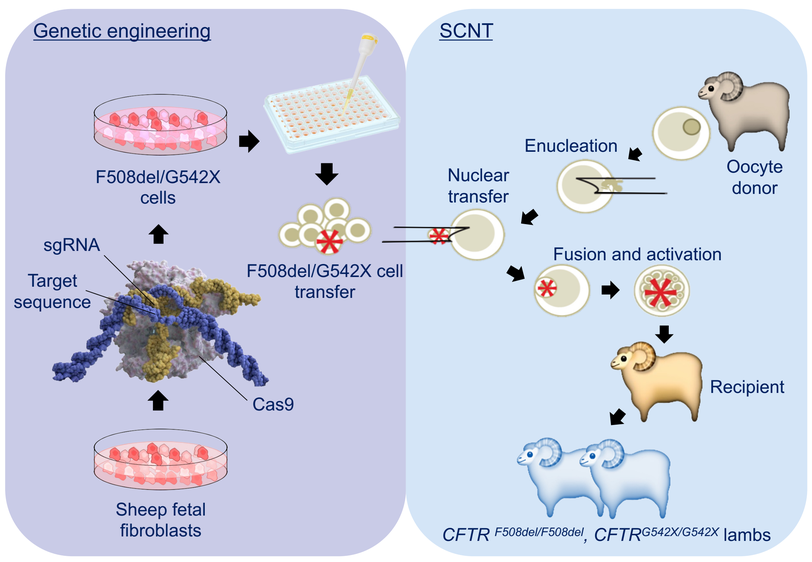

Figure 2. Production of *CFTR*$^{F508del/F508del}$ and *CFTR*$^{G542X/G542X}$ lambs.

Objectives

- We hypothesized that the absence of a functional CFTR protein will cause structural abnormalities in the airway such as cartilage defects, altered smooth muscle bunds, and hypoplastic submucosal glands.
- In this study, we evaluated if sheep may be an effective model to study the development of pulmonary structural airways and impacts of nonfunctional CFTR proteins

Methods

Tissue Collection: CF sheep were produced through cloning using CRISPR/Cas9 and SCNT techniques. Fetal tissues were collected on the following days of development; 50, 65, 80, 100, and 120. Multiple samples were taken from both CF sheep and non-CF sheep

Slide Preparation: histochemical staining with hematoxylin and eosin (HE) stain. Tissues that were abnormal, insufficient, or irregular plane of sectioning were excluded to avoid bias.

Morphometry: Lungs were evaluated at key developmental periods. In order to achieve a large sampling number airways sampled per tissue ranged between 300-350 structures except when limited by tissue size. To examine the lumen diameters, the length of the minor diameter was consistently selected for each airway in cross sections to provide consistency. All airways were measured including those in which the lumen was not observed and consequently given a value of zero. To evaluate the incidence of unexpanded airways the percentage of the total airways with a diameter value of <6 μm were determined. Lastly, stained samples were based on appearance where; 0 – none, 1 – eosinophilic, 2 – mixture of eosinophilic and basophilic, 3 – basophilic material

Results

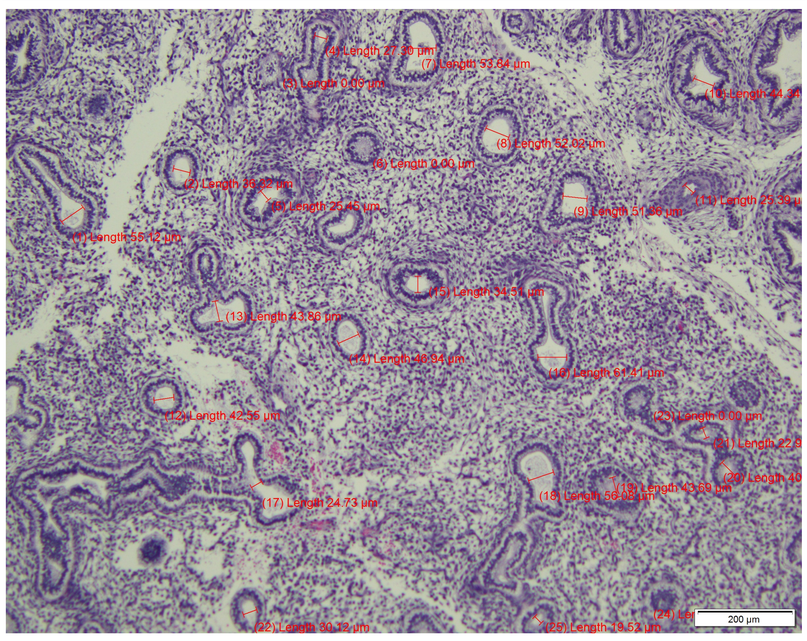

Figure 3: 50-day sheep impacted by Cystic Fibrosis with minor diameter selected in cross sections taken at 20x magnification.

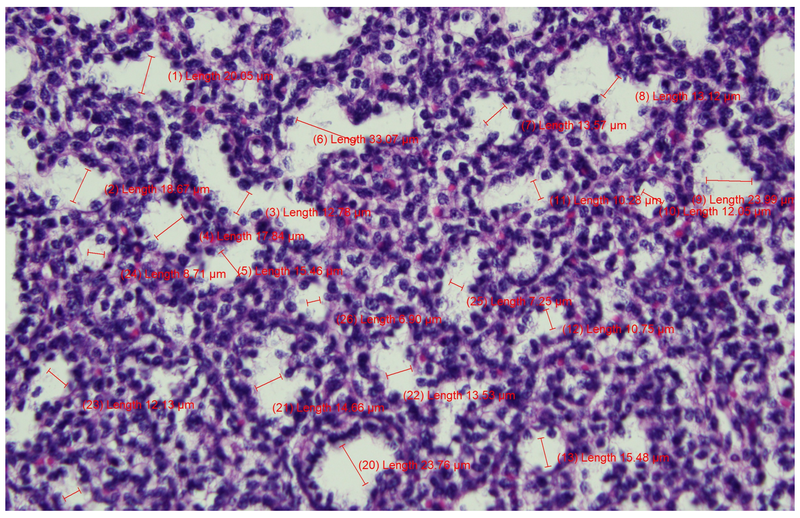

Figure 4. 80-day non-CF sheep with minor diameter selected in cross sections taken at 40x magnification.

50 days: CF 19x9-2 (x20 mag)					
Image #	Airway #	Luminal diameter (um)	% acini <6	Lumen filing score	Staining score
1	1	57.70		3	N/A
	2	22.44		1	2
	3	0.00		N/A	N/A
	4	3.72		0	N/A
	5	15.07		1	2
	6	0.00		N/A	N/A

Figure 5: Example of data collected on each airway including Luminal diameter, if less than 6μm marked in green, Lumen filling score where; : 0 – none, 1 – 1 to 25%, 2- 26 to 50%, 3 – 51 to 75%, and 4 – 76 to 100% or complete obstruction, and lastly staining score marked according to methods.

Table 1: Comparison of average values of Luminal Diameter and Luminal Filling Score comparing Wild-type and Cystic Fibrosis sheep.

	Average Luminal Diameter (um)			
	50d	65d	80d	100d
WT	20.08	13.39	10.49	15.40115
CF	24.35	16.54	8.87	8.52

	Average Luminal Filling Score			
	50d	65d	80d	100d
WT	1.33	1.07	0.46	0.323988
CF	1.09	1.03	0.91702	1.092105

We studied tissues from wild-type and CF fetal sheep. We found that upon reaching the 80-day mark CF sheep had a significantly higher percentage of "hypo-distended" airways (i.e. < 6μm diameter). Additionally crossing the 80-day mark CF lumen material had higher scores indicative of more basophilic staining than that of wild-type sheep. This is likely due to several factors such as pH and the local environmental.

Due to the similar values prior to day 80, there is potentially a key developmental step in which a functional CFTR protein is critical. This key event is represented by the drastic divergence of both the luminal diameter and luminal filling score between the two groups. This data suggests that CFTR contributes to, but is not essential for, lung development during fetal growth.

Summary

Questions concerning a role of CFTR during early fetal life have long persisted in the CF community but have not been possible to study. The introductions of animal models such as sheep allow for study and better potential in generating a cure.

References

1. Elborn, J. S. (2016). Cystic fibrosis. *The lancet*, *388*(10059), 2519-2531.
2. Meyerholz, D. K., Stoltz, D. A., Gansemer, N. D., Ernst, S. E., Cook, D. P., Strub, M. D., ... & McCray, P. B. (2018). Lack of cystic fibrosis transmembrane conductance regulator disrupts fetal airway development in pigs. *Laboratory Investigation*, *98*(6), 825-838.

Acknowledgements

This work was supported by Utah Agricultural Experiment Station project 1343 and grants from Cystic Fibrosis Foundation (POLEJA17XX0 and POLEJA18G0 and an ADVS undergraduate summer research internship.