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Review article

Lessons learned from the cystic fibrosis pig

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ABSTRACT

Deficient function in the anion channel cystic fibrosis (CF) transmembrane conductance regulator is the fundamental cause for CF. This is a monogenic condition that causes lesions in several organs including the respiratory tract, pancreas, liver, intestines, and reproductive tract. Lung disease is most notable, given it is the leading cause of morbidity and mortality in people with CF. Shortly after the identification of CF transmembrane conductance regulator, CF mouse models were developed that did not show spontaneous lung disease as seen in humans, and this spurred development of additional CF animal models. Pig models were considered a leading choice for several reasons including their similarity to humans in respiratory anatomy, physiology, and in size for translational imaging. The first CF pig models were reported in 2008 and have been extremely valuable to help clarify persistent questions in the field and advance understanding of disease pathogenesis. Because CF pigs are susceptible to lung disease like humans, they have direct utility in translational research. In addition, CF pig models are useful to compare and contrast with current CF mouse models, human clinical studies, and even newer CF animal models being characterized. This "triangulation" strategy could help identify genetic differences that underlie phenotypic variations, so as to focus and accelerate translational research.

1. Introduction

Cystic fibrosis (CF) is a recessive, monogenic disease caused by mutations in the gene encoding an anion channel, cystic fibrosis transmembrane conductance regulator (CFTR) [1]. Well over a 1000 mutations have been reported, but the most common mutation is a deletion of phenylalanine in position 508 (ΔF508) [2]. The ΔF508 mutant protein has a processing defect permitting only a small portion of the CFTR to reach and function along the apical membrane [3–6]. Given the high incidence of this mutation and the recent advent of modulator therapies, there is scientific interest in the potential of using ΔF508 models to study novel management strategies and therapies.

CF begins early in life and was considered a disease of young children, but thanks to decades of medical advances a newborn CF baby is now predicted to have a median survival of nearly 40 years (www.cff.org). The disease affects several organs including respiratory tract, pancreas, liver/gallbladder, intestines, reproductive tract, and sweat glands [4,7–9]. Respiratory disease is the most recognized clinical feature in CF because it is the leading cause of morbidity and mortality in CF patients. Lung disease is characterized by persistent airways infection, chronic inflammation, and tissue remodeling [10] (Fig. 1).

2. CF pig development

Clinical studies on humans are often limited by variations in treatment, secondary disease changes/remodeling, lack of adequate controls, and appropriate ethical constraints. Thus, animal models serve as a useful surrogate for study in CF. Shortly after the *CFTR* gene was identified, several CF mouse models were developed; however, these models consistently lacked characteristic lesions including

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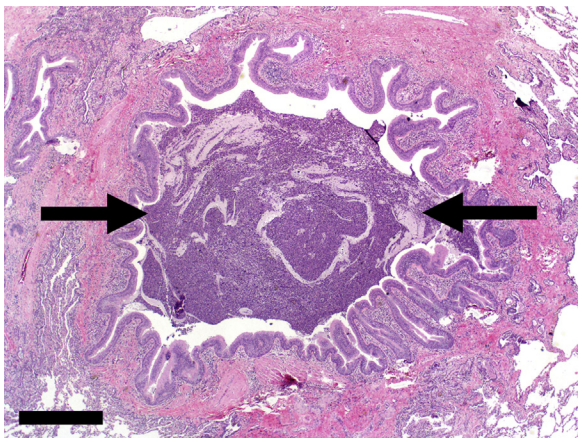

Fig. 1. Human CF lung from an archival autopsy tissue block. Note the CF airway lumen is obstructed (arrows) by neutrophilic cellular inflammation and some mucus. Bar = 800 μm.

spontaneous respiratory disease as seen in humans. Why might CF mice have lacked disease? Several theories have been proposed [11]. Some of these include a short life span (~2 years), small body size, lack of submucosal glands in lung airways, differences in modifier gene expression, etc. Since then, several other species have been targeted for study of CF including the pig [12], ferret [13], zebrafish [14], the rat [15], and others such as the sheep are being developed [16].

2.1. Pigs are an excellent candidate species for CF study

Pigs were considered a leading species candidate for genetic manipulation of CFTR because of several reasons [17,18]. The pig has similar size, anatomy, and physiology to humans. Pigs can live much longer (10–15+ years) than rodents with a generation interval of about 12 months and year-long breeding capacity. More specific to modeling CF lung disease [19], pigs have been used as models of pneumonia including viral [20] and bacterial [21] diseases, pigs have lung tissue markers that are similar to humans [22], and pigs are of similar size for translational lung imaging [19,23]. Anatomically, pigs have submucosal glands and relevant target tissues for CF pathogenesis, which extend along cartilaginous airways into the pulmonary parenchyma [24,25]. These features help exemplify why it would make a useful model species for CF lung research.

2.2. CF pig models

The first CF pigs were developed using a stop codon in exon 10 of CFTR. Somatic cell nuclear transfer was used to make heterozygous pigs, and through subsequent heterozygous × heterozygous breeding, CFTR-null pigs were born [12]. At birth, CF pigs were characterized by severe intestinal obstruction (i.e., meconium ileus) that required surgical intervention, similar to about half of meconium ileus cases in CF infants [8,26]. Another homozygous CFTR-null pig model was made using a STOP box that stopped message and protein synthesis in exon 1 and it displayed a similar phenotype [27]. The ΔF508 mutation is common in people with CF and it confers partial CFTR function at the apical surface of cells; thus, a ΔF508 pig was developed with hopes that it might be able to partially overcome the meconium ileus phenotype seen in CFTR-null pigs. The ΔF508-pig had a similar to slightly less severe meconium ileus phenotype compared with CF-null pigs [28]. Importantly, the porcine mutant ΔF508 CFTR had a processing defect—similar to humans—but with only ~6% CFTR activity compared with non-CF in airway epithelia, and this was insufficient correction to prevent CF airway disease. With the advent of modulator therapeutics [29], the ΔF508 pig could provide an attractive large animal model to test therapeutic strategies or drug combinations during various stages of lung disease.

To overcome the meconium ileus seen in newborn CF-null and CF-ΔF508 pig models, a gut-corrected model was later generated using a transgene for porcine CFTR under the fatty acid–binding protein 2 (FABP2) promoter. The results of this model suggested that raising the levels of intestinal CFTR message to as little as 20% of non-CF could mitigate severe meconium ileus at birth, and importantly, these pigs retained lesions in other organs consistent with CF for use in postnatal studies [30].

2.3. CF pig phenotype

Phenotyping of CF pig models were needed to confirm that these animals mimicked the human condition. At birth, CF pigs had destruction of the exocrine pancreas, focal biliary cirrhosis, micro-gallbladder, segmental absence of the vas deferens, impaired glucose tolerance, deficient insulin-like growth factor-1, and meconium ileus obstruction. These features exemplify a broad scope of changes that are consistent with human CF. This emphasizes that CF pigs mimic the human condition to overcome several of the deficiencies found in CF mouse models [9,12,26,28,30–34].

Although newborn pigs had severe changes in many organs, it was surprising that the lungs of newborn CF pigs lacked evidence of inflammation. Even so, CF large airways had structural changes with reduced circularity, smaller caliber, hypoplastic submucosal glands, and lesions in smooth muscle and cartilage rings [12,28,30,35].

3. CF pigs and understanding of CF lung disease

Lung disease is the leading cause of morbidity and mortality in CF patients [10]; therefore, the remainder of this review will emphasize how the CF pig model has helped to clarify our understanding of CF respiratory disease. Although this targeted review will not necessarily discuss all the clinical features of CF lung disease (*Pseudomonas* infection, leukocytes, etc.), it will focus only on those that the CF pig model has significantly increased scientific understanding and perspective. For additional information on CF, including its clinical features, lesions, and animal models useful for translational studies, the reader is also encouraged to see recent publications [3,7,8,10].