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Animal and Mouse Models for Cystic Fibrosis

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***Abstract*: Cystic fibrosis mouse models are tools that enable us to study and understand the mechanisms and complexities of cystic fibrosis. Mouse models are better, and usually preferred when compared to other animal models, because of their high reproduction rate, low cost, and easy maintenance. A Mouse can be easily genetically manipulated by making it a transgenic or knockout mouse. However, as there are anatomic and immunological differences between mice and humans, these models have inherent limitations which need to be accounted for when analyzing the results obtained from experiments. This review focuses on the different CF mouse models which represent diverse phenotypes observed in humans with cystic fibrosis. This can potentially help researchers interpret the diverse functions of the CFTR protein.**

***Keywords*: Cystic fibrosis, Mouse models, CFTR protein, Animal models, Genetically engineered mouse.**

1. Introduction

Cystic Fibrosis is a respiratory disease that mainly affects the lungs. It can cause problems to other organs as well. It also affects various glands, e.g. sweat glands. It's an inherited disease. It is mainly caused by mutations in the CFTR (cystic fibrosis transmembrane regulator) gene.

A. CFTR Protein

The CFTR genes provide information to their respective proteins. These proteins (CFTR) are present in all the places where mucus is produced, mainly in glands such as the pancreas, intestines, etc. These mutations in the gene make it difficult for proteins to understand the instructions and thus result in malfunctioning. This causes very sticky mucus which then blocks the lungs and digestive system.

B. Mouse Models

It basically means using mice as a model (organism) to experiment, observe and identify various aspects of diseases caused to humans. The mouse is selected, and breeding is done. This helps us get the progeny of the mouse with favourable characteristics. Thus, scientists use these genetically modified mice to study and stimulate inherited diseases. What is the significance of using mouse models? As mentioned above, mouse models are used for experiments because they are similarly related to humans. They help us provide insights into the mechanisms of various diseases. One can know the efficacy of certain drugs after understanding the mechanism and thus can also predict the human responses to these medications.

C. Animal Models

Similar to mouse models, animal models are used to study and observe the effect of various medicines and drugs on their bodies. Since human experiments are prohibited and can have a severe penalty if done without permission. Scientists have opted for this model for further understanding the process as they show similarities in anatomy as well as physiological features. One such example is the use of monkeys as an animal model to study the after- effects of certain drugs and predict the after effect on humans. Animal models (monkey) also show similar immune responses and thus, are a much more effective method than mouse models

2. Cystic Fibrosis

Mutations in the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) gene present on human chromosome 7 can cause cystic fibrosis (CF). These mutations can cause CFTR protein dysfunction or complete loss, resulting in multiple organ dysfunction. There are many manifestations of CF disease, including meconium intestinal obstruction and peritonitis, stunting, nasal polyposis, pancreatitis, malabsorption, liver cirrhosis, and repeated and persistent lung infections, such as *Staphylococcus aureus* and *Pseudomonas aeruginosa* bacteria. Because mutations can cause such variable disease manifestations, it is difficult to create animal models that mimic human diseases with high accuracy. However, many attempts have been made with varying degrees of success. The following review will evaluate various mouse models and their value to researchers in understanding the many functions of the CFTR protein.

3. Genotypes

The CFTR gene was identified and cloned in the year 1989, and only three years later, the first CF mouse model was reported (Snouwaert et al, 1992). To date, 11 CF mouse models have been characterized and reported (Table 1). Gene targeting methods in embryonic stem cells have been used to disrupt the endogenous CFTR gene, resulting in several CF mouse models. These models have established animal models for the human mutations that are the two most common ones ΔF508 and G551D.

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TABLE 1. CYSTIC FIBROSIS MOUSE MODELS

	Mutation	Usefulness
CFTR^{tm1UNC}	Exon 10 replacement No CFTR mRNA detectable	Survival rates, using a liquid-nutrient diet and colyte Transgenic mice containing FABP-hCFTR gene to correct intestinal disease Susceptibility to *S. aureus, B. cepacia, P. aeruginosa* Resistance to *V. cholerae* Congenic strain B6 (lung disease) and BALB/C
CFTR^{tm1HGU}	Exon 10 insertional 10% of WT CFTR mRNA	Susceptibility to *S. aureus, B. cepacia, P.aeruginosa*
CFTR^{tm1CAM}	Exon 10 replacement No CFTR mRNA detectable	Transgenic mice containing hCFTR gene to correct intestinal pathology Resistance to *V. cholerae*
CFTR^{tm1BAY}	Exon 3 insertional duplication < 2% WT CFTR mRNA	
CFTR^{tm3BAY}	Exon 2 replacement No CFTR mRNA detectable	
CFTR^{tm1HSC}	Exon 1 replacement No CFTR mRNA detectable	Modifying genes for meconium ileus
CFTR^{tm1EUR}	ΔF508 exon 10 insertional "hit and run" Mutant CFTR mRNA normal levels	
CFTR^{tm2CAM}	ΔF508 exon 10 replacement Mutant CFTR mRNA 30% of WT levels	Resistance to *S. typhi*
CFTR^{tm1KTH}	ΔF508 exon 10 replacement Mutant CFTR mRNA Low in intestine	Susceptibility to *P. aeruginosa*
CFTR^{tm1G551D}	G551D exon 11 replacement Mutant CFTR mRNA 53% of WT levels	Susceptibility to *P. aeruginosa*
CFTR^{tm2HGU}	G480C exon 10 insertional "hit and run" Mutant CFTR mRNA normal levels	

Definition of abbreviations: CFTR, cystic fibrosis transmembrane conductance regulator; WT, wild type.

The first CF mouse model was created at the University of North Carolina in 1992 (Clarke et al, 1992). By introducing a stop codon in exon 10, targeting the endogenous CFTR gene in the mouse embryonic stem cell line leads to the destruction of the CFTR gene. The targeted cell line was inserted into early C57BL/6/129 mouse embryos and was transferred to B6D2/129 pseudo-pregnant foster mothers. The offspring were then mated to C57BL/6/129, BALB/C/129, or B6D2/129 mice for generating heterozygotes, which were crossed for producing homozygous offspring, termed CFTR^{tm1UNC} knockout (KO) mice. The rate of survival of these mice was really low as only less than 5% survived to maturity.

Kent and colleagues theorized that the mixed genetic background of the original strain of CFTR^{tm1UNC} mice affected the development of lung pathology. For testing this theory, the CFTR^{tm1UNC} mice which were heterozygous at the *Cftr* locus were backcrossed to a C57BL/6 background. This new strain of the same kind was named B6-CFTR^{tm1UNC}/CFTR^{tm1UNC} knockout mice (Kent et al, 1997). This mouse model has been used to characterize the multi-organ involvement associated with CFTR dysfunction (Durie et al, 2004), as well as the pulmonary inflammatory response to bacterial infections using less invasive infection techniques (Guilbault et al, 2005).

At about the same time that CFTR^{tm1UNC} knockout mice were being produced, Dorin and colleagues used insertion mutagenesis targeting exon 10 of the *Cftr* gene to generate another knockout mouse strain under the MF1/129 genetic background, named CFTR^{tm1HGU} (Dorin et al, 1992). However, due to the utilization of targeting strategies, exon skipping, and abnormal splicing created 10% of normal *Cftr* mRNA, resulting in a much milder disease phenotype. 95% of these mice survived to maturity.

Another *Cftr* mutant mouse strain was produced by Ratcliff and colleagues in a mixed genetic background of C57BL/6/129 and MF1/129, resulting in null mutations (Ratcliff et al, 1993). The phenotype of these mice named CFTR^{tm1CAM} has similarity to that of CFTR^{tm1UNC} knockout mice, except that these mice also showed lacrimal gland pathology.

Researchers in Texas and Iowa created a CF mouse on a mixed C57BL/6/129 background by duplicating exon 3 in the mouse *Cftr* gene and named it CFTR^{tm1BAY}. These mice produced wild-type (WT) mRNA 2% below normal levels and showed a severe phenotype with high mortality; only 40% of *Cftr*-KO mice survived after day 7 (O'Neal et al, 1993). After two years, Hasty and colleagues produced another null mutation in mice by replacing exon 2, named CFTR^{tm3BAY}. CFTR^{tm3BAY} knockout mice at 1 month of age had a 40% survival rate (Hasty et al, 1995).

Rozmahel and colleagues created another strain of *Cftr*-deficient mice by disrupting exon 1 of the *Cftr* gene, called CFTR^{tm1HSC} knockout mice. Initially, these mice were produced in a mixed genetic background and showed a severe phenotype, with a survival rate of only 30% (Rozmahel et al, 1996). In subsequent studies, the founder mice were crossed with different inbred lines to produce F1 mice with different genetic backgrounds, and heterozygous F1 mice were crossed to produce homozygous CFTR^{tm1HSC}/CFTR^{tm1HSC} knockout mice. As per their genetic background, these knockouts showed different disease severity degrees and rates of survival.

Generally speaking, most CFTR mutations in humans result in loss of function due to abnormal protein processing and failure to insert CFTR into the plasma membrane (Kartner et al,1992). The most common human CF mutation ΔF508 mouse model has been generated by introducing this mutation into the endogenous mouse *Cftr* gene. The ΔF508 mouse model was created by van Doorninck and colleagues using double homologous recombination technology to insert mutations into exon 10 (van Doorninck et al, 1995). The mice produced under the background of FVB/129 and named CFTR^{tm1EUR} were viable but did not exhibit serious disease. It may be because the expression level of the mutant CFTR protein is close to the normal level, so it can provide enough residual function to improve Adverse phenotypic changes (Gray et al, 1994). Several studies have shown that the ΔF508 CFTR protein exhibits a partial function as a Cl^- channel and has a similar conductivity level, but the probability of opening the channel is reduced (Dalemans et al, 1991). Colledge and colleagues also created ΔF508 knockout mice by replacing exon 10 on the background of C57BL/6/129. These F508 mutants, named CFTR^{tm2CAM}, showed a survival rate of 65% compared to null mutants (CFTR^{tm1CAM}) that showed only a 20% survival rate (Zeiher et al, 1995). This higher survival rate is due to CFTR^{tm2CAM} mice expressing 30% of normal levels of mutant *Cftr* mRNA. Zeiher and his collaborators used the same method as Colledge and colleagues to generate ΔF508 knockout mice by replacing exon 10 on the C57BL/6/129 background. However, the knockout mouse named CFTR^{tm1KTH} has a survival rate of only 40% and expresses almost no mutant mRNA levels in the intestine.

The G551D mutation is a class III mutation that affects the regulatory domain of the CFTR protein (Delaney et al, 1996). CFTR^{tm1G551D} was produced by replacing exon 11 of CD1/129 mice, and the survival rate of these mice was 27%. Mutant *Cftr* mRNA expression resulted in 4% residual activity.

The latest mouse model was developed to simulate the human G480C mutation (Dickinson et al, 2002). Dickinson and colleagues used double homologous recombination to insert

mutations into exon 10 to create a G480C mouse model. The mouse named CFTR^{tm2HGU} was born on the background of C57BL/6/129 and survived to maturity under normal animal feeding conditions. Like the CFTR^{tm1EUR} strains, their high survival rate may be related to the use of the "hit and run" technique, resulting in the normal expression of the mutant CFTR protein.

It has been shown that the survival rate of different mouse models is affected by diet and housing conditions. Placing CFTR^{tm1UNC} KO mice on a liquid nutrient-rich diet improves their survival rate greatly and reduces intestinal obstruction (Kent et al, 1996). Additionally, placing CFTR^{tm1G551D} mice under SPF conditions can limit their intestinal obstruction and improve their rate of survival from 27% in traditional animal facilities to 60% under SPF conditions.

4. Phenotypes

A. Intestinal Disease

The intestinal phenotype seems to be the hallmark of most CF mouse models. Except for CFTR^{tm1HGU}, CFTR^{tm1EUR}, and CFTR^{tm2HGU}, all models showed quite a serious pathology. Most mouse models exhibit abnormal electrophysiological characteristics, running and dysplasia, goblet cell hyperplasia, mucin accumulation in Lieberkuhn crypts, crypt expansion, intestinal obstruction leading to perforation, peritonitis, and death. Intestinal obstruction is physiologically very similar to meconium intestinal obstruction observed in people with CF. Overall, the mouse CF model seems to show the bowel disease observed in humans.

Recently, Walker and colleagues studied the effect of talniflumate (LOMUCIN) on CF mice (CFTR^{tm1UNC} and CFTR^{tm1Kth}) with distal intestinal obstruction syndrome (DIOS). DIOS involves insufficient hydration of mucus and debris on the mucosal surface due to abnormal transepithelial electrolyte and water transport due to weakened or absent CFTR activity. They observed that oral talniflumate reduces the absorption of NaCl in the small intestine by inhibiting the apical membrane Cl^-/HCO_3^- exchanger, thereby having a beneficial effect on the survival of CF mice (Walker et al, 2006).

B. Pancreatic Disease

Snouwaert and colleagues review that in the pancreas of CFTR^{tm1UNC} mice there is a relative lack of pathological changes. Another study of CFTR^{tm1UNC}/CFTR^{tm1UNC} mice reported lumen dilation and accumulation of zymogen particles on top of ductal epithelial cells. CFTR^{tm1CAM} CF mice showed some small pancreatic duct obstruction in 50% of the mice tested, although the lesions were considered severe enough to change pancreatic function. Mice with CFTR^{tm3BAY} and CFTR^{tm1BAY} mutations show acinar atrophy, which becomes more severe as the mice age. Mice with the CFTR^{tm1HGU} mutation did not show pancreatic pathology, possibly due to the expression of large amounts of WT CFTR. In addition, these mice showed no evidence of malabsorption or other gastrointestinal problems. In addition, neither ΔF508 nor G551D models showed any obvious pancreatic pathology. Compared with CF patients, the milder pancreatic pathology observed in the CF mouse model seems to depend on two factors. First, some mouse models show enough CFTR residual activity to possess a normal Cl^- secretion pathway. Secondly, it has been proposed that the murine pancreas contains an alternative channel for CFTR, which is a Ca^{2+}mediated Cl^- conduction channel that is expressed in both WT and CF mice.

C. Hepatobiliary Disease

In most mouse models of CF, there is no obvious liver pathology. However, most of the mice studied are very young, and if they are studied later in life, they may suffer from liver disease, just like humans. In CFTR^{tm1G551D} mice, 20% of mice have been reported to exhibit bile duct epithelial hyperplasia (23). The gallbladder of CF mice appears to exhibit more abnormalities than in the liver. However, the pathological changes are great. Some CF mouse models (CFTR^{tm1G551D}, CFTR^{tm1UNC}, and CFTR^{tm1Bay}) have a swollen gallbladder. The gallbladder of CFTR^{tm1UNC} and CFTR^{tm1G551D} mice were filled with black bile, and the gallbladder wall was infiltrated with neutrophils, indicating the presence of continuous inflammatory response. Understanding the pathology of the gallbladder seen in CF mice may help to find ways to treat the frequent formation of gallstones and gallbladder malformations observed in human CF patients.

D. Lung Disease and Inflammation

The first batch of CFTR^{tm1UNC} KO mice showed pathological changes in the upper respiratory tract, but there were no signs of inflammation or bacterial infection in the lungs. It is assumed that the mixed genetic background of the original strain affects the development of lung pathology. The modified gene may encode an alternative Cl^- channel that can compensate for the defective CFTR (3). Therefore, Kent and colleagues reported the development of a similar strain named B6-CFTR^{tm1UNC}/CFTR^{tm1UNC}. Although there is no spontaneous lung infection, B6-CFTR^{tm1UNC}/CFTR^{tm1UNC} mice maintained under specific pathogen-free (SPF) conditions will develop spontaneous and progressive lung diseases. The main characteristics of lung disease include the inability to effectively remove the mucous cilia, hyperinflation of the alveoli after bronchiole, and thickening of the parenchymal interstitial thickening, accompanied by evidence of inflammatory cell recruitment and fibrosis. An early feature of human CF, Acinar, and alveolar hyperinflation is consistent with obstructive small airway disease. CFTR^{tm1HGU} KO mice did not exhibit severe lung disease at birth but exhibited cytokine abnormalities when reared in standard animal facilities (Davidson et al, 1995). Further studies have shown that the mucociliary transport of inert particles *in vivo* is significantly impaired, and the submucosal glands have changed (Borthwick et al, 1999). In mice carrying the G551D mutation, approximately one-third of the animals exhibit concentrated eosinophils in the lumen of the submucosal glands of the pharynx. As many as one-third of mice homozygous for the CFTR^{tm1G551D} mutation have abnormal regulation of lung inflammation. Additionally, the mice with the CFTR^{tm1G551D}

mutation showed increased initial susceptibility and impaired lung clearance to Pseudomonas aeruginosa. Another interesting phenotype observed in CF mice has reduced lung levels of iNOS that has already been implicated in the CF lung disease's pathogenesis. In a mixed background strain of homozygous $CFTR^{tm1KTH}$ and $CFTR^{tm1UNC}$ KO mice, the expression of iNOS was significantly reduced (Kelley et al, 1995). All other mouse models examined had normal lung histology and no mucus blockage, showing no signs of lung pathology. Therefore, except for B6-$CFTR^{tm1UNC}$ mice, no other CF mouse models developed spontaneous pulmonary inflammation, nor did they develop the "spontaneous" chronic bacterial infection and/or inflammation observed in human CF patients. The reason for this difference is not yet clear, but part of the reason may be that there are relatively fewer submucosal glands in the mouse trachea and main bronchus compared to human airways, and part of the reason may be the expression of alternative (non-CFTR) chlorides Channels in mouse airway epithelial cells.

E. Nasal and Tracheal Electrophysiologic Profiles

Mouse nasal mucosa is composed of 40% olfactory epithelium and 60% respiratory epithelium. In contrast, the human nasal mucosa is composed of more than 95% of the respiratory epithelium. However, the nasal mucosa of the CF mouse model accurately replicates the characteristics of the human body and also shows excessive Na^+ absorption and Cl^- transport defects (Grubb et al, 1996). The overabsorption of Na^+ is detected by the significant negative value of the baseline nasal potential difference (PD) *in vivo* and the response to amiloride (a drug that blocks the epithelial sodium channel ENaC). In response to amiloride, all CF mouse models showed increased potential differences compared to non-CF controls. In human airway tissue, Cl^- secretion is almost mediated by CFTR channels and alternative Ca^{2+} regulatory channels in the apical membrane. In human CF tissues, although the cAMP-stimulated CFTR pathway is defective, the Ca^{2+}-mediated Cl^- secretion pathway is functional and is sometimes up-regulated. In addition, nasal potential differences in patients with severe CF genotypes also showed that CFTR-corrected CFBE41o airway cells and A2 adenosine (Ado) receptors in human subjects are CFTR-dependent. These results indicate that Ado is an effective Cl^- secretory agent with little effect on cAMP levels, even though it has a strong effect on CFTR-dependent short-circuit current and nasal Cl^- transmission.

In normal mouse nasal mucosa, CFTR is the main Cl^- secretion pathway. In CF nasal mucosa that does not express CFTR, the Ca^{2+} mediated Cl^- secretion pathway is upregulated. Except for mice carrying the $CFTR^{tm1HGU}$ and $CFTR^{tm1EUR}$ mutations, all CF mice exhibited a decrease in cAMP-mediated Cl^- response and an increased Ca^{2+} mediated Cl^- secretion response.

F. Other Manifestations of the Disease

Several studies using various CF mouse models report that male fertility is normal and there is no pathological change in the male reproductive tract. It is speculated that the normal fertility of CF male mice is due to the presence of Ca^{2+}-mediated Cl^- secretion pathways in the epididymis and seminal vesicles. There were no obvious pathological changes in the reproductive tract of CF female mice. However, $CFTR^{tm1UNC}$ female mice take more time to become pregnant than normal littermate mice, which suggests that fertility may be reduced, and the CF knockout females in B6-$CFTR^{tm1UNC}$ are almost completely sterile (unpublished Observation results).

The height of CF patients is usually below average, and it is difficult to recover once they lose weight. In addition to poor absorption of fat and protein, other factors, such as increased caloric requirements to fight infection and difficulty breathing, can also cause weight loss. All CF mouse models except $CFTR^{tm2HGU}$ and $CFTR^{tm1HGU}$ have lower body weight than their WT littermates. The lower body weight of CF mice is thought to be caused by intestinal disease because CF mice whose defects were corrected by tissue-specific CFTR transgene have the same body weight as non-CF littermate control mice (Haston et al, 2002).

With the development of CF research, treatment can help extend the median survival age of CF patients. Decreased bone mineral density and vertebral compression fractures that lead to kyphosis are further complications that become more pronounced as the survival rate of CF patients increases. These phenotypes cause pathological fractures in children and adults. The link between CFTR dysfunction and osteopenia/osteoporosis has not been determined, as it may depend on many accompanying disease factors, including pancreatic insufficiency, calcium or vitamin D nutritional deficiencies, reduced exercise capacity, and glucocorticoid therapy, Delayed puberty, and chronic lung infections. A study of histomorphometry analysis of CF mouse genetic model bones found that CFTR mutations are associated with severe osteopenia. Compared with the littermate control group, the bone mineral density (BMD) of the whole body and individual bones was significantly reduced. The CFTR mutant showed a 50% reduction in cortical bone width, thinner bone trabeculae, and a significant reduction in bone formation, accompanied by a strong increase in bone resorption.

5. Conclusion

In conclusion, there are various animal models for cystic fibrosis that target different organs, but there is no specific model for humans. Every model has its own advantage and combining the findings from these models, we find important information for our understanding of the CF disease pathogenesis and it's progression.

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