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Understanding Human Cystic Fibrosis Pathology in Sheep Models Using CRISPR/Cas9 Technology

Cystic fibrosis is a genetic disorder that causes damage to the lungs and other organs such as pancreas, liver, gall bladder, kidney, and digestive tract. This is due to a mutation of the F508del in the cystic fibrosis transmembrane conductase regulator (CFTR) gene which causes a deletion of the phenylalanine residue at the position 508 of the protein that causes a misfolding. G542X is a nonsense mutation that introduces a premature stop codon leading to the lack of production of the CFTR protein. The goal of this study was to use CRISPR/Cas9 along with single-stranded oligonucleotide (ssODN) and somatic cell nuclear transfer (SCNT) to sheep models of F508del and G542X mutations to show how they respond to the mutations to improve the health of human patients who suffer from this disorder. Following the transfer, the models gave birth to CF lambs that develop the same pathology to $CFTR^{-/-}$ sheep and human patients. These newborn CF lambs underwent necropsy, tissue samples and genomic DNA were extracted to make sure that the transfer was successful and that the mutations were present. The small intestines, pancreas, liver, gallbladder, vas deferens, lung, and kidney of the $CFTR^{-/-}$, $CFTR^{F508del/F508del}$, and $CFTR^{G542X/G542X}$ were evaluated, and it showed that the small intestines of the three models exhibited obstruction and were filled with meconium, 100% of hypoplasia, acini duct inflammation, and minimal inflammation was evident on the pancreas of $CFTR^{F508del/F508del}$ and $CFTR^{G542X/G542X}$, and 40% on the $CFTR^{-/-}$ model. The liver of both $CFTR^{F508del/F508del}$ and $CFTR^{G542X/G542X}$ showed 100% portal ductular reaction with mild fibrosis intrahepatic cholestasis while the $CFTR^{-/-}$ model showed 78.6% of portal ductular reaction and 85.7% intrahepatic cholestasis. The gallbladder showed hypoplasia, 50% on the $CFTR^{F508del/F508del}$ model, 100% on $CFTR^{G542X/G542X}$ model, and 80% on $CFTR^{-/-}$ model. All three models exhibited 100% aplasia or atrophy on their vas deferens and 100% cryptorchidism on both $CFTR^{F508del/F508del}$ and $CFTR^{G542X/G542X}$ models. The lungs of all three models were unremarkable. Kidneys of both $CFTR^{F508del/F508del}$ and $CFTR^{G542X/G542X}$ models exhibited 100% hydronephrosis and 86% on the $CFTR^{-/-}$ model. These characteristics were also found in the phenotype of human CF patients. The result of this study established that they have somehow successfully shown the similarities that the human CF patients exhibit and that these sheep models have a high potential to enhance the development of treatments whether that be therapy or drugs. These models were then treated with correctors: VX-661, VX-809, and VX-445, which promotes correct folding of F508del hCFTR, and potentiator VX-770. The results showed that combination of VX-445, VX-661 following the addition of VX-770 were the most potent and showed that CFTR channel was restored.

The generation of animal models like sheep, pigs, and rats, provides useful resources in understanding early pathology and developing new therapeutic advances for a disease like cystic fibrosis. However, most animal models fail to mimic some human disease as accurate as possible, and this is a hurdle that most scientist encounter up to this day. It was believed several years ago that animals like sheep could potentially be a great model for understanding these diseases and until the development of CRISPR/Cas9, it was made easier to

target the CFTR protein. Sheep models and human CF patients both fail to exhibit any lung discrepancies but when tissues of the CF sheep are further examined, it could reveal and provide more information that human CF patients fail to demonstrate in vivo. The further advancement of CRISPR/Cas9 technology can also be a useful platform to decipher early onset etiology not only in CF but in other emerging diseases and disorders. Especially with diseases that are genetically inherited, people usually will have no idea if they possess this disease unless they undergo gene testing where they could detect it early on. However, it might not be accessible for everyone as it can be costly if it is not covered by insurance. It is important to establish a model that can showcase the effects of CF during early fetal development to develop therapeutic advances. Overall, this study, the technological advances of CRISPR/Cas9 and the development of animal models demonstrates that we are one step ahead to uncover a treatment for this disease.

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