Molecular delivery truck serves gene therapy cocktail

August 15 2011

In a kind of molecular gymnastics, scientists at the University of North Carolina at Chapel Hill School of Medicine have devised a gene therapy cocktail that has the potential to treat some inherited diseases associated with "misfolded" proteins.

Like strings of beads attached end-to-end on a chain, a given sequence of a protein's amino acids usually folds into a characteristic, three-dimensional structure. When "misfolded," a mutant protein's natural biological role may be compromised, sometimes with implications for disease development.

This is one of the challenging research arenas chosen by R. Jude Samulski, PhD, director of the UNC Gene Therapy Center and a professor of pharmacology. "Among the roughly 5,000 genetic disorders for which the majority of genes have been mapped, there's a subset in which the mutant or misfolded protein by itself can cause disease symptoms - this is in addition to the lack of a normal gene," he says. "And that has added another layer of complication faced by the clinical research community when trying to develop and test new treatment approaches to disorders that result from toxicity associated with cellular accumulation of misfolded proteins."

Among these disorders are cystic fibrosis, Huntington disease, amyotrophic lateral sclerosis (ALS, or Lou Gehrig's disease), and Alzheimer's disease.

The report published in the on-line Early Edition of the *Proceedings of the National Academy of Sciences* during the week of August 15, 2011, reveals that the Samulski lab has focused a gene therapy approach on a protein deficiency that causes serious lung and liver disease in children and adults: alpha-1 antitrypsin (AAT) deficiency, or alpha-1.

This inherited condition is caused by an abnormal AAT protein that is mainly produced by the liver. An estimated 1 out every 2,500 people in the U.S. have the condition, which is often misdiagnosed as asthma or smoking-related emphysema. (See http://www.alpha1.org/). Scarring of healthy liver tissue (cirrhosis) also may affect infants as well as adults diagnosed with the condition.

Studies suggest that a build-up in liver cells of "misfolded" abnormal AAT is responsible for alpha-1. It is thought that the misfolded protein builds up in the cellular endoplasmic reticulum, the part of the cell that manufactures proteins, and is unable to move out of the liver and into the bloodstream.

"Alpha-1 antitrypsin plays a very important role in the health of the lungs, preventing fluid build-up, protecting against infections," Samulski said. "But in some individuals, the protein mutation they've acquired actually creates additional toxicity in the liver. And so, there's a liver pathology in addition to the lung damage. You have two complications going on, and not just one involving a lack of alpha-1 antitrypsin's protective role in the airway."

In the study, first- and co-corresponding author with Samulski, Chengwen Li, PhD, research assistant professor of pediatrics, conducted a series of gene therapy experiments using a mouse model of alpha-1 disorder. All involved the adeno-associated virus (AAV) vector as a molecular delivery truck.

Samulski, also a member of the UNC Lineberger Comprehensive Cancer Center, has long pioneered methodologies for using viruses to deliver genes effectively and safely to various targets in the body, including the brain, lungs, heart and muscle. As a graduate student at the University of Florida in the early 1980s, his thesis project was understanding and developing AAV as a vector for therapeutic genes. This work eventually led to development of AAV type-2 as a viral vector, which has been used for gene therapy trials in cystic fibrosis, hemophilia, Parkinson's disease, retinal disorders and in several other settings, including the first clinical trial of gene therapy for muscular dystrophy in the United States.

"In essence, we engineered this sophisticated molecular Fed-Ex truck that delivers two payloads simultaneously. One payload involves a genetic approach that disables the mutant protein so that it no longer causes toxicity, and the other payload provides a new gene to replace the protein activity that is missing," Samulski said. "In this way, Chengwen packaged both strategies into the same vector, a single therapeutic approach that would resolve both problems."

The researchers delivered the gene therapy cocktail via the bloodstream, and targeted it to the liver. Once there, the replacement gene payload and the other payload for disabling the misfolded protein acted independently, and successfully. The authors observed "over 90 percent knockdown of the mutant AAT along with a 13- to 30-fold increase" of therapeutic AAT in the blood circulation.

"I believe we've validated a path to go forward and test this cocktail cassette approach in a clinical trial," Samulski said. "This general approach has potential application to other diseases associated with misfolded proteins, such as Huntington's disease and ALS, among others."

Provided by University of North Carolina School of Medicine