

## **ENGL1101 (Life Sciences Learning Community)**

**Student:** William C. Mitchell

**Instructor:** Dr. Sharon McCoy

### **Overcoming Proteasomes: One Step Closer to a Cure for Cystic Fibrosis**

Take a deep breath and consider how easy it was to do so. Now picture struggling and gasping for air everyday; normal, easy tasks to the average person prove to be quite challenging to a patient of cystic fibrosis. Scientists have always been daunted by this fatal genetic disease that affects the body by excreting a “thick mucus” in the lungs, making breathing difficult and blocking the “ducts” leading from the pancreas, causing “poor digestion of food” (1). Until recently, there was nothing that scientists could do to provide a long-term cure, but even though scientists cannot cure patients one hundred percent, they can increase their life expectancy. Despite having discovered the cystic fibrosis gene’s location in 1989, numerous underlying obstacles prevent complete success (2). The primary obstacle that prevents scientists from making gene therapy an effective cure is the placement of the healthy genes into long-term cells, the cells that remain long enough to be replicated. The replacement of the healthy gene into long-term cells is necessary because these cells make the new cells, thereby distributing the healthy DNA throughout the body. The properly functioning gene is attached to a vector or “carrying molecule” that will transport “the therapeutic gene to the patient’s target cells” (2). Currently, the most common vector is a virus because it can easily capture the gene and deliver it into the cell; the virus infiltrates the corrupt cells and places the healthy gene into the nucleus which then transforms the corrupt cell into one which operates properly (2). The only difficulty is that the body’s natural immune system provides many barriers for viruses trying to enter the body. Scientists have managed to stop parts, but not all, of the immune system from breaking down the viruses that carry the healthy gene, giving scientists an opportunity to develop a method for inserting the healthy genes into long-term cells.

Since scientists started using a virus as the carrying molecule, the virus has shown considerable effectiveness on the cells that is inserted into; however, repeated doses are necessary. Some commonly used viruses are “Retroviruses, Adenoviruses, Adeno-associated viruses, and Herpes simplex viruses” (2). The primary virus used in gene therapy is the “adeno-associated virus” (AAV), because they are able to “insert their genetic material at a specific site on [the] chromosome” (2). And even though “trials experimenting with gene therapy” as a possible cure for cystic fibrosis have been unable to show that “gene transfer efficiency” is significant enough for “clinical benefit”, which is due to the natural bodily defenses in the “airway,” this does not mean this method cannot be perfected (2).

The obstacles preventing scientists from developing a cure include finding a way to overcome the short life-span of cells with newly inserted genes, inhibiting “immune response[s]”, and overcoming difficulties with “viral vectors” (7.1073). Also, repeated administration of the virus has been difficult because of the body’s ability to build antibodies against it; however, these aren’t the only challenges faced by scientists trying to cure cystic fibrosis (2). Others include overcoming “DNA degradation, nuclear import, and the ability to maintain long-term transgene expression” (1;2). The most important of these is long-term transgene expression because the genes have to get into the cell before the others can be dealt with. The insertion of genes into long-term cells is essential because these are the cells that will be replicated and produce the new cells. The new cells will have the correct DNA to be

distributed throughout the body. If the viruses insert the correct gene into a cell that dies, it has no lasting effect in the body; all this cell will accomplish is a temporary improvement in the side effects of cystic fibrosis. Therefore, to obtain “long-term transgene expression” scientists must first create a manner for inhibiting “proteasomes” which currently break down any viruses inserted into the body (2). A proteasome is “a giant protein complex that recognizes and destroys proteins tagged for elimination by the small protein ubiquitin” (7.1062). These viruses are not only blocked by the proteasomes but also by other obstacles along the human respiratory tract, preventing airborne pathogens from entering the body. These include “mucus, lack of receptors, immune surveillance, etc.” that are designed to “prevent penetration” of materials, even “gene therapy vectors,” from entering an opening in the throat (2). These are the exterior obstacles that prevent the virus from entering the epithelial cells, with proteasomes acting as an interior defense.

Viruses have been the main source of gene therapy because “repeated AAV2 administration caused an increase in AAV2-neutralizing antibodies, but did not induce lung inflammation” (7.1072). A new virus called “Sendai virus (SeV)” is an effective means for “gene transfer” and can also “correct Chlorine transport defect in CF knockout mice,” but needs “repeat[ed]” doses (7.1073). A CF knockout mouse is a specimen that has had the cystic fibrosis gene deactivated so that it cannot function in the mouse. Cystic fibrosis patients have a defect in the “Cystic Fibrosis Transmembrane Regulator protein” that operates “as a channel that allows cells to release chloride and other ions”; therefore, when this protein doesn’t function properly, a build-up of chloride ion creates an imbalance of salt in the cell, causing the production of mucus in the lungs (5). The Sendai virus still has trouble inserting the healthy gene into the cell due to proteasomes. Therefore, scientists who advocate the use of viruses must first block the proteasomes, which will allow the virus to implant its healthy gene into the body.

“Proteasomes” play a critical role in “processing . . . viral particles used for gene delivery” (2). If this ability can be controlled, then viruses will be able to deposit their healthy gene into the respiratory tract (2). Scientists have been able to show that when proteasomes are “in the presence of proteasome inhibitors, adeno-associated virus (AAV) mediated 60-fold higher gene expression in cell culture and enhanced the level of AAV-mediated transduction in the liver and lung” (4.1681). This means that controlling what the different proteasomes metabolize can lead to increased effectiveness of AAV2 by inserting the healthy gene that the viruses are carrying into the defective cells (7.1072). Due to this information, scientists created several peptides to inhibit the proteasomes and allow the viral particles to penetrate and enter the epithelial cells (4.1687). The test result showed that blocking the proteasomes increases “gene expression” through the use of “a viral vector” in cystic fibrosis cells (4.1687). For scientists to analyze a way of inserting the healthy genes into long-term cells, the virus must first be able to enter. This has been a challenge for scientists until now because the proteasomes blocked the viruses from entering the cells. Now, scientists can focus on a method for the insertion of the healthy gene into long-term cells instead of temporary cells that eventually degrade. This evidence shows that viruses can be used more effectively when proteasomes are blocked by peptides, leading to increased insertion of the healthy gene into cells.

Perfecting gene therapy into a cure for cystic fibrosis is a task scientists are currently trying to accomplish. Scientists have been able to show that viruses are able to insert their genes into the epithelial tract, creating a temporary decrease in the amount of mucus produced. The overall goal in creating a cure for cystic fibrosis is to create long-term gene expression. Scientists can now focus on the latter goal because scientists have stopped proteasomes from

eliminating the viruses carrying the healthy genes. A cure for cystic fibrosis is still going to be a challenge, but scientists have come one step further toward their goal.

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