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Genomics and Medicine

March 8, 2011

The Progress of Gene Therapy

Almost 12 million Americans have cancer, and almost 81 million Americans have cardiovascular disease. What is it that is causing these diseases in so many Americans? Science has proven that proteins are the foundation for all biological processes and that genes in our DNA encode for those proteins. Most disease stem from gene mutations, which then cause protein malfunctions, significantly disrupting important biological processes. Researchers have spent countless hours trying to find the cure for several diseases, and some have turned to gene therapy as a solution. Gene therapy is a technique for correcting defective genes that can be implemented in several different ways; however, there are certain dangers to using gene therapy, and researchers are currently attempting to make gene therapy a more successful method of treatment.

There are several different gene therapy techniques used to correct defective genes. Some of these include a) inserting a normal gene into the genome to replace a nonfunctional gene, b) repairing the abnormal gene, c) the regulation of a particular gene could be altered, or d) an abnormal gene could be swapped for a normal gene through homologous recombination[1]. The most common technique

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1 http://www.ornl.gov/sci/techresources/Human_Genome/medicine/genetherapy.shtml

used is the first one mentioned—inserting a normal gene into the genome to replace a nonfunctional gene. To insert a normal gene into the genome, a vector must be used to deliver the gene to target cells, and viruses are the primary vectors because of their pathogenic ability to insert DNA into human cells. These viruses must be genetically altered to carry human DNA. According to Mark Kay, genomics researcher at Stanford, the most promising vectors are the adeno-associated viruses (AAV) because they lack pathogenicity and toxicity, have the ability to infect dividing and quiescent cells [cells in the G_0 phase] of various tissue origins, and have the potential for site-specific integration into the host chromosome, which can result in long-term gene expression[2]. These properties are extremely important because other viral vectors don't have these properties, and using them causes great concern. There is always the fear that a viral vector, once inside the patient, may recover its ability to be pathogenic and cause disease, and the toxicity of a viral vector may cause an immune response which would greatly reduce the effectiveness of gene therapy due to immunological memory of viruses. The potential for site-specific integration is significant because gene therapy has been known to cause other genetic diseases through non-site-specific integration of a therapeutic gene.

Site-specific integration of therapeutic genes is a novel property because non-specific integration of genes has caused disease in past clinical gene therapy trials. When a therapeutic gene has non-site-specific integration, it is possible for it to integrate within another gene and disrupt the production of those functional

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[2] http://kaylab.stanford.edu/manuscripts/CGT03Grimm.pdf

proteins. For example, 18-month old Rhys Evans of England was born with X-linked SCID or Severe Combined Immunodeficiency Disorder, a potentially fatal bone marrow condition that left his body incapable of fighting infection. Evans successfully underwent gene therapy for the defective gene gamma c, but later he was diagnosed with leukemia[3]. Researchers discovered that the transferred gamma c gene had interrupted the function of a gene that normally helps regulate the rate at which cells divide, causing leukemia[4]. Because of these safety issues, the Food and Drug Administration (FDA) has put gene therapy clinical trials on hold until further investigation[5].

Other than therapeutic genes interrupting other gene functions, there are several other reasons that gene therapy is not a more prevalent method of treatment. Gene therapy is more likely to be successful when there is only one mutated gene as the root of a disease, but, unfortunately, some of the most common diseases like heart disease, arthritis, diabetes, and Alzheimer's are the result of multiple mutated genes. These disorders would be much more difficult to effectively treat with gene therapy. Another reason that gene therapy is not widely used as a treatment is because of its short-lived nature. The rapidly dividing nature of many cells prevents long-term effects of gene therapy. For gene therapy to be permanently effective, the DNA introduced into target cells must remain functional, and the cells containing the therapeutic DNA must be long-lived and stable[1].

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[3] http://www.guardian.co.uk/uk/2002/apr/04/medicalscience.genetics

[4] http://learn.genetics.utah.edu/content/tech/genetherapy/gtchallenges/

[5] http://www.nature.com/nbt/journal/v21/n3/full/nbt0303-217.html

Otherwise, patients would need continuous treatments of gene therapy, which would prove to be painful and costly.

However, many developments in gene therapy are being made and proving that it can be a successful treatment. For example, UK researchers from the UCL Institute of Ophthalmology and Moorfields Eye Hospital NIHR Biomedical Research Centre have published results to show that gene therapy is effective for a type of inherited blindness. The treatment involved young patients with Leber's Congenital Amaurosis, an eye disease caused by a mutation in the gene RPE65. There are no effective treatments for this disease, and it's a condition that appears at birth or in the first few months of life and causes loss of vision[6]. An important discovery made in the clinical trial was that the gene therapy caused no side effects. All patients achieved levels of vision at least equivalent to before the operation, but one patient's night vision significantly improved[5]. This success provides a milestone for gene therapy since it was a successful experiment without side effects, and it may lead to further developments in gene therapy treatments for a broader range of retinal disease.

Another area of gene therapy that is making tremendous progress is the ability to repair a mutated gene. By repairing a mutated gene instead of inserting a therapeutic gene, it bypasses the concern of oncogenic risks where the therapeutic gene may insert itself within another gene and disrupt that gene's function, possibly causing oncogenic disease. Once again, adeno-associated viruses have emerged as the best vector for gene repair. According to Mark A. Kay's article, "Adeno-

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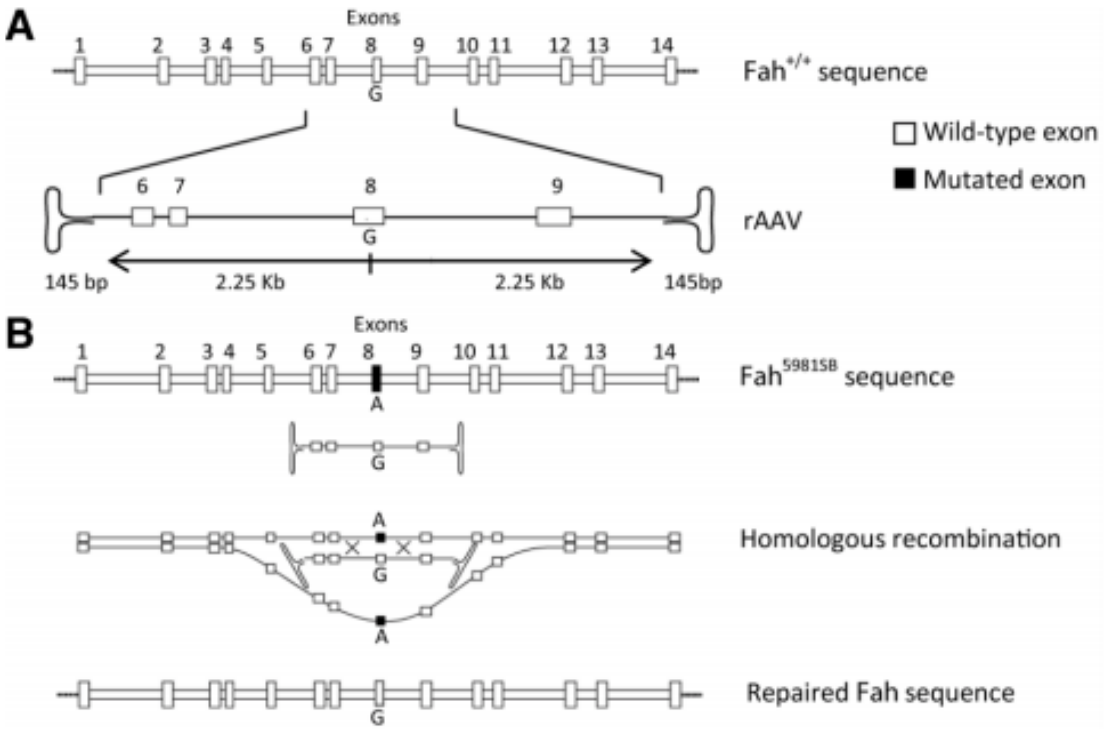
[6] http://www.ucl.ac.uk/media/library/Genetherapyblind

Associated Virus Gene Repair Corrects a Mouse Model of Hereditary Tyrosinemia *In Vivo*," "numerous *in vitro* studies have shown AAV capable of correcting various types of mutations (insertions, deletions, substitutions) by vector-mediated homologous recombination. AAV vectors engineered to perform gene repair have the ability to target multiple different genomic loci, show both targeted and stable expression through integration, and have an increased number of applicable human diseases.[7]" Diseases that result from mutations in one or a few nucleotides are the most frequent genetic abnormality, and these diseases are the best targets for gene repair. The success of gene repair was demonstrated in mice with a hereditary disease, tyrosinemia type I (HTI). HTI is caused by the lack of fumarylacetoacetate hydrolase (FAH), the terminal enzyme in the tyrosine catabolic pathway (kay mouse 1201). The *Fah*^{5981SB} mouse was used in the experiment, and it has a point-mutation at the final nucleotide of exon 8 within the *Fah* gene. This mutation creates a premature stop codon, which results in an unstable FAH protein that ends up getting degraded. An AAV was used as the vector to carry out homologous recombination, and the experiment successfully resulted in a G→A transversion (as shown in the figure below).[6]

[7] http://kaylab.stanford.edu/manuscripts/HEP-2010-PAULK.pdf



Successes in gene therapy trials like these two that were just mentioned provide hope that gene therapy may become a more prevalent method of treatment for genetic diseases. The discoveries of the adeno-associated viruses as a promising vector has helped lead to a successful experiment involving gene repair of point-mutations. Much progress still needs to be made before it becomes an accepted treatment for all genetic diseases, but recent developments indicate that some day, we may even be able to use gene therapy for multi-gene diseases like heart disease, diabetes, arthritis, and Alzheimer's.