What Doesn't Kill You Makes You Stronger: Using Diseases to Treat Patients

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Abstract:
Genetic disorders are the most common diseases that are diagnosed today. However, genetic disorders are difficult to treat and often the disorder is incurable. This research paper presents a new treatment option for genetic diseases. The new treatment uses viruses to treat these diseases. The mechanism of virotherapy is examined in several studies of the use of virotherapy on cancer cells. By understanding the mechanism of virotherapy, scientists can then find ways to use this mechanism to treat genetic disorders. The paper looks into detail of a genetic disorder, cystic fibrosis, and how research is being conducted of virotherapy on cystic fibrosis. Finally, this project presents disease treatment in an innovative way by changing the perspective of viruses and changing the from something harmful to something life-saving.

Key Words: Oncolytic Virotherapy, Innovative Research, Genetic Disorders, Clinical Trials, Mutations

The body is composed of over thirty-seven trillion microscopic bodies known as cells. Each cell has its own unique job, such as insulin-producing cells in the pancreas or dopamine producing cells in the brain. While each cell has a different job, they each work together in a coherent mechanism that allows the body to grow, work, and reproduce. Interestingly, the complexity of the human body can be easily broken down by something as simple as a change in a letter in the genetic code. Just as a typo has the ability to alter the coding in a computer system, a mutation from one gene to another presents the body with a variety of problems. These mutations in the genes translate into diseases that attack and destroy the body. According to the National Human Genome Research Institute, genetic mutations are the cause of almost all diseases.[1] These diseases are often hard to treat because one letter change causes a cascade of problems that affect the body in different ways. For instance, Cystic Fibrosis, a genetic disorder in which the membranes in the organs are altered and cannot transport chlorine into the cells, not only affects the lungs but also the pancreas, reproductive organs, and digestive organs. Due to this complexity, treatment for Cystic Fibrosis is not contained to one "miracle" pill, but instead the patient has to take multiple medications to aid in breathing, mucus clearing, and insulin production.[2] Taking multiple medications is expensive and a hassle to the patient and the patient's family; furthermore, researchers are constantly looking for a more streamlined approach to treatment. In a recent study published by the *Scientific American Journal*, scientists have begun to research the effect of viruses on mutated cancer cells. This new treatment, oncolytic virotherapy, uses viruses to attack the cancer cells without harming the healthy cells around.[3]Viruses are known to harm the body by infecting cells and releasing harmful antibodies that multiply eventually taking over the body. In this study, viruses are not viewed as potential

threats to the body, but instead the viruses are perceived as a potential treatment for cancer. This radical change in perspective could also aid in the treatment of complex genetic disorders.

Oncolytic virotherapy is a unique approach to cancer treatment because it only targets and destroys cancer cells. Typical cancer treatments such as radiation and chemotherapy destroy both cancer cells and the healthy functioning cells around the tumor. Cancer is the uncontrollable growth of cells in the body. This rapid growth of cells forms solid cell masses called tumors which can be benign or malignant. Benign tumors are tumors that are of slow growth and are localized to a certain area whereas malignant tumors are fast-growing and spread throughout the body. Malignant tumors are deadlier than benign tumors and are the leading cause of cancer-related deaths.[4] Cancer slowly takes over the body until the body is no longer able to support itself and eventually dies. Current cancer treatment revolves around chemotherapy, radiation, and/or surgery. Chemotherapy uses drugs to treat cancer and works by targeting cells that are currently reproducing and growing. By doing this, the drugs are able to stop the cancer before it even grows. While this treatment option seems ideal, chemo drugs cannot distinguish between cancerous and healthy cells thus harming healthy cells as well.[5] Using chemotherapy is like using a weed eater to clean weeds from a garden. Even though the weed eater cleans the weeds out, there is risk of destroying the vegetable plants in the process. Radiation works in a similar manner to chemotherapy by shooting high-energy light waves at the cancer cells. These high-energy waves break down the cancer cells internally and stop the reproduction of cells. As in chemotherapy, healthy cells that are near the tumor are broken down and destroyed in radiation treatment.[6] Surgical procedures are a less invasive treatment option for cancer. However, surgery has limitations as well. Due to the rapid growth of cancer cells, surgery is only beneficial if the cancer is caught early and has yet to start growing. If the cancer is already malignant, surgery is

useless in an attempt to treat the patient.[4] While all these treatments are effective in treating cancer, scientists are looking for new treatment styles that only attack cancer cells. In the past forty years, a new technique known as oncolytic virotherapy is being used to treat cancer.

Oncolytic virotherapy is a new cancer treatment that uses genetically-altered viruses to attack and destroy cancer cells. Viruses are infectious diseases that are neither classified as alive or dead. This concept can be hard to grasp and understand when something that is neither alive or dead causes several problems in the body. Viruses work in the body by invading a host cell and depositing their DNA into the cell. This virus continues to replicate inside of the cell until eventually the cell gets overwhelmed and bursts open, spreading the virus to the surrounding cells. These cells spread throughout the body causing sickness, disease, and in extreme cases death.[7] Viruses cannot be treated with antibiotics and thus are usually incurable. In oncolytic virotherapy, scientists have genetically altered the DNA in the virus' core to selectively target cancer cells in the infected area. Cancer research with virotherapy began in the early 1950s. A doctor named William Coley reported that his patients who developed infections after surgery lived longer than cancer patients who did not get infected. Coley and scientists began trials by administering bacteria and viruses into tumors and noticed that the tumors subsided.[8] At the time, the scientists did not know the mechanism behind the tumor regression and due to this, research stopped for nearly forty years. In the late 1980s, Dr. Robert Martuza began research with a genetically-modified Herpes Simplex Virus-1. His research showed that modifying the virus to inactive a protein prevented healthy cells around the tumor to be infected and harmed.[9] This sparked a research revolution and oncolytic virotherapy became the new craze. This new technique changed the perspectives of scientists, doctors, and researchers around the world. A virus, once considered detrimental to human health, is now being perceived as a possible life-

saving medicine. This change of perspective opens new doors to view things scientists once thought as harmful as potentially lifesaving.[3]

The next step was to fully understand how virotherapy worked on the cancer cells. The cellular mechanism of oncolytic therapy starts by genetically-altering the DNA of the virus. HSV-1 was the first virus to be used in virotherapy, but as research has increased, several viruses like adenovirus, poliovirus, vaccinia virus, and others are being used and genetically altered.[10] The main mutations that scientists are focusing on are mutations in thymine kinase, ribonucleotide reductase, and the γ34.5 gene.[11] These mutations all aid in destroying the membranes of the cancer cells causing the cells to lyse or break apart. The lysing of the cell allows for the virus to travel to neighboring cancer cells that recognize and destroy the cells. When a virus enters the body and infects a cell, the cell produces antigens which mark the virus and copy the code in order for the body to recognize the virus and destroy it more easily.[7] Inserting the virus into cancer cells causes the body to produce antigens against the tumor cells which signals the body's immune system to destroy the tumors.[8] The antigen production of the body's immune system also allows for the virus to not attack healthy functioning cells around the tumor. Figure 1 illustrates the process of oncolytic virotherapy. The virus enters the cell through the bloodstream

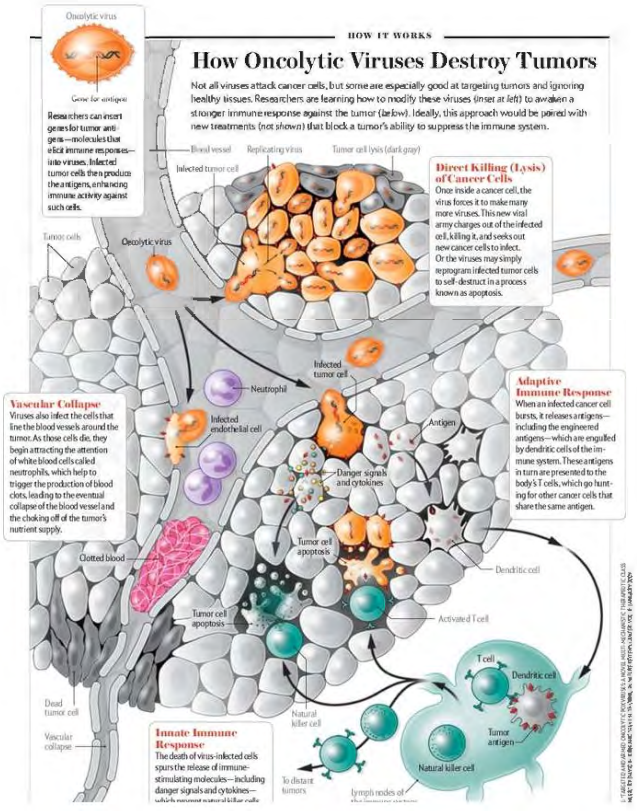

Figure 1
The process of Oncolytic Virotherapy in Cancer Cells

and begins replication. The replication continues until the cell lyses causing antigens to be formed. These antigens release into the cell causing the body's immune system to take over. White blood cells, T-cells, and B-memory cells flood the cancer cell. T-cells and B-cells are cells in the body's immunes system the destroy foreign invaders. The cells also create antibodies against the tumor cells which allows the body to begin killing unwanted tumor cells.[3] By understanding the mechanism of virotherapy, scientists can use this knowledge to treat cancer patients as well as other genetic disorders.

Once the cellular mechanism of virotherapy was known, clinical trials began to test the new treatment of cancer patients. The main concerns in clinical trials were the efficacy and toxicity of the virus. In a 1999 clinical trial conducted by Lynda K. Hawkins and David Kirn, both scientists agreed that efficacy and toxicity would be dependent on multiple factors including (1) the inherent ability of a given tumor to replicate and shed the virus (2) the location of the tumor to be treated (e.g., intracranial vs. peripheral), and (3) the route of administration of the virus.[10] Hawkins and Kirn administered the virus in three different ways: intratumoral, intraperitoneal, and intravenously in which the virus is inserted straight into the tumor, into the abdominal cavity, and into the bloodstream respectively. The doses were given in amounts of $2x10^{12}$ particles. All three administrations were well tolerated with side effects ranging from fever to slight abdominal pain. In this study, a few patients showed decreased tumor growth whereas the others showed no sign of remission.[10] While these results seem discouraging, this was an early study and further research on gene therapy had yet to be conducted. To improve cancer research, scientists had to change their perspectives on viruses and increase research of virotherapy.

In a later study conducted in 2007 in China, Wang Yu and Hu Fang found a higher response rate to the virus treatment. In a phase II clinical trial, Yu and Fang administered a $5.0x10^{11}$ vp/day dosage intratumorally for five consecutive days.[12] This trial was an open-label trial which means the researchers know which group receives the virus and which group receives the placebo control. The fact that the trial was open labeled raises some suspicion to a biased trial, but nevertheless, the trial is credited and trustworthy. The virus used in this study was a mutated H101 adenovirus, common cold viruses. Fifty-three patients with a variety of cancers participated in the clinical trial in which seven people dropped out of due to illness or unknown circumstances. A control group was set up as well to ensure that the virus was indeed helping the

Lesion	n	Median size (cm2)	CR	PR	SD	PD	Response rate (%)
H101	46	12.5	3	11	24	8	30.4
Control	46	11.3	1	5	28	12	13.0

Figure 2. Efficacy of H101 on Injected Lesion and Control Lesion in Phase II

patients. Like in the previous study, side effects included flu like symptoms and injection pain. Figure 2 shows the efficacy results of the trial. The results show that there was a 17.4% increase in efficacy of the virus and the control. This shows that the virus was working on cancer cells in the patients.[12] Yu and Fang's study shows that virotherapy does help decrease tumor size in cancer patients. The study also proves that further research and improvements in technology allow for improvements in clinical trials. With more studies like this one, scientists can begin to understand disease therapy and use this knowledge to combat genetic disorders.

While oncolytic virotherapy is producing promising results, concerns have arisen in the usage of this treatment option. One concern is the high dosage of virus administered to the patient.[13] The concern for this is that the insertion of this high dosage of virus may cause detrimental effects to the patient. To put it into perspective, a dosage of HSV-1 virus in

virotherapy is about 10^6 pfu whereas the vaccine for HSV-1 virus is about 10^3 pfu. The virotherapy dosage is 1000 times stronger than the typical vaccine.[13] What does this do to the patient? Is too much virus harmful to the patient or could it possibly increase immunity? Without further research and trials, it is difficult to answer these questions. Another concern for the use of viruses is increased immunity to the virus. With constant exposure to a virus, eventually the cancer builds up enough antigens and antibodies to which the drug no longer works. This is the same concept as antibiotic treatment. Overtime, the bacteria becomes resistant to the antibiotics and no longer die. To counter the antibiotic resistance, scientist have begun "arming" the viruses to prevent resistance. Scientists have added defense mechanisms such as memory T-cells. Cytokines, prodrugs, and anti-angiogenic factors to prevent the cancer cells from becoming immune to the virus therapy.[13] These factors work by preventing the cancer cells from producing antigens and memory receptors to the virus. By doing this, cancer cells are not able to become immune to virus being used for therapy. Although there are some setbacks to virotherapy, further research is being conducted to work on these problems and increase the efficacy of the virus.

Future implications of oncolytic virotherapy include systemic versus local treatment, combination therapy, and the incorporation of virotherapy into other disorders.[9] In fact, studies are currently being conducted where viruses are being used to treat various genetic disorders. The composition of the entire body is composed of around $3.6x10^{22}$ letters. These letters make up the genetic code of the human body. Gene sequences code for amino acids that then code for specific proteins that make up the entirety of the body. Although the DNA sequence in a human is big and complex, it only takes one change of a letter in the code to disrupt the homeostatic environment of the body. These disruptions in sequences are known as genetic disorders. Genetic disorders are caused by changes, known as mutations, in the genetic code. Changes in genetic

material can be inherited, environmental, or random.[14] Because genetic material can be altered in numerous ways, genetic disorders are common and almost all disease are caused by genetic mutations. Mutations that occur in the code happen in three different ways: base substitutions, deletions, and insertions. In a genetic code, every set of three letters known as codons translate into a specific amino acid. In base substitutions, a single letter is changed causing a change in the amino acid code. Figure 3 shows examples of different types of base substitutions in genetic codes. Deletions are mutations in which an entire codon is taken out of the code. This deletion causes a variety of problems from the insertion of a different amino acid to the incompletion of the genetic sequence. Insertions act in the opposite manner and codons or base pairs are added into the code shifting the code and altering the sequence.[15]Despite this, the body has mechanisms in which the DNA fixes itself when mutations occur. However, when these mutations are not fixed, genetic disorders occur.

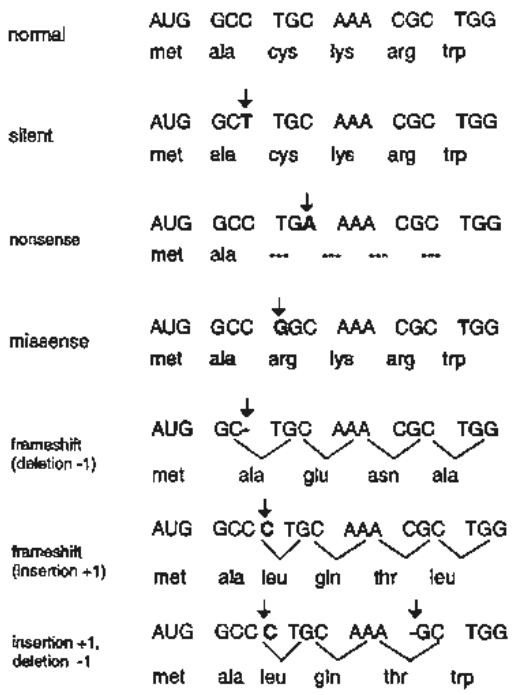

Figure 3. Different Base Substitution Mutations

Genetic disorders can benefit from use of virotherapy as treatment. Genetic codes that have been mutated can be reimplemented using viruses to inject genetic codes into the affected cells. This new technique opens a new perspective for the future of medicine. Instead of looking at diseases as threats to the body, they can be transformed into medicines and preventative treatments. Research on genetic disorders needs to continue in order for the knowledge of genetic mechanisms to continue to grow. Without knowing the mechanisms of genetic disorders,

scientists cannot fully hypothesize and design new treatment plans to effectively treat the disease. Scientists are currently in progress of looking at diseases to treat genetic disorders. One of the most recent studies involves the use of adenoviruses to treat cystic fibrosis.

Cystic fibrosis (CF) is a genetic disorder in which the cystic fibrosis transmembrane conductance regulator (CFTR) gene is altered causing the CFTR protein to malfunction.[2] A cell is surrounded by a layer of fatty acid chains known as the cell membrane. Embedded in this membrane are protein channels that help facilitate substance across the membrane and into and out of the cell. The most common substance that are facilitated across the membrane are ions. Ions are positively or negatively charged particles that help in different functions in the body. For instance, sodium and potassium ions aid in muscle contractions. In cystic fibrosis, the CFTR protein helps facilitate chloride ions across the membrane. Chloride ions aid in the formation of salt in the body. Without the proper transportation of chloride ions, water cannot form at the cell surface causing a buildup of mucus in the cells.[2] The buildup of mucus in the organs cause an increase in bacterial colonization. The most common bacteria species are *Pseudomonas, Haemophilus influenza, and Staphylococcus aureus.*[16] Bacteria colonies usually form in the lungs causing infections. Figure 4 shows a chest radiograph of a CF patient's lungs. Notice the large amount of red mucus in the lungs.

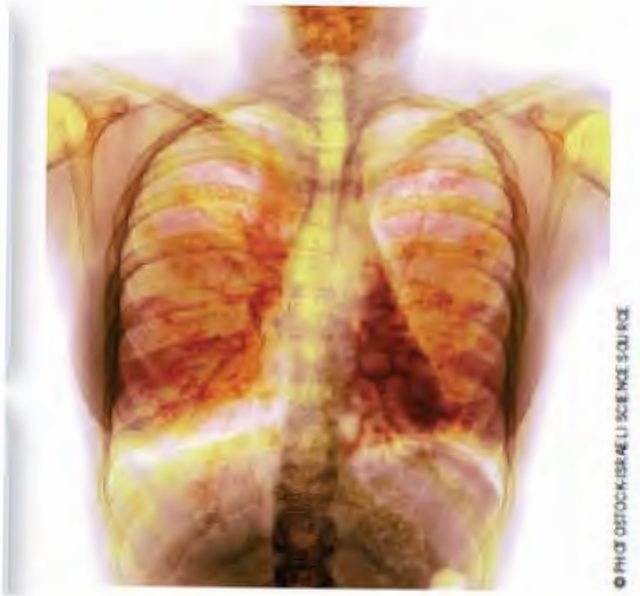

Figure 4. Radiograph showing excess mucus in a pair of lungs from a CF patient.

Cystic fibrosis is diagnosed early on; in fact, a sweat chloride test for CF is conducted on newborns. An abnormal amount of salt on the skin is a marker for CF diagnostics because the mutation of the CTFR gene causes the chloride ions to stay on the surface of the cell resulting in an increased salty environment.[16] Other diagnostic tests include genetic mapping of both the parents and patient. CF is a recessive trait which means both parents require a copy of the recessive gene in order for the child to contract CF.

Treatment for CF is complicated for two reasons. The first reason is that CF is not caused by just one mutation in the CTFR gene. In fact, more than 2,000 different CFTR gene mutations have been discovered in the past forty years, which prevents development of a single treatment.[16] The second reason that CF is hard to treat is that the disease is not just localized to one organ. For instance, patients with CF experience lung problems, CF-related diabetes, cardiac problems, and others.[16] Treatment now becomes complicated and multiple medications must be given to control all the problems presented with CF.

In a study conducted in 1994, researchers at the University of North Carolina at Chapel Hill conducted a Phase I clinical trial in which they tested the effects of adenovirus on CF. The researchers have found that genetic therapy is hard to conduct effectively. Gene therapy requires the gene carrier to be a non-replicating cell, and that the recombinant gene needs to be in the target area for a prolonged amount of time.[17] The researchers discovered that adenoviruses are adequate vessels for gene therapy. The virus was altered to carry a functioning CFTR gene to be inserted into nasal epithelial cells. The virus would be inserted into the nose through and inhalation device. The effects of the drug would be monitored as well as the CF patient's daily routine.[17] The only problem with this study is that it was all theoretical and a proposed plan of action. No human trials were actually conducted in this study. Despite this setback, the study did

create the virus that contained the CFTR gene. This research in and of itself is a step forward in CF research. It is important for researchers to begin human trials on this study to find out if the CFTR gene in the adenovirus will actually transfer to the membrane. Researchers are hopeful that the CFTR gene recombination will successfully happen. If promising results occur, then we are one step closer to finding a cure for cystic fibrosis.

About twenty years later, a similar study to the one conducted at Chapel Hill was actually performed. Researchers at the National Heart, Lung, and Blood Institute successfully inserted the first adenovirus containing the CFTR gene into a human. Just like in oncolytic virotherapy, the adenovirus transfer in CF patients faced the same problem of immunogenicity.[18] To fix this problem, the researchers basically stripped the adenovirus of all of its internal DNA and inserted the desired genome into the virus. At this point, the virus was just the vessel for the desired genetic data. The chart in Figure 5 shows the effects of the adenovirus on CFTR distribution. As seen in the graph, the 5% dotted line represents the target concentration of CFTR gene in the nasal cavity. The adenovirus was able to get enough of the gene into the body, however it only lasted for about three days until the concentrations dropped down again.[18] Although the virus worked in transporting the gene, further research needs to be conducted to find a way to modify the virus to prolong the amount of time the gene is over the concentration amount. Furthermore,

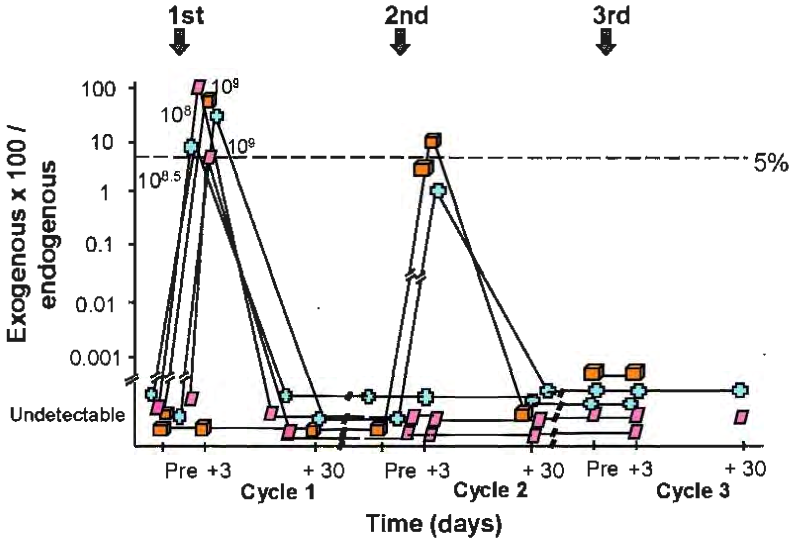

Figure 5. Assessment of the effect of adenovirus on transporting CFTR.

research needs to be conducted to understand why stripping the adenovirus of its DNA worked better than the whole virus. By doing this, scientists can begin to understand more about genetic recombinant therapy, viral mechanisms and structures, and cystic fibrosis.

Genetic mutations are constantly forming and evolving throughout time and we have barely hit the surface of identifying all the genetic mutations that have occurred. As new genetic disorders evolve, our ways of treating these disorders must evolve as well. In the past twenty years, virotherapy has surfaced as a new technique to treating various diseases. As seen in the paper, virotherapy is helping reduce symptoms in both cancer patients and cystic fibrosis patients. Virotherapy is beginning to be used in the treatment of other disease as well. Trials are currently being conducted for the use of virotherapy to help rebuild vascular walls in damaged heart muscles; similarly, virotherapy is being used to rapidly grow hair follicles to treat balding in people.[18] The versatility of a virus allows it to be used to treat a variety of diseases; in fact, diseases are not the only thing that virotherapy is good for either. This treatment is also being used to strengthen vaccinations, synthesize vaccinations for possible bioterrorism acts, and develop vaccinations against addictive drugs.[18] By looking at a harmful virus as a potential life saver, scientists were able to create a new treatment for several different diseases. Even though scientists have changed their perspective on viruses, the general public is not too keen on the idea of inserting a virus into their body. The next step in the use of oncolytic virotherapy is encouraging the public to accept this change of perspective as well. Innovative marketing techniques and transparency must be used to allow people to open up to the idea of virotherapy. By being open to new imaginative ways in cancer and genetic therapy, both the scientists and the patients have the ability to one day find cures for these diseases.

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