The Implementation of CRISPR In Accordance with Brain Cancer and The Moral Concerns of Its Progression in The Future

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CRISPR-Cas 9 was discovered by Emmanuelle Charpentier and Jennifer Doudna who later received a Nobel prize for their efforts. It was dubbed "genetic scissors" and they have now progressed to be able to be used in trials on cancer and various other genetic mutations, in this paper specifically progressions in brain cancer. A recent experiment has addressed many major CRISPR concerns like the ability of the mutation to spread and infect healthy cells and the difficulty of inserting the code into the cell in the first place. Another main concern deals with the ethical and moral implications of CRISPR and how the general public feels about CRISPR moving to human trials/the experiments on embryos. This paper will touch on the moral progressions of CRISPR and what the most recent progressions are as a whole.

Introduction

Clustered regularly interspaced short palindromic repeats, shortened as "CRISPR" was able to affect DNA directly by cutting a segment and inserting its own code. The associated Cas-9 protein is an enzyme produced by the CRISPR system. "When the target DNA is found, Cas9 – one of the enzymes produced by the CRISPR system – binds to the DNA and cuts it, shutting the targeted gene off. Using modified versions of Cas9, researchers can activate gene expression instead of cutting the DNA" [1].

It was discovered in 1987 from Escherichia coli bacteria by Emmanuelle Charpentier and Jennifer Doudna who later received a Nobel prize in 2020 for their discoveries [2]. CRISPR is often used on cancerous cells to correct the cell without causing severe harm or damage. CRISPR is inserted by injection, sometimes directly into the cells but others into the general direction of the mutation.

Its use is currently limited due to two major factors, the first and most concerning one being that CRISPR can spread and mutate healthy cells and because most tumors have numerous genetic mutations, making it incredibly difficult to insert a code and difficult to insert in general. Second, CRISPR is inserted using a virus, which cells can eventually build n immunity to. Recent advancements of CRISPR include the amount of code now needed to correct a mutation. 419 nucleotides used to be needed and that amount has now shortened to around 19. Other recent advancements include the median life span after CRISPR was injected as well as a lower risk of off-target gene editing.

History of Application Methods for CRISPR

CRISPR has completely revolutionized since its founding in 1987 in many ways, one of which being its application. "Its mechanism is as follows: sgRNA(single guide RNA) composed of 20–24 variable bases recognizes the DNA target sequence protospacer in the way of RNA–DNA matching and leads Cas9 endonuclease [8] to cut the target sequence by recognizing the PAM (protospacer adjacent motif) sequence flanking the target DNA sequence." [3] The body then repairs the DNA through non-homologous end-joining or homologous recombination to create the correct mutations. It's incredibly important that during this end-joining, the PAM (protospacer adjacent motif) sequence is recognized; if not, the Cas9-mediated cleavage process will not be able to occur.

There is much difficulty in the actual insertion of CRISPR into a specific part of the body. The two most common ways are as follows: a viral vector method, a non-viral vector method/ a physical method. A viral vector method uses a non-dangerous virus to infiltrate the cell in order to change its genetic code. This is done by 'flooding' the targeted area, which means inserting by dispersing it without a specific boundary. This leads to many dangers because of the mutations that are caused in healthy cells. It also leads to immunogenicity, invoking an immune response to the virus in the body, making the virus less effective or completely ineffective once implanted. "Non-viral vectors are DNA plasmids that can be delivered to the target cells as naked DNA or in association with different compounds such as liposomes, gelatin or polyamine nanospheres" [4]. These methods include microinjection and hydrodynamic delivery, which remain to be the most common forms on injection.

Microinjections are extremely easy and efficient and have

one of the highest rates of successful insertion. The benefits of microinjection are that it's more suitable for in-vitro insertion, it can be used in highly specific areas, and it can be completely controlled by the injector. The negatives are its impracticality due to how difficult it is to insert into a cell: tediously one-by-one. Hydrodynamic delivery is much simpler but does not remain efficient. Drugs/CRISPR is/are directly injected into the veins through syringes. Hydrodynamic delivery is also a form of 'flooding' and due to how much this could spread and mutate other cells, it is not currently the best for insertion.

CRISPR Integration as Seen in Natural Settings

Archaea and bacteria have been seen integrating aspects of CRISPR because of their immune systems being so adaptive but their genetic adaptations seem to be more advanced than initially thought. They contain domains that "are characteristic of several nucleases, a polymerase, and various RNA-binding proteins."[5] When this was initially discovered, it was thought that it was an extremely ideal repair system but upon further investigation, some of the coded DNA, also referred to by the authors as a "CRISPR spacer", are actually direct lines of code from various viruses. This led to the conclusion that CRISPR-Cas 9 is used in order for the bacteria and archaea to defend itself against a dangerous virus.

It was proposed that it incorporated a system similar to that of a eukaryote but there is a severe difference in their ways of incorporation. Eukaryotic cells have RNA systems where genes are transcribed in multiple different RNA polymerases in various different gene classes. The other crucial difference is their way of binding to sequences. Eukaryotic RNA polymerases "need to interact with a variety of additional proteins to specifically initiate transcription."[6] This allows them to function at a more advanced level needed for multicellular organisms.

Bacteria and archaea use CRISPR to integrate a piece of DNA from the nucleic acid into the DNA sequence which leads to an eventual immunity against their invader. The bacteria this was most recently found in was Streptococcus thermophilus that was able to build a resistance to the cognate page. If the code was not perfectly inserted using the target sequence, there would not be any resistance created and it would be repealed. This takes a 3-step process that is broken down into two major parts - the "information processing system" and the "executive system". The information processing is as it sounds: the processing of the genetic code from the targeted sequence and then the execution of it. The execution of it includes interpreting the code and integrating it in the actual DNA[7].

Brain Cancer and Its Treatments

Brain cancer has a tremendous effect on a patient's quality of life due to the body being f life due to the body completely reliant on the health of the brain. The five-year survival rate for patients ages 15 through 39 is roughly 72% but this drastically changes for patients ages 40 and up. The survival rate becomes 21% due to the decrease in brain elasticity[8]. The likelihood of being diagnosed with brain cancer also rises with age, making the survival rate fall a considerable amount. The most common form of treatment is surgery and it's usually sufficient for removal. However, tumors like glioblastoma are not easy to remove and pieces of the tumor are often left behind that can continue to multiply and expand.

Chemotherapy is used in these cases and has very dangerous side effects on the quality of life of the patient. Chemotherapy, in short, damages the cells' DNA. There is no way for it to dictate which cells it will harm and because of this, it ends up attacking the majority of the cells in the centralized area in which it is applied or in extreme cases, the entire body. The goal of chemotherapy is for it to spread through the body, or centralized area, and take out the mutated cells as they are more susceptible to damage. Through this process, it often attacks more than just those cells leaving the patient feeling incredibly weak. CRISPR would be able to attack the tumor directly and fix the mutations with little to no side effects and successfully turn the mutated cells into their healthy state leaving the patient without such a big loss of cells[9].

As stated, surgery is currently the most common treatment for any cancer in the brain and spine. The procedure following the surgery is typically radiation which is applied directly to the areas that have experienced the cancer. Radioactivity to the brain and spine is extremely dangerous due to their hypersensitivity and is usually only used post-operatively to ensure all of the cancerous cells are removed. Radioactivity is also used preoperatively to shrink the tumor before surgery is done.

Chemotherapy is the most common form of cancer treatment in general, but it is also used postoperatively due to the negative effects it can have on a patient's quality of life[10]. Spinal tumors treated with chemotherapy also don't often lead to positive results. Spinal tumors actually seem to usually be not reacting to chemo. Chemotherapy has many side effects, especially when its use is directed towards the brain. Patients will have trouble performing tasks and will have many problems with memory/have a weakened state in general. Oftentimes, patients will feel sickness and/or dizziness. No matter the case, surgery is usually involved in the treatment plan for the patient. In cases where surgery is not possible, it is oftentimes a race for the tumor to be shrunken until operation can be performed. In areas that it cannot be performed at all like the brain stem, radiation and chemotherapy will be applied in hopes of the cancer to be removed.

CRISPR would be able to ultimately tackle the problem where it began: the mutated cells. Tumors are made of hundreds or thousands or even millions of cells that had difficulty copying the correct string of DNA. CRISPR would be inserted into each of these with a virus in order to be able to infiltrate it and "cut" the incorrect code of DNA to replace with the corrected one as has been seen most recently in bacteria and archaea.

Limitations

As stated previously, CRISPR has two main limiting factors. One of them being the difficulty of the actual insertion. Bodies automatically build immunity to viruses so bypassing the cell will progressively become more difficult. Difficulty of insertion also narrows down to the actual cell mutation. These hundreds or thousands of millions of cells will have slightly different mutations. The very first cell mutation will copy its DNA to hundreds of cells. Those hundreds of cells are now more susceptible to change and so the DNA they copy could be slightly different. Now within one tumor could be many different mutations which means the injector of CRISPR will need to find these cell mutations and adjust accordingly.

The second limiting factor will be the possibility of mutation to healthy cells. In trials, CRISPR is inserted into the healthy cells of the organism in order to mutate them to become cancerous. Something similar can occur when trying to effectively cure the tumor. When CRISPR is injected, it can spread to various healthy cells and change their genetic structure and mutate them. In a recent study by using mice injected with glioblastoma, however, this seemed to only affect .05 of the testing subjects and had an efficiency of 38.1%. The median survival time for the mice was 68 days versus non-functionals g-RNA treated mice having a median survival of 24 days. "To evaluate whether the nanocapsules can achieve specific gene disruption, we used luciferase as a model gene and encapsulated firefly luciferase guide RNA (sgLuc) as the targeting sequence in ANCSS(Cas9/sgLuc) nanocapsules. The Cell Counting Kit-8 (CCK-8) cell proliferation assay and a luciferase knockdown assay were used to assess the cell conditions after treating with the nanocapsules in luciferase-expressing U87MG cells constitutively (U87MG-Luc). The CCK-8 assay showed that the nanocapsules were nontoxic (fig. S7), which may be ascribed to their small size and nearly neutral surface charge (table S1). ANCSS(Cas9/sgLuc) treatment produced a 42.0% reduction in luciferase protein expression, a significantly larger reduction than that achieved by nonreducible nanocapsule ANC(Cas9/sgLuc) or by nontargeting nanocapsule NCSS(Cas9/sgLuc) at 17.2 and 25.1%, respectively (Fig. 1G). Nanocapsules containing Cas9 with scrambled guide RNA (sgScr) did not reduce luciferase expression, indicating that gene silencing by ANCSS(Cas9/sgLuc) was sgRNA sequence specific." Nanocapsules' abilities to disrupt specific genes were tested by encapsulating the firefly luciferase guide RNA (sgLuc) and assessing their effects on luciferase expressing U87MG cells.The (CCK-8) showed that the nanocapsules were effective and safe due to their size and nearly neutral surface charge. With this treatment, there was 42% reduction of in luciferase protein expression, a much more significant reduction than seen by nonreducible nanocapsules [11].

This was achieved by a newer system that "combines Cas9 nuclease with single-guide RNA (sgRNA) to bind and cut target DNA for gene editing (1, 2) and applications in the treatment of genetic disorders (3, 4)." This rivaled the most current form of CRISPR that uses viral vectors but the biggest concern with them is the amount that they spread. The possibility of mutation far outweighs the tremendous benefits it has.

The conductors of the experiment state the following "We addressed these challenges by developing a new CRISPR-Cas9 brain delivery platform that satisfies the following design criteria: ease of formulation, high loading content, small and uniform size, stability with a long plasma lifetime, BBB permeability, active targeting of the brain and brain tumor cells, rapid intracellular release, efficient gene editing, and negligible off-target effects." They were especially successful in attacking the brain, tumor cells, and the negligible off-targets. They specifically attacked the PLK1 (polo-like kinase 1) gene.

PLK1 is a serine/threonine protein kinase and it plays multiple critical roles in centrosome maturation and mitotic chromosome separation. In simpler terms, it allows cells to divide and it occurs during the G2-M stage of cellular division. This is also the time where unhealthy cells are more likely to die from the inability to split and attacking PLK1 is doing exactly that. Once CRISPR successfully attacks PLK1, as done explicitly in this experiment, the mutated cells are no longer able to divide and they will die in their attempt to do so. This experiment was able to attack PLK1 specifically and create a manner of the injection of CRISPR that prevents it from spreading and mutating the healthy cells of the mice. Once the cells began to multiply, the tumor was gone but it did take away one of the positive aspects of CRISPR: the ability to revert unhealthy cells to healthy cells leaving a difficult hole to patch up in the brain.

Other limitations include cost-effectiveness and the fight to move to clinical trials. CRISPR research and trials are fairly expensive with the Yale Genome Editing Center charging $11,000 for CRISPR/Cas KO, $14,500 for CRISPR/Cas point mutation, simple knockin, and $5,250 for a transgenic mouse. In order to run the numerous trials required, the costs can easily reach up to six figures.

Even so, there is a necessity to try any and all treatments possible, even if they seem impossible, in order to achieve the desired results in the future. Potential risks in clinical trials, however, are extremely dangerous.

Gene therapy did not begin with CRISPR. Before CRISPR, gene therapy used viral vector deliveries of therapeutic transgenes for many different mutations. "Jesse Gelsinger, an 18-

year-old with a mild form of the genetic disease ornithine transcarboxylase (OTC) deficiency, participated in a clinical trial which delivered a non-mutated OTC gene to the liver through a hepatic artery injection of the recombinant adenoviral vector housing the therapeutic gene. Unfortunately, Jesse passed away 4 days after treatment. The adenovirus vector triggered a much stronger immune response in Jesse than it had in other patients, causing a chain of multiple organ failures that ultimately led to his death. At the time of the trial, adenoviral vectors were considered reasonably safe. In preclinical development, however, two of the rhesus monkeys treated with the therapy developed a similar pattern of fatal hepatocellular necrosis."[12]

"After the FDA determines that the proposed study will not place human subjects under unreasonable risk of harm, the CRISPR system must go through three stages of clinical trials. First, the CRISPR system must undergo phase I trials. Phase I trials are conducted on healthy human volunteers. During Phase II trials, investigators collect data on whether the CRISPR system actually works as intended. Those enrolled in phase II trials have been diagnosed with the condition the CRISPR system intends to treat. Finally, large-scale phase III studies are conducted. During these trials between 300 and 3000 subjects diagnosed with the target condition are enrolled to gather more robust data on safety and effectiveness. Only after all of those hurdles are successfully cleared will a CRISPR system obtain FDA approval for use in a clinical setting." Although there is no absolute way to ensure absolutely no unreasonable harm is done, there are many limitations put in place to prioritize life over advancements. The protection of individual rights will always be assured.

Morality and Execution

Progression to trials on humans is closer than one might think. Already testing is progressing for congenital vision disease, something seemingly small but will be the beginning of tests to see whether or not cells in the human body can truly be mutated. With this, comes, of course, moral implications. When CRISPR first landed to the widespread public, it subjected itself to much religious debate as the majority of the world classifies themselves to be religious. The general public can't help but wonder whether or not this will do much good and how it aligns with their religious and moral beliefs. However, this also serves as a reminder that the majority of the world has not researched or truly knows what CRISPR and its capabilities are, following a blind devotion to their beliefs. This is especially dangerous as it is seen more often than not as of late. A general statement such as "CRISPR mutates DNA and can allow parents to customize their baby" sounds inherently dangerous and can be. CRISPR will most likely not be used to do so and will be used in different life saving medical instances.

However, a major concern with the advent of CRISPR is the possibility that people will use it to perpetuate ableist ideals. Genetic predispositions would have major involvement with CRISPR. In the future, CRISPR could target predispositions while the fetus still remains in the womb. It would be able to take away the hereditary gene responsible for cancer and replace it with a healthier gene. Virtually all genetic predispositions in the womb could be replaced with a healthy copy of the gene. The major worry is that it is morally wrong to do so.

The line between implicit bias and ableism would be difficult to draw. If changing the person's DNA as a fetus will improve quality of life then there are little to no arguments against the project as a whole. However, again, it's very difficult to know in what way the decision is being made. If CRISPR were to progress to a point where DNA as a fetus is completely alterable, the moral dilemma will quickly cause it to hit a stopping point.

Scientific American states, "As disability studies scholars and women with genetic differences who are experts in thinking about the consequences this technology will have for actual human beings, we have grave worries that the use of these "genetic scissors" will, in the future, cut people like us out of existence without others even noticing. Scientists who use CRISPR could see editing genes such as ours out of the gene pool as entirely uncontroversial."[13]. The fear is that if disabilities are completely cut out socially, the consequences for those who are alive could be devastating. CRISPR poses many grave questions for all of society: is it okay to eliminate an entire group of people if it is believed to be in their best interest? And, why should people without disabilities be able to make that decision? If the answer were that, yes, it is okay to genetically modify fetuses, would it actually be for the greater good, or would it be perpetuating ableist internal biases?

In another case, CRISPR causes serious damage to human embryos that have the mutation for hereditary blindness. Around half of the embryos had a mutation and some of the cells were so badly damaged that they were unfixable according to the New York Times. Many could have a moral loyalty to these cells but they are only embryos and only have the possibility of human life. After all, an eye has been turned away from testing when it comes to mice, rats, rabbits, and even chimpanzees[14].

A debate that has been recurring recently is when a fetus becomes a human life. There is a belief that it becomes living at conception while others believe it is at the first heartbeat or maybe even just the second trimester in general. Overturning Roe v Wade erupted new found opinions on the value put on human life. In its overturn, there is a heavier weight put on a fetus than on the life of its mother. Does the pattern of the sacrifice of human lives continue on the morality of not performing on an unborn human embryo? Either way, in order to have a society with no genetic mutations or genetic predispositions, there would need to be testing on human life.

There is no doubt CRISPR would save and revolutionize millions of lives but it will also revolutionize our way of living and

the current way of thinking. With its continued implementation, biases and moral values will need to continue to be pushed. Many will continue to be upset at the mutation of genes and the general public will question what they do not have a full grasp of, as is the norm. However, I believe that there is a common goal: humanity and the preservation of life. That is the ultimate goal of CRISPR-Cas 9 in whichever way it is applied.

Moving Forward

There are many different pathways for improving CRISPR in terms of brain cancer. The most imperative, however, is its application. In the experiment previously stated, there is already a huge jump in effectiveness. A median survival time of 24 days versus one of 68 is a vast development; however, more paths must be taken.

Viral vector methods would be too dangerous as flooding mutates numerous healthy cells, and a body part as important as the brain cannot face such a risk. There also lies the problem of the immunity the body automatically builds when faced with a virus. The body will eventually reject the method entirely and mutate the healthy cells in the surrounding areas.

The best possible route for progression to clinical trials would be using a non-viral vector method like microinjections. Although tedious and extremely difficult, this would limit the risk in the surrounding areas of the brain as well as ensure the cells will mutate in a healthy manner. When dealing with an organ as fragile as the brain, the only way to progress is to face it with extreme precision, making a non-viral vector method the most logical route to follow.

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