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CRISPR/Cas9 Gene Editing: An Approach to Prevent Hereditary Cancers

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ABSTRACT

Mutations in oncogenes and tumor-suppressor genes can give rise to a variety of genetic cancers. The use of CRISPR/Cas9 can be applicable in the prevention of these cancers through the knockout of oncogenes and the upregulation of tumor-suppressor genes. This paper proposes this use of the technology by reviewing previous research and successes of CRISPR in treating genetic diseases and the application of those methods to preventing cancer. CRISPR/Cas9 can alter mutated DNA; in the case of oncogenes, CRISPR/Cas9 would restore the cell's ability to grow and divide, while it would restore the tumor-suppressor gene's ability to induce apoptosis. This paper also discusses the limitations of this technology, including off-target effects and the delivery of CRISPR into cells.

Introduction

Inherited genetic changes are estimated to be responsible for as much as 10% of all cancer cases (1). Inherited cancers are caused by mutated genes, passed down through a parent's egg or sperm cells. There are two categories of genes that contribute to cancer. Tumor suppressor genes, when functioning properly, slow down cell division and cause programmed cell death. In the absence of these genes, cells can grow uncontrollably, leading to cancer. TP53 is an example of one such tumor suppressor gene. Coding for the p53 protein, which regulates cell division, a mutated TP53 gene can result in rapid cell division. Inherited changes in this gene can lead to Li-Fraumeni syndrome, an inherited predisposition for several types of cancer. Another class of genes related to cancer is proto-oncogenes, which normally help cells grow, divide, and stay alive. A mutated proto-oncogene can activate at an improper time, causing the cell to grow out of control, which can lead to cancer. Most oncogenic mutations occur during an individual's lifetime, rather than being inherited (2). A mutated BRCA1 or BRCA2 gene passed down from a parent can increase the child's risk of developing breast and ovarian cancers. Inherited changes in the APC gene, which regulates the turnover of beta-catenin within the Wnt pathway, can lead to familial adenomatous polyposis, a hereditary cancer predisposition syndrome leading to adenomatous polyps. If untreated, these can lead to the development of colon and rectal cancers (1).

Current cancer preventions include maintaining a healthy weight with physical activity and a proper diet, and limiting fast food, red meat, and alcohol consumption (3). Some claim that various vitamins and dietary supplements can prevent cancer, but no reliable evidence exists to support this (4). Common cancer treatments include chemotherapy, which kills cancer cells via chemicals, hormone therapy, which treats cancers that use hormones to grow, radiation therapy, which uses radiation to kill cancer cells and shrink tumors, and surgery, which removes cancer from the body (5). The current survival rate for all cancers, even with these therapies, is about 67% five or more years after diagnosis (6). These therapies also induce painful side effects, such as pain, fatigue, nausea, vomiting, and anemia (7).

In order to prevent the long and painful journey of cancer treatment, this paper introduces a method of preventative care to reduce the risk of cancer given a patient with a family history of the disease and known inherited gene mutations. This method includes altering the mutated gene via gene editing. Introduced in the

late 1900s, scientists have studied genome editing for its potential ability to prevent and treat genetic diseases. Several gene editing technologies exist, such as clustered regularly interspaced short palindromic repeats - CRISPR-associated protein 9 (CRISPR/Cas9), zinc-finger nucleases (ZFNs), and transcription activator-like effector nucleases (TALENs). These technologies are all adapted from naturally occurring biological processes (8). This paper will focus on CRISPR as it is cheaper, more efficient, and can modify several genomic sites simultaneously. CRISPR is a defense mechanism in prokaryotic organisms, used by researchers for targeted gene editing. For these purposes, DNA is programmed to target specific DNA sequences. A guide RNA (gRNA) recognizes a specific site in the genome, and then recruits a Cas protein, which will edit DNA at the designated target. It precisely cuts these sequences, and then lets the natural DNA repair process take over (9). Since the discovery of this technology, many new techniques have been developed for various applications (Fig. 1).

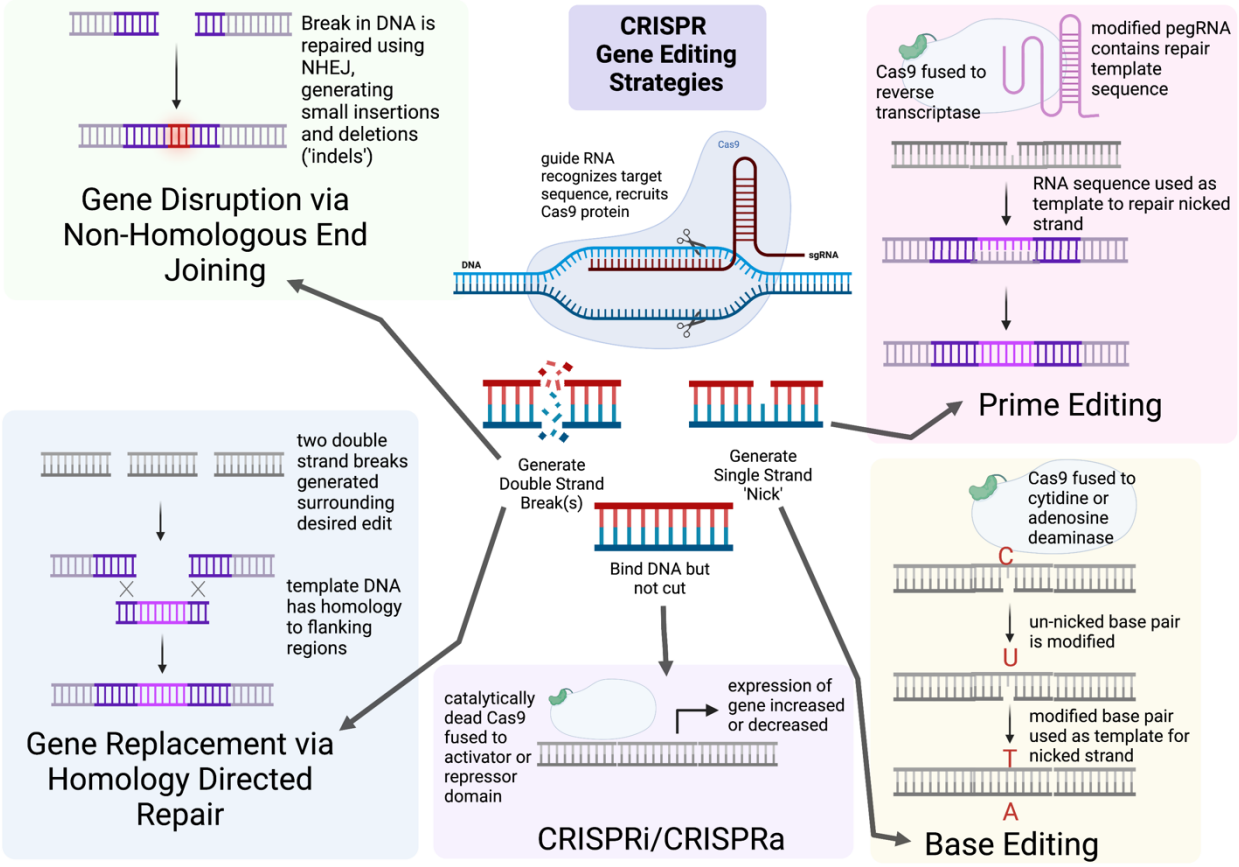

Figure 1. CRISPR Gene Editing Strategies. Gene disruption via non-homologous end joining is a method in which CRISPR generates a double-strand break, causing the insertions or deletions of genetic code. Gene replacement via homology-directed repair uses CRISPR to generate double-strand breaks around the targeted section of DNA, which is then repaired by homologous recombination and a DNA template. CRISPR interference (CRISPRi) and CRISPR activation (CRISPRa) use a deactivated Cas9 fused to an effector domain to increase or decrease the expression of the gene. Base editing uses CRISPR to nick a nitrogenous base on DNA and makes a matching base change, and the cell repairs the nicked section. Prime editing is a method that uses CRISPR to nick a base and introduce an RNA sequence as a template to repair the nick. Taken with permission from Maani et. al 2021 (35).

CRISPR/Cas9 Technology

The CRISPR/Cas9 system is currently being used in clinical trials aiming to cure genetic diseases. Recently, a trial targeting sickle cell disease and transfusion-dependent beta-thalassemia, two genetic disorders affecting the hemoglobin in red blood cells, used CRISPR to introduce extra fetal hemoglobin. This trial saw a reduction in symptoms relating to the two diseases in most patients (10). CRISPR Therapeutics has also shown promising results in clinical testing with allogeneic CRISPR-modified CAR T-cells. With this technology, T-cells are genetically engineered to have a receptor capable of identifying cancer cells, prompting them to initiate an attack. CRISPR Therapeutics has reported an approximate 40% complete remission rate in aggressive large B-cell lymphomas using this treatment (10). Although CAR T-cell therapy is seemingly promising, these therapies have serious side effects. This includes cytokine release syndrome, in which large amounts of cytokines are released into the blood due to an excess amount of CAR T-cells. This can cause headaches, fevers, trouble breathing, nausea, vomiting, a fast heartbeat, and muscle and joint pain. This treatment can also cause nervous system problems, which can result in headaches, changes in consciousness, seizures, tremors, trouble speaking, and loss of balance (11). CRISPR therapy for existing diseases involves many side effects. These CRISPR trials prove the success of the technology, but because of the unwanted reactions to the therapy, our focus should lie in the prevention of these diseases.

Base editing is another type of CRISPR technology. This involves targeting mutations caused by point mutations. A point mutation is a genetic mutation involving the replacement, addition, or deletion of a single nucleotide base in a DNA sequence. Some point mutations may not result in any change in the protein's amino acid sequence, while others can be missense mutations, meaning the mutation leads to the incorporation of a different amino acid in the protein, which may affect its structure. The structure of a protein determines its purpose, meaning that the proper formation of a protein is essential to the overall function of an organism. Some point mutations can cause a nonsense mutation, which introduces a premature stop codon, causing premature termination of the protein synthesis. A point mutation in a BRCA1 or BRCA2 gene can disrupt its function, leading to damaged DNA repair processes. This can lead to an accumulation of genetic errors in cells, which makes them more susceptible to further mutations, which can cause them to grow uncontrollably and form a tumor (12). Base editing via the CRISPR/Cas9 technology allows for the introduction of point mutations in the DNA, reversing the harmful inherited point mutations (13) (Fig. 2). This technology does not cause double-strand breaks, which lead to mutations and chromosome rearrangements that can result in the formation of a tumor or death (14). There is a wide variety of genetic mutations causing cancer, meaning many different methods using CRISPR are applicable for these different mutations.

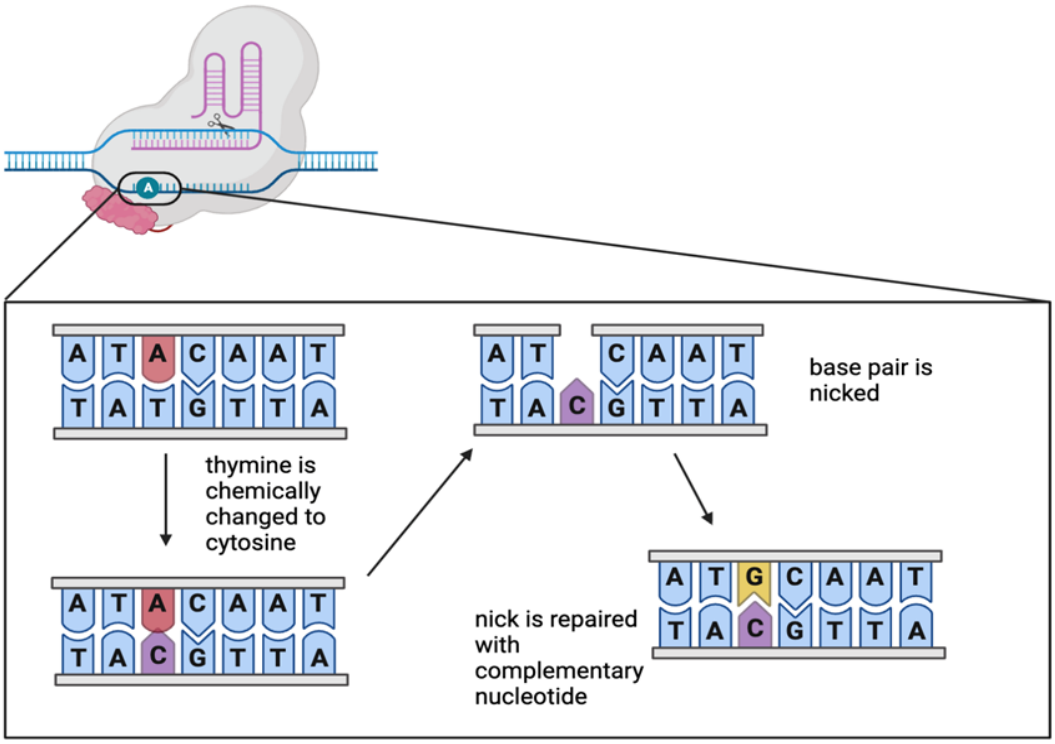

Figure 2. Base editing altering nucleotide base pair A-T to G-C. A guide RNA targets the sequence of interest. A protein fused to Cas9 chemically changes thymine to cytosine, CRIPSR/Cas9 nicks the targeted adenine, and the nick is repaired, using the modified strand as a template and adding a guanine nucleotide.

Knockout of Oncogenes

A common cause of cancer is the mutation of a proto-oncogene. Non-mutated proto-oncogenes regulate cell division. When mutated, the cell can divide and grow uncontrollably, forming a tumor (15). These mutations can arise from a variety of mutations, which can cause increased transcription and higher expressions of the proto-oncogene. (16). Some proto-oncogenes that have been successfully targeted using CRISPR/Cas9 include the epidermal growth factor receptor (EGFR), focal adhesion kinase (FAK) and E3 ubiquitin ligase (UBR5). These genes are discussed in greater depth below.

Researchers have targeted the epidermal growth factor receptor (*EGFR*) gene, which codes for a cell surface protein that binds to epidermal growth factor, triggering processes that cause cell proliferation. A mutated *EGFR* gene can promote uncontrolled cell proliferation, and thus, tumor growth (17). The use of the CRISPR/Cas9 system to target a mutated *EGFR* gene with a single-nucleotide missense mutation led to a precise knockdown of these oncogenes in the site administered (18). Utilizing CRISPR/Cas9 to target an oncogenic mutant allele can effectively stop tumor growth. The knockdown of mutant alleles prevents the expression of oncogenes, thereby stopping future tumor growth. In the case of prevention, the use of CRISPR in the knockdown of mutated *EGFR* genes could potentially inhibit tumorigenesis, thereby preventing tumor growth.

Another common proto-oncogene is *KRAS*. *KRAS* mutations exist in about 20-25% of lung cancers, and they are the most commonly mutated oncogene in non-small cell lung cancer (NSCLC) (19). A mutant *KRAS* positively regulates focal adhesion kinase (FAK). FAK is a protein enzyme, typically responsible for cell adhesion and migration. Overexpression of FAK can lead to various cancers, including lung, breast, colon, and

ovarian. In a study of the effect of FAK inhibition in NSCLC, researchers used CRISPR/CAS9 to knock out FAK; this elimination led to the death of mutant *KRAS* NSCLC cells (20).

Researchers identified E3 ubiquitin ligase UBR5 as a significant carcinogen in the development of triple-negative breast cancers (TNBC). Strong amplification and overexpression of the *UBR5* gene has been associated with this cancer. In a CRISPR/Cas9-mediated deletion of *UBR5*, tumor growth was terminated through increased necrosis and apoptosis and decreased proliferation (21). The results of this study prove that the deletion of an oncogene, in this case, *UBR5*, leads to the cessation of tumor growth. This is applicable to the prevention of tumor growth, specifically to avert TNBC, as the deletion of the mutated oncogene decreases proliferation, which may prevent tumorigenesis.

Activation of Tumor-Suppressor Genes

Many cancers lack oncogenes and are instead caused by mutations in tumor-suppressor genes. Tumor-suppressor genes are genes that normally function to inhibit cell proliferation and induce apoptosis, thereby preventing a mass of mutated cells, which could grow into a tumor. Cancers caused by mutations in these genes include breast, ovarian, colorectal, pancreatic, and lung cancers. CRISPR/Cas9 can be applied for the correct expression of these genes. By activating tumor suppressor genes, cells will be more capable of apoptosis, which can prevent the growth of tumors through the death of mutated cells (22).

The role of tumor suppressors is perhaps best illustrated by the fact that researchers have created new mouse models of cancer by knocking out numerous tumor suppressor genes using CRISPR/Cas9. These tumor suppressor genes included Ptch1, p53, Pten, and Nf1. The deletion of the single gene Ptch1 led to the growth of a medulloblastoma, a brain tumor. The deletion of the genes p53, Pten, and Nf1, all at the same time, caused the development of a glioblastoma, which is an aggressive form of brain cancer (23). This research shows the tumorigenic capability of mutated tumor-suppressor genes. A preventative effect should therefore be possible when restoring the function of these tumor-suppressor genes, specifically through the use of CRISPR. CRISPR technology could target mutated tumor-suppressors, repair the mutation, and restore the gene's function to increase apoptosis of mutated cells.

Another tumor suppressor gene, phosphatase and tensin homolog (PTEN) encodes an enzyme found in most tissues in the body. In many cancers, the expression of this gene is lost. In an attempt to activate the expression of the PTEN gene, researchers fused dCas9 (a deactivated form of Cas9 that maintains the ability to change sgRNA sequence at a targeted location but does not induce double-strand breaks) to a transcriptional activator (VPR). Transcriptional activators are necessary for the expression of genes. This approach was successful in cancer cell lines with low PTEN expression; the dCas9-VPR increased PTEN expression in these cell lines. Common mutations in the PTEN cell lines are truncating mutations, chromosomal deletions, and missense mutations (24). Considering these common mutations, CRISPR/Cas9 methods that could be used in the activation of a mutated PTEN include homology-directed repair, CRISPRa, and base editing, respectively (Fig. 3).

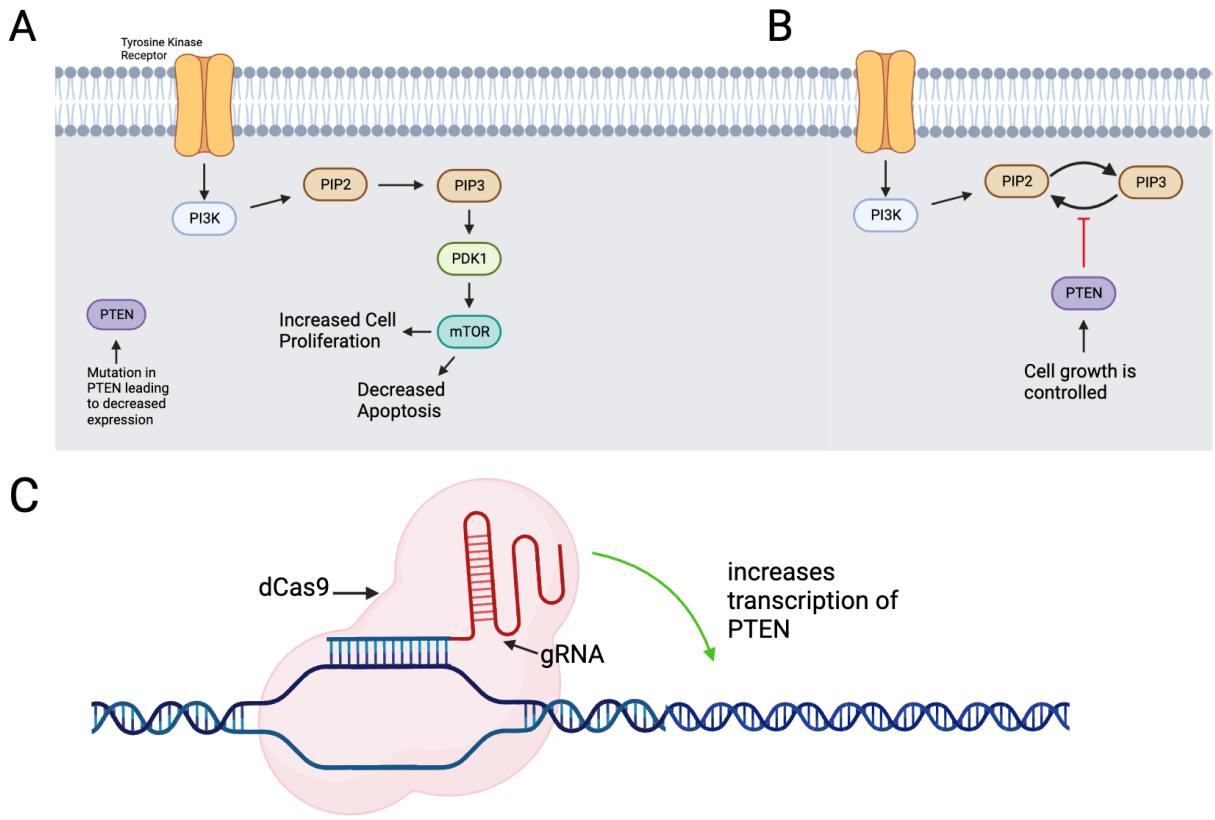

Figure 3. Using dCas9 to increase PTEN transcription and block the PI3K-PKB/Akt Pathway.

A. PI3K-PKB/Akt Pathway in the absence of PTEN. Without PTEN, the conversion of PIP2 to PIP3 is not blocked. The downstream effects include increased cell proliferation and decreased apoptosis.

B. PI3K-PKB/Akt Pathway in the presence of PTEN. PTEN inhibits the conversion of PIP2 to PIP3, thereby blocking the downstream effects of this pathway, decreasing cell proliferation and increasing apoptosis.

C. dCas9 system leading to increased transcription of PTEN. A gRNA recruits an inactive Cas9, leading to increased transcription of the target gene.

Many other cancers develop through the suppression of tumor-suppressor genes. Retinoblastoma (RB1) gene, for example, encodes the protein pRB. A mutated RB1 gene is often associated with breast and prostate cancers (25). One of the most common mutations discovered is a single-base mutation (26). If, in a genome study, RB1 is found to be mutated, base editing via CRISPR/Cas9 editing could be used in the correction of the RB1 gene.

A more well-known example of a mutated tumor suppressor gene is BRCA1, which, when mutated, can lead to breast, ovarian, and prostate cancers. Many types of mutations in this gene are common (27). In the application of CRISPR/Cas9 gene editing, base editing could be used for single-base mutations, similar to the aforementioned usage for mutated RB1 genes. Missense mutations in BRCA1 could similarly be targeted by CRISPR through the method of base editing. Initiating a double-strand break and providing a template with the correct DNA sequence could be useful in targeting nonsense mutations of BRCA1.

Limitations of CRISPR/Cas9

Before seeking preventative care for inherited diseases, people must determine whether they have inherited specific genetic traits and mutations from their parents. There are many different types of genetic testing that can identify inherited mutations. An early detection method is prenatal diagnostic testing, which tests for genetic mutations and diseases in unborn babies. However, this testing can increase the risk of a miscarriage (28). Similarly, "newborn screening" is genetic testing performed on newborns. This testing is performed on nearly every newborn in the US, taking blood from the infant and screening it for genetic mutations and diseases (29). For other individuals, predictive testing is used to determine genetic mutations in asymptomatic cases. This testing can determine how likely a person is to develop a certain disease, including cancer, and with the knowledge of the mutation, preventative care can begin (30). Genetic testing can detect mutations and can determine if gene editing is the right method for each person when preventing inheritable cancers. However, some people may not have a family history of certain genetic cancers, meaning they are not aware they should pursue genomic testing. Once their cancer is detected, it may be too late to harness the capabilities of preventative care.

There are also limitations of the CRISPR/Cas9 technology itself. This includes off-target effects, which are caused by Cas9 cleaving untargeted genomic sites, leading to unwanted changes in the genome. However, computer programs have proven useful in determining the likelihood of these effects. Using these algorithms, researchers can better code their RNA sequence to guide the DNA to the correct location (31). Another limitation of CRISPR is the efficiency of the editing. Homology-directed repair, a pathway to repair a double-strand break using a template strand of DNA, has low efficiency. Researchers have aimed to increase its efficiency by suppressing the non-homologous end-joining pathway, but the chemical inhibitors used to accomplish this may not be the best choice for *in vivo* treatment (32).

Another consideration is the delivery of CRISPR; whether transport is necessary to every cell in the body or just one specific organ, and how methods would change accordingly. This paper focuses on preventing cancer-prone mutations, meaning the delivery to specific organs is not as beneficial as systemic delivery. One method to deliver CRISPR systemically, to the whole body, uses adeno-associated viral (AAV) vector. These small viruses know how to infect cells, and are therefore capable of bringing CRISPR into the cell. However, the association of AAV and the immune response means that this therapy is susceptible to the boy's natural response to foreign factors.

The delivery of CRISPR can similarly be accomplished via extracellular vesicles. These are membrane-enclosed structures released from cells, responsible for cell communication and the delivery of cargo from cell to cell. Extracellular vesicles already exist within the body, meaning they are successful at entering cells, making it easier for the therapy to reach the genome (Fig. 4). Scientists create lipid nanoparticles to mimic these extracellular vesicles for the purpose of delivery into the cell. This may be a promising method to initially reach the cells, but in places where cell growth is ongoing, such as the musculature, lasting effects would be difficult to obtain (33).

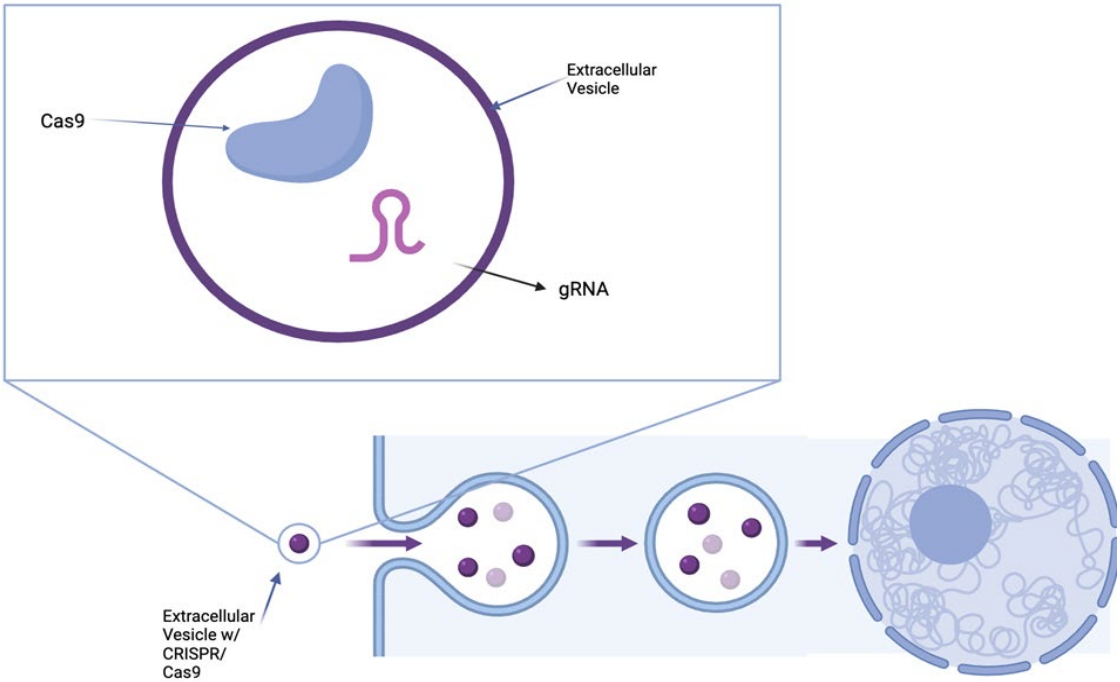

Figure 4. Extracellular vesicle carrying Cas9 and gRNA. The extracellular vesicle merges with the cell membrane, moving into the cell. Once it enters the nucleus, it will release its cargo. The gRNA and Cas9 can then make the designated edits.

Discussion

Given the adverse side effects of existing cancer treatments, researching preventative cancer therapy could prove advantageous. In the case of cancers arising from genomic mutations, gene editing technology could be applied systemically to fix the mutations. In preclinical research, CRISPR/Cas9 has had high efficacy in editing DNA for a lower cost than other gene-editing technologies. However, *in vivo* testing utilizing CRISPR/Cas9, specifically when reversing the effects of genetic diseases, produced an array of harmful side effects. The focus on genetic diseases should therefore lie in prevention, where CRISPR/Cas9 could be used to edit mutated genes before cancer begins.

This paper focuses on two main types of genes susceptible to carcinogenic mutations: proto-oncogenes and tumor-suppressor genes. Proto-oncogenes help the growth and formation of new cells. When mutated, these genes are called oncogenes and can cause rapid cell proliferation, which can result in a tumor. Clinical trials have shown the ability of CRISPR/Cas9 to knockout oncogenes, which stops the growth of tumors. This technology should also be applicable in preventing the growth of tumors; CRISPR/Cas9 can knockout oncogenes systemically, thereby preventing tumorigenesis.

Tumor-suppressor genes inhibit the development of tumors and prevent cell proliferation. Mutated tumor-suppressor genes are not capable of coding for the correct proteins, and therefore damage the cell's ability for apoptosis. Researchers found that CRISPR/Cas9 could repair a mutated tumor-suppressor gene and restore a cell's ability to induce apoptosis in mutated cells. This technology should be applicable in prevention; CRISPR/Cas9 can systemically fix a mutated tumor-suppressor, which can prevent the initial growth of tumors by increasing the cell's ability to control cell proliferation.

This method relies upon the knowledge of mutated genes within an organism. The use of CRISPR/Cas9 to prevent the growth of tumors is, therefore, more suitable for inherited cancers; an individual with an inherited cancer would likely have a family history of that cancer and would know to get genome testing to identify genetic mutations. This means that those with genetic mutations coming about in their lifetime, as opposed to inherited, may not know about their mutations until they begin to feel symptoms of their cancer, at which point it would be too late for this therapy.

Further limitations to this proposed technology lie in the CRISPR technology itself. This includes the potential for CRISPR itself to cause mutations, and as researchers found, for large portions of genetic material to be deleted from a cell (34). Additionally, as CRISPR is a newer technology, its long-term effects have not yet been studied. It is uncertain if, over decades, the effects of CRISPR/Cas9 may wear off as cells continue to grow and divide in living organisms.

Another consideration is the point at which this technology would be administered. In the case of inherited mutations, gene editing could be performed on a fetus, as the fetus' genome would already have the mutations. This technology could also be used later in life, both for individuals with inherited mutations or for those with known mutations arising over their lifetime.

The application of gene editing raises significant ethical considerations that must be reviewed before changing a genome. Since the introduction of gene editing technology, researchers have questioned the ethical ability to change an individual's DNA. Scientists and lawmakers continue to debate whether gene editing should be permitted on babies. These ethical questions arise in the consideration of when to administer this CRISPR/Cas-9 technology: should the parent have the right to decide they want to prevent genetic cancer as early as possible, or should the child have the right to decide if their DNA is altered?

Cancer's threat as one of the leading causes of death in the United States gives rise to new considerations on how to prevent its detrimental effects. For years, researchers have devoted their lives to the study of curing cancer. With the development of new technologies, specifically CRISPR/Cas9, applying new techniques becomes increasingly important. Similarly, preventing the effects of genetic cancers should be studied through the prevention of the cancers themselves, not just curing them. Utilizing CRISPR/Cas9 technology in the early prevention of inherited genetic diseases could revolutionize the way in which we look at cancer, switching our focus from treating the disease to preventing it altogether. Furthermore, the use of CRISPR for prevention purposes is applicable to not just cancer, but all genetic diseases, and using this technology could prevent a significant array of genetic diseases, even prior to the onset of symptoms.

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