Gene therapy and gene editing in human disease: CRISPR and beyond

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How genetic mutations cause disease in Williams Syndrome

Williams Syndrome is an autosomal dominant disorder, meaning the gene affected is not a sex gene and only one parental copy is necessary to pass down the disease to offspring. This syndrome is caused by a deletion mutation on chromosome 7. This mutation involves the deletion of 25-27 genes including ELN, GTF2I, GTF2IRD1, and LIMK1. This deletion has a drastic effect on protein function and can lead to the disease's numerous symptoms. For example, the ELN gene is important in the creation of connective tissue and cardiovascular function. Without this crucial gene, individuals with William's syndrome typically experience connective tissue abnormalities and cardiovascular disease. Likewise, the GTF2IRD1 gene is responsible for creating facial features. Without this gene, individuals typically have facial deformities and distinctive abnormal facial features. Other effects of William's syndrome include intellectual disabilities, learning problems, delayed development and unique personality characteristics (ex: overfriendliness, social disinhibition, excessive empathy, attention problem). Overall, the deletion mutation on chromosome 7 has a drastic impact on many different bodily functions, causing this syndrome.

Gene editing tools: CRISPR-Cas9

CRISPR-Cas9 is a revolutionary gene editing tool that allows for the editing of parts of the genome. It works using two main components: the Cas9 enzyme and the guide RNA (gRNA). The gRNA is composed of the specific RNA bases that correspond with the DNA bases at the targeted location in the genome. This allows the gRNA to guide Cas9 to the targeted location, where Cas9 can then bind to the DNA through the PAM site and cut the DNA. After Cas9 cuts the DNA, a double strand break is formed k which sends a signal to the cell that the DNA needs to be repaired. The cell utilizes two major pathways of repair: homology directed repair (HDR) and nonhomologous end joining (NHEJ). When NHEJ is used, it is more prone to produce mutations at the repair site. These mutations alter the DNA sequence and can cause the gene to not be expressed, which is why it is helpful in dominant diseases. CRISPR-Cas9 can also be used to delete sections of DNA by having two CRISPR-Cas9 molecules with different gRNA sequences that attach to opposing sides of the desired deleted section. The gRNA will cut the flanking sites, and the repair process can join the two cleaved ends, therefore deleting the middle section. The last way CRISPR-Cas9 can edit the genome is by creating gene knock-ins. When a donor DNA template is present, the cell can use the HDR repair pathway, which is very precise and uses a template to fix the break, whether that is the original "correct" DNA sequence or a new gene. Overall, CRISPR-Cas9 uses the Cas9 and gRNA to edit the genome through insertion, deletion or gene knock-ins.

Current progress in gene therapy

Gene therapy is a life-changing treatment that introduces new genetic information into the body by using viruses as carriers of this genetic material to treat genetic disorders and diseases. The first discussions of gene therapy began in the 1980s, with clinical trials starting in 1990 and results published in 1995. The first successful gene therapy trial involved Ashanthi de Silva, a 4 year old with adenosine deaminase deficiency, a type of severe combined immunodeficiency

(ADA-SCID), and lacked a functional copy of the ADA gene. The gene therapy trial that she enrolled in involved the delivery of a normal, exogenous copy of the ADA gene. This trial was successful, although it required frequent repeated treatments, and Ashanthi is still alive today. This inspired many more clinical trials and a rush in the genetics field towards gene therapy. However, in 1999, the death of a fairly healthy Jesse Gelsinger during a clinical trial spurred mass skepticism and fear, causing a halt in gene therapy. The 2010s saw a revival of gene therapy with improvements to the technology such as promoters, enhancers and better viral vectors.

Duchenne's Muscular Dystrophy (DMD) is an X-linked autosomal recessive genetic disorder that causes the body's muscles to weaken and waste away over time. It is caused by a mutation that makes the gene that creates the protein dystrophin defective. Previously, there were only treatments used to target the symptoms of the disorder and not the root cause, such as medication to increase muscular strength and exercise programs. However, on June 22nd 2023, the FDA approved Elevidys, a gene therapy treatment targeting the genetic cause of DMD. This therapy delivers a gene into the body that produces a shortened version of the missing dystrophin protein. The treatment underwent numerous successful clinical trials leading to the eventual FDA approval on June 22, 2023. This treatment provides hope for individuals with DMD and demonstrates the incredible possibilities of gene therapy treatments.

Current progress in leukemia gene editing trials

Current treatments for pediatric B cell acute lymphoblastic leukemia are time-consuming and require detailed personalization due to differing antibody reactions and toxicity. CRISPR offers a new alternative to the current autologous (obtained from the same individual) chimeric antigen receptor T (CAR T) cells. The current treatment uses these immune cells that are genetically altered in a lab to target and destroy cancer cells. However, due to previously discussed issues with this treatment gene editing is being considered as a possibility. The way this gene editing works is that CRISPR Cas-9 disrupts the T cell receptor A chain and removes CD52 (a glycoprotein) in CAR19 T cells. This treatment is way more efficient because it is a universal cell therapy and it is currently being tested in a clinical trial. In the trial, researchers treated six children each with relapsed/refractory CD19-positive B cell acute lymphoblastic leukemia. This trial was phase one, open label, and not randomized. Overall, the trial was moderately successful, and four of the patients saw promising signs such as cell expansion and flow cytometric remission. This is promising because it means the therapy is targeting the cancer and making an impact. These patients then received allogeneic stem cell transplantation, which replaces faulty bone marrow with healthy blood stem cells. However the other two patients experienced unfortunate effects such as grade II cytokine release syndrome, transient grade IV neurotoxicity, and skin Graft-versus-Host Disease (GVHD). Apart from these effects, other complications were within standard expectation and the safety requirements were met, providing hope for this CRISPR treatment for leukemia.

Safety and ethical considerations of gene editing for leukemia

Currently, genetic editing is in clinical trials for pediatric B cell acute lymphoblastic leukemia treatment and has not yet been fully approved. While there have been some promising results, many safety concerns have arisen due to unexpected side effects. For example, two of the patients developed grade II cytokine release syndrome, one of the patients developed transient grade IV neurotoxicity, and another patient experienced skin GVHD. While primary safety

objectives were met, these negative reactions raise concern for the benefit of these treatments and those undergoing them must weigh the positive and negative results. In general, one of the biggest concerns with gene editing treatments such as this CRISPR one are the unpredictable off-target effects, which scientists are still working to prevent. As for ethical concerns, there are two methods of gene editing treatments, targeting somatic cells and targeting germline cells. This leukemia treatment targets somatic cells meaning it has much less ethical considerations because it is a lifesaving treatment that would only affect the individual being treated. The defective somatic cells are simply removed from the body, edited and then returned to the body, not affecting the genome of the patient's children. However, there are also treatment methods altering germline genes in which case the gene responsible for leukemia could be cut out of the embryo's DNA, fixed, and replaced with a healthy gene. This method would not only be editing a non-consenting embryo, but it would also pass down edits made to future children of the embryo, preventing leukemia development in them as well. This raises more ethical questions because it is treatment without informed consent, it could be seen as enhancement rather than just treatment, and it may not be available to all due to high costs and limited accessibility. There is not a common consensus on the morally correct way to go about either of these treatments, germline or somatic, but many more scientists and students are in favor of somatic gene editing due to numerous ethical concerns with germline gene editing.

References

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