Retrons, CRISPR, and Genome Editing

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Genome editing is an important and quickly emerging type of gene therapy in which newly inserted genes can replace faulty ones to improve a cell's ability to fight disease. Double-stranded breaks in DNA sequences (DSBs) are one of the most dangerous types of DNA damage, with ionizing radiation and chemotherapeutics serving as their leading causes (Patrick et al., 2023). DSBs are one reason for cancer relapses because the treatment can damage the cell's DNA enough to create cancer-causing mutations. CRISPR Cas9 and retron therapies are both advancing types of gene therapy that can disable virus functions in bacterial cells with the end goal of successfully treating diseases in humans. CRISPR Cas9 genome editing has already begun treatment for humans, while retron therapies are progressing quickly but haven't been implemented as much in gene therapy.

Retrons are prokaryotic genetic retroelements, or elements that are transcribed into RNA, reverse-transcribed into DNA, and then inserted into a new site in the cell's genome. Retrons contain a reverse transcriptase, an enzyme for converting RNA into DNA, and a non-coding RNA, which is a sequence that is not transcribed into proteins. Found in bacterial cells, their function is to produce single-stranded DNA fragments (ssDNA), which scientists have discovered can detect whether a virus has infected a cell. Retrons' abilities to produce large amounts of single-stranded DNA have been exploited by scientists for genome editing, just as scientists at Harvard Medical School (HMS) have created a retron-based gene editing tool. Named Retron Library Recombineering (RLR), the tool simultaneously generates millions of mutations while keeping track of every mutant cell to easily analyze the data (Brownell, 2022). Retrons' genetic sequences are also easy to track as "barcodes" for seamless data analysis, which

also contributes to retrons' quick advancement toward competing with CRISPR Cas9 for the title of best genome editing technology.

CRISPR Cas-9 is a gene-editing tool that is used to cut specific pieces of DNA to create a mutation, which tricks the cell into using a different piece of DNA to repair the break. It was developed from a naturally occurring genome editing system present in bacteria for defense, and it was further innovated by Dr. Emmanuelle Charpentier and Dr. Jennifer Doudna, who both won the Nobel Prize in Chemistry in 2020, with their team (Ledford & Callaway, 2020). It has two parts: the Cas9 enzyme and a guide RNA, and is used via a process called Homology Directed Repair (HDR) (Patrick et al., 2023). This process uses homologous sequences as templates for DNA repair and is the most accurate and error-free type of DSB repair. This process is also able to intentionally create mutations in the DNA to repair it instead of just replicating the previously non-damaged copy. CRISPR Cas9 also allows the bacterial cells to "remember" viruses that they come in contact with (or very closely related ones) to be ready for attack if the virus returns. The bacteria is able to produce RNA segments that recognize and attach to the virus's DNA, along with using Cas9 to cut the DNA apart to deactivate it. It is much more useful than other previously used genome-editing technologies because it is much simpler, cheaper, and works faster than previous methods.

Unfortunately, CRISPR Cas9 can be toxic to cells because it can cut off parts of DNA not included in the target sites, which can cause extreme damage or accidental mutations in the cell, especially in stem cells as they have a higher risk of off-target gene editing (Hsu et al., 2014). There have also been controversies about the ethics of genome editing, especially in human embryos - it is against the law to change the DNA of an unborn baby, one of the reasons being to

prevent the child from having unnatural genetic benefits or increased abilities that wouldn't have been present otherwise (MedlinePlus, 2022).

Retron and CRISPR Cas9 genome editing technologies are ever-changing forms of gene therapies to battle viruses and treat diseases caused by genetic mutations. Currently, CRISPR Cas9 is a more effective option because there has been more research conducted in the practice, but retron therapies have the potential to edit DNA more precisely in the future. In an experiment with E. Coli, retrons were able to disable the virus, which is a huge step in the right direction toward gene therapy in humans, though more research definitely needs to be done (Shimamoto et al., 1993). When retron therapy reaches the stage of successful therapies in humans, it will most likely surpass CRISPR Cas9 because of its superior accuracy in cutting targeted pieces of DNA, which will lead to lower levels of off-target cleavage.

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