Understanding the Basis of Genome Editing to Target Neurological Disorders

By Anber Ansari

There are a number of neurological disorders that are caused by DNA changes called mutations, including X-linked intellectual disability (XLID), Huntington's disease, and Angelman Syndrome. The mutations associated with these disorders have become a target for therapy development. Researchers have been experimenting with different biological tools to artificially edit DNA to fix the disease-causing mutations.

To create these biological tools, researchers have been utilizing human induced pluripotent stem cells (hiPSCs). hiPSCs are type of stem cell created by reprogramming an existing adult cell (like a skin cell) to form a “pluripotent” stem cell. Pluripotent stem cells can form almost any cell type of the human body, except sex cells like an egg or sperm cell (Petazzi et al., 2020). Since hiPSCs can change into any cell type, they have been utilized in a variety of ways in research, including for modelling diseases, personalized therapy plans, biological tool analysis, drug screening, and more (Petazzi et al., 2020). These reprogrammed cells have also opened up avenues for therapies and research centered on DNA targeting.

All cells in the human body contain the same DNA. So what exactly is the reason for the different cells, tissues and organs in our body? Sequences in the DNA called genes! Each gene within our DNA encodes for a different protein with a specialized function performed within the cells of our body (Feher, 2017). Different genes are expressed in different cells, which the reason why a muscle cell looks and acts differently than a bone cell or a blood cell (Feher, 2017). In every cell, some genes are turned off and some are turned on (Feher, 2017).

In cells, genes are packaged into small structures called chromosomes. Humans have 23 pairs of chromosomes (so, 46 chromosomes total); they are referred to by numbers 1 – 22, plus the X and the Y chromosome. Typically, men have one X and one Y chromosome in their cells, while women have two X chromosomes.

Although Women carry two X chromosomes in their cells, only one X chromosome is “active,” meaning the genes on the chromosome can be expressed and generate the proteins they encode. The other X chromosome undergoes random “inactivation"; this X-chromosome is silenced to match the singular X chromosome that men possess. Having only one X active between the two sexes for equal distribution of genes (Boeren & Gribnau, 2021). As a result, women carry two copies of X-linked genes—one on the

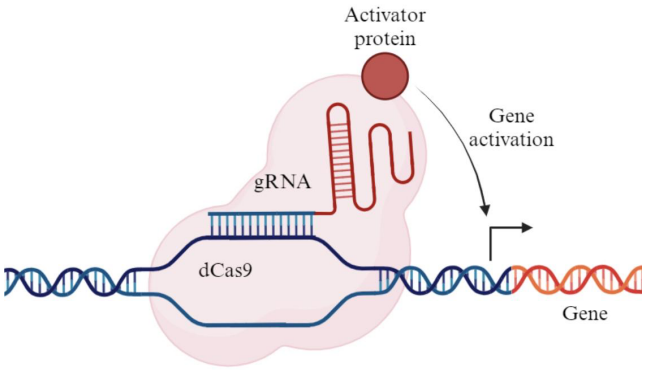
active X chromosome and one on the inactive X chromosome. In XLID, the gene(s) on the active X can be mutated, causing harmful proteins to be produced. Meanwhile, a normal copy of the gene on the inactive X can be present, but we do not observe gene or protein expression.

Interestingly, some genes escape X inactivation, meaning that they are expressed on the "inactive" X chromosome (Tukiainen et al., 2017). Mechanistically, there is something going on that allows certain genes to be expressed despite the widespread inactivation on the rest of the chromosome. Researchers are trying to better understand this mechanism in order to create gene activating tools that will enable the activation of genes located on the silenced X, but without changing the underlying DNA sequences (Halmai et al., 2020).

What may turn genes on and off without changing the sequences? These changes may be caused by small molecules called methyl groups on DNA that can affect which genes get turned off and on (Feher, 2017). Methyl groups may act as blockers (Feher, 2017) or interact with other molecules on the histone proteins that DNA is wrapped around (Handy et al., 2011). DNA is wrapped around histone proteins to compact DNA in the nucleus; importantly, modifying histones with chemical subgroups (like methyl groups) can affect their interaction with DNA in a manner that can influence gene expression (Handy et al., 2011). If DNA is being blocked, genes cannot be expressed to produce their encoded proteins (Feher, 2017). In contrast, if these methyl groups are removed, the genes within the DNA can be expressed and the encoded proteins will be synthesized (Feher, 2017).

Methyl groups are a type of epigenetic marker that can be edited. Epigenetics is the study of changes in gene expression rather than changing the sequence itself. The success of human stem cell-based therapies rely on epigenetic editing (Petazzi et al., 2020). These methyl groups can be artificially changed in order to change gene expression through tools created experimentally.

CRISPR-dCas9 is a molecular tool that has been shown to successfully edit markers (e.g., methyl groups) on the genome without changing the underlying DNA sequence (Halmai et al., 2020). This system allows scientists to target a very specific region on a chromosome in order to introduce modifying proteins to the DNA that can activate or inhibit gene expression at the targeted region. Fig 1. illustrates how a protein may fuse to the CRISPR-dCas9 tool to induce changes, in this case, gene expression is being induced.



Gene activating proteins and demethylating proteins can be fused to the dCas9 protein to be delivered to a target gene (Halmai et al., 2020). Halmai et al. illustrated that they could remove methyl groups *and* add activating proteins to reactive genes on the X chromosome (Halmai et al., 2020). This is in contrast to *just* introducing activating proteins. They were able to target a gene, CDKL5, that is responsible for neurological complications when mutated (Van Bergen et al., 2022). The normal copy of CDKL5 from the silenced X-chromosome was reactivated after methyl groups were removed and a gene activator protein was introduced (Halmai et al., 2020). The activating protein they used was VP64 and the demethylating protein was TET1 (Halmai et al., 2020). Halmai et al. demonstrated that gene activation was strong when TET1 and VP64 were added at the same time indicating they may work together (Halmai et al., 2020).

The end goal is to find a therapeutic treatment that is long-term, stable and can be applicable to many diseases. There are many mutated genes that produce non-functional or toxic proteins leading to many neurological disorders. However, newly reactivated genes can counteract the production of toxic proteins with further tool development. Activating genes and maintaining their expression could further research for therapy development for genetic neurological disorders.

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