'CRISPR' science: Newer genome editing tool shows promise in engineering human stem cells

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A powerful "genome editing" technology known as CRISPR has been used by researchers since 2012 to trim, disrupt, replace or add to sequences of an organism's DNA. Now, scientists at Johns Hopkins Medicine have shown that the system also precisely and efficiently alters human stem cells.

In a recent online report on the work in *Molecular Therapy*, the Johns Hopkins team says the findings could streamline and speed efforts to modify and tailor human-induced pluripotent stem cells (iPSCs) for use as treatments or in the development of model systems to study diseases and test drugs.

"Stem cell technology is quickly advancing, and we think that the days when we can use iPSCs for human therapy aren't that far away," says Zhaohui Ye, Ph.D., an instructor of medicine at the Johns Hopkins University School of Medicine. "This is one of the first studies to detail the use of CRISPR in human iPSCs, showcasing its potential in these cells."

CRISPR originated from a microbial immune system that contains DNA segments known as clustered regularly interspaced short palindromic repeats. The engineered editing system makes use of an enzyme that nicks together DNA with a piece of small RNA that guides the tool to where researchers want to introduce cuts or other changes in the

genome.

Previous research has shown that CRISPR can generate genomic changes or mutations through these interventions far more efficiently than other gene editing techniques, such as TALEN, short for transcription activator-like effector nuclease.

Despite CRISPR's advantages, a recent study suggested that it might also produce a large number of "off-target" effects in human cancer cell lines, specifically modification of genes that researchers didn't mean to change.

To see if this unwanted effect occurred in other human cell types, Ye; Linzhao Cheng, Ph.D., a professor of medicine and oncology in the Johns Hopkins University School of Medicine; and their colleagues pitted CRISPR against TALEN in human iPSCs, adult cells reprogrammed to act like embryonic stem cells. Human iPSCs have already shown enormous promise for treating and studying disease.

The researchers compared the ability of both genome editing systems to either cut out pieces of known genes in iPSCs or cut out a piece of these genes and replace it with another. As model genes, the researchers used JAK2, a gene that when mutated causes a bone marrow disorder known as polycythemia vera; SERPINA1, a gene that when mutated causes alpha1-antitrypsin deficiency, an inherited disorder that may cause lung and liver disease; and AAVS1, a gene that's been recently discovered to be a "safe harbor" in the human genome for inserting foreign genes.

Their comparison found that when simply cutting out portions of genes, the CRISPR system was significantly more efficient than TALEN in all three gene systems, inducing up to 100 times more cuts. However, when using these genome editing tools for replacing portions of the genes, such as the disease-causing mutations in JAK2 and SERPINA1 genes,

CRISPR and TALEN showed about the same efficiency in patient-derived iPSCs, the researchers report.

Contrary to results of the human cancer cell line study, both CRISPR and TALEN had the same targeting specificity in human iPSCs, hitting only the genes they were designed to affect, the team says. The researchers also found that the CRISPR system has an advantage over TALEN: It can be designed to target only the mutation-containing gene without affecting the healthy gene in patients, where only one copy of a gene is affected.

The findings, together with a related study that was published earlier in a leading journal of stem cell research (*Cell Stem Cell*), offer reassurance that CRISPR will be a useful tool for editing the genes of human iPSCs with little risk of off-target effects, say Ye and Cheng.

"CRISPR-mediated genome editing opens the door to many genetic applications in biologically relevant cells that can lead to better understanding of and potential cures for human diseases," says Cheng.

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