Study shows patient's own cells may hold therapeutic promise after reprogramming, gene correction

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Scientists from the Morgridge Institute for Research, the University of Wisconsin-Madison, the University of California and the WiCell Research Institute moved gene therapy one step closer to clinical reality by determining that the process of correcting a genetic defect does not substantially increase the number of potentially cancer-causing mutations in induced pluripotent stem cells.

Their work, scheduled for publication the week of April 4 in the online edition of the journal Proceedings of the National Academy of Sciences and funded by a Wynn-Gund Translational Award from the Foundation Fighting Blindness, suggests that human induced pluripotent stem cells altered to correct a genetic defect may be cultured into subsequent generations of cells that remain free of the initial disease. However, although the gene correction itself does not increase the instability or the number of observed mutations in the cells, the study reinforced other recent findings that induced pluripotent stem cells themselves carry a significant number of genetic mutations.

"This study showed that the process of gene correction is compatible with therapeutic use," says Sara Howden, primary author of the study, who serves as a postdoctoral research associate in James Thomson's lab at the Morgridge Institute for Research. "It also was the first to demonstrate that correction of a defective gene in patient-derived cells via homologous recombination is possible."

Like human embryonic stem cells, induced pluripotent stem cells can become any of the 220 mature cell types in the human body. Induced pluripotent stem cells are created when skin or other mature cells are reprogrammed to a pluripotent state through exposure to select combinations of genes or proteins.

Since they can be derived from a patient's own cells, induced pluripotent stem cells may offer some clinical advantages over human embryonic stem cells by avoiding problems with rejection. However, scientists are still working to understand subtle differences between human embryonic and induced pluripotent stem cells, including a higher rate of genetic mutations among the induced pluripotent cells and evidence that the cells may retain some "memory" of their previous lineage.

Gene therapy using induced pluripotent stem cells holds promise for treating many inherited and acquired diseases such as Huntington's disease, degenerative retinal disease or diabetes. The patient in this study suffers from a degenerative eye disease known as gyrate atrophy, which is characterized by progressive loss of visual acuity and night vision leading to eventual blindness.

While diseases such as genetic retinal disorders and diabetes offer attractive targets for induced pluripotent stem cell-based transplant therapies, concerns have been raised over the commonly occurring mutations in the cells and their potential to become cancerous.

Howden says that because gene targeting to correct specific genetic defects typically requires an extended culture period beyond initial induced pluripotent stem cell generation, researchers have been interested to learn whether the process would increase the number of mutations in the cells. The team set out to determine if it was possible to correct defects without introducing a level of mutations that would be incompatible with clinical applications.

In the study, the researchers used a technique called episomal reprogramming to generate the induced pluripotent stem cells. In contrast to techniques that use retroviruses, episomal reprogramming doesn't involve inserting DNA into the genome. This technique allowed them to produce cells that were free of potentially harmful transgene sequences.

The scientists then corrected the actual retinal disease-causing gene defect using a technique called homologous recombination. The stem cells were extensively "characterized" or studied before and after the process to assess whether they developed significant additional mutations or variations. The results showed that the culture conditions required to correct a genetic defect did not substantially increase the number of mutations.

"By showing that the process of correcting a genetic defect in patient-derived induced pluripotent cells is compatible with therapeutic use, we eliminated one barrier to gene therapy based on these cells," Howden says. "There is still much work to be done."

David Gamm, an author of the study and an assistant professor with the Department of Ophthalmology and the Waisman Center Stem Cell Research Program, says the ability to correct gene defects in a patient's own induced pluripotent stem cells should increase the appeal of stem cell technology to researchers striving to improve vision in patients with inherited blinding disorders.

"Although further development certainly is needed before such techniques may reach the clinical trial stage, our findings offer reason for continued hope," Gamm says. "Dr. Howden and our collaborative group have overcome an important hurdle which, when considered in the context of other recent developments, may lead to personalized stem cell therapies that benefit people with genetic visual disorders."

Provided by University of Wisconsin-Madison