Scientists reveal key barrier to reprogramming human zygotes

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(Medical Xpress) -- New research from Howard Hughes Medical Institute (HHMI) scientists pinpoints a biological barrier that has thus far slowed progress in creating disease-specific stem cell lines using a technique known as nuclear transfer. Identifying the problem opens up new opportunities for scientists to address and possibly overcome it.

Stem cells created from a patient's adult cells offer scientists the opportunity to study disease processes as cells bearing the patient's own set of genes grow and develop. In a report published online October 4, 2011, in the journal *Nature Communications*, HHMI scientists offer a biological explanation for why nuclear transfer has so far failed in human cells, even though it has been used successfully to reprogram animal cells.

Using nuclear transfer, scientists can introduce a set of genes from an adult animal into another cell – usually an unfertilized egg – whose nucleus has been removed. Using the technique in animal cells, scientists have successfully prompted egg cells with transferred nuclei to develop into embryos genetically identical to the individual from which the nucleus was taken.

The technique holds promise for creating lines of stem cells with genes identical to those of individual patients – offering the opportunity to reproduce a patient's disease in the laboratory and learn how his or her genetic makeup affects the function of specific cells and tissues. Many researchers hope that eventually, disease-specific stem cell lines might

be directed to develop into healthy tissue to treat diseases such as Parkinson's disease, diabetes, or Amyotrophic lateral sclerosis (ALS, or Lou Gehrig's disease).

So far, however, nuclear transfer has not yet been successful in human cells. Many scientists have turned instead to stem cell lines created by reprogramming adult cells – usually skin cells -- taken from patients. Rather than introducing a patients' genes to an egg or zygote, a set of genetic reprogramming factors are introduced to the adult cells, creating cells known as induced pluripotent stem (iPS) cells, which, like all stem cells, can be coaxed them into becoming a variety of cell types. The first human iPS cells were created in 2007.

HHMI early career scientist Kevin Eggan uses iPS cells to study the neurodegeneration that occurs in patients with ALS or spinal muscular atrophy (SMA). But he acknowledges that recently, several studies have "reengaged the question" of whether iPS cells might differ from embryonic stem cells in important ways. "Progress is the area of nuclear transfer is a way of making cell lines that could be used to address that question," he says.

In 2006, before iPS cells were available for human cells, Eggan and two colleagues at the Harvard Stem Cell Institute -- HHMI investigator Doug Melton and George Daley, who is now also an HHMI investigator –-- launched an effort to create disease-specific stem cell lines using somatic cell nuclear transfer. "At that time," Eggan explains, "that was the only way to think about making patient-specific stem cells."

Their plan was to adapt the nuclear transfer techniques that had been successful in animals to transfer DNA from skin cells donated by patients suffering from diabetes, blood diseases, and neurodegenerative diseases into unfertilized human eggs.

In accordance with guidelines set forth by the National Academy of Sciences recommending that eggs donated for research should be obtained only from women willing to donate without compensation, donors were offered reimbursement only for expenses they directly incurred by participating in the study. More than 239 women responded to the scientists' request for eggs – but in the end, only one woman committed to the necessary medical procedures. The team's attempts at nuclear transfer with the six eggs that woman was able to provide were unsuccessful.

Because of the challenges in obtaining eggs, Eggan's team, in collaboration with Melton's lab, decided to pursue an alternative: rather than transferring a patient's DNA into an unfertilized egg, they would see if they could achieve the same result by transferring the DNA into an embryo. "We did have success in finding people willing to give us their fertilized, one-cell embryos," he says. In fact, Eggan and his colleagues received more than 4,000 donated embryos from couples who had had embryos frozen after in vitro fertilization procedures. Such procedures typically result in the creation of multiple embryos, with many that are never implanted and not needed clinically.

For their experiments, the researchers isolated the embryos that were still single cells—fertilized eggs that had not yet developed any further. When they transferred nuclear DNA into the embryos, development continued though its early stages, but came to a halt about four days later. This was in contrast to what they observed when they used the identical technique to transfer DNA to single-cell mouse embryos, which developed into more mature embryos expressing the transferred genes.

With further experiments, the team traced the developmental arrest to a specific cellular failure: the embryos never turned on the genes contained in the transferred nucleus. It's not clear what prevents this essential activity from occurring in the human cells, but Eggan says his

team's finding could focus the attention of other researchers as they work to make somatic cell nuclear transfer a viable method for producing disease-specific stem cell lines. "We've closed the loop on the experiments that we said in 2006 that we'd do," Eggan says. "We've taken it as far as figuring out some of things that have been causing nuclear transplantation to fail in human cells."

Both Eggan and Melton now plan to focus their attention on iPS stem cell lines – but they encourage ongoing research into somatic cell nuclear transfer as an alternative method of creating disease-specific stem cell lines, which could turn out to be functionally different from iPS cells in physiologically important ways. That research will require researchers to obtain human eggs or embryos to use for their experiments.

While Eggan's team was able to carry out the current study thanks to the couples undergoing assisted reproduction who donated frozen embryos – about 11 percent of which were at the single-cell stage -- he says experiments using unfertilized eggs will also be important going forward. But such studies will require researchers to obtain donated eggs from women who must undergo hormone treatments and surgery which, the researchers say, is a major obstacle due to current guidelines that recommend women not be financially compensated for these procedures.

In a paper published October 7, 2011 in the journal *Cell Stem Cell*, Eggan, Melton, and colleagues detail the challenges of recruiting egg donors under the current NAS guidelines. When the scientists surveyed 52 women who responded to their initial appeal for donors – but ultimately chose not to participate – the absence of financial compensation was mentioned most frequently. The necessary medical procedures – medication, injections, surgery, and side effects – were also a significant concern. Hesitation to participate in stem cell research was not mentioned, the scientists say.

In contrast to the guidelines provided by the National Academy of Sciences, The International Society for Stem Cell Research and the Ethics Committee of the American Society for Reproductive Medicine have published guidelines that support financial compensation for women who donate eggs for research purposes, the authors of the Cell Stem Cell paper say. In light of their own difficulty in recruiting altruistic egg donors for nuclear transfer experiments, they conclude that wider adoption of these guidelines by academic institutions and funding agencies could significantly accelerate stem cell research.

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