Finding a home for nuclear transfer

By Kai-Jye Lou, Senior Writer

Oregon Health & Science University researchers have for the first time generated stable lines of human embryonic stem cells via somatic cell nuclear transfer.[1] Whether the platform can carve a niche among existing stem cell–based techniques will hinge on how the cells compare with those generated through other approaches.

Generating embryonic stem cells (ESCs) via somatic cell nuclear transfer (SCNT) involves taking an unfertilized oocyte, removing its nucleus and then transplanting the nucleus of a somatic cell into the enucleated oocyte. The resulting cell is then activated and allowed to divide until a blastocyst is formed. ESCs are collected from this blastocyst and used to establish cell lines.

Researchers in the stem cell space have previously reported on the use of SCNT to generate ESC lines from a range of lab animals,[2] including nonhuman primates in a study led by Shoukhrat Mitalipov and published in *Nature* in 2007.[3]

Mitalipov is an associate scientist in the Division of Reproductive and Developmental Sciences in the **Oregon National Primate Research Center** at OHSU.

The next hurdle was to use SCNT to generate ESC lines from human cells. However, early attempts to do so were not successful because human cells generated through SCNT typically stopped dividing after only a few rounds—a phenomenon called early embryonic arrest.[4,5]

SCNT had been further sidelined owing to the limited supply of donor oocytes and the advent of induced pluripotent stem (iPS) cell technologies.[6] The latter are easier to use, more scalable and subject to fewer funding restrictions and ethical considerations than SCNT.

Now, Mitalipov's group at OHSU has reported a protocol that enables the generation of stable human ESC (hESC) lines from cells obtained via SCNT. The researchers used SCNT to fuse fibroblasts from a human cell line with enucleated donor oocytes and then activated the resulting cells.

In culture medium containing caffeine, a subset of the activated cells continued to divide past the early embryonic stage and formed blastocysts. The researchers were able to derive stable ESC lines from these blastocysts. The OHSU group reported in 2007 that adding caffeine to culture medium improved the development of SCNT-generated nonhuman primate cells into blastocysts.[7]

In the current study, the resulting ESCs expressed known pluripotency markers, formed teratomas when injected into mice and inherited the genome of the donor fibroblast.

Importantly, and contrary to earlier assumptions that deriving an hESC line by SCNT would require unfeasible quantities of donor oocytes,[8] the researchers were able to derive at least one ESC line per round of oocyte donation. The result suggests that the SCNT approach could be scalable.

The team's results were published in *Cell*.

Comparative metrics

The key question is what role SCNT-derived ESCs might have among existing stem cell–based techniques (*see* **Table 1, "Stem cell types and methods for stem cell generation"**). It is probable that the SCNT approach will need to play catch-up with its peers.

iPS cells have already begun to take root in disease modeling and drug screening. For example, **Cellular Dynamics International Inc.** markets a suite of human iPS cell–derived cell lines and related services for such applications, and **iPierian Inc.** is using its in-house iPS cell technology platforms to aid the discovery of new therapies.

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> **—Susan Solomon, The New York Stem Cell Foundation**

Researchers will generally want to see whether SCNT-derived hESCs have properties that would make them superior to iPS cells, other hESC lines or tissue-specific stem cells in a particular therapeutic or nontherapeutic setting.

To sort this out, researchers need to first comprehensively characterize SCNT-derived hESCs and compare them with stem cells generated by other approaches.

The New York Stem Cell Foundation CEO Susan Solomon told *SciBX* that the foundation and its collaborators are doing just that. "We're already conducting comparative cell studies on SCNT-derived ESCs, iPS cells and other ESCs to characterize their similarities and differences," she said. Solomon declined to disclose details about the origin of the SCNT-derived ESCs.

One area in which SCNT-derived hESCs could potentially shine is in the generation of genetically matched tissues for transplantation. Cells derived from current ESC lines are not genetically matched to the patient, which decreases their suitability for use in long-term grafts, in which transplanted cells need to persist and remain functional.

Indeed, Solomon thinks that hematopoietic stem cell transplantation might be an area in which SCNT-derived hESCs could have utility given the high cost and difficulty of finding a genetic match.

"If researchers were able to develop a way to safely derive hematopoietic stem cells from patient-matched ESCs, it would likely result in a less costly and more efficient approach than trying to find a match through a registry," she told *SciBX*.

Another niche area to consider—and one in which SCNT-derived hESCs could have a potential advantage over iPS cells—would be in

Table 1. Stem cell types and methods for stem cell generation. There are three major classes of stem cells: embryonic stem cells (ESCs), induced pluripotent stem (iPS) cells and tissue-specific stem cells. Each class has distinct advantages and drawbacks, including how the cells are generated or extracted.
Source: Refs. 1, 8–12; BCIQ: BioCentury Online Intelligence; California Institute of Regenerative Medicine; EuroStemCell

	Advantages	Drawbacks
ESCs	- Can differentiate into any cell type - Can self-renew indefinitely	- Number of lines available is limited - Derivation requires the use of donor oocytes - Many older lines are unsuitable for therapeutic use owing to contamination - Use carries risk of teratoma - Cells derived from ESCs may not recapitulate adult cell phenotype - Use faces major ethical, regulatory and funding obstacles
Methods for generating ESCs		
Derivation from *in vitro* fertilized embryos	- Easier to apply than nuclear transfer - Protocols are well established - Therapeutic candidates have entered clinical trials	- Donor and recipient cells are genetically distinct
Somatic cell nuclear transfer	- Genetically matched to somatic cell donor, except for mitochondrial DNA - Uses unfertilized oocytes	- Methods are technically cumbersome - Protocols need further optimization - Scalability and efficiency of approach still need to be determined
iPS cells	- Can differentiate into any cell type - Can self-renew indefinitely - Donor and recipient cells can be genetically matched - Source cells are plentiful and easy to obtain - Reprogramming protocols are highly scalable - Use faces fewer ethical and funding obstacles than with ESCs	- Use carries risk of teratoma - Cells derived from iPS cells may not recapitulate adult cell phenotype - Immunogenicity is possible even if cells are genetically matched - iPS cell–derived cell therapies have not yet entered clinical trials - Therapeutic development assumed to carry higher risk than ESCs
Methods for generating iPS cells		
Generated with integrating, nonexcisable DNA–based vectors	- Reprogramming efficiency is average to high, depending on vector - Reprogramming factor transgenes are silenced after reprogramming step - Some vectors (such as inducible lentivirus) use inducible transgene expression systems to provide fine control of reprogramming factor expression	- Genomic integration raises additional safety concerns - Transgene silencing may be incomplete
Generated with integrating, excisable DNA–based vectors	- Reprogramming efficiency is average - Transgenes are removed from host cell genome after reprogramming step	- Additional steps are needed to confirm transgene removal in cells - Some vectors (such as lentivirus with floxed transgenes) still leave sequences in host cell genome
Generated with nonintegrating DNA–based vectors	- Genomic integration does not occur under normal circumstances	- Reprogramming efficiency is low - Vector DNA still has a low potential to integrate with host cell genome - Additional steps are needed to check for possible genomic integration
Generated with nonintegrating RNA–based vectors	- Reprogramming efficiency is high - Genomic integration does not occur - Some vectors (such as Sendai virus) can stimulate very high levels of reprogramming factor production - Some vectors (such as microRNAs) might be able to reprogram somatic cells refractory to other reprogramming approaches	- Replicating viral vector must be removed after reprogramming step - Reprogramming with nonviral vectors may require multiple rounds of transfection
Generated with proteins and/or small molecule cocktails	- Genomic integration does not occur	- Reprogramming efficiency is low - Need for constant supply of reprogramming factors can be expensive
Tissue-specific stem cells	- Marketed therapies have been shown not to cause tumors - Approved therapies that contain tissue-specific stem cells already exist - Cells usually recapitulate adult cell phenotype - Certain types (such as umbilical) can be frozen and stored - Use faces few ethical and funding obstacles compared with ESCs	- Cells can differentiate into a limited number of cell types - Capacity for self-renewal is limited - Cells are present in small quantities in source tissues - Cells are less scalable than iPS cells
Methods for generating tissue-specific stem cells		
Extraction from autologous tissues	- Cells are genetically matched to the patient - Protocols are well established	- Autologous source tissues are limited in supply
Extraction from allogeneic tissues	- Allogeneic source tissues may be more plentiful than autologous tissues - Method could enable the development of off-the-shelf therapies - Protocols are well established	- Donor and recipient cells are genetically distinct

patients who have diseases caused by mutations in mitochondrial DNA, said Natalie DeWitt, special projects officer at the **California Institute for Regenerative Medicine**.

She noted that SCNT-derived ESCs generated from such patients should remain genetically matched but have the oocyte donor's mitochondrial DNA, which would presumably be free of disease-causing mutations. In contrast, iPS cells generated from such patients would retain their mutant mitochondrial DNA.

DeWitt said that she wanted to see studies that involve generating SCNT-derived ESC lines and iPS cell lines from the same individual followed by detailed characterization studies of the cells. She noted that such studies will help the field to better understand the pathways and mechanisms that mediate the reprogramming of cells to a pluripotent state and also provide insights on how to improve iPS cell reprogramming.

She also wanted to see comparisons between the genomic integrity of SCNT-derived ESCs and that of iPS cells.

DeWitt thinks that one of the major barriers to the development of a commercially viable platform for generating SCNT-derived hESCs is the need for large quantities of human donor oocytes.

"Unless a way to create large quantities of human oocytes *in vitro* is also developed, I think it would be tough to build a commercially viable platform based on nuclear transfer," she told *SciBX*.

Solomon added that the efficiency of the SCNT approach will also be a key determinant of whether others in this space will want to pick up the technology.

OHSU has pending patents covering the use of SCNT to generate stem cells for therapeutic application. The technology is available for licensing.

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COMPANIES AND INSTITUTIONS MENTIONED

California Institute for Regenerative Medicine, San Francisco, Calif.
Cellular Dynamics International Inc., Madison, Wis.
iPierian Inc., South San Francisco, Calif.
The New York Stem Cell Foundation, New York, N.Y.
Oregon Health & Science University, Portland, Ore.
Oregon National Primate Research Center, Portland, Ore.