Somatic Cell Nuclear Transfer in Oregon: Expanding the Pluripotent Space and Informing Research Ethics

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ABSTRACT

In May, Oregon Health and Science University (OHSU) announced the successful derivation, by the Mitalipov laboratory, of embryonic stem cells by somatic cell nuclear transfer. This experiment was recognized as a “formidable technical feat” and potentially a key step toward developing cell-based therapies. The OHSU report is also an example of how a scientific breakthrough can inform research ethics. This article suggests ways that nuclear transfer embryonic stem cell lines may contribute to research ethics by adding rigor to studies addressing pressing research questions important to the development of cell-based therapies.

Somatic cell nuclear transfer and variability in the pluripotent space

Sir William Osler described medicine as “a science of uncertainty and an art of probability” [101]. Uncertainty is a fundamental challenge in the development of cell-based therapies from pluripotent cells—human embryonic stem cells (hESCs), induced pluripotent stem cells (iPSCs), and nuclear transfer embryonic stem cells (NT-ESCs) [1]. Current clinical models envisage starting with a pluripotent cell culture followed by considerable propagation and then differentiation to a therapeutic cell type or product [2]. Clin-

ical application of this model requires the development of standardized tests for product consistency, potency, stability, tumorigenicity, toxicity, and immunogenicity. However, standardization has proven challenging because cellular products are often heterogeneous and every type of stem cell can vary in its underlying biology and behavior [3].

A pressing question is the extent to which variability will affect the usefulness of cell products in regenerative medicine [4]. Variability exists both among and between cell lines and types being contemplated for clinical applications. Comparative studies among the same types of lines—for instance, hESC to hESC—and between types—hESC to iPSC—are ongoing. The categories, magnitude, mechanistic origins, and implications of these differences are the subject of intensive research and scholarly review [4–6].

The advantages of any particular cell type for clinical applications must be considered against the nature of the disease and the timing of treatment.

Until recently, the human pluripotent space has been defined by hESCs and iPSCs (Fig. 1). While hESCs are often described as the gold standard for evaluating pluripotency and differentiation potential [102], iPSCs are viewed as having great promise, especially for autologous (derived or transferred from the same individual’s body) transplantation to reduce the potential of histoincompatibility and risks from immune rejection [7]. Differences in differentiation potential and immunogenicity have been identified as critical factors for clinical applications and are being evaluated in comparative (hESC vs. iPSC) studies [4,8].

One unique consideration for iPSCs is the extent to which the induction (reprogramming) process converts the somatic (adult) cell to an embryonic state. Experiments demonstrate that genes that are

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FIG. 1. The pluripotent space before nuclear transfer lines derivation.

Prior to the derivation of NT-ESCs the pluripotent space included iPSCs and hESCs, area in blue. Comparative studies have focused on differentiation potential among and between cell types.

FIG. 2. The pluripotent space with nuclear transfer lines derivation.

The derivation of NT-ESCs expands the pluripotent space, area in blue. Comparative studies can now expand their focus to include reprogramming efficiency and blastocysts characteristics among and between cell types.

normally silenced in hES cells can remain active in iPS cells [9]. The significance of this phenomenon, also known as incomplete DNA methylation, is not known, but it may impact subsequent propagation and differentiation.

The derivation of human NT-ESC lines now expands the pluripotent space (Fig. 2).^{*} As Mitalipov suggests, NT-ESCs

^{*}These figures are for illustrative purposes. Recent studies suggest rare somatic cells

offer a new platform for the development of cell-based therapies [1]. However, the resulting NT-ESC lines also provide a comparator (iPSC vs. NT-ESC) that may be particularly useful for identifying factors relating to somatic cell reprogramming efficiency and effectiveness. Further, the Oregon Health and Science University (OHSU) team derived iPSCs from the NT-ESC somatic cell donor, so an iPSC–NT-ESC comparator exists for somatic cells obtained from the same individual, thus adding another important dimension to research aimed at optimizing the reprogramming process.

Somatic cell nuclear transfer, cell-based therapies, and ethics

The advantages of any particular cell type for clinical applications must be considered against other issues, including the nature of the disease and the timing of treatment. There are compelling reasons to advance a variety of therapeutic approaches involving multiple cell types, as evidenced by the numerous translational research studies supported by the California Institute for Regenerative Medicine's therapy development projects [103].

As therapy development progresses toward clinical trials, research ethics demand that risks to patients be minimized. Expanding the pluripotent space enables studies that were previously not possible, thus allowing consideration of clinical trial design based on more complete information. Susan Solomon, CEO of the New York Stem Cell Foundation, recently noted, "We currently conduct comparative studies between iPSCs and embryonic stem cells derived via nuclear transfer to understand similarities and differences—a crucial step before any cell therapies can reach patients" [104]. Comparative studies do not eliminate uncertainty, but they pro-

exhibiting pluripotent characteristics exist. See www.pnas.org/content/110/12/4598.full?sid=2b11e7c0-ef54-40dd-913e-283b53cb1edf

vide additional knowledge. Issues and questions under consideration with regard to the therapeutic application of iPSCs are identified in Table 1.

To be ethical, clinical research must be conducted in a methodologically rigorous manner and have a favorable benefit-to-risk ratio for the individual receiving treatment. Determining favorable benefit is particularly challenging with therapies originating from pluripotent cells because clinical experience is limited. Under these conditions and in the interest of methodological rigor, we are compelled to develop a body of evidence in preclinical studies performed in animal models. Testament to this point is the 22,000-plus-page Investigational New Drug application produced by Geron Corporation for its hESC-based GRNOPC1 product [10].

In the case of iPSC-based products, it is plausible that NT-ESC lines will play a substantial role in evaluating the efficacy of somatic cell reprogramming and differentiation, thus supporting benefit-to-risk assessments. To date we have no clinical experience with iPSC-based products (one trial has been proposed [105]), suggesting, as with Geron's GRNOPC1 product, the need for extensive preclinical studies. One might argue that these conditions create an ethical imperative to incorporate knowledge gained from research involving NT-ESC lines into preclinical evaluation of iPSC-based products.

CONCLUSION

In a recent perspective on the state of stem cell science, Zachary Russ writes, "The roadblocks to research are quick to appear and slow to dissipate, but they are inevitably overcome" [106]. The example of somatic cell nuclear transfer for generating human cell lines is a case in point. After a decade of effort, OHSU researchers have reported an efficient and effective means of generating NT-ESC lines. As a result, the pluripotent space now includes a set of comparator lines for addressing a range of issues and questions related to safe and effective application

Table 1. Issues and Questions for Evaluating Therapeutic Applications

Reprogramming efficiency and effectiveness

- How does reprogramming efficiency vary between iPSC and NT-ESC, particularly for cells obtained from the same individual?
- Is epigenetic memory retained?
- Can differences be attributed to molecular factors?
- Can the efficiency and speed of iPSC reprogramming be improved?

Differentiation potential

- Does gene expression vary between iPSC and NT-ESC derived from the same individual?
- Does the ability to differentiate to mature cell types vary by source of line (e.g., hESC, iPSC, and NT-ESC)?
- What is the nature of this variation?

Genetic stability in culture

- How does the stability of NT-ESC compare to iPSC lines that have been reported to be genetically unstable in culture?

Engraftment

- Do NT-derived cell lines differ from hESC or iPSC lines in their detection by immune surveillance, which could impact the engraftment efficiency and durability of cells or derivatives?

hESC, human embryonic stem cell; iPSC, induced pluripotent stem cell; NT-ESC, nuclear transfer embryonic stem cell.

of cell-based therapies. This breakthrough comes just as cell-based therapies from pluripotent cells are entering clinical application. The additional knowledge provided by NT-ESC lines

will support research ethics by enhancing the empirical basis for evaluating risks and benefits in future clinical trials.

Author disclosure statement

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The pluripotent space now includes a set of comparator lines for addressing a range of issues related to safe and effective applications of cell based therapies.

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