

CLINICAL IMPLICATIONS OF BASIC RESEARCH

Progress in Human Somatic-Cell Nuclear Transfer

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In June 2005, Hwang and coworkers at Seoul National University<sup>1</sup> reported that pluripotent human embryonic stem cells can efficiently be generated by nuclear transfer from a wide variety of patients (Fig. 1). The authors transferred somatic-cell nuclei from eight male and three female donors, 2 to 56 years of age, into oocytes whose nuclear genomes had been removed. To underscore the clinical relevance of their work, they used donors who had conditions that are potentially amenable to stem-cell therapy: congenital hypogammaglobulinemia, spinal cord injury, and juvenile diabetes.

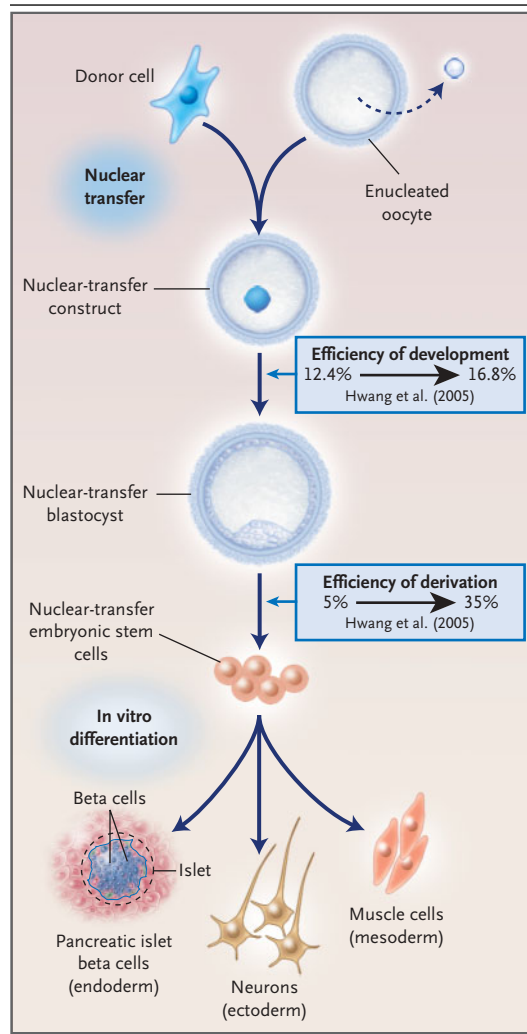
Cells containing nuclei from nine donors developed to the blastocyst stage, whereas cells containing nuclei from the other two donors failed to do so. Blastocysts from each of the nine patients yielded 1 or 2 embryonic stem cell lines (referred to here by the generic term “nuclear-transfer embryonic stem cells” to denote that they were derived from nuclear-transfer constructs), for a total of 11 embryonic stem cell lines from 31 blastocysts. On average, one

cell line was established per 16.8 oocytes, an efficiency of 6.0 percent. This reflects an increase in efficiency by a factor of more than 14 as compared with the report last year by the same group,<sup>2</sup> in which a single human nuclear-transfer embryonic stem cell line was derived from 242 oocytes. This improvement is attributed in part to the use of oocytes from younger donors in the present study.

With this improved efficiency, the line has been crossed between viewing the derivation of human

**Figure 1. Derivation of Patient-Specific Therapeutic Cells through Nuclear Transfer.**

Nuclear-transfer embryonic stem cells that carry the nuclear DNA of an existing person can be obtained by injecting a nucleus from a patient into an enucleated ovum. The nuclear-transfer construct is then activated, whereupon it undergoes cell division and forms a blastocyst. Explantation of the blastocyst to cell culture yields a line of cells that can differentiate into ectodermal, mesodermal, and endodermal cells (i.e., cell lines that have the potential to develop into any type of cell). A bottleneck in the procedure has been the low efficiency with which nuclear-transfer embryonic stem cells are derived from blastocysts. Hwang et al.<sup>1</sup> recently reported a dramatic improvement in the efficiency of stem-cell derivation from blastocysts: from 5 percent to 35 percent. They went on to show that the stem cell lines could differentiate into somatic cells of the ectoderm, mesoderm, and endoderm lineages, including cells with characteristics of the skin, striated muscle, smooth muscle, neuroepithelium, cartilage, renal tissue, gastrointestinal epithelium, respiratory epithelium, colonic epithelium, and bone. The data in the blue boxes indicate the improvement in the efficiency of the specific process since 2004.



nuclear-transfer embryonic stem cells as an experimental system and viewing it as a viable clinical proposition. Scientifically speaking, this is a pedestrian crossing. The derivation of embryonic stem cells from the inner cell mass of blastocyst-stage embryos was first achieved in mice nearly 25 years ago, in primates 10 years ago, and in humans in 1998. Elements of this work showed that in general, it was possible to maintain embryonic stem cells in culture for prolonged periods in an undifferentiated state, although this has yet to be shown for human nuclear-transfer embryonic stem cells. Like the human nuclear-transfer embryonic stem cells described by Hwang et al., undifferentiated embryonic stem cells from a variety of species are pluripotent, in that they can give rise to each of the three founding germ layers of an early embryo.

The next step in assessing the therapeutic potential of human nuclear-transfer embryonic stem cells will be to derive pure populations of clinically relevant cells from them in vitro. It is now possible to induce mouse embryonic stem cells to differentiate into many types of cells (though fewer than 10 percent of the estimated total types of cells), including pancreatic beta cells, cardiomyocytes, and specific subgroups of neurons. There is still only one reported study in which such differentiation has been shown to produce cells that have not been genetically altered and yet are able to correct a deficit after being transplanted — in this case, midbrain dopaminergic neurons in a mouse model of Parkinson's disease.<sup>3</sup> Prescriptive differentiation into cells with neuronal specifications has also been shown for human nuclear-transfer embryonic stem

cells<sup>4</sup> with the first report of the behavior of neuronal precursors derived from such cells after transplantation.<sup>5</sup> The derivation of human nuclear-transfer embryonic stem cells is a prelude to the arguably more difficult and time-consuming work ahead and yet brings us closer to the prospect of patient-specific cell therapy.

The importance of the report by Hwang et al. goes beyond this, for it gives the clearest indication yet that the United States has lost the initiative in the human nuclear-transfer debate. In March, the majority of the Asian countries voting on a U.S.-backed, nonbinding statement by the United Nations calling for a ban on all forms of human cloning this past spring rejected it; among them were Cambodia, China, India, North Korea, South Korea, Japan, Singapore, and Thailand. While the United States remains rooted in atavism, Hwang and co-workers have shown that Asia is moving forward.

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1. Hwang WS, Roh SI, Lee BC, et al. Patient-specific embryonic stem cells derived from human SCNT blastocysts. *Science* (in press).
2. Hwang WS, Ryu YJ, Park JH, et al. Evidence of a pluripotent human embryonic stem cell line derived from a cloned blastocyst. *Science* 2004;303:1669-74.
3. Barberi T, Klivenyi P, Calingasan NY, et al. Neural subtype specification of fertilization and nuclear transfer embryonic stem cells and application in parkinsonian mice. *Nat Biotechnol* 2003;21:1200-7.
4. Perrier AL, Tabar V, Barberi T, et al. Derivation of midbrain dopamine neurons from human embryonic stem cells. *Proc Natl Acad Sci U S A* 2004;101:12543-8.
5. Tabar V, Panagiotakos G, Greenberg ED, et al. Migration and differentiation of neural precursors derived from human embryonic stem cells in the rat brain. *Nat Biotechnol* 2005;23:601-6.

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**Editor's Note:** The scientific reports by Hwang et al.<sup>1,2</sup> that are described in this article were retracted by *Science* on January 12, 2006. See Snyder and Loring (*N Engl J Med* 2006; 354:321-4) for further information.

**CORRECTION**

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Progress in Human Somatic-Cell Nuclear Transfer . With regard to the last two sentences in the first full paragraph on page 88, it should be noted that the embryonic stem cells used in the studies by Perrier et al.<sup>4</sup> and Tabar et al.<sup>5</sup> were derived by the standard method, rather than by nuclear transfer. In addition, the article that was discussed, a report by Woo-Suk Hwang et al. (Hwang WS, Roh SI, Lee BC, et al. Patient-specific embryonic stem cells derived from human SCNT blastocysts. *Science* 2005;308:1777-83.), has been retracted.