PERSPECTIVES: CELL BIOLOGY

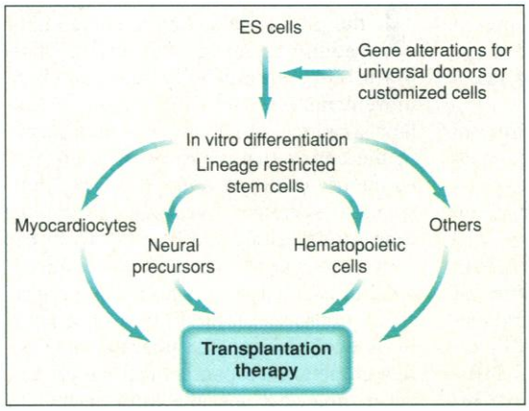
New Potential for Human Embryonic Stem Cells

John Gearhart

Pluripotential stem cells, present in the early stages of embryo development, can generate all of the cell types in a fetus and in the adult and are capable of self-renewal. A renewable, tissue culture source of human cells capable of differentiating into a wide variety of cell types would have broad applications in basic research and transplantation therapies. A major step in realizing this goal has now been taken with the demonstration that human embryonic stem cells can be grown in culture. These stem cells have been derived in culture from two embryonic tissues: inner cell masses of blastocysts (those cells within the conceptus that form the embryo proper) and primordial germ cells. Embryonic stem (ES) cells were first derived from the inner cell masses of mouse blastocysts in the early 1980s (*1*, *2*). More recently, primordial germ cell cultures were found to give rise to cells with characteristics of ES cells and were designated EG (embryonic germ) to distinguish their tissue of origin (*3*, *4*). ES and EG cells have now been derived from embryos of other mammals, including primates (*5*–*10*). Now on page 1145 of this issue, Thomson *et al.* (*11*) report the derivation of ES cell lines from human blastocysts.

Pluripotential stem cells, primarily ES cells, have been used extensively in studies of embryogenesis, gene function, and development in the mouse (*11*). ES and EG cells transferred to a mouse blastocyst can contribute substantially to all differentiated cell types in the fetus, including the germ line. Consequently, gene targeting within ES cells has enabled both whole-animal studies of gene function and the production of mouse models of human genetic diseases and abnormalities. ES and EG cells have also been used to study the differentiation of various cell types and tissues in vitro, such as neural cells (*12*–*16*), hematopoietic lineages (*17*–*19*), and cardiomyocytes (*20*). ES-derived cells have been successfully transplanted into fetal and adult mice, where they have demonstrated morphological and functional integration (*19*–*23*).

Thomson's group at the Wisconsin Regional Primate Research Center in Madison, in collaboration with the Departments of Obstetrics and Gynecology at the Rambam Medical Center in Haifa, Israel, and the University of Wisconsin, reports the derivation of five independent cell lines from the inner cell masses of 14 blastocysts (*11*). The ES cell lines were continuously cultured for 5 to 6 months and expressed high levels of telomerase activity, characteristic of cells with high replicative lifespan. The cell lines had normal karyotypes (two male and three female) and expressed cell surface markers characteristic of ES cells. Four cell lines tested produced teratomas when grown in immunocompromised mice. Histology of the tumors revealed differentiated cells derived from all three embryonic germ layers (ectoderm, mesoderm, and definitive endoderm)—a result consistent with pluripotency.



Embryonic stem cell differentiation. Possible applications of ES cells to transplantation therapy.

This report of the derivation of ES cells from human blastocysts represents a major technical achievement with great importance for human biology. Although ES cells have been derived from several mammalian species, other species have proved refractory in yielding ES cells. It was, therefore, not a foregone conclusion that ES cells could be derived from human embryos. Earlier publications from the Thomson group reporting the derivation of ES cells from nonhuman primates increased the expectation that such cells could be derived from human blastocysts. In a related report, it now appears that human embryos are also amenable to EG cell derivation from primordial germ cells (*24*).

The derivation of human ES cells now raises a whole new set of expectations. On the basis of the use and study of mouse ES cells, the research and clinical potential for human ES cells is enormous. They will be important for in vitro studies of normal human embryogenesis, abnormal development (through the generation of cell lines with targeted gene alterations and engineered chromosomes), human gene discovery, and drug and teratogen testing and as a renewable source of cells for tissue transplantation, cell replacement, and gene therapies. These latter applications could eventually preclude the direct use of fetal tissue in transplantation therapies.

It is exciting to speculate on how human ES lines could be used in tissue transplantation therapies (see figure) (*25*). Obvious clinical targets would include neurodegenerative disorders, diabetes, spinal cord injury, and hematopoietic repopulation. In addition to possibly providing large numbers of pure populations of cells for transplantation, ES cells would also lend themselves to several strategies for the prevention of immunological tissue rejection after transplantation, including (i) banking of multiple ES cell lines representing a spectrum of major histocompatibility complex (MHC) alleles to serve as a source for MHC matching; (ii) creation of universal donor lines, in which the MHC genes could be genetically altered so rejection would not occur, an approach that has been tried with moderate success in the mouse; (iii) customization of ES cells through transgenesis and gene targeting so that a potential recipient's MHC genes are introduced into ES cells through homologous recombination; and (iv) production of ES lines containing the genome of the prospective recipient. Blastocysts obtained through nuclear transfers would be used to generate ES cells, which then could be differentiated to specific lineages for transfer to the nuclear donor (*26*). Because EG cells have been shown to reprogram adult nuclei (*27*) after cell hybrid formation, it may eventually be possible to do nuclear transfers into pluripotent stem cells, which could then be expanded and differentiated.

To realize the full potential of human pluripotent stem cells, challenging research lies ahead and several practical issues must be resolved. The conditions necessary to derive human ES cells efficiently and reliably must be defined. How did the Thomson group succeed when, on the surface,

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their protocol is so similar to that of other investigators? Their previous experience with nonhuman primate ES cell derivation was certainly critical for this success. Thomson *et al.* (*11*) report that of 14 inner cell masses placed in culture, five ES cell lines were established. This result is excellent, but could it be better? Are there ways of assaying blastocysts for their potential of yielding ES cells? As in the mouse, are there predisposing genes for this property? Are there other extrinsic or intrinsic factors that may lead to a greater success rate?

The conditions for directed, lineage-restricted differentiation of ES cells must be defined. Studies to date on ES cell differentiation in vitro rely primarily on the selection and enrichment of specific lineages from the many that may be present when cell differentiation is induced. Also, strategies must be developed to obtain the large numbers of pure populations of cells that would be required for engraftments. In the short term, feeder cell-independent lines will have to be derived and methods for complete cell disaggregation developed. It must also be determined if the cells are amenable to transfections, enabling selection and gene-targeting strategies.

Reports on the isolation of human pluripotent stem cells will no doubt catch the public eye, and there will be expressions of concern, rekindling the debate on human embryo research. The debate will encompass the source of the cells, human cloning potential, and the possibilities of germ line modifications. Four years ago, the Human Embryo Research Panel's report to the director of the National Institutes of Health (NIH) concluded that research deriving ES cells is acceptable as long as embryos are not created expressly for research purposes. Several issues will have to be resolved to permit the appropriate exploitation of the uniqueness and potential of these cells. Currently, as broadly written, U.S. federal law bans the use of federal funds for the derivation of these cells [Public Law 105-78, Section 513(a)]. To date, research in this area has been sponsored through private and corporate funding, with hospital and academic institutional internal review board approval and informed patient consent. It is not clear whether NIH funding necessary to realize the biomedical potential of the cells will be available to support studies using the derived ES cells. Federal legislation and funding policies should be reexamined in light of the biomedical potential of human ES cells, now made more imminent by the Thomson *et al.* report. Federal guidelines must be established so that ES cell research can be funded after appropriate peer review and oversight.

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PERSPECTIVES: NEUROSCIENCE

Long-Term Change of Mind

Michael Merzenich

The removal of an arm or leg causes the grand-scale functional reorganization of maps on the surface of the brain representing body sensations and movements (see the figure) (*1–3*). In human amputees, many square centimeters of the cortex that were formerly engaged by the use of the missing limb come to respond to elaborated inputs from the intact limb stump, trunk, and face (*2, 3*). In parallel, the sensory sensitivity and ability to discriminate different sensations progressively improves on the limb stump; sensations evoked from the phantom hand or foot come to be topographically represented in somewhat unstable form on the amputation stump and, in many patients, on the face; and the perceived body form is distorted, with "phantom" fingers or toes moving progressively toward the arm or leg stump, often ultimately being perceived as being located near, on, or even within the stump (*4*). Enduring pain often parallels these changes, replaying the impact of the crushing machinery or the shark's bite that caused the amputation. The magnitude of this phantom limb pain is directly correlated with the extent of representational remodeling recorded within the cerebral cortex: The greater the changes in the cortex, the greater the pain experienced (*5*).

What mechanisms account for this massive representational cortical remodeling? Can this powerful capacity for representational translocation and functional revision be harnessed to prevent the genesis of phantom limb pain, or to improve rehabilitation after major peripheral or central nervous system injuries? Two reports in this issue (*6, 7*) shed new light on these questions, describing provocative large-scale changes in the morphology of the thalamus and in cortical network connectivity in these monkeys with long-term injuries.

Jones and Pons (page 1121) have examined the "Silver Spring monkeys," macaque monkeys that many years in their past suffered limb deafferentation by the cutting of sensory nerve roots as they enter the spinal cord (*1, 8*). These monkeys show degeneration of sectors of brainstem and thalamic nuclei formerly excited by sensory inputs from their long-insensate limb. In the reorganized thalamus, the representation of the face directly abuts that of the body trunk, just as in the remodeled cerebral cortex (*1, 2*).

How did this dramatic functional reorganization arise? The authors argue that thalamic shrinkage resulting from extensive cell death and subsequent physical rearrangement, combined with a probable sprouting of inputs from the face to neurons formerly representing the missing hand and arm, are the causes. From one perspective, sprouting would not appear to be on a very large scale, because the thalamic representations of the body and original face zones are both substantially shrunken, indicating that the thalamic organization recorded so many years after injury may primarily reflect a simple physical rearrangement of this deep brain nucleus. Alternatively, it is possible that this "rearrangement" has been achieved specifically through sprouting, by which a reinnervation of neurons formerly representing

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