A Scientific Rationale for Human Embryonic Stem Cell Research

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Stem cell biology has recently been at the forefront of a national discussion combining science, politics, and ethics. Few aspects of medicine and scientific research have been the subjects of a frenzy like that surrounding human embryonic stem (ES) cell research. Often lost amidst the opinions of pundits and op-ed writers in articles about research on human ES cells is: (1) the scientific basis of this research; and (2) the reasons why scientists and physicians are so interested in pursuing these studies. Quite simply, human ES cells are uniquely suited for research that uncovers the fundamental basis of human developmental biology. They might revolutionize areas of medicine such as transplantation medicine or gene therapy, and research on them will likely impact a wide variety of other fields. Indeed, in describing human ES cells, Harold Varmus, former director of the National Institutes of Health (NIH), said “[t]here is almost no realm of medicine that might not be touched by this innovation.” Under Varmus, the NIH released a report in which nineteen of its institutes each answered the question, “What would you hope to achieve from human pluripotent stem cell research?” The health conditions potentially better understood or treated range from cancer to neurological diseases, to HIV and AIDS, to burns and trauma, to hearing and sight, and to drug abuse and mental illness. The scientific and medical impact of this research is almost endless.

The federal government, primarily through the NIH, provides the largest single source of funding for basic biological and medical research in the country. Whether or not the NIH is allowed to fund studies of human ES cells will determine how quickly scientific research on human ES cells will progress. On August 9, 2001, President George W. Bush gave his first nationally televised address since his inauguration. This speech...

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addressed solely human ES cell research and the role the federal government should play in funding studies of these cells. In general, the President agreed that federal funding of research involving human ES cells would be permitted, but only on sixty or so human ES cell lines created prior to his speech. While this compromise did not fully satisfy either supporters or opponents of this research, it did set the stage to use federal dollars to move this research forward more rapidly.

I. STEM CELL BASICS

The most important basic concept about stem cells (and a point often not well understood) is that not all stem cells are the same. In general terms, a stem cell is defined as a cell that has two important characteristics: it can undergo self-renewal, and it can differentiate into two or more other cell types. Self-renewal refers to the property of these cells to divide without undergoing differentiation—as the cell divides and replicates to make more cells, each of these cells maintains an undifferentiated, multipotent, stem cell potential. However, in the proper environment or with the proper stimuli, a stem cell retains the ability to form more specialized cells, such as blood, muscle, liver, or skin cells. Two main categories of stem cells exist: adult stem cells and ES cells. Adult stem cells are those present within tissues of the body after birth. They are responsible for the continued growth of a single tissue or organ. For example, hepatic stem cells are in the liver, hematopoietic (blood) stem cells are in the bone marrow, and skin stem cells are in the basal layer of the epidermis. To maintain the integrity of a particular tissue or organ, these adult stem cells continually produce new cells to replace cells that are lost, diseased, or damaged. Hematopoietic stem cells (HSC) in bone marrow produce billions of blood cells daily. Some of these mature blood cells will survive months (red blood cells) or years (lymphocytes), while others only survive for a few hours (neutrophils). While HSCs make up no more than 0.1% of all bone marrow cells, their ability both to self-renew and to differentiate into a variety of cell types (like red blood cells, white blood cells, and platelets) enables the production of these billions of cells each day.

In contrast to these adult stem cell populations, ES cells are not normally found in the body after birth. ES cells are derived from a cluster of cells called the inner cell mass (ICM) that exists for only a few hours at an early stage of mammalian development. The cells of the ICM normally differentiate into more specialized cells that form all the cells, tissues, and organs of the fetal and adult (post-natal) body. Under proper conditions, ICM cells can be placed in a tissue culture environment that allows them to
be maintained as undifferentiated cells (now described as ES cells) that retain this potential to form any cell of the adult body. In this manner, ES cells were first derived from mouse blastocysts twenty years ago. Studies of these mouse ES cells have been instrumental to advances in mammalian developmental biology (e.g., providing an understanding of how specialized cells grow and develop from embryo to adult). However, the early stages of mouse development markedly differ from human development, so not all lessons learned from mouse ES cells may apply to human biology. It therefore became desirable to derive human ES cells. Many groups tried, but none succeeded until James Thomson and colleagues published a paper describing the derivation of human ES cells in 1998.

Human ES cells have many of the same characteristics as mouse ES cells. Both can be maintained in culture for months or years without evidence of differentiation and without genetic (karyotypic) abnormalities, and both can be induced to differentiate into a variety of cells or tissues. Therefore, human ES cells are capable of both self-renewal and differentiation. However, researchers immediately noted differences between mouse and human ES cells. For example, the human ES cells grew more slowly and had a different morphology compared to mouse ES cells. Moreover, the conditions required for maintenance of undifferentiated growth are different between these cell types. These findings re-emphasize the fact that not all discoveries regarding mouse ES cells apply to humans. Whereas mouse models were previously regarded as optimal to learn about mammalian (and therefore human) biology, human ES cells must now be considered the "gold standard" to learn about human developmental biology.

II. BASIC SCIENCE AND HUMAN ES CELLS

The most obvious and important scientific reason to study human ES cells is to learn the basics of human developmental biology—how humans develop from a fertilized egg to an adult organism. As a researcher interested in blood development, I am interested in this system because it permits intricate studies of genes and proteins as blood cells develop from ES cells. Samples can be taken every day, every hour, or even every minute to understand in fine detail the changes that may occur as these cells grow and differentiate. The environment can also be altered to define what signals control whether an ES cell undergoes self-renewal to create more ES cells, or becomes stimulated to form blood or other cells of interest. There is no other method to examine closely these early developmental steps in a human system without using human ES cells. These cells promise
to open new vistas to show how humans develop from a single cell. Similar studies cannot be done using human adult stem cells, as these cells are already committed to a specific developmental lineage. Even if some of these adult stem cells are capable of changing their lineage fidelity (as discussed below), there is no way to determine how they reach that stage in the first place. That is, the early steps of development will remain a black box if research is confined to only adult stem cells.

Eventually, using human ES cells to understand better these earliest stages of human development will likely translate into clinical therapies and improved drug development. For example, it is impossible to test for harmful (teratogenic) effects of newly designed medications on pregnant women and the developing fetus. While these teratogenic effects can be tested in animals, the absence of adverse outcomes in a pregnant animal does not preclude the possibility that the medication would still be harmful to a developing human. This point was tragically demonstrated by the use of thalidomide by pregnant women in the 1950s, which resulted in limb deformities in children exposed to this drug in utero. Now, however, a potentially harmful drug might be added to an ES cell culture to see if it prevents normal growth of a few cell types of interest. For example, blood, neural, and muscle development can be monitored from ES cells in culture. These crucial lessons can be best learned from using ES cells as a culture model of embryonic growth. In the future, this model will likely prevent untoward teratogenic side effects of pharmacological therapies.

III. CLINICAL THERAPIES AND TRANSPANTATION MEDICINE

Studies of human ES cells will teach us how cells develop and grow. This knowledge can then be applied to derive new ES cell-based therapies to treat a host of degenerative, malignant, and genetic diseases. The clinical field of hematopoietic cell transplantation (HCT) (commonly called bone marrow transplantation (BMT)) offers an excellent example of why human ES cell research is needed and the types of patients who will benefit from it. HCT is the only routine “stem cell therapy” currently performed in medicine. Thousands of patients a year undergo HCT typically to treat hematologic (blood) malignancies such as leukemia, lymphoma, or multiple myeloma. The greatest chance to cure these otherwise fatal diseases is by allogeneic HCT where HSCs from a healthy person are transplanted into the patient. When successful, these transplanted blood cells will grow in the new host as a means to eradicate malignant cells. Due to immunologic barriers, the donor and the host must be closely matched for tissue antigens (HLA molecules). Without this close matching, either the host will reject the donor cells or the
transplanted cells will cause overwhelming graft-versus-host disease that could be fatal to the patient. Optimal allogeneic HCT uses cells from an HLA-matched sibling of the patient; there is a 25% chance that any one sibling will be a perfect HLA match for the patient.8 If siblings do not match, then bone marrow donor registries are searched. While there are now over five million people listed worldwide in these donor registries, many racial and ethnic groups remain underrepresented.9 Studies have found that because it is so difficult to find an appropriate donor, only about one-third of patients who would benefit from an allogeneic donor actually receive a transplant.10 Patients without a suitable match may either undergo autologous HCT, where the patient’s own hematopoietic stem cells are used, or receive additional chemotherapy without a transplant. While these treatments are often effective, the probability of curing the disease is typically less than with an allogeneic HCT.11 Despite more than twenty years of clinical experience with this type of adult stem cell therapy, obvious deficiencies exist and patients are dying who might otherwise live with new treatment options.

Human ES cells offer a novel source of cells to treat patients who do not have a suitable donor for an allogeneic HCT. Already, our research has demonstrated it is possible to derive hematopoietic cells from human ES cells.12 Red blood cells, white blood cells, and megakaryocytes (platelets) can all be derived. The ability to transplant these ES cell-derived blood cells will be an important step to establishing these cells as a source of blood cells for patients without a donor. Even if these ES cell-derived blood cells are not directly transplanted into patients, the lessons learned by studying how blood cells grow and develop from human ES cells may provide insights that could be applied to help patients. For example, another potential source of cells for allogeneic HCT comes from umbilical cord blood. Although such cells come from the umbilical cord of a newborn baby, these cells are considered adult stem cells since they are committed to form only more blood cells. Cord blood cells seem to have unique properties when compared to other hematopoietic stem cells, and these cells could be successfully used for allogeneic HCT.13 However, only a limited number of cells can be isolated from an individual cord. While this number of cord blood cells is suitable for transplant into a child, there are often not enough of them for an effective transplant into an adult. Considerable research to find ways to expand the number of hematopoietic stem cells in a cord blood unit is underway. To date, however, researchers have not yet found the means to accomplish this ex vivo expansion routinely. Research on blood development from human ES cells may lead to scientific breakthroughs leading to more widespread use
of cord blood for clinical purposes. For example, these studies may define specific proteins and genes that are essential for growth of transplantable HSCs. We may then be able to identify a new protein, X, that leads to dramatic expansion of HSCs without differentiation. Addition of this protein, X, to cultures of cord blood may allow more successful ex vivo expansion of these cells. Eventually, this may permit more cord blood transplants and the ability to cure patients of devastating diseases that may otherwise be fatal.

IV. GENE THERAPY

Hundreds of diseases are caused by mutations or deficiencies of a single gene (a segment of DNA). Gene therapy refers to an area of science and medicine that attempts to treat disease by replacing abnormal DNA with normal DNA. Many vectors have been developed to insert new pieces of DNA into cells or tissues. For example, genetically engineered viruses can be inhaled or injected, leading to expression of a normal gene and potential improvement in the underlying disorder. Unfortunately, despite almost twenty years of studies and hundreds of clinical trials, this strategy has not been very effective, and few clinical successes have been published.

Cell-based therapies have been more successful at treating certain genetic disorders. Children affected by severe combined immunodeficiencies (SCID) lack functioning immune systems. These children are very susceptible to infections and will usually die at a young age without treatment. To date, the most effective means to treat SCID patients is with allogeneic HCT (as described above). This therapy replaces the abnormal blood cells with bone marrow-derived cells from an unaffected individual, leading to engraftment of a normal immune system and dramatically improved survival. Again, for reasons described above, many patients who would benefit from allogeneic HCT do not have a suitable donor. For some of these patients, gene therapy has successfully inserted the defective gene into cells of the immune system, leading to effective treatment, but this method remains difficult.

Human ES cell research could improve the treatment of such genetic diseases. Careful studies with mouse ES cells have shown that any cell in the body can be derived from undifferentiated ES cells. If we can learn how to derive specific cells and tissues from human ES cells, we could develop novel cell-based therapies. For example, blood cells could be grown for transplantation and treatment of immunodeficiencies. Hepatocytes (liver cells) could be derived to treat other enzyme deficiency diseases. Muscle cells, neurons, or any cell type in the body could be derived from human ES cells to treat better a variety of genetic or
degenerative diseases. If needed, a gene could be directly inserted into human ES cells so that cells derived from these modified ES cells can sufficiently produce a protein that is lacking in a patient with a particular genetic abnormality. In this manner, the ES cells (or ES cell-derived cells or tissues) could become an optimal vector for gene replacement therapies.

Many barriers will have to be surmounted before the human ES cell-based therapies reach clinical practice. For example, methods to prevent immune-mediated rejection of transplanted foreign cells need to be established. However, the unique properties of human ES cells will spur scientists to overcome these difficulties.\textsuperscript{17}

V. STEM CELL PLASTICITY

Some opponents of human ES cell research argue that human ES cell studies are unnecessary because adult stem cells are a suitable option for all proposed cell-based therapies. Several recent studies suggest that adult stem cells may have more versatility or plasticity than previously thought.\textsuperscript{18} Previous studies indicated that adult stem cells could differentiate into only a limited spectrum of tissue-specific cells.\textsuperscript{19} For example, bone marrow stem cells gave rise only to blood cells; neural stem cells gave rise only to neural and glial cells; and satellite cells in muscle cells gave rise only to muscle. Recent work suggests this may not be the case—adult stem cells may be more multipotent than previously believed. Such studies claim that bone marrow-derived cells develop into neurons and glial cells,\textsuperscript{20} hepatocytes,\textsuperscript{21} and skeletal\textsuperscript{22} and cardiac muscle.\textsuperscript{23} Muscle\textsuperscript{24} and neural-derived\textsuperscript{25} cells may produce blood, and skin-derived cells may produce neurons.\textsuperscript{26} Under the right conditions, a single neural stem cell or bone marrow cell may differentiate into multiple tissue types.\textsuperscript{27}

While these experiments are intriguing, they do not obviate the need for human ES cell research. The scientific and clinical implications of these studies on adult stem cell plasticity remain unclear. While certain adult tissues may transdifferentiate into another cell type when placed in a suitable environment, other interpretations must be considered. For instance, it is possible that multipotent stem cells are found in minute amounts in multiple tissues. Placing these uncommitted cells in a particular environment may cue the observed results. Other possible interpretations also exist.\textsuperscript{28}

These studies on potential adult stem cell plasticity further highlight the need to make human ES cells a gold standard for stem cell-based research. We know that mouse ES cells can be grown in culture for years as undifferentiated cells, and yet retain their pluripotent capabilities.\textsuperscript{29} In
contrast, adult stem cells typically cannot be maintained for more than a few weeks in culture. Certain sources of these adult stem cells, like bone marrow or neural tissue, are difficult to obtain in suitable amounts, and the purity of these cell populations may be questionable. The ability to grow large numbers of well characterized human ES cells will best allow for studies to determine which specific genes and proteins regulate the development of particular tissues. As discussed above, the most important implication of the derivation of human ES cells is not the potential of these cells to be used for future therapeutic purposes. Rather, it is to understand better basic human developmental biology.

Basic scientific studies in fields such as genetics and developmental biology demonstrate the need to use multiple model systems. Yeast (Saccharomyces cerevisiae), worms (Caenorhabditis elegans), fruit flies (Drosophila melanogaster), fish (Danio rerio), and mice (Mus musculus) each have particular strengths and weaknesses as investigational models to define the mechanisms by which cells and organisms grow and develop. Only by working on these alternative models have researchers obtained the basic knowledge that led to understanding normal human growth and development, eventually resulting in better therapies for human disease. Research on human ES cells will add another important piece to this puzzle. Eliminating our ability to use human ES cells might prevent a full understanding of the earliest stages of human development.

VI. THE BUSH DECISION AND THE FUTURE OF HUMAN ES CELL RESEARCH

President Bush’s decision to allow federal funding of human ES cell research is a step in the right direction. While this announcement seemingly set an arbitrary date by which human ES cell lines needed to be derived in order to qualify for federal funding, the decision validates the role of the federal government in supporting and promoting advances in this exciting field. The NIH recently released a registry of sixty-seven human ES cell lines that are available to laboratories throughout the world. With NIH funds available to expedite this research, progress will be made more rapidly than if only private funds were available to study these cells.

CONCLUSION

No one knows if these sixty or so human ES cell lines that qualify for federal funds will be enough to bring human ES cell-based therapies into clinical practice. If the goal of these studies is to derive transplantable cells to treat genetic, degenerative, or malignant diseases, then this limited
number of cell lines is unlikely to be sufficient. However, if the goal is to use human ES cells to understand better the basics of human developmental biology, then this may be a reasonable number of cell lines for the near future. Scientific research does not always (or often) proceed in a straightforward, linear manner. Information must be gathered from multiple sources before conclusions can be reached. Human ES cells now provide a crucial model adding to the pool of information about human growth and development. Knowledge obtained from the basic study of these cells may likely be applied to other systems to improve clinical therapies directly or indirectly. There is no doubt that research on human ES cells will lead to progress in clinical medicine, but exactly how this research will impact future therapies for patients is difficult to predict.
References


7. Id. at 1146; James A. Thomson et al., Primate Embryonic Stem Cells, 38 CURRENT TOPICS IN DEV. BIOLOGY 133 (1998).


11. Richard T. Silver et al., An Evidence-Based Analysis of the Effect of Busulfan, Hydroxyurea, Interferon, and Allogeneic Bone Marrow Transplantation in Treating the Chronic Phase of Chronic Myeloid Leukemia: Developed for the American Society of Hematology, 94 BLOOD 1517 (1999); Keith E. Stockerl-Goldstein & Karl G. Blume, Allogeneic Hematopoietic Cell Transplantation for Adult Patients with Acute Myeloid Leukemia, in 2 HEMATOPOIETIC CELL TRANSPLANTATION (E. Donnall Thomas et al. eds., 1999).


16. András Nagy et al., Derivation of Completely Cell Culture-Derived Mice from Early-
Passage Embryonic Stem Cells, 90 PROC. NAT'L ACAD. SCI. 8424 (1993).


19. NAT'L INST. OF HEALTH, id. at 23-42.

20. See Timothy R. Brazelton et al., From Marrow to Brain: Expression of Neuronal Phenotypes in Adult Mice, 290 SCI. 1775 (2000); Eva Mezey et al., Turning Blood into Brain: Cells Bearing Neuronal Antigens Generated In Vivo from Bone Marrow, 290 SCI. 1779 (2000).


23. See Donald Orlic et al., Bone Marrow Cells Regenerate Infarcted Myocardium, 410 NATURE 701 (2001).


25. See Christopher R. Bjornson et al., Turning Brain into Blood: A Hematopoietic Fate Adopted by Adult Neural Stem Cells In Vivo, 283 SCI. 534 (1999).

26. Jean G. Toma et al., Isolation of Multipotent Adult Stem Cells from the Dermis of Mammalian Skin, 3 NATURE CELL BIOLOGY 778 (2001).


28. Blau et al., supra note 18; Fuchs & Segre, supra note 18; Lagasse et al., supra note 18.

