Stem Cells: Current Challenges and Future Promise

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When stem cells divide, they can either produce more copies of themselves (self-renewal) or they can produce daughter cells able to differentiate into one or more adult cell types. Human embryonic stem cells (hESCs) appear to have an unlimited capacity to self-renew in cell culture and can differentiate into hundreds of adult cell types. Scientists hope to use hESC lines to identify molecular mechanisms of cell fate determination, to culture human cells for use in new drug discovery and toxicity testing, and to generate differentiated cells for novel transplantation therapies. The National Institutes of Health (NIH) identified some roadblocks to progress in stem cell research and developed funding opportunities designed to overcome them. NIH recognizes the unparalleled promise offered by stem cell research, and has made investments in this field a priority for the foreseeable future.

What are stem cells?

Two characteristics distinguish stem cells: (1) they can divide to produce more copies of themselves (self-renewal) under appropriate *in vitro* or *in vivo* conditions, and (2) they are pluripotent, or able to differentiate into most, if not all, mature cell types. Stem cells derived from an early stage human blastocyst (an *in vitro* fertilized embryo after about five days in culture) have the capacity to renew indefinitely, and can theoretically provide an unlimited supply of cells. It is also possible to derive stem cells from non-embryonic tissues, including amniotic fluid, placenta, umbilical cord, brain, gut, bone marrow, and liver. These stem cells are sometimes called "adult" stem cells, and they are typically rare in the tissue of origin. Experts estimate that only one cell in 1,000 to 10,000 cells of an adult organ or tissue is actually a stem cell. Because so-called adult stem cells include cells from the placenta and other early stages of development, they are more correctly termed "non-embryonic stem cells." Non-embryonic stem cells are more limited in their capacity to self renew in the laboratory, making it more difficult to generate a large number of stem cells for a specific experimental or therapeutic application. Under normal conditions, non-embryonic stem cells serve as a repair pool for the body, so they typically differentiate only into the cell

types found in the organ of origin. So far, there is little compelling evidence for trans-differentiation, whereby a stem cell from one organ differentiates into a mature cell type of a different organ. Many studies provide gene expression profiles or antigen staining rather than demonstrating the appropriate mature cell function. New discoveries may one day overcome any or all of these limitations of stem cells derived from non-embryonic sources. In fact, research directed towards this goal is currently underway in a number of laboratories. If we can learn to reverse the differentiation process to generate pluripotent cells from differentiated cells, the research community could study these remarkable cells without destroying human embryos.

What is the medical promise of stem cell research?

Scientists are leveraging the potentials of human pluripotent, self-renewing cell cultures to make a significant impact on biomedical research. These cells are enabling them to understand the molecular mechanisms that determine how a pluripotent, self-renewing cell differentiates into a specialized cell type (Figure 1). These mechanisms include epigenetic changes in the chromatin structure, developmental changes in gene expression, exposure to growth factors, and interactions between adjacent cells. Understanding these basic mechanisms of stem cells may enable us to someday mobilize and differentiate endogenous populations of pluripotent cells to replace a cell type ravaged by injury or one of many cellular degenerative diseases, including Type 1 diabetes mellitus, Parkinson's disease, myocardial infarction, and spinal cord injury, to name a few prominent examples. Alternatively, scientists may some day be able to coax human pluripotent cells grown in the laboratory to become a specific type of specialized cell, which physicians might be able to subsequently transplant into a patient to replace cells damaged by these same disease processes.

Scientists are gradually learning to direct the differentiation of pluripotent cell cultures into a specific type of cell, which can then be used as cellular models of human disease for drug discovery or toxicity studies. In the case of the fatal neurodegenerative disorder Huntington's disease (an autosomal dominant genetic disease), one could imagine that pluripotent cells differentiated into neurons in culture could be used to test drugs to delay or prevent degeneration. It is impossible to predict the many ways that a basic understanding of stem cell differentiation may lead to new approaches for treating patients with cellular degenerative diseases.

What are the sources of human pluripotent cell lines?

Currently, there are at least 6 sources that have already been used to establish human pluripotent stem cell lines. These include the "traditional" IVF-embryo method first reported by James Thomson in 1998, human primordial germ cells reported by John Gearhart, "naturally dead" embryos, chromosomally abnormal embryos, single

cell embryo biopsy, and parthenogenesis. Scientists are actively pursuing alternative sources of pluripotent stem cells, some of which are being attempted using human tissue, and some which are only being done in animals at present.

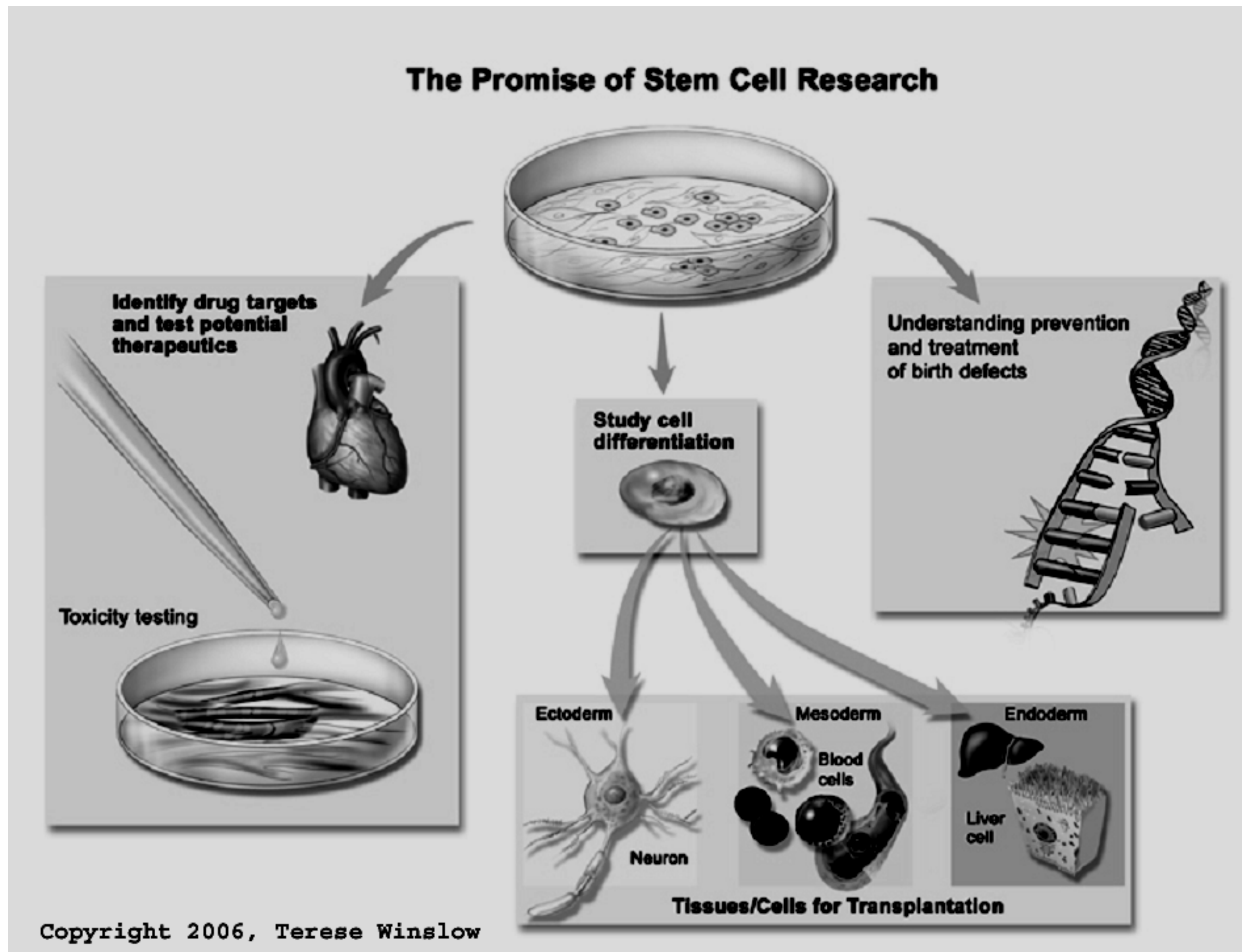


Figure 1. The Promise of Stem Cell Research
Stem cell research provides a useful tool for unraveling the molecular mechanisms that determine the differentiation fate of a pluripotent cell, and for understanding the gene expression properties and epigenetic modifications essential to maintain the pluripotent state. This knowledge may someday be used to generate cells for transplantation therapies, whereby a specific cell population compromised by disease is replaced with new, functional cells. Differentiated derivatives of human pluripotent cells may also prove to be useful as cellular disease models for understanding the biology of disease, and also for developing new drugs, particularly when there is no animal model for the disease being studied. These cells will also lead to improvements in human health in ways that we cannot predict.

Sources that have already been used to establish human pluripotent stem cell lines:

1) Traditional hESC Line Generation

In 1998, James Thomson, Ph.D., VMD at the University of Wisconsin was the first to succeed in generating human embryonic stem cell (hESC) lines[1]. His studies were built on approximately twenty years of cumulative scientific experience using mouse embryonic stem cells. The "traditional" IVF-embryo method for generating human embryonic stem cell lines uses embryos generated for in vitro fertilization (IVF) that are no longer needed for reproductive purposes. During IVF, medical professionals typically produce more embryos than a couple attempting to start a family may actually need. These extra, or spare, embryos are typically stored in a freezer for possible future attempts for additional children in the future. It is estimated that there are about 400,000 such spare embryos worldwide. If these embryos are never used by the couple, they will either remain in storage or be discarded as medical waste. Alternatively, these embryos could potentially be used to generate a hESC line.

To generate a hESC line, scientists begin with a donated blastocyst stage embryo, at about five days after it was fertilized in vitro (Figure 2A). The blastocyst consists of approximately 150-200 cells that form a hollow sphere of cells, the outer layer of which is called the trophectoderm. During normal development, the trophoblast would become the placenta and umbilical cord. At one pole of this hollow sphere, 30 to 50 cells form a cluster that is called the inner cell mass (ICM), which would give rise to the developing fetus. ICM cells are pluripotent, possessing the capacity to become any of the several hundred specialized cell types found in a developed human, with the exception of the placenta and umbilical cord.

Scientists remove the ICM from the donated blastocyst and place these cells into a specialized culture medium. In about one out of five attempts, a hESC line begins to grow. The conditions for culturing the cells are critical for maintenance of the self-renewing and pluripotent properties of these remarkable cells.

2) hESC Lines from Human Primordial Germ Cells

The laboratory of John Gearhart, Ph.D., at The Johns Hopkins Medical School, published a second method for generating human pluripotent stem cell lines in 1998[2]. They isolated specialized cells known as primordial germ cells (PGCs) from a 5-7 week old embryo and placed these cells into culture (Figure 2). PGCs are destined to become either oocytes or sperm cells, depending on the sex of the developing embryo. The resulting cell lines are called embryonic germ cell lines, and they share many of the same properties as embryonic stem cells. Scientists are actively working to define the similarities and differences between these two types of human pluripotent cells.

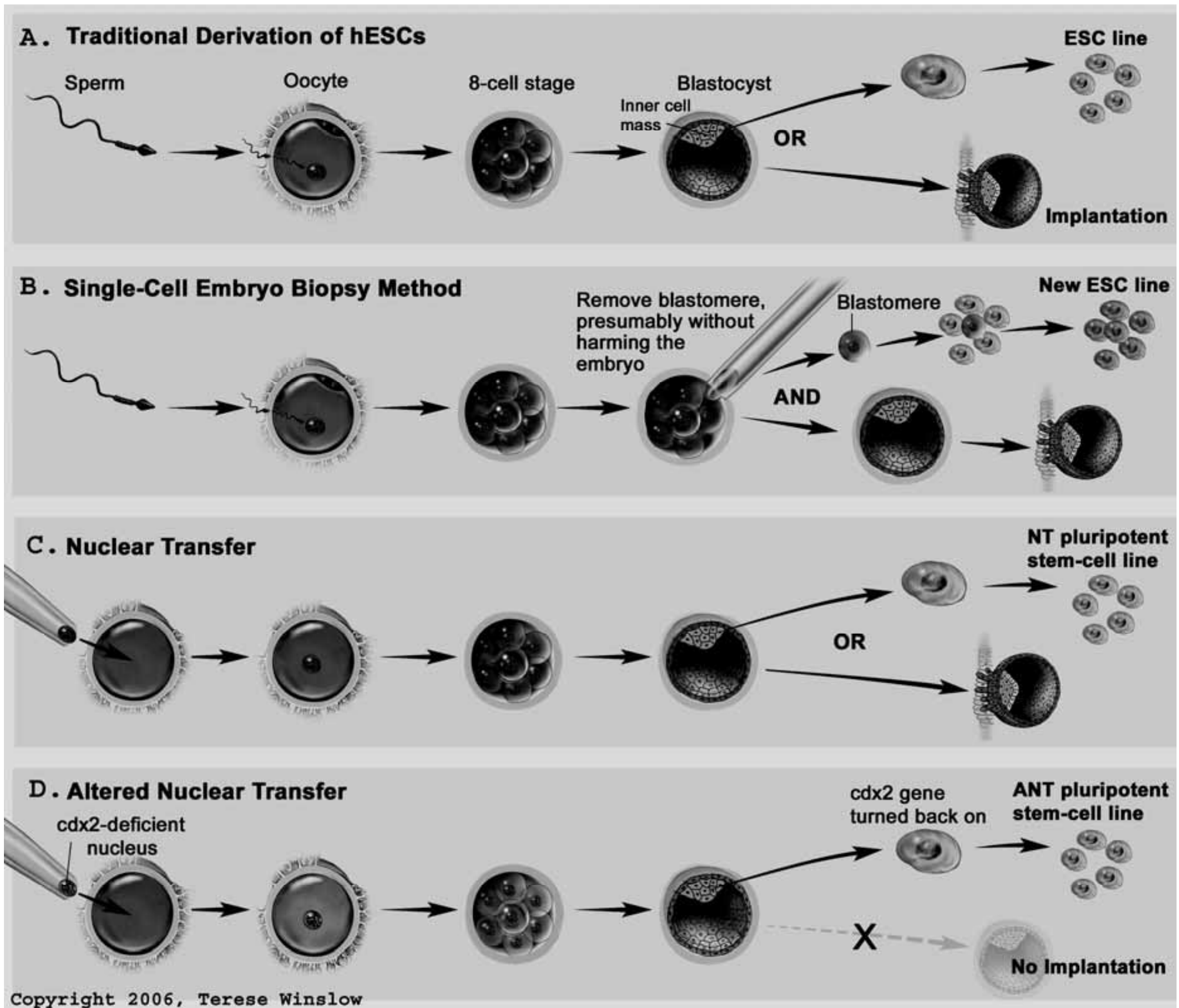


Figure 2. Alternative Methods to Create Pluripotent Stem Cells
Human pluripotent stem cells can be generated from embryos in IVF clinics, from the primordial germ cells found in a 5-7 week fetus, by somatic cell nuclear transfer (SCNT) into a donated human oocyte

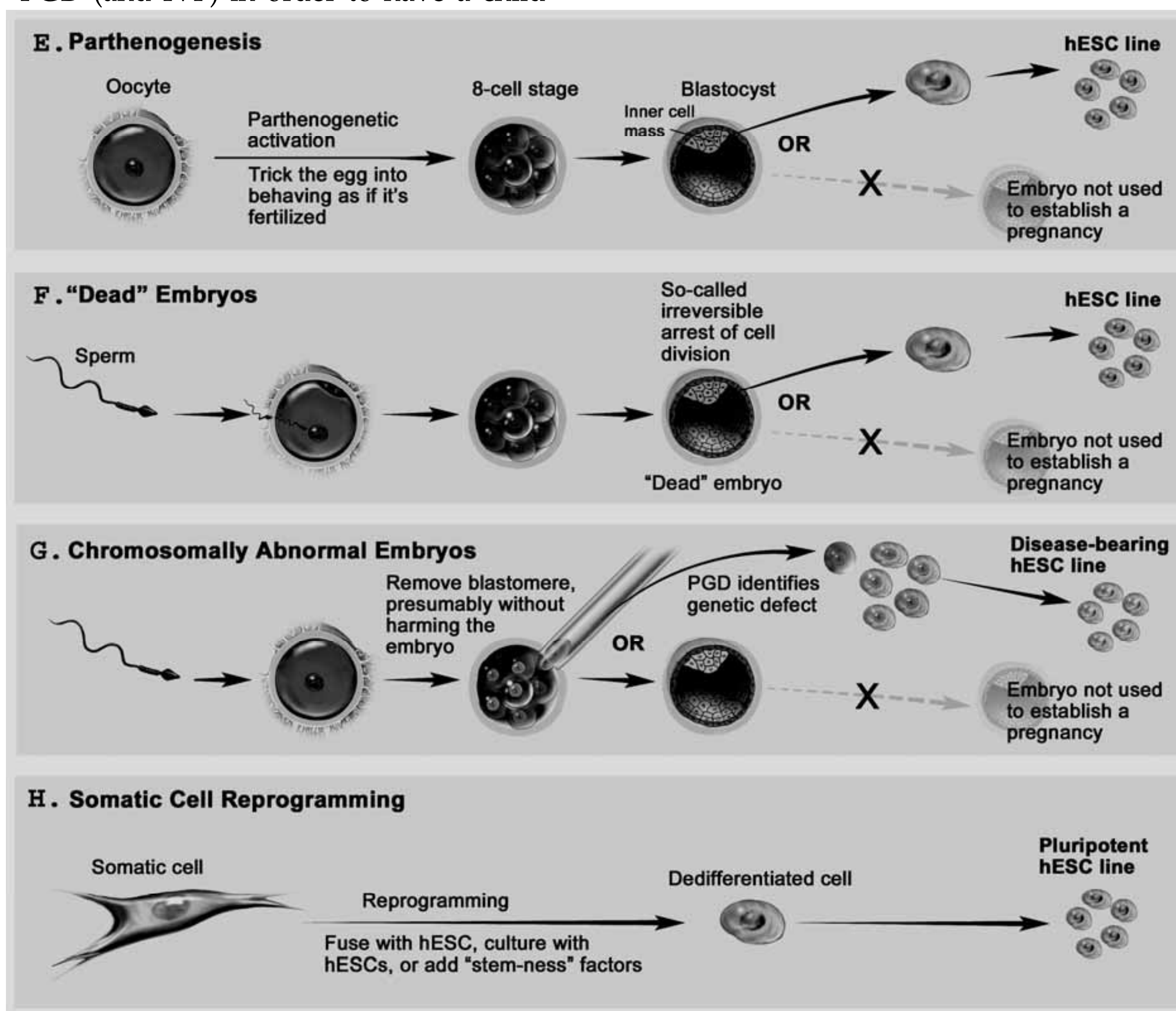
3) hESC Lines from Dead Embryos

Embryos that stop dividing after being fertilized *in vitro* are not preferentially selected for implantation in a woman undergoing fertility treatment. These embryos are typically either frozen for future use, or discarded as medical waste. In 2007, scientists at the University of Newcastle, United Kingdom, generated hESC lines from IVF embryos that had stopped dividing[9]. These scientists used similar methods as described above under "Traditional hESC Line Generation", except that their source material was so-called "dead" IVF embryos (Figure 2F). The human stem cells created using this technique behaved like pluripotent stem cells, including making proteins critical for "stemness" and being able to produce cells from all three germ layers. Proponents of this technique suggest that when an IVF embryo dies naturally, the embryo could not develop into a human being, and thus could potentially

be used to derive human embryonic stem cells without having to destroy a living embryo. However, ethical considerations make it uncertain whether scientists will equate this procedure of deriving hESC lines to organ donation. That is, scientists can remove live cells from dead embryos in the same way that they remove live organs from individuals who have donated them after death.

4) hESC Lines from Chromosomally Abnormal Embryos

Couples who have learned that they carry a genetic disorder sometimes use PGD (and IVF) in order to have a child that does not carry the disorder. PGD requires scientists to remove one cell from a very early IVF human embryo and test it for diseases known to be carried by the hopeful couple. Normally, embryos identified with genetic disorders would be discarded as medical waste. However, scientists at the Reproductive Genetics Institute in Chicago have capitalized on these embryos as a way to further our understanding of the diseases they carry (Figure 2G) -- they derived hESC lines from them[4]. The new stem cell lines can now be used to help scientists



understand how things go wrong in diseases such as thalessemia, Fanconi's anemia, muscular dystrophy, Huntington's disease, Marfan syndrome, adrenoleukodystrophy, and neuro-fibromatosis. Scientists plan to add to this list by deriving hESCs from embryos that carry additional genetic disorders.

5) hESC Lines from Single Cell Embryo Biopsy

In 2006, Dr. Robert Lanza and his colleagues at Advanced Cell Technologies (ACT) demonstrated that it was possible to remove a single cell from a pre-implantation mouse embryo and generate a mouse embryonic stem cell line[5]. Later that same year, Dr. Lanza's laboratory reported that they had successfully established hESC lines (Figure 2B) from single cells taken from pre-implantation human embryos[6]. The human stem cells created using this technique behaved like pluripotent stem cells, including making proteins critical for "stemness" and being able to produce cells from all three germ layers. Proponents of this technique suggest that since it requires only one cell from the embryo, the remaining cells may yet be implanted in the womb and develop into a human being. Thus scientists could potentially derive human embryonic stem cells without having to destroy an embryo. However, ethical considerations make it uncertain whether scientists will ever test if the cells remaining after removal of a single cell can develop into a human being, at least in embryos that are not at risk for carrying a genetic disorder. In addition, it is not clear whether or not the single cell used to generate a pluripotent stem cell line has the capacity to become a human being.

6) hESC Lines Created Via Parthenogenesis

Parthenogenesis is defined as an embryo created without fertilizing the egg with a sperm, thus omitting the sperm's genetic contributions. Instead, scientists "trick" the egg into believing it is fertilized, so that it will begin to divide and form a blastocyst (Figure 2E). In 2007, scientists at Lifeline Cell Technology in Walkersville, Maryland reported that they successfully used parthenogenesis to derive hESCs[13]. These stem cell lines retained the genetic information of the egg donor and demonstrated characteristics of pluripotency. They were also derived and grown on a human feeder cell layer. This technique may lead to the ability to generate tissue-matched cells for transplantation to treat women who are willing to provide their own egg cells. It could also offer an alternative method for deriving tissue-matched hESCs that do not require destruction of a fertilized embryo.

Human Stem Cell Lines Whose Potency is Still Being Determined:

The scientific literature also reports the isolation of human stem cells that have not yet been conclusively established as pluripotent. Two prominent examples of these putative pluripotent human stem cells include amniotic fluid stem cells and cell lines generated by somatic cell reprogramming.

1) Human Stem Cell Lines from Amniotic Fluid

Amniotic fluid surrounding the developing fetus contains cells shed by the fetus and is regularly collected from pregnant women during amniocentesis. Scientists have previously reported that some of these cells can differentiate into fat, muscle, bone, and nerve cells. Dr. Anthony Atala and his colleagues at the Wake Forest University have generated non-embryonic stem cell lines from cells found in both human and rat amniotic fluid[10]. They named the cells amniotic fluid-derived stem cells (AFS). Experiments demonstrate that AFS can produce cells that originate from each of the three embryonic germ layers. The cells are self-renewing and maintain the normal number of chromosomes after a long time in culture. However, undifferentiated AFS did not make all of the proteins expected in pluripotent cells, and they were not capable of forming a teratoma. The scientists developed *in vitro* conditions that enabled them to produce nerve cells, liver cells, and bone-forming cells from AFS. AFS-derived human nerve cells could make proteins typical of specialized nerve cells and were able to integrate into a mouse brain and survive for at least two months. Cultured AFS-derived human liver cells secreted urea and made proteins characteristic of normal human liver cells. Cultured AFS-derived human bone cells made proteins expected of human bone cells and formed bone in mice when seeded onto 3-D scaffolds and implanted under the mouse's skin. Although scientists do not yet know how many different cell types AFS can generate, AFS may one day allow scientists to establish a bank of cells for transplantation into human beings.

2) Human Cell Lines Generated by Somatic Cell Reprogramming – the Eggan Method

In 2005, Dr. Kevin Eggan and colleagues at Harvard University reported that they had fused cultured adult human skin cells with hESCs (Figure 2H)[3]. The resulting "hybrid" cells had many characteristics of hESCs—they grew and divided in a similar manner and manufactured proteins that are typically made in hESCs. Some as-yet unknown factor(s) within the hESCs enabled them to "reprogram" the adult skin cells to behave as hESCs. The cells still raise a significant technical barrier that must be overcome before they can be used to treat patients. Because fused cells are tetraploid (they contain four copies of the cellular DNA rather than the normal two copies), scientists must develop a method to remove the extra DNA without eliminating their hESC-like properties. If this hurdle can be overcome, this technique may one day allow scientists to create patient-specific stem cells without using human eggs. At present, this new approach to creating stem cells is a useful model system for studying how stem cells "reprogram" adult cells to have properties of pluripotent cells.

Pluripotent Stem Cell Lines Derived in animals

A third category of pluripotent stem cells includes those that have been isolated in animal models, but have not yet been generated from human tissues. Among these are pluripotent cells

created by somatic cell nuclear transfer (SCNT), those derived by altered nuclear transfer, and those created by a form of reprogramming that drives cells to express genes characteristic of hESCs.

1) Stem Cell Lines from SCNT

SCNT (Figure 2C) is also referred to as therapeutic cloning, a term that causes confusion given the multiple uses of the word "cloning." Cloning refers to making an identical copy of anything—a molecule, cell, or, in this case, an animal. SCNT begins with the collection of human oocytes (eggs) from a female volunteer donor. The collection procedure carries some risks to the donor since she is asked to take drugs that stimulate the production of more than one oocyte during her menstrual cycle. Scientists then delicately remove the cell nucleus from the donated oocyte and replace it with the nucleus from a somatic cell -- an adult cell from elsewhere in the body,—hence the name somatic cell nuclear transfer. The oocyte with the newly transferred nucleus is stimulated to develop through a process called parthenogenesis—as defined previously. The oocyte may only develop if the transplanted nucleus—which came from a differentiated cell—is returned to the pluripotent state by factors found in the oocyte cytoplasm. This alteration in the state of the mature nucleus is called nuclear reprogramming. Understanding the molecular mechanisms that facilitate this process is another active area of research.

When parthenogenesis progresses to the blastocyst stage, the inner cell mass is removed and placed into culture in an attempt to establish a pluripotent stem cell line. Embryos generated by SCNT have successfully produced pluripotent cell lines in mice that appear to behave exactly the same as pluripotent cell lines generated from mouse blastocysts created by IVF. At this time, there are no published reports in which human embryos generated by SCNT have been used to generate a pluripotent cell line, although scientists worldwide are actively pursuing this area of research.

Why are scientists interested in using embryos generated by SCNT to create pluripotent cell lines? The nuclear genes of such a pluripotent cell line will be identical to the genes in the donor nucleus. If such a nucleus came from a cell that carries a gene mutation underlying a human genetic disease such as Huntington's disease, then all cells derived from the pluripotent cell line would carry this mutation. Cellular models of human genetic disease could be developed with this procedure, both to explore the underlying biology of disease and to develop drugs to slow or halt disease progression. Alternatively, if the cell providing the donor nucleus comes from a specific patient, all cells derived from the resulting pluripotent cell line would be a genetic match to the patient with respect to the nuclear genome. If these cells were used in transplantation therapy, the likelihood that the patient's immune system would recognize the transplanted cells as foreign and initiate tissue rejection would be reduced. However, because mitochondria also contain DNA, the donor oocyte will be the source of the mitochondrial genome, which is likely to carry mitochondrial gene differences

from the patient which may still lead to tissue rejection.

A new technique reported in 2007 by Dr. Kevin Eggan and colleagues at Harvard University may expand scientists' options when trying to "reprogram" an adult cell's DNA[11]. Previously, successful SCNT relied upon the use of an unfertilized egg. Now, the Harvard scientists have demonstrated that by using a drug to stop cell division in a fertilized mouse egg (zygote) during mitosis, they can successfully reprogram an adult mouse skin cell by taking advantage of the "reprogramming factors" that are active in the zygote at mitosis. They removed the chromosomes from the single-celled zygote's nucleus and replaced them with the adult donor cell's chromosomes. The active reprogramming factors present in the zygote turned genes on and off in the adult donor chromosomes, to make them behave like the chromosomes of a normally fertilized zygote. After the zygote was stimulated to divide, the cloned mouse embryo developed to the blastocyst stage, and the scientists were able to harvest embryonic stem cells from the resulting blastocyst. When the scientists applied their new method to abnormal mouse zygotes, they succeeded at reprogramming adult mouse skin cells and harvesting stem cells. If this technique can be repeated with abnormal human zygotes created in excess after IVF procedures, scientists could use them for research instead of discarding them as medical waste. Human embryonic stem cells generated in this way would be an excellent match for the chromosome donor, helping to avoid the problem of transplant rejection. In addition, use of excess IVF zygotes for SCNT would eliminate the need for human egg donations. This technique may overcome some ethical objections to deriving stem cells from 5-day-old human embryos, since the abnormal zygotes that would be used for this technique are not believed capable of surviving until birth.

2) Stem Cell Lines Generated by Altered Nuclear Transfer (ANT)

Altered Nuclear Transfer (ANT) is a variation on standard SCNT that proposes to create patient-specific stem cells without destroying an embryo. In ANT, scientists turn off a gene needed for implantation in the uterus (*Cdx2*) in the patient cell nucleus before it is transferred into the donor egg (Figure 2D). The proponents of ANT attempt to address concerns about embryo destruction by suggesting that because the entity created is unable to implant in the uterus, it is not a true embryo. Early in 2006, Dr. Rudolph Jaenisch and colleagues at MIT reported proof of principle tests that ANT works in mice[7]. Mouse ANT entities whose *Cdx2* gene is switched off are unable to implant in the uterus and do not survive to birth. However, scientists used ANT to create viable stem cell lines capable of producing almost all cell types. The authors point out that this technique must still be tested with monkey and human embryos, and the manipulation needed to control *Cdx2* expression introduces another logistical hurdle that may complicate the use of ANT to derive embryonic stem cells.

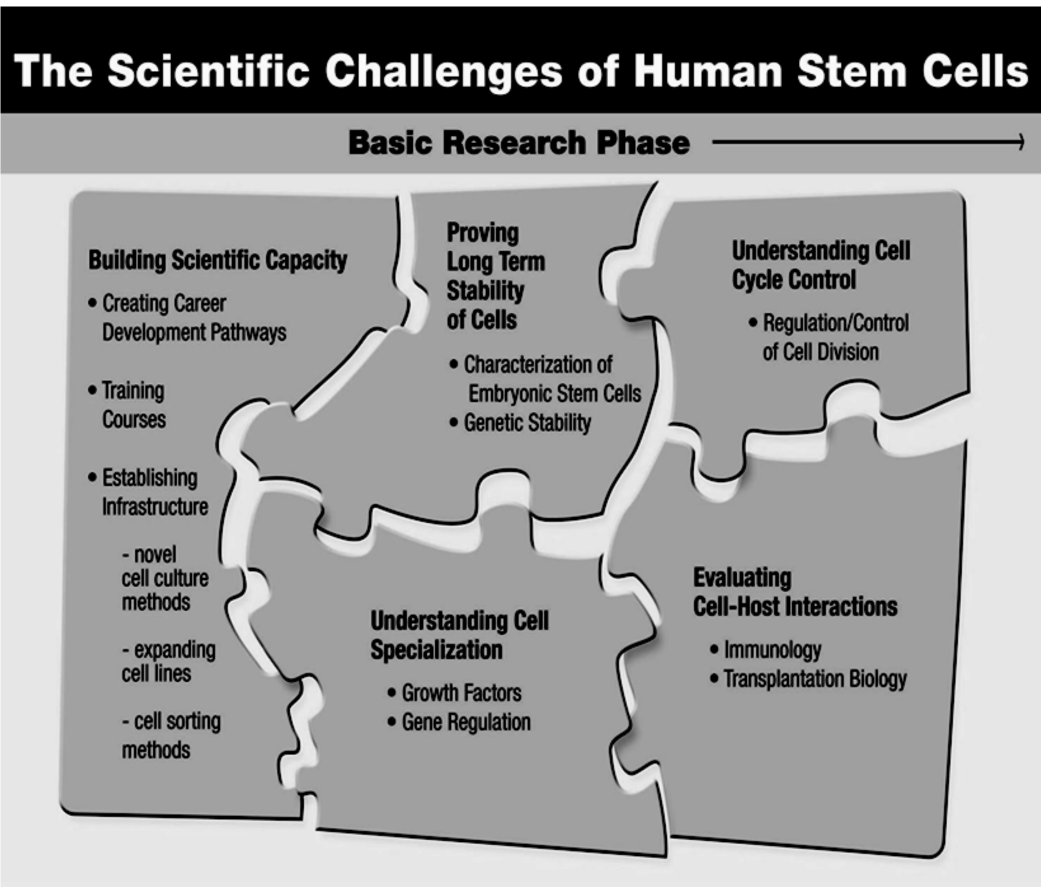


Figure 3. The Scientific Challenge of Human Stem Cells
The state of the science currently lies in the development of fundamental knowledge about the properties of human pluripotent cells. The scientific capacity needs to be built, an understanding of the molecular mechanisms that drive cell specialization needs to be advanced, the nature and regulation of interaction between host and transplanted cells needs to be explored and understood, cell division needs to be understood and regulated, and the long-term stability of the function in transplanted cells needs to be established.

3) Stem Cell Lines Generated by Reprogramming – the Yamanaka Method

In 2006, Dr. Shinya Yamanaka and colleagues at Kyoto University in Japan reported that they could use a virus to introduce four important stem cell factors into adult mouse cells and reprogram them to behave like embryonic stem (ES) cells (Figure 2H)[8]. They called the reprogrammed cells iPS, for induced pluripotent stem cells. However, iPS produced using the original technique could not do everything that ES cells can do. Notably, the original iPS cells do not make sperm and egg cells when injected into an early mouse blastocyst, and they do not make some changes to their DNA that help silence genes. Now the same scientists have modified their original technique, and they report that they can select for iPS that can make sperm and eggs[12]. Their 2007 report is accompanied by another from Dr. Rudolph Jaenisch and colleagues at MIT, which successfully

reproduced the Japanese group's results[14]. In addition, the MIT scientists determined that iPS DNA is modified in a manner similar to ES cells, and important stem cell genes are expressed at similar levels. They also demonstrated that iPS injected into an early mouse blastocyst can produce all cell types within the developing embryo, and such embryos can complete gestation and are born alive. These research advances were made in mice, and scientists must still determine if the same techniques can reprogram cells of adult humans. If this can be accomplished, scientists should be able to develop stem cell lines from patients who suffer from genetic diseases, such as Huntington's Disease, spinal muscular atrophy, muscular dystrophy, and thalessemia. Such lines would be invaluable research tools for understanding specific diseases and testing potential drugs to treat them. A second use of reprogrammed cells would be to repair damaged tissues in the human body. The Japanese scientists noted that the virus used to introduce the stem cell factors sometimes caused cancers in the mice. This represents a significant obstacle that must be overcome before the technique can lead to useful treatments for humans. This work suggests an additional method for creating pluripotent stem cells that, together with studies of other types of pluripotent stem cells, will help scientists learn how to reprogram cells to repair damaged tissues in the human body.

What is the state of the science of human pluripotent cells?

The research community is currently in the stage of fundamental discovery, or the basic science phase of understanding the properties of human pluripotent cells. Researchers are gradually learning how to direct these cells to differentiate into specialized cell types of interest for research, and using these human cell types for attempts at drug discovery and transplantation therapy (Figure 3). Scientists require a more complete understanding of the molecular mechanisms that drive pluripotent cells into differentiated cells before they can attempt to use stem cell derivatives for clinical applications. Scientists will need to pilot experimental transplantation therapies in animal model systems to assess the safety and long-term stable functioning of transplanted cells. In particular, they must be certain that any transplanted cells do not continue to self-renew in an unregulated fashion after transplantation, which may result in a teratoma, or stem cell tumor. In addition, scientists will need to make sure that cells transplanted into a patient are not recognized as foreign by the patient's immune system and rejected. At present, there are no clinical trials using cells generated by differentiating human embryonic stem cells, although scientists are hopeful that such trials will commence in the not-too-distant future. Human blood-forming (hematopoietic) stem cells from the umbilical cord and bone marrow are currently being used to treat patients with disorders that require replacement of cells made by the bone marrow, including Fanconi's anemia and chemotherapy-induced bone marrow failure after cancer treatment.

What Federal laws and policies affect human embryonic stem cell research in the United States?

At present, there is no Federal law that limits research involving human embryos and embryonic stem cell research. For example, no Federal law prohibits attempts at cloning humans for reproductive purposes using SCNT—an activity that many individuals believe is morally repugnant since animal SCNT frequently produces abnormal fetuses and animals that are born dead or die soon thereafter. Several states have adopted laws that limit the scope of research within their borders, and a number of states have passed laws that provide state-based support for stem cell research.

However, limits have been placed on Federal funding for scientific research. On August 9, 2001, in his first televised nationally televised address, President Bush set forth his policy placing limits on the use of Federal funds for human embryonic stem cell research. The President announced that he would, for the first time, allow the use of Federal funds for study of embryonic stem cell line so long as prior to his announcement: (1) the derivation process (which commences with the removal of the inner cell mass from the blastocyst) had already been initiated; and (2) the embryo from which the stem cell line was derived no longer had the possibility of development as a human being. In addition, the President indicated that these additional conditions must be met: (1) the stem cells must have been derived from an embryo that was created for reproductive purposes; (2) the embryo was no longer needed for these purposes; (3) informed consent was obtained for the donation of the embryo; and (4) no financial inducements were provided for donation of the embryo. The President's policy does not pertain to human embryonic germ cell lines, whereby the pluripotent cells are derived from the primordial germ cells from a 5-7 week fetus rather than a human embryo. In addition, the President's policy does not pertain to the use of private or state funds for embryonic stem cells. Scientists may still pursue research that may not be funded by the Federal government, so long as they procure non-Federal funds for such work.

To help scientists identify stem cell lines eligible to receive Federal funding, the NIH created the Human Embryonic Stem Cell Registry (the Registry). The Registry lists all human embryonic stem cell lines—at varying stages of development—that meet the President's eligibility criteria. Seventy-eight such derivations were subsequently located at private institutions around the world, seven of which are duplicates located at Geron, a biotechnology company, and WiCell, a nonprofit research subsidiary of the Wisconsin Alumni Research Fund. Since all 78 derivations are owned by private entities, the owners are under no obligation to provide human embryonic stem cells to the research community and NIH has no authority to insist that this limited resource be shared. In addition, since some of the derivations are little more than a frozen inner cell mass, there is no guarantee that the cells

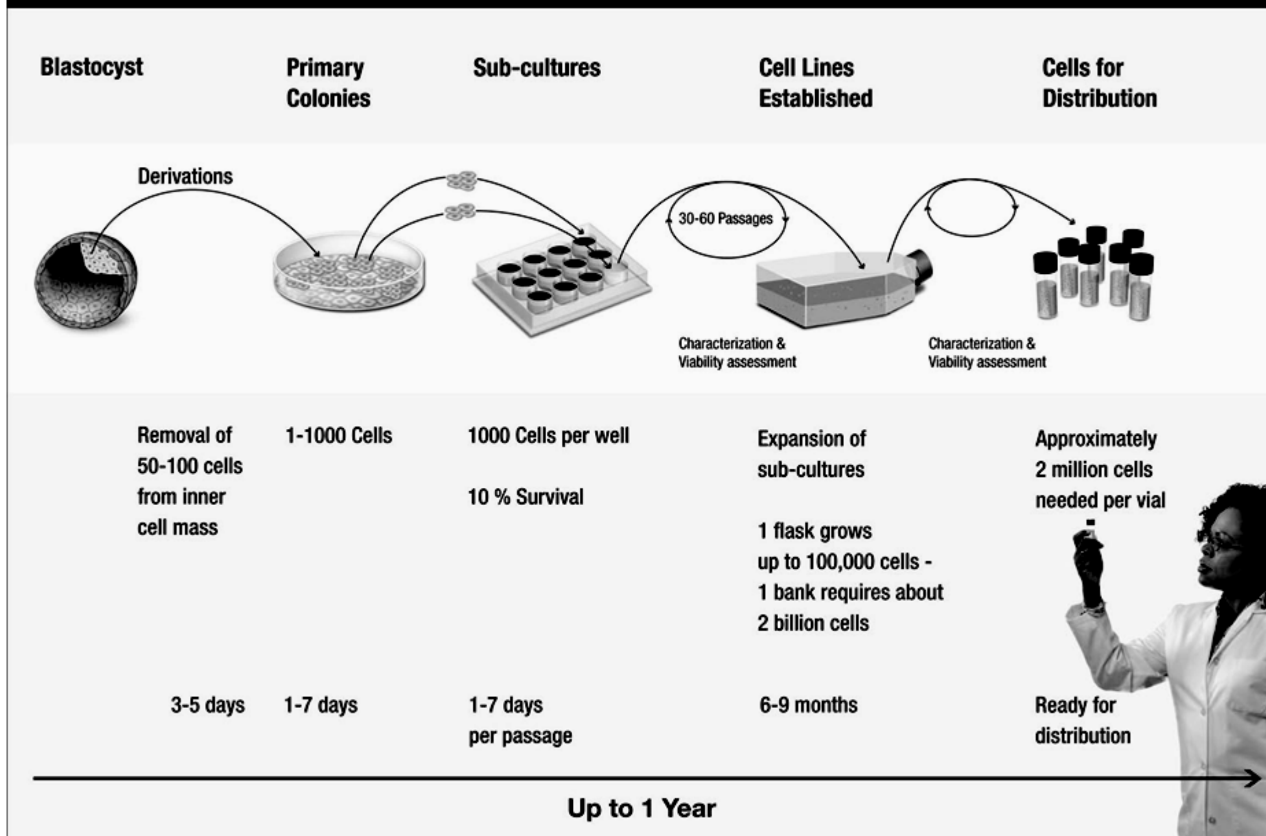


Figure 4. Establishing Human Embryonic Stem Cell Lines
The process of developing a human embryonic stem cell line is both time- and labor-consuming. It can take up to a year to progress from removal of the inner cell mass to achieving a well-characterized, scaled-up cell line ready to be distributed to the research community for study.

will propagate as a cell line when thawed.

The scale-up and characterization effort required to distribute cell lines is both time- and resource-intensive (Figure 4). In an effort to maximize the availability of cell lines eligible for Federal funding to the research community, NIH provided support through Infrastructure Grant Awards to allow private institutions with derivations on the Registry to prepare, expand, and characterize cell lines for responsible distribution to the community. Of the 15 private entities that own the 78 derivations eligible for Federal funding, nine have applied for and received NIH Infrastructure Awards with the intent of generating distribution-ready human embryonic stem cell lines from their derivations. These nine private entities control 40 of the 78 derivations eligible for Federal funding. Of these 40 derivations, 16 have failed to expand into pluripotent, self-renewing human embryonic stem cell lines, and one derivation was withdrawn by the donors. At the present

time, 21 cell lines have been scaled up and characterized to the point at which they can be distributed to the research community for human embryonic stem cell research supported by Federal funding.

A second limitation was placed on the use of NIH budget authority by the Legislative Branch of the Federal Government for research that involves human embryos. Beginning in 1996 and every year thereafter, the Human Embryo Research Ban (also called the Dickey Amendment) to the Department of Health and Human Services (DHHS) annual Appropriation Act prohibits the use of funds appropriated to DHHS to support the creation of a human embryo for research purposes or research in which a human embryo is destroyed, discarded, or subjected to risk of injury or death greater than that allowed under Federal requirements for fetuses *in utero*. For the purposes of this prohibition, the definition of a human embryo is very broad including embryos generated by parthenogenesis. Since NIH budget authority falls under the DHHS appropriation, NIH funds cannot be used to create a new human embryonic stem cell line, because a human embryo created by either IVF or SCNT would be destroyed in the process.

Policies and laws in other countries are sometimes more permissive and other times more restrictive than in the United States, depending on the country in question. This complex issue is beyond the scope of this discussion, although it is fair to note that the United States is unique in having a policy that restricts activities funded by the Federal government but places no restrictions on research funded by other sources.

What factors are limiting progress in hESC research?

hESC lines are extremely difficult to grow in culture. The cells require highly specialized growth media, containing essential ingredients of variable quality. In addition, most hESC lines are grown in the presence of a feeder cell line, a layer of cells from a mouse or human source on which stem cells can grow and obtain nutrients but which has been treated so the feeder cells cannot divide. Proper preparation of the feeder cells is essential for successful culture conditions. hESC lines used to produce human cells for transplantation therapies may need to be propagated on a human feeder cell layer in order to reduce the risk of contamination by harmful mouse viruses or other proteins that may cause rejection.

Human embryonic stem cell cultures must be expanded using an exacting protocol, or the cells will either die or begin to differentiate spontaneously and lose their pluripotency and self-renewal properties. Since only a few laboratories in the United States are growing these cells, there is a shortage of people well-versed in the art and science of successful hESC culture. In order to expand this rate-limiting human resource, NIH offers training grants for institutions to provide hands-on training in the techniques needed to culture hESCs. In 2003, five such courses were established, and about 200 scientists were trained. In 2006, the number of courses was increased to seven, and NIH plans to continue to support this activity as long as the demand is evident. In

addition, the NIH is supporting training in many independent laboratories funded to perform investigator-initiated hESC research.

Stem cell scientists frequently cite a compelling need for simplified, cost-effective and uniform cell culture conditions that will support the growth and pluripotency of most if not all hESC cultures. Optimally, these conditions would replace the feeder cell layer with purified stocks of necessary growth factors. Feeder cells add more steps to cell culture protocols, and may be problematic if an undesirable biological agent or molecule is unwittingly transmitted from the feeder layer to the cultured hESCs. This issue could result in additional safety concerns on the part of the U. S. Food and Drug Administration when they receive the first application proposing clinical trials involving transplantation of cells differentiated from hESCs. NIH is supporting efforts at several different institutions to establish culture conditions using only well-defined components. The University of Wisconsin has reported progress towards eliminating the need for feeder cells either to establish or propagate hESC lines. It will be essential to determine if the protocols for culture developed at Wisconsin can be simplified further or rendered less costly, since it requires the addition of purified growth factors that are very expensive. In addition, the cells must be monitored for genetic stability, sustained pluripotency, and continuous self-renewal over many passages in the new culture conditions.

Availability of hESC lines is another potential impediment to research progress. NIH-supported Infrastructure Grant Awards have resulted in the generation of 21 human embryonic stem cell lines that are eligible for Federal funding and are ready to be shipped to investigators. However, these cell lines are scattered among a variety of different providers, each specifying different requirements to be satisfied before shipment. In addition, a $5,000 licensing was required from all not-for-profit entities who requested a human embryonic stem cell line, since the intellectual property for derivation of human embryonic stem cell lines is currently held by WiCell, a biotechnology spin-off company started by the Wisconsin Alumni Research Fund. NIH conducted a competitive review of applications, and ultimately initiated a research and development contract to fund a National Stem Cell Bank (NSCB) at WiCell. It is the responsibility of the NSCB to consolidate as many of the 21 available lines as possible in one location, standardize quality control, and reduce the cost of the cells provided to researchers. The hope is that this step will simplify efforts on the part of the research community to obtain human embryonic stem cells for research, and reduce the cost to obtain the cells to $500.

In the short term, some of the challenges include the development of more robust culture conditions and protocols, understanding the molecular mechanisms that direct differentiation into specific cell types, and developing the human infrastructure to advance this exciting new scientific opportunity.

Once these challenges have been met, scientists will need to conduct transplantation studies in animal models (rodent and non-human primates) to demonstrate safety, effectiveness, and long-term benefit before stem cell therapies may enter into clinical trials. The risks and benefits of transplantation therapies will need to be very carefully considered, as these interventions represent a lifelong experiment with unknown consequences. While it is clear that transplantation-based therapies using hESCs are far from imminent, we can never know the full potential of these remarkable cells unless we embark on this important area of biomedical research.

Research opportunities and advances, as well as links to other information about stem cell research can be found on the NIH Stem Cell Information Web site: stemcells.nih.gov.

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