Preface

Human embryonic stem cells are derived from the earliest stages of blastocyst development after the union of human gametes. Prior to fertilization, the oocyte first requires timed completion of meiosis. This vital step does not occur throughout a woman's life; rather, oocytes are arrested at the first meiotic division until puberty when small numbers mature competitively during the reproductive years. Maturation is complete at the one-day event of ovulation that occurs in a regular, approximately monthly cycle. In humans, oocytes can be successfully fertilized only during a short period after ovulation; oocytes that are not fertilized are not retained.

Sperm cells mature from spermatic stem cells through a sequential process that has been well characterized. Spermatic stem cells are in turn generated from primordial germ cells set aside during early embryonic development. Generally, tens of millions of sperm are present in an ejaculate of which only one will successfully fertilize the mature oocyte. Sperm that fail to fertilize are discarded.

Fertilization initiates the process of cell differentiation. Because embryonic transcription is not initiated until later, the earliest developmental events are regulated primarily by maternally inherited mRNA. Once the sperm enters the egg, its DNA-associated proteins are replaced by oocyte histones. The two pronuclei become enveloped with oocyte-derived membranes, which fuse and begin the zygote's mitotic cell cycle. Embryonic development starts with a series of cleavages to produce eight undetermined and essentially equivalent blastomeres. The pattern of cleavage is well coordinated by cytoplasmic factors and in mammalian eggs is holoblastic and rotational. Though genomic DNA is inherited from both parents, mitochondrial DNA is inherited from only the mother. Paternal mitochondria transferred at the time of sperm entry are discarded by a little-understood process.

Human eggs, with a diameter of 100 μm, are generally smaller than eggs of other species. They are normally fertilized within the fallopian tubes and undergo cellular division in a defined milieu as they migrate toward the uterus. Over the first few days, cellular division follows a predictable 12–18-h cycle resulting in 2- to 16-cell pre-embryos. The

sperm centrosome controls the first mitotic divisions until day 4, when genomic activation occurs within the morula stage. The individual blastomeres are initially totipotent until the morula begins compaction and the cells initiate polarization. During compaction, cell boundaries become tightly opposed and cells are no longer equivalent. Cells in the inner cell mass (ICM) contribute to the embryo proper, whereas cells on the outside contribute to the trophoectoderm. The blastocyst forms approx 24 h after the morula stage by the development of an inner fluid-filled cavity, the blastocell.

Implantation is the process through which the compact zonula is thinned and the blastocyst released to implant. The blastocyst must first hatch from the thinning zona pellucida by alternating expansion and contraction; this process of hatching is critical to further development. Implantation of the hatched blastocyst requires several steps, including apposition, attachment, penetration, and trophoblast invasion, and cannot occur until the first cell specification into trophoectoderm has occurred.

As the trophoblast is developing to form the fetal component of the placenta, the endometrial lining of the maternal uterus is undergoing a decidual reaction to generate the maternal component of the placenta. Simultaneously the inner cell mass undergoes gastrulation, defined as a process of complex, orchestrated cell movements that vary widely among species, but include the same basic movements. These include epiboly, invagination, involution, ingression, and delamination.

Thus, several major developmental events have taken place as the fertilized egg migrates from the site of fertilization (fallopian tubes) to the body of the uterus over a period of 4 d. At all stages the egg is shielded from the external environment, initially by the zonula and subsequently by the trophoectoderm, but is accessible to *ex utero* manipulation (*see* Fig. 1). It is important to emphasize that these critical developmental events have occurred prior to implantation and well before blood vessel growth and heart development. The early stage fertilized egg that has not yet been implanted has been termed a pre-embryo to distinguish it from the implanted embryo. Manipulation of the preimplanted embryo has been feasible for the past three decades, and detailed rules governing the use of blastocysts have been developed.

After implantation, the ICM proliferates and undergoes differentiation. Several results suggest that lineage-specific genes are operating in a totipotent blastocyst cell prior to lineage commitment, and strongly support the concept that stem cells express a multilineage transcriptosome. Most genes (including tissue-specific genes) are

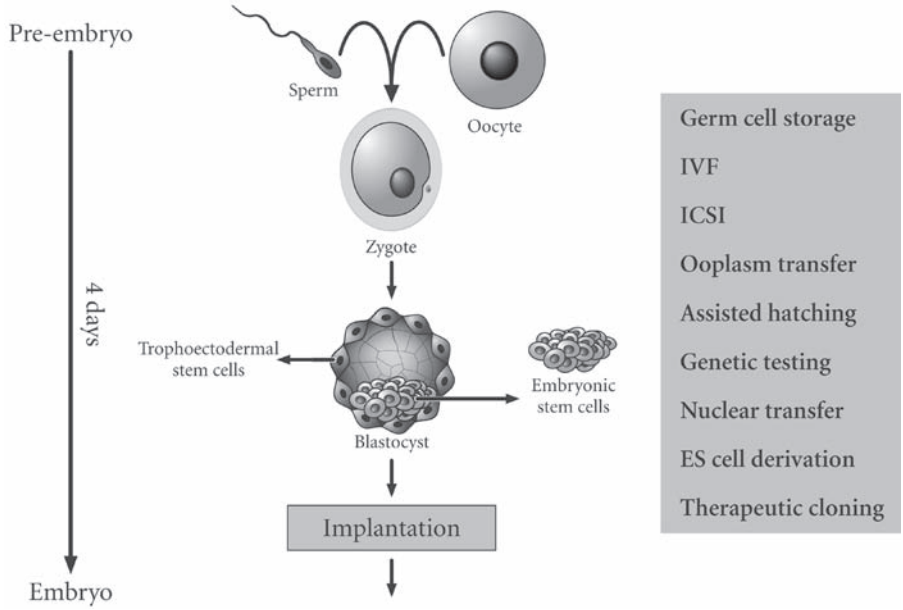

Fig. 1. Many techniques have been devised to manipulate the process of fertilization and maturation prior to implantation (summarized at right). The recent development of techniques to generate embryonic stem cell lines and perform somatic nuclear transfer has increased our ability to understand the process of development and intervene therapeutically. Note that the fertilized egg and early stage zygote are accessible to manipulation prior to implantation.

maintained in an open state with low but detectable levels of transcription with higher levels of specific transcription seen in appropriate cell types. Maintenance of an open transcriptosome in multipotent cells likely requires both the presence of positive factors as well as the absence of negative regulators. Factors that maintain an open transcriptosome include as yet unidentified agents such as demethylases, reprogramming molecules present in blastocyst cytoplasm, and regulators of heterochromatin modeling. Global activators, global repressors, and master regulatory genes play important regulatory roles in switching on or off cassettes of genes, whereas methylation and perhaps small interfering RNA (siRNA) maintain a stable phenotype by specifically regulating the overall transcriptional status of a cell. Allelic inactivation and genome shuffling further sculpt the overall genome profile to generate sex, organ, and cell-type specification.

Few genes have been identified that are required for the maintenance of the epiblast population. *Oct4*-/- embryos die before the egg cylinder stage and embryonic stem (ES) cells cannot be established from *Oct4*-/- cells. Levels of Oct 4 expression are critical to the fate of the cells. Low cell levels lead to differentiation into trophoblast giant cells, whereas high levels cause differentiation into primitive endoderm and mesoderm. FGF4 is required for formation of the egg cylinder and FGF 4-/- embryos fail to develop after implantation and ICM cells do not proliferate in vitro. *Foxd3/Genesis* is another transcription factor that may be required for early embryonic development. TGF-β/SMADs, Wnts, and FGFs are thought to play an important role in the process of gastrulation. BMP4 is essential for the formation of extraembryonic mesoderm and the formation of primordial germ cells. Nodal expression is required for mesoderm expansion, maintenance of the primitive streak, and setting up the anterior–posterior and proximo–distal axis. FGF4 is secreted by epiblast cells and is required for the maintenance of the trophoblast.

As our understanding of early developmental events has increased, our ability to safely manipulate the reproductive process has also increased. In vitro fertilization is now a relatively commonplace procedure that has been performed for more than 20 yr. Today, there are over a thousand established clinics worldwide. Even such technically complex procedures as intracytoplasmic sperm injection (ICSI), ooplasm transfer, assisted hatching, intrauterine genomic analysis, intrauterine surgery, and organ transplants are becoming more commonplace. More then 70 human embryonic cell lines have been established and their ability to differentiate into ectoderm, endoderm, and mesoderm repeatedly demonstrated. Nuclear transfer has become feasible, and the potential of combining ES cell technology with somatic nuclear transfer to clone individuals has caught the attention of people worldwide. At each stage of technological sophistication, profound ethical issues have been raised and publicly debated. Perhaps the most recent technological breakthroughs are the ones that have created the most controversy, primarily because of their potential to be used on a large scale. The ability to generate human ES (hES) cells and the ability to perform somatic nuclear transfer and successfully clone mammalian species raise fears often fueled by limited information.

An additional potential paradigm shift has been the suggestion that pluripotent ES-like cells may exist and indeed may persist into adulthood (*see* Fig. 2). These cells, while differing subtly from ES cells, may be functionally equivalent for therapeutic applications. The possible

existence of such adult cells with ES cell properties has fanned the debate and fueled a drive to assess the properties of all classes of pluripotent cells and to understand their underlying differences.

In *Human Embryonic Stem Cells* we invited leaders in the field to present their work in an unbiased way so that readers can assess the potential of stem cells and the current state of the science. The first section covers issues that regulate the use of human pluripotent cells. Chapters 1–3 begin with a summary of the ethical debate surrounding the derivation of human stem cells, and the current policies governing their use in the United States and abroad. The presidential announcement of August 2001 heralded a change in policy enabling federal support of research with hES cells that meet specific criteria. In Chapters 2 and 3, representatives from the National Institutes of Health (NIH) discuss the rules and conditions regulating federal funding, and issues of intellectual property regarding the use of hES cells. Chapter 2 delves into what constitutes "allowable" research and provides a guide to researchers interested in acquiring funding from US federal agencies such as the NIH for studies in this field.

Part II describes the types of human pluripotent cells that are currently being studied, their sources, methods of derivation, and maintenance. Many tissues are constantly renewed by the activities of resident, multipotent precursor or progenitor cells that have the ability to produce several different mature phenotypes. In the well-characterized hematopoietic system, T and B lymphocytes are derived from the lymphoid stem cell, whereas the myeloid stem cell can generate a host of red and white blood cells, including monocytes, eosinophils, platelets, and erythrocytes. However, both the myeloid and lymphoid stem cells are committed precursors, unable to differentiate along other pathways. There are only a few examples of truly pluripotent stem cells with the developmental capacity to generate cells representing all three germ layers (*see* Fig. 2) . Four types of such pluripotent stem cells are discussed in this section. In Chapter 4, Draper, Moore, and Andrews review the tumorigenic origins of embryonal carcinoma (EC) cells and their developmental counterparts, embryonic germ (EG) cells, present in the germinal ridges of young fetuses. Although there are many claims that pluripotent and highly plastic stem cells reside in adult tissues, the best characterized are those present in bone marrow. Cardozo and Verfaille summarize studies demonstrating their pluripotency in Chapter 5.

The high degree of interest in hES cells arise from two properties: their ability to self-renew essentially indefinitely and to be maintained

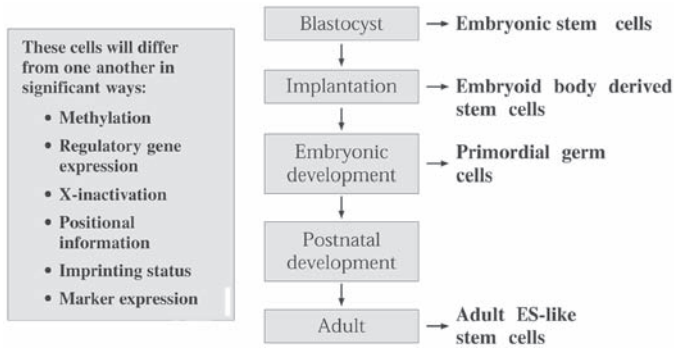

Fig. 2. Many different cell populations have been isolated that appear ES-like in their ability to contribute to chimeras after blastocyst injection, and to differentiate into ectoderm, endoderm, and mesoderm lineages in vitro. These distinct populations, although superficially similar, are likely to differ from each other when examined in more detail.

in an immature state, and their ability to differentiate into a wide range of mature tissues and cells. This enables the same population of cells to be studied under a variety of conditions; their properties, behavior, and fates can be reproduced and predicted. This capacity to standardize, predict, and reproduce results using a particular cell line will in turn greatly enhance the development of treatments and assays. For these reasons, much work is currently focused on methods for the expansion of hES cells and protocols to regulate their differentiation down selective lineages toward defined fates. Procedures for the growth, subcloning, and maintenance of hES cells are presented in Chapters 6 and 7 by three groups that are pioneers in this endeavor.

The following five chapters in Part III focus on specific methods that drive their differentiation into neuroepithelium, pancreatic islet cells, cardiomyocytes, vascular cells, and hematopoietic progenitors. Because much of the groundbreaking work was first conducted on murine ES cells, these initial animal studies are described and compared with the behavior of their human counterparts.

Part IV focuses on the potential uses of human stem cells in a variety of applications. In Chapter 13, Harley and Rao compare the advantages and disadvantages of using hES cells versus stem cells acquired from adult tissues for transplantation therapies. More complex applications to generate cells with the desired genetic composition include genetic manipulation of hES cells (Chapter 14) and somatic cell nuclear transfer (also called therapeutic cloning) (Chapter 15) to produce hES

cells with the patient's genetic composition for autologous transplants. In Chapter 16, Kamb and Rao discuss possible uses of human stem cells as tools for drug and gene discovery in vitro, and as therapeutic agents in vivo. The latter include cell, tissue, and organ replacement and regeneration, as well as the use of cells as peptide manufacturers and as delivery systems. They also explore what is needed to generate a donor cell that is universally accepted.

Most cell transplantation treatments will require the oversight of and approval by the Food and Drug Administration (FDA). In Chapter 17, Fink reviews the regulatory role of the FDA in ensuring the safety, purity, potency, and efficacy of new therapies involving human stem cells. Although the main interest in stem cell research resides in their great potential for cell replacement therapy to treat a long list of diseases that are currently incurable, there are at present no stem cell treatments in use, and only a rare few in clinical studies. In the last chapter, Reier and colleagues review preclinical and clinical studies conducted with neuron-like cells derived from one of the best-studied human EC cell lines, the NT-2 cells. This discussion introduces the complexities involved with in vivo studies, and the behavioral and functional analyses following cell transplantation.

Finally, we include a series of appendices that will provide additional information on useful websites, stem cell patents, and examples of Material Transfer Agreements to facilitate the sharing of cells. We hope that the readers will find the contents of *Human Embryonic Stem Cells* useful, and we welcome comments proposing additions or deletions to what we hope will become the standard reference book in the field of hES cell biology.

Arlene Y. Chiu
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A Researcher's Guide to Federally Funded Human Embryonic Stem Cell Research in the United States

Gregory J. Downing

1. INTRODUCTION

The issuance of the first grant awards for human embryonic stem (hES) cell research by the National Institutes of Health (NIH) in 2002 represents a milestone in this emerging field of biomedical investigation. Based on a policy announced on August 9, 2001 by President George W. Bush, scientists can now apply for and receive federal funds for research to use certain hES cell lines in their laboratory investigations. This chapter provides information often sought by researchers regarding federal policies, regulations, procedures, and opportunities for pursuing studies using hES cells. Although research that results in the creation or destruction of human embryos is prohibited from receiving federal support, there are no legal restrictions on performing these research activities with private sources of funding. In 1998, a research group led by Thomson published their privately funded studies on hES cells, which were obtained after removal of the inner cell mass from an early blastocyst *(1)*. Also in 1998, Gearhart and co-workers published their privately funded studies on human embryonic germ (hEG) cells, which were derived from the primordial ridge of early-gestation fetal tissue *(2)*. Even though hES and hEG cells are isolated from distinctly different stages in human development, both cell lines were found to have the capacity for infinite proliferation in an undifferentiated state and development into specialized cells with properties similar to those found in many different types of tissues. Both hES and hEG cells are described as "pluripotent," inferring that the undifferentiated cells are capable of developing into cells typically found in specialized tissues representing all three germ layers (the endoderm, mesoderm, and ectoderm) in the course of normal development of an

From: *Human Embryonic Stem Cells*
Edited by: A. Chiu and M. S. Rao © Humana Press Inc., Totowa, NJ

organism.[a] These discoveries sparked intense interest among the scientific community because of the enormous potential for basic research and development of cell-based therapies, and they led to the establishment of policy for the use of public resources for hES cell research. It is important to note that the presidential decision of August 9, 2001 dealt strictly with hES cell research and did not directly affect the existing guidelines pertaining to the use of federal funds for hEG cell research *(3)*. The main objective of this chapter is to discuss conditions whereby research with hES and hEG cells can be supported by the federal government, and it focuses primarily on the regulations that apply to obtaining funding from the NIH. Other important issues such as those involving patents and intellectual property are addressed in the next chapter.

2. CONDUCTING HUMAN EMBRYONIC STEM CELL RESEARCH IN THE UNITED STATES

Over the past 20 yr, research to develop and use murine *(4,5)* and nonhuman primate embryonic stem cells *(6)* was supported through public and private resources, and this contributed indirectly to fundamental advances in hES cell research. However, none of the innovative work leading to the derivation of hES and hEG stem cell lines to date has been supported with US federal funds; they were developed solely with private resources.[b] Publicly financed stem cell research is widely recognized as important because of the need for extensive basic research on the unique biological properties of hES cells such as studies to understand the regulation of genes involved in the control of proliferation and differentiation. In general, basic research is not the major target of the private sector, which is generally more focused on technology development and cellular engineering. An important point about the funding of hES research in the United States is that whereas there are specific research activities that cannot be carried out with the use of public funds, these same activities *can* be conducted with support from private resources, such as companies, foundations, or universities.[c] A second

[a]The term *pluripotent stem cell* has been used to describe cells that are derived from human embryos or from the primordial ridge in fetal tissues. The discussion in this chapter uses the term to refer solely to hES and hEG cells. However, recent scientific evidence suggests that some "adult-type" stem cells may also have pluripotent capabilities, but research activities using them are not subject to the policies, procedures, and guidances presented here.

[b]In this chapter, a distinction is made between federally and publicly sponsored research activities. In some cases, state government funding has been authorized to support research for the development of hES cell lines.

[c]Many states have laws and regulations regarding human embryos and fetal tissue and the use of them in research. Researchers are recommended to confer with institutional administrative officials regarding local and state requirements. A resource for current state embryonic and fetal research laws and pending legislation may be found at the National Conference of State Legislatures website (www.ncsl.org/programs/health/genetics/embfet).

important distinction is the difference in policies regulating the use of public funds for the development or use of hES cell lines versus those regulating hEG cells because the former are derived from human embryos and the latter are from fetal tissue. Investigators who perform research with US federal funding need to understand these key distinctions in order to remain in compliance with regulations and guidances.

2.1. Human Embryo Research

The existing policy governing the use of public resources for hES cell research in the United States is linked to legal interpretations of the federal prohibition of funding for investigations that use human embryos. Since 1993, NIH has been prohibited from supporting research that result in the creation or destruction of human embryos. This prohibition was recently reaffirmed in the congressional appropriations for NIH in fiscal year 2002 as Public Law 107-116 of the Departments of Labor, Health and Human Services, and Education, and Related Agencies Appropriations Act, 2002 Sec. 510. which states:

> (a) None of the funds made available in this Act may be used for (1) the creation of a human embryo or embryos for research purposes; or (2) research in which a human embryo or embryos are destroyed, discarded, or knowingly subjected to risk of injury or death greater than that allowed for research on fetuses in utero under 45 CFR 46.208(a)(2) and section 498(b) of the Public Health Service Act (42 U.S.C. 289g(b)).
>
> (b) For purposes of this section, the term "human embryo or embryos" includes any organism, not protected as a human subject under 45 CFR 46 as of the date of the enactment of this Act, that is derived by fertilization, parthenogenesis, cloning, or any other means from one or more human gametes or human diploid cells.

Clearly, federal funds may not be used for the derivation of hES cell lines, a process that requires the destruction of a human embryo. In 1999, a legal interpretation of the prohibition by the Department of Health and Human Services Office of General Council determined that research *using* hES cell lines did not constitute human embryo research as long as federal funds were not used for the actual derivation of the cells. This interpretation opened the door for the development of NIH guidelines, issued in August 2000, pertaining to the use of pluripotent stem cells in federally sponsored research *(3)*. In 2001, a year after these guidelines were issued, President George W. Bush announced a policy that, in effect, eliminated portions of these guidelines covering hES cell research. The next two sections detail current application of these guidelines.

2.2. Use of Human Embryonic Stem Cells in Research

President George W. Bush announced the current policy for federal funding of hES cell research on August 9, 2001. The new policy allowed federal-expenditures for research conducted *only* on cell lines developed prior to the presidential announcement. This means that funding for any new cell lines established by the destruction of an embryo(s) after August 9, 2001 is prohibited. On the other hand, federal funds may now be used for research on existing hES cell lines if (1) the derivation process (which commences with the removal of the inner cell mass from the blastocyst) had already been initiated prior to the announcement on August 9, 2001 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, President Bush's announcement identified four requirements that must be met regarding the acquisition of the embryo for derivation of the hES cells:

- The embryo was originally created for reproductive purposes.
- The embryo was no longer needed for reproductive purposes.
- Informed consent must have been obtained for the donation of the embryo.
- No financial inducements were provided for donation of the embryo.

In November 2001, an NIH Human Embryonic Stem Cell Registry (escr.nih.gov) was developed to facilitate hES cells research by providing investigators with the list of the hES cells that meet the eligibility criteria and with contact information so that they could acquire cells that are available. Each of the sources of the 78 cell lines currently described on the Registry provided NIH with documents and assurances that they were in compliance with these eligibility criteria. The next chapter provides an in-depth discussion of issues dealing with intellectual property, acquisition costs for cell lines, and material transfer agreements with the sources of cells. Table 1 summarizes a series of steps to guide investigators seeking NIH funding for research using human pluripotent cells.

2.3. Use of Human Embryonic Germ Cells in Research

The presidential decision of August 9, 2001 dealt strictly with hES cells. It did not change the rules for federal funding of hEG cell research; however, their use is still regulated by the NIH Guidelines for Research using Human Pluripotent Stem Cells *(3)*. Despite the fact that no prohibition or policy has ever prevented federal funding of hEG research, it is noteworthy that the first hEG cell lines *(2)* were not developed with the use of federal funds. The NIH guidelines, which apply to the use of hEG cells, are closely aligned with provisions governing the use of human fetal tissue in research,

Table 1
Applying for Federal Funding of Research Using Embryonic Stem Cells and Embryonic Germ Cells[a]

A. Human Embryonic Stem Cell Research
• Include statement in grant proposal indicating which cell lines from NIH Registry (escr.nih.gov) will be used in research activities—cite the NIH identifying code for the cell line(s) to be used in the proposal
• Indicate in the proposal that the research will not include prohibited activities
• Obtain cell lines from eligible sources using material transfer agreement
• Contact institutional officials regarding need for IRB review and approval—refer to OHRP guidance (ohrp.osophs.dhhs.gov/humansubjects/guidance/stemcell)
• Contact appropriate NIH extramural program officer with technical questions about research plan (grants1.nih.gov/grants/stem_cell_contacts)
B. Human Embryonic Germ Cell Research
• Include description of cell lines to be used in research protocol in the grant proposal
• Indicate in the proposal that the research will not include prohibited activities
• Provide documentation of compliance with criteria described in Section II.B.2 of the NIH Guidelines for Research Using Human Pluripotent Stem Cells (www.nih.gov/news/stemcell/stemcellguidelines)
• Submit supplemental application of research assurances to Human Pluripotent Stem Cell Review Group *(7)*
• Contact institutional officials regarding need for IRB review and approval—refer to OHRP guidance (ohrp.osophs.dhhs.gov/humansubjects/guidance/stemcell)
• Contact appropriate NIH extramural program officer with technical questions about research plan (grants1.nih.gov/grants/stem_cell_contacts)

[a]Research activities for basic, in vitro, or animal studies. Future clinical studies with stem cells may have additional submission and approval requirements by NIH and US Food and Drug Administration.

as listed below. The policies regulating hEG and hES cell research differ in five important points:

- Federal funding may be used in the derivation of the hEG cells from fetal tissue.
- Research may be conducted with federal funds for hEG cell lines established *after* August 9, 2001.
- Federal funding may be used only for those hEG cells that are in compliance with the NIH Guidelines for Research Using Pluripotent Stem Cells (Section II.B.2).
- The use of hEG cells in federally sponsored research requires the approval by

an NIH committee known as the Human Pluripotent Stem Cell Review Group (HPSCRG) as described in the NIH Guidelines *(3)*.
- Eligible hEG cells are not listed on the NIH Human Embryonic Stem Cell Registry.

Individuals planning to use hEG cells in research should also consider two important aspects of federal laws and regulations. The first pertains to the legal aspects of human subjects protections that are associated with acquiring human fetal tissue for use in research. Section 42 U.S. Code 289g-2 (www.nih.gov/news/stemcell/42-289g2) describes conditions, prohibitions, and penalties associated with the acquisition of human fetal tissue. Research conducted with human fetal tissue is also subject to state and local regulations, as specified in 45 Code of Federal Regulations 46.210 (www.nih.gov/news/stemcell/45).

If federal funding for conducting hEG cell research is sought, the application must comply with the NIH Guidelines for Research Using Pluripotent Stem Cells *(3)*. An NIH guide notice *(7)* has been issued that describes in detail procedures for submitting materials that must accompany a grant application proposing to use hEG cells. These procedures also apply to funded grantees who would like to include the use of hEG cells in their existing grant awards. The following eight criteria must be fulfilled and documentation submitted to NIH for review by the HPSCRG prior to final approval and award of funding by the NIH:

- An assurance, signed by the responsible institutional official, that the hEG cells were derived from human fetal tissue in accordance with the conditions set forth in Section II.B.2 of the NIH guidelines and that the institution will maintain documentation in support of the assurance.
- A sample informed consent document (with patient identifier information removed) and a description of the informed consent process that meet the criteria for informed consent described in the following paragraph.
- An abstract of the scientific protocol used to derive hEG cells from fetal tissue.
- Documentation of institutional review board (IRB) approval of the derivation protocol.
- An assurance that the hEG cells to be used in the research were or will be obtained through a donation or through a payment that does not exceed the reasonable costs associated with the transportation, processing, preservation, quality control, and storage of the stem cells.
- The title of the research proposal or specific subproject that proposes the use of hEG cells.
- An assurance that the proposed research using hEG cells is not a class of research that is ineligible for NIH funding as set forth in Section III of the NIH guidelines.
- The Principal Investigator's written consent to the disclosure of all material submitted to the NIH as necessary to carry out the public review and other oversight procedures set forth in the NIH guidelines.

There are aspects of the informed consent procedures that must be considered by investigators who have derived hEG cells from fetal tissue, regardless of whether this was carried out with federal funding. Informed consent documents should include the following information:

- A statement that fetal tissue will be used to derive stem cells for research that may include human transplantation research.
- A statement that the donation is made without any restriction or direction regarding the individual(s) who may be the recipient(s) of transplantation of cells derived from the fetal tissue.
- A statement as to whether or not information that could identify the donors of the fetal tissue, directly or through identifiers linked to the donors, will be removed prior to the derivation or the use of human pluripotent stem cells.
- A statement that derived cells and/or cell lines may be kept for many years.
- A disclosure about the possibility that the results of research on the stem cells may have commercial potential, and a statement that the donor will not receive financial or any other benefits from any such future commercial development.
- A statement that the research is not intended to provide direct medical benefit to the donor.

The above materials must be submitted to the HPSCRG by investigators seeking federal funds to use hEG cells in their research before approval for funding can be granted *(7)*. If a research proposal has been determined to merit federal funding, the assurance documents will be reviewed by HPSCRG in a public meeting for compliance with the NIH guidelines. For investigators proposing to use hEG cells that have received prior HPSCRG approval, there is an expedited review and approval process that does not require discussion at a public meeting. Under these circumstances, researchers should provide the HPSCRG with a copy of the approval letter from the source of the cells, a letter specifying their request, and accompanying documents. Researchers planning to use hEG cells in their investigations should contact and consult with the appropriate program officials at NIH before submitting their grant applications and assurance documents.

3. RESEARCH ACTIVITIES THAT CANNOT BE SUPPORTED WITH US FEDERAL FUNDS

The current policy on federally funded research also identifies research activities that are not eligible for support. These prohibited activities, first described in the 2000 NIH guidelines *(6)* (Section III, Areas of Research Involving Human Pluripotent Stem Cells that are Ineligible for NIH Funding), were not affected by the president's August 2001 policy decision and include the following:

Table 2
Types of Stem Cell Research Activities That Are Not Subject to Special Requirements for Federally Funded Research

• Studies proposing to use animal sources of adult stem cell, embryonic stem cell, or embryonic germ cell lines
• Basic (nonclinical) research studies involving human adult stem cells (such as hematopoietic stem cells)
• Basic research (nonclinical) using nonpluripotent stem cells derived from human fetal tissue (e.g., stem cells found in tissues other than primordial ridge, such as hematopoietic and neuronal stem cells)

- The derivation of pluripotent stem cells from human embryos
- Research in which human pluripotent stem cells are utilized to create or contribute to a human embryo
- Research utilizing pluripotent stem cells that were derived from human embryos created for research purposes, rather than for fertility treatment
- Research in which human pluripotent stem cells are derived using somatic cell nuclear transfer (i.e., the transfer of a human somatic cell nucleus into a human or animal egg)
- Research utilizing human pluripotent stem cells that were derived using somatic cell nuclear transfer (i.e., the transfer of a human somatic cell nucleus into a human or animal egg)
- Research in which human pluripotent stem cells are combined with an animal embryo
- Research in which human pluripotent stem cells are used in combination with somatic cell nuclear transfer for the purposes of reproductive cloning of a human

Investigators who are conducting federally sponsored research and have questions about eligible research activities should consult with their NIH program officer.

4. FEDERAL REQUIREMENTS FOR REVIEW AND APPROVAL BY INSTITUTIONAL REVIEW BOARD OF BASIC RESEARCH INVOLVING USE OF hES AND hEG CELLS

Investigators and institutional officials should be aware of the requirements for IRB assessment and approval of research that proposes to use hES and hEG cells. Recognizing that the use of these cells in human subjects in clinical trials may be years away, researchers are sometimes uncertain whether IRB review is required for their research. For basic research applications that only involve in vitro studies or studies in animals, the human subjects concerns focus on protections for the donor(s) of the embryos or fetal tissue that served as the source of the cell lines.

Recently, the Office of Human Research Protections (OHRP) developed guidelines for researchers and research institutions with information about circumstances that require IRB action for basic hES and hEG cell research applications (Guidance for Investigators and Institutional Review Boards Regarding Research Involving Human Embryonic Stem Cells, Germ Cells and Related Test Articles [ohrp.osophs.dhhs.gov/humansubjects/guidance/stemcell.pdf]). Under circumstances described later in this section, research supported by the Department of Health and Human Services (HHS) may be subject to HHS human subjects protection regulations described in Title 45 Code of Federal Regulations (CFR) Part 46 "Protection of Human Subjects," including Subpart B, 45 CFR 46.206.

Of concern is the possibility that identifying information (i.e., information that could reveal the identities of the donors of the embryos or fetal tissue), might become available to the researchers who work with cell lines derived from these sources. Some cell lines have been developed "anonymously," without any information that could link the donors to the cells *(1)*. In the case of other cell lines, donor information has been retained, either by the source of the embryos or by the laboratories where the cell lines were originally developed. If federally sponsored research is conducted with cells where identifying information could be made available to investigators, such research is governed by 45 CFR Part 46 because the donors are considered human subjects, and, therefore, IRB review and approval is required. However, 45 CFR Part 46 does not apply if (1) the investigator and the research institution do not have access to the identifiable private information related to the cell line and (2) a written agreement is obtained from the holder of the information linking the donor to the cell line, indicating that such information will not be released to the investigator under any circumstances. If these conditions are met, an institution or an IRB can decide to waive IRB review of the research using the cell line.

Researchers can determine whether a cell line has identifying information linked to it in a number of ways. In many cases, the material transfer agreements between investigators and the sources of the hES and hEG cells will delineate the anonymity of sources or specify the limitations of access by the researcher to identifying information about the donor(s). In addition, researchers should recognize that their institutions and IRBs might require review and approval of basic research use of these cells even under circumstances that are not required by the OHRP guidance. Therefore, investigators are encouraged to contact their institutional research administrative officials to assure compliance with their policies in addition to federal regulations.

As investigators begin to contemplate obtaining federal support for clinical research (i.e., studies conducted on human subjects) using hES and hEG

cells, the OHRP guidance provides additional information about other regulations that apply. Although beyond the scope of this discussion, researchers should be aware that in addition to the OHRP guidance for stem cell research, use of these cells in *clinical* research is also subject to the US Food and Drug Administration IRB and informed consent regulations (Title 21 CFR Parts 50 and 56).

5. FEDERAL FUNDING OPPORTUNITIES FOR STEM CELL RESEARCH

Opportunities for NIH funding of research with hES and hEG cells can be found at a central NIH website with information on this subject (www.nih.gov/news/stemcell/index) as well as related topics such as information about the acquisition of cells, contact information for NIH extramural program staff, and frequently asked questions. The NIH also posts requests for applications (RFAs) and program announcements (PAs) soliciting research applications on specific aspects of stem cell research; these are updated weekly in the NIH Guide for Grants and Contracts (grants.nih.gov/grants/guide/index). Finally, the NIH provides administrative supplements for investigators with existing grants to enable them to acquire the skills and cells for initiating research in this field.

Some investigators have asked under what circumstances can they conduct research in their NIH-funded laboratories on cell lines that do not fulfill all of the federal criteria, such as hES cells that are not listed on the NIH Registry. They may have private resources from industry or foundations to conduct research that is prohibited with federal funds. In laboratories receiving both federal and nonfederal sources of funding, investigators and their staff must segregate allowable and unallowable activities so that the costs incurred by each type of research is charged to the appropriate funding source. For instance, the time and effort of laboratory personnel working on ineligible stem cells may not be charged to any federal grant. Acquisition of equipment, use of cell and tissue culture supplies in the project, and travel to conferences to discuss or present work on prohibited activities likewise may not be supported with federal funds. Finally, it is the institution's responsibility to provide clear instructions to investigators who conduct research that is "unallowable" under federal research funding policy.

6. CONCLUSIONS

Research using hES and hEG cells is now underway in the United States with both public and private support. The NIH is supporting stem cell research by providing extensive information to the research community on

the acquisition of stem cells, guidelines and legal requirements, funding opportunities, and technical assistance about the grant application process. A high priority for the NIH has been the development of the science infrastructure to facilitate investigator-initiated basic research studies. This infrastructure includes enhancing the distribution network for cell lines, improving their characterization and quality assurance measures, supporting laboratory training programs, workshops, and conferences, and establishing career development pathways to ensure that a highly skilled scientific workforce is in place. Investigators who apply for federal funding in the United States are encouraged to learn about the framework for conducting hES and hEG cell research. Once fully informed, researchers will find that there are minimal burdens and many opportunities for successful research programs using these unique cells.

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