Embryonic Stem Cell Lines Derived from Human Blastocysts
James A. Thomson, et al.
Science 282, 1145 (1998);
DOI: 10.1126/science.282.5391.1145

The following resources related to this article are available online at www.sciencemag.org (this information is current as of October 23, 2009):

Updated information and services, including high-resolution figures, can be found in the online version of this article at:
http://www.sciencemag.org/cgi/content/full/282/5391/1145

This article cites 21 articles, 9 of which can be accessed for free:
http://www.sciencemag.org/cgi/content/full/282/5391/1145#otherarticles

This article has been cited by 2128 article(s) on the ISI Web of Science.

This article has been cited by 84 articles hosted by HighWire Press; see:
http://www.sciencemag.org/cgi/content/full/282/5391/1145#otherarticles

This article appears in the following subject collections:
Development
http://www.sciencemag.org/cgi/collection/development

Information about obtaining reprints of this article or about obtaining permission to reproduce this article in whole or in part can be found at:
http://www.sciencemag.org/about/permissions.dtl
Embryonic Stem Cell Lines Derived from Human Blastocysts

James A. Thomson,* Joseph Itskovitz-Eldor, Sander S. Shapiro, Michelle A. Waknitz, Jennifer J. Swiergiel, Vivienne S. Marshall, Jeffrey M. Jones

Human blastocyst-derived, pluripotent cell lines are described that have normal karyotypes, express high levels of telomerase activity, and express cell surface markers that characterize primate embryonic stem cells but do not characterize other early lineages. After undifferentiated proliferation in vitro for 4 to 5 months, these cells still maintained the developmental potential to form trophectoderm and derivatives of all three embryonic germ layers, including gut epithelium (endoderm); cartilage, bone, smooth muscle, and striated muscle (mesoderm); and neural epithelium, embryonic ganglia, and stratified squamous epithelium (ectoderm). These cell lines should be useful in human developmental biology, drug discovery, and transplantation medicine.

Embryonic stem (ES) cells are derived from totipotent cells of the early mammalian embryo and are capable of unlimited, undifferentiated proliferation in vitro (1, 2). In chimeras with intact embryos, mouse ES cells contribute to a wide range of adult tissues, including germ cells, providing a powerful approach for introducing specific genetic changes into the mouse germ line (3). The term “ES cell” was introduced to distinguish these embryo-derived pluripotent cells from teratocarcinoma-derived pluripotent embryonal carcinoma (EC) cells (2). Given the historical introduction of the term “ES cell” and the properties of mouse ES cells, we proposed that the essential characteristics of primate ES cells should include (i) derivation from the preimplantation or periimplantation embryo, (ii) prolonged undifferentiated proliferation, and (iii) stable developmental potential to form derivatives of all three embryonic germ layers even after prolonged culture (4). For ethical and practical reasons, in many primate species, including humans, the ability of ES cells to contribute to the germ line in chimeras is not a testable property. Nonhuman primate ES cell lines provide an accurate in vitro model for understanding the differentiation of human tissues (4, 5). We now describe human cell lines that fulfill our proposed criteria to define primate ES cells.

Fresh or frozen cleavage stage human embryos, produced by in vitro fertilization (IVF) for clinical purposes, were donated by individuals after informed consent and after institutional review board approval. Embryos were cultured to the blastocyst stage, 14 inner cell masses were isolated, and five ES cell lines originating from five separate embryos were derived, essentially as described for nonhuman primate ES cells (5, 6). The resulting cells had a high ratio of nucleus to cytoplasm, prominent nucleoli, and a colony morphology similar to that of rhesus monkey ES cells (Fig. 1). Three cell lines (H1, H113, and H114) had a normal XY karyotype, and two cell lines (H7 and H9) had a normal XX karyotype. Each of the cell lines was successfully cryopreserved and thawed. Four of the cell lines were cryopreserved after 5 to 6 months of continuous undifferentiated proliferation. The other cell line, H9, retained a normal XX karyotype after 6 months of culture and has now been passaged continuously for more than 8 months (32 passages). A period of replicative crisis was not observed for any of the cell lines.

The human ES cell lines expressed high levels of telomerase activity (Fig. 2). Telomerase is a ribonucleoprotein that adds telomere repeats to chromosome ends and is involved in maintaining telomere length, which plays an important role in replicative life-span (7, 8). Telomerase expression is highly correlated with immortality in human cell lines, and reintroduction of telomerase activity into some diploid human somatic cell lines extends replicative life-span (9). Diploid human somatic cells do not express telomerase, have shortened telomeres with age, and enter replicative senescence after a finite proliferative life-span in tissue culture (10–13). In contrast, telomerase is present at high levels in germ line and embryonic tissues (14). The high level of telomerase activity expressed by the human ES cell lines therefore suggests that their replicative life-span will exceed that of somatic cells.

The human ES cell lines expressed cell surface markers that characterize undifferentiated nonhuman primate ES cells and human EC cells, including stage-specific embryonic antigen (SSEA)-3, SSEA-4, TRA-1-60, TRA-1-81, and alkaline phosphatase (Fig. 3) (4, 5, 15, 16). The globo-series glycolipid GL7, which carries the SSEA-4 epitope, is formed by the addition of sialic acid to the globo-series glycolipid Gb5, which carries the SSEA-3 epitope (17, 18). Thus, GL7 reacts with antibodies to both SSEA-3 and SSEA-4 (17, 18). Staining intensity for SSEA-4 on the human ES cell lines was consistently strong, but staining intensity for SSEA-3 was weak and varied both within and among colonies (Fig. 3, D and C). Because GL7 carries both the SSEA-4 and SSEA-3 epitopes and because staining for SSEA-4 was consistently strong, the relatively weak staining for

*To whom correspondence should be addressed.

J. A. Thomson, M. A. Waknitz, J. J. Swiergiel, V. S. Marshall, Wisconsin Regional Primate Research Center, University of Wisconsin, Madison, WI 53715, USA. J. Itskovitz-Eldor, Department of Obstetrics and Gynecology, Rambam Medical Center, Faculty of Medicine, Technion, Haifa 31096, Israel. S. S. Shapiro and J. M. Jones, Department of Obstetrics and Gynecology, University of Wisconsin, Madison, WI 53715, USA.

Fig. 1. Derivation of the H9 cell line. (A) Inner cell mass–derived cells attached to mouse embryonic fibroblast feeder layer after 8 days of culture, 24 hours before first dissociation. Scale bar, 100 μm. (B) H9 colony. Scale bar, 100 μm. (C) H9 cells. Scale bar, 50 μm. (D) Differentiated H9 cells, cultured for 5 days in the absence of mouse embryonic fibroblasts, but in the presence of human LIF (20 ng/mL; Sigma). Scale bar, 100 μm.
H9 cells were allowed to differentiate for 2 even in the presence of fibroblasts. After ES cell lines differentiated spontaneously allowed to pile up in the culture dish, the (Fig. 1). When grown to confluence and human leukemia inhibitory factor (LIF) ers, both in the presence and absence of of mouse embryonic fibroblast feeder lay- (endoderm); and neural epithelium, embryonic smooth muscle, and striated muscle (meso- (endoderm and trophoblast differentiation conditioned culture medium, indicating (hCG, 46.7 mIU/ml) and human chorionic gonadotropin (350.9 ± 14.2 IU/ml) and human chorionic gonadotropin (hCG, 46.7 ± 5.6 mIU/ml) were detected in conditioned culture medium, indicating endoderm and trophoblast differentiation (20).

Human ES cells should offer insights into developmental events that cannot be studied directly in the intact human embryo but that have important consequences in clinical areas, including birth defects, infertility, and pregnancy loss. Particularly in the early postimplantation period, knowledge of normal human development is largely restricted to the description of a limited number of sectioned embryos and to analyses drawn from the experimental embryology of other species (21). Although the mouse is the mainstay of experimental mammalian embryology, early structures including the placenta, extraembryonic membranes, and the egg cylinder all differ substantially from the corresponding structure of the human embryo. Human ES cells will be particularly valuable for the study of the development and function of tissues that differ between mice and humans. Screens based on the in vitro differentiation of human ES cells to specific lineages could identify gene targets for new drugs, genes that could be used for tissue regeneration therapies, and teratogenic or toxic compounds.

Elucidating the mechanisms that control differentiation will facilitate the efficient, directed differentiation of ES cells to specific cell types. The standardized production of large, purified populations of euploid human cells such as cardiomyocytes and neurons will provide a potentially limitless source of cells for drug discovery and
transplantation therapies. Many diseases, such as Parkinson’s disease and juvenile-onset diabetes mellitus, result from the death or dysfunction of just one or a few cell types. The replacement of those cells could offer lifelong treatment. Strategies to prevent immune rejection of the transplanted cells need to be developed but could include banking ES cells with defined major histocompatibility complex backgrounds or genetically manipulating ES cells to reduce or actively combat immune rejection. Because of the similarities to humans and human ES cells, rhesus monkeys and rhesus ES cells provide an accurate model for developing strategies to prevent immune rejection of transplanted cells and for demonstrating the safety and efficacy of ES cell–based therapies. Substantial advances in basic developmental biology are required to direct ES cells efficiently to transplantation of just one or a few cell types. The replacement of those cells could offer lifelong treatment. Strategies to prevent immune rejection of the transplanted cells need to be developed but could include banking ES cells with defined major histocompatibility complex backgrounds or genetically manipulating ES cells to reduce or actively combat immune rejection. Because of the similarities to humans and human ES cells, rhesus monkeys and rhesus ES cells provide an accurate model for developing strategies to prevent immune rejection of transplanted cells and for demonstrating the safety and efficacy of ES cell–based therapies. Substantial advances in basic developmental biology are required to direct ES cells efficiently to transplantation of just one or a few cell types. The replacement of those cells could offer lifelong treatment.