Embryonic Stem Cell Lines Derived from Human Blastocysts

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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 pre-implantation or peri-implantation 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 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

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SSEA-3 suggests a restricted access of the antibody to the SSEA-3 epitope. In common with human EC cells, the undifferentiated human ES cell lines did not stain for SSEA-1, but differentiated cells stained strongly for SSEA-1 (13) (Fig. 3). Mouse inner cell mass cells, ES cells, and EC cells express SSEA-1 but do not express SSEA-3 or SSEA-4 (17, 19), suggesting basic species differences between early mouse and human development.

The human ES cell lines were derived by the selection and expansion of individual colonies of a uniform, undifferentiated morphology, but none of the ES cell lines was derived by the clonal expansion of a single cell. The uniform undifferentiated morphology that is shared by human ES and nonhuman primate ES cells and the consistent expression by the human ES cell lines of cell surface markers that uniquely characterize primate ES and human EC cells make it extremely unlikely that a mixed population of precursor cells was expanded. However, because the cell lines were not cloned from a single cell, we cannot rule out the possibility that there is some variation in developmental potential among the undifferentiated cells, in spite of their homogeneous appearance.

The human ES cell lines maintained the potential to form derivatives of all three embryonic germ layers. All five cell lines produced teratomas after injection into severe combined immunodeficient (SCID)–beige mice. Each injected mouse formed a teratoma, and all teratomas included gut epithelium (endoderm); cartilage, bone, smooth muscle, and striated muscle (mesoderm); and neural epithelium, embryonic ganglia, and stratified squamous epithelium (ectoderm) (Fig. 4). In vitro, the ES cells differentiated when cultured in the absence of mouse embryonic fibroblast feeder layers, both in the presence and absence of human leukemia inhibitory factor (LIF) (Fig. 1). When grown to confluence and allowed to pile up in the culture dish, the ES cell lines differentiated spontaneously even in the presence of fibroblasts. After H9 cells were allowed to differentiate for 2 weeks, both α-fetoprotein (350.9 ± 14.2 IU/ml) and human chorionic gonadotropin (hCG, 46.7 ± 5.6 μIU/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 analogies drawn from the experimental embryology of other species (21). Although the mouse is the mainstay of experimental mammalian embryology, early structures including the placenta, extrabronchiomembranes, 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 clinical use.
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 modifying 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 even more closely to the prevention and treatment of human disease.

Fig. 4. Teratomas formed by the human ES cell lines in SCID-beige mice. Human ES cells after 4 to 5 months of culture (passages 14 to 16) from about 50% confluent six-well plates were injected into the rear leg muscles of 4-week-old male SCID-beige mice (two or more mice per cell line). Seven to eight weeks after injection, the resulting teratomas were examined histologically. (A) Gutlike structures. Cell line H9. Scale bar, 400 μm. (B) Rosettes of neural epithelium. Cell line H14. Scale bar, 200 μm. (C) Bone. Cell line H14. Scale bar, 100 μm. (D) Cartilage. Cell line H9. Scale bar, 100 μm. (E) Striated muscle. Cell line H13. Scale bar, 25 μm. (F) Tubules interspersed with structures resembling fetal glomeruli. Cell line H9. Scale bar, 25 μm.