Embryonic Stem Cells

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Key points

- Introduction
- What is Embryonic Stem Cell
- What is Murine ES cell
- What is Human ES cell

It is 20 years since murine embryonic stem (ES) cells were first reported when they were segregated and grown in vitro. When assimilated into chimeras with intact mouse embryos at the morula or blastocyst stage, ES cells were found to contribute to a variety of lineages including the germ line. In vitro studies have shown that murine ES cells can be propagated indefinitely in an undifferentiated state but have the capacity, when provided with the definite signals, to differentiate, presumably via the formation of precursor cells, to almost all mature cell phenotypes. This discovery was appreciated, of course, as a breakthrough for developmental biologists, as it provided a model system to study cellular differentiation and to manipulate the genome. However, it also held the promise of something else. If the pluripotent differentiation potential of murine ES cells could be harnessed in vitro, it might be a means of deriving cell or tissue types virtually to order. If human ES cells responded in a similar trend, this would provide a radical new approach to the treatment of a wide variety of conditions where age, disease or trauma has led to tissue damage or dysfunction. Three years ago, the derivation of human ES cells made this a real possibility. This article reviews what is known about ES cells, with particular emphasis being placed on their potential use in the field of regenerative medicine. 1,2

Embryonic stem cells

For many years, the trials encountered in accessing peri-implantation embryos limited study of the cellular interactions that occur during the initial stages of mammalian development. Therefore, in-vitro techniques were developed to circumvent this problem. Pluripotent embryonic stem (ES) cells, like embryonal carcinoma cells before them, were first used as a vehicle to examine in detail early differentiation. However, the properties of ES cells identified them as being highly suitable for the generation in vitro of specific cell lineages. ES cells are harvested from the inner cell mass of the preimplantation blastocyst and have been derived from rodents, primates, and human beings.3 Murine ES cells remain undifferentiated when grown in the presence of leukaemia inhibitory factor (LIF) and, for some lines, culture on murine embryonic fibroblasts (MEFs). LIF does not have the same effect on human ES cells and, to maintain them in an undifferentiated state, these require culture on MEF feeder layers in the presence of basic fibroblast growth factor (bFGF), or on Matrigel or laminin in MEF-conditioned medium. When LIF or feeder cells are withdrawn, most types of ES cells differentiate spontaneously to form aggregates known, in view of their similarity to post-implantation embryonic tissues, as embryoid bodies. These spherical structures are comprised of derivatives of all three germ layers. Synchronous formation of embryoid bodies, a prerequisite for gene expression studies of differentiating ES cells, can be achieved by removal of the feeder cell layer or LIF followed by suspension culture (hanging drop method) or by cultivation in methyl cellulose containing medium.4

Murine ES cells

Differentiation potential Continued in vitro culture of murine ES cells as embryoid bodies leads to the formation of a range of differentiated cell types including cardiomyocytes, hematopoietic cells, endothelial cells, nerves, skeletal muscle, chondrocytes, adipocytes, liver, and pancreatic islets7. In each case, despite the use of, for example, growth factors favoring the differentiation of a particular cell type, the resulting cultures were heterogeneous. Our own studies have shown that it is feasible to procure cultures from murine ES cells that are enriched for osteoblasts or lung epithelium. Initially, osteoblasts were generated from embryoid bodies which were dispersed 5 days after removal of LIF and grown in a culture medium designed for the maintenance and growth of explanted osteoblasts.8 Further experimentation revealed that not only does the biochemical composition of the medium promote the

differentiation to an osteoblast phenotype, but also that the time at which a particular stimulus is administered can enhance the effect. Thus, by providing the cells with dexamethasone 14 days following the dispersal of embryoid bodies, it was possible to increase the yield of osteoblasts seven-fold. Using a similar approach, we have also been able to derive type II pneumocytes, identified by their expression of surfactant protein C and the presence of lamellar bodies, from murine ES cells. 9,10

Cell selection

As none of the approaches used on murine ES cells has yet been shown to give 100% yield of cells with the required phenotype, strategies to further purify the populations are required. Methods such as FACS (fluorescence activated cell sorting) or MACS (magnetic-activated cell sorting) allow such purification but are dependent on the cell type of interest expressing a surface marker that can be recognized by a fluorescence or magnetic microbead tagged antibody. To be fully constructive, the marker needs to be distinct to the desired cell lineage. Where either a specific cell surface marker or satisfactory antibodies are not available, ES cells can be transduced with a lineage-specific transgene with a marker, for example green fluorescent protein. Alternatively, the insert could carry one of several resistance genes that allow for preferential selection of cell subpopulations that should be restricted to the lineage of interest. This type of approach has been used to select neural and cardiomyocyte phenotypes. In the latter example, the selected cardiomyocytes were subsequently implanted into the damaged hearts of mice and shown to form stable grafts. 6

Human ES cells

To derive human ES cells, cleavage-stage embryos are grown into blastocysts and the inner cell mass is withdrawn. The embryos from which the initial human ES cell lines were derived were produced by in vitro fertilization and donated with the informed consent of the parents. The use of such 'spare' embryos is widely held to be acceptable; they are created for reproductive purposes for which they are no longer required, and it is ethically superior to use them for medical research rather than destroying them or storing them indefinitely. Another approach to deriving human embryonic stem cells is somatic nuclear transfer or cloning. This procedure uses the transfer of a somatic cell nucleus from an individual into an enucleated oocyte. The cells are then allowed to undergo embryonic development to the blastocyst stage prior to the isolation from the inner cell mass of ES cells that would be genetically matched to the tissues of the donor of the nucleus. Although there has been a recent report of the cloning of a human embryo, not everyone agrees with this claim. The main objection is that the authors only took the embryos to the six-cell stage, whereas an egg contains RNA and protein, made during the egg's formation in the ovary, which can support development up to the eight-cell stage without any signals from the transferred nuclear DNA. In addition, not only is this technique notoriously inefficient, but also the creation of a human embryo in this way raises significant ethical issues 6,8. The group that claimed to clone human embryos has also recently reported that they have derived stem cells parthenogenetically from non-human primates. They suggest that their approach provides an alternative to somatic cell nuclear transfer to generate autologous human embryonic stem cells.12 Human ES cells show several morphological and behavioural differences from murine ES cells; they grow more steadily and tend to form flat rather than spherical colonies. Several lines of human ES cells have been produced, including some that were clonally derived. These clonal lines were found to have all the characteristics of the parent cell line, including rapid growth rate, normal karyotype, and pluripotency, thereby establishing that the multilineage potential of ES cells occurs at the single-cell level. There are a rapidly increasing number of reports describing their differentiation. Examination of differentiating human ES cell cultures reveals cells with the distinct morphology of neurons, epithelium, and beating cardiomyocytes. In addition, spontaneous in vitro formation of functionally active pancreatic b cells and cardiomyocytes has been observed. As with murine ES cells, modification of the culture medium in which human ES cells are grown can encourage the differentiation of certain lineages. For example, enriched populations of proliferating neural progenitors have been obtained by supplementation of the culture medium with specific growth factors. Providing specific local influences, through co-culture with mature cells, can also encourage the formation of a particular lineage. For example, ES cells grown with bone marrow cells or yolk sac endothelium form hematopoietic precursors.11,12.

Conclusions

The potential use of human ES cells to create cell populations, tissues or organs for implantation stands to revolutionize medicine by providing unlimited material to order, that is, using therapeutic nuclear transfer or other methods, totally compatible with the patient's own tissues. In addition, the cells may be used as an in vitro system for not only study of the differentiation of specific cell types, but also evaluation of the effects of new drugs and the identification of genes as potential therapeutic targets. However, it is early days, and many hurdles must be cleared before the research reaches a point where clinical trials can begin.5

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