Special Article

Potential Applications of Human Embryonic Stem Cells

Supat Sinawat MD, MSc, FRTCOG, FICS*

** Reproductive Biology Unit, Department of Obstetrics and Gynecology, Faculty of Medicine, Khon Kaen University, Khon Kaen*

Advances in reproductive technologies provided opportunity for scientists to be able to grow human embryos in vitro for more than two decades. Skills and knowledge derived from in vitro fertilization and in vitro culture of mammalian embryos opened the chance for scientists to develop the strategies to derive embryonic stem cell lines from mammalian and human embryos. This achievement has initiated a new era in the fields of biotechnology, pharmacology, basic scientific research, and cell-based medicine. To date, scientists have made some progress in optimizing regimens in deriving ES cell lines from human embryos but much more research and development are still required especially in the aspect of directing stem cells into the specific cells of potential clinical use. Collaboration among clinicians and scientists from diverse fields, together with the public awareness of how useful this technology could be to modern medicine, will result in the accumulation of knowledge in this field and, in the near future, a progress in cell-based therapy.

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Stem cell is a cell that has the ability to divide (self-replicate) for indefinite periods. Under the right conditions, or given the right signals, a stem cell can give rise (differentiate) to many different cell types that make up the organism. Stem cells, therefore, have the potential to develop into mature cells that have characteristics, shapes, and specialized functions, such as muscle cells, neurons or skin cells.

A pluripotent stem cell is an undifferentiated cell that has the potential to develop into virtually any cell types in the body. Pluripotent stem cells are transiently present during embryogenesis, in pre-implantation embryos and fetal gonads. They can also be maintained as established cell lines, derived from either pre-implantation embryos, primordial germ cells, or germ cell tumors.

Embryonic stem cells

Embryonic stem (ES) cell lines are certain types of pluripotent stem cell lines that have been derived by the isolation and propagation of inner cell mass (ICM) cells of blastocyst stage embryos. These unique cell lines can develop into a wide range of cell types *in vitro* and *in vivo*. In addition, they are immortal. They can be grown continuously in culture without losing their properties or their wide development potential. These two features, pluripotency and unlimited self-renewal, have made ES cells extremely interesting and important to basic and applied research, especially to cell-based therapy and the study of early embryonic development.

The derivation of ES cell lines in mammals was first demonstrated in mice[1,2] in which basic methods for their isolation, propagation, and genetic manipulation were established. The accumulated experience in the mouse has allowed scientists to better define the properties of ES cells, that:

* derive from ICM/epiblast of blastocysts,

* are capable of undergoing unlimited number of symmetrical cell divisions without differentiating

* maintain a normal karyotype,

* can give rise to differentiated cells of ectoderm, mesoderm, and endoderm origin *in vitro* and *in vivo* within teratoma/teratocarcinoma tumors following engraftment into immunodeficient mice,

* can colonize all fetal tissues, including the

Correspondence to : Sinawat S, Reproductive Biology Unit, Department of Obstetrics and Gynecology, Faculty of Medicine, Khon Kaen University, Khon Kaen 40002, Thailand.

germ line, during embryonic development following their injection into host blastocysts,

* are clonogenic; each single cell can give rise to many other genetically identical cells that share the same properties and potentials as the original,

* specifically express the transcription factor Oct4, a regulatory molecule characteristic of pluripotential cells at different developmental stages.

Based on the accumulated experience both with mouse ES cells and with human embryonal carcinoma (EC) cells[3,4], which are pluripotent and resemble ES cells in many aspects, ES cell lines were successfully derived from non-human primates (common marmoset and rhesus monkeys)[5,6]. These studies have set the stage for the derivation of human ES cells in human, first by Thomson et al[7], Reubinoff et al[8], and later by other groups[9-11]. The described cell lines were derived from ICM cells of normal surplus blastocysts donated by couples undergoing in vitro fertilization (IVF). Some regulatory policies allow the use for research of embryos generated for reproductive purposes but lacking parental project, including the derivation of ES cell lines. Many reports have been published on the availability of embryos for research and different patient's attitudes have been described[12-15]. Ethics committees from scientific societies have made recommendations on this topic and the European Society for Human Reproduction and Embryology (ESHRE) Task Force on Ethics and Law and the Ethics Committee of the American Society for Reproductive Medicine have established guidelines for the donation of oocytes and embryos for research, including stem cell derivation[16,17].

The human ES cells proliferate for extended periods *in vitro*, maintain a normal karyotype, differentiate spontaneously into somatic cell lineages of all three primary germ layers, and form teratomas when injected into immunodeficient mice. Moreover, they express a panel of markers which are typical to non-human primate ES cells as well as to other types of human pluripotent stem cell lines (embryonic carcinoma (EC) cells and embryonic germ (EG) cells)[18].

Derivation method for embryonic stem cells is today highly empirical, and various protocols are used in the different steps of the process, including feeder cell preparation (if feeder cells are used), inner cell mass isolation, and initial steps of derivation. Nowadays derivation of human embryonic stem cells have been made possible in feeder-[19,20] and animal-free[21]conditions as well as in chemically defined culture conditions[22,23].

As human ES cell research advances, scientists and clinicians now better appreciate the far-reaching potential of these cells. It is, thus, not surprising that many of the IVF clinics worldwide are now aiming to set the required system and skills for the establishment of new ES cell lines from human embryos.

Potential applications of embryonic stem cells

1. Cell source for transplantation

Since human ES cells can be grown indefinitely in culture without losing their basic characteristics, and have the potential to develop into practically any cell types *in vitro*, they may be used as an unlimited cell source for cell transplantation. Once efficient protocols for induced differentiation are established, it will be possible to generate specific cell types in large numbers for the repair of degenerating or damaged tissues in humans. This will reduce the current supply problems of tissues available for transplantation. Indeed, it has been demonstrated in mice, and to certain extent in humans, that ES cell-derived progeny can proliferate and integrate, following their transplantation into adult animals[24-29]. Moreover, in the mouse ES cell system, transplanted progeny were shown to be functional and could improve behavioral deficits in animal models of diseases. Mouse ES cell derived cardiomyocytes were able to form stable functioning intracardiac grafts[30], and glial precursor derivatives formed myelinating transplants in the brain and spinal cords of myelin deficient rats[31]. In addition, insulin-secreting cells derived from ES cells normalized glycemia in streptozotocin-induced diabetic mice[32] and, in addition, transplanted functional dopaminergic neurons corrected motor asymmetry following transplantation into the animal model of Parkinson's disease[33].

While these results are promising, many more experiments are required to test the functionality and safety of human ES cell differentiated derivatives in animal models before they can be considered appropriate for clinical use. In addition, there will be a need to overcome the difficulty of graft rejection as a result of the immune response[34]. There are several possibilities that can be applied for minimizing graft rejection of ES cell derivatives. One possibility is to establish a bank that will include a large number of ES cell lines that differ in their major histocompatibility complex (MHC) expressed molecules, thus allowing major histocompatibility complex matching between the donor cell line and the recipient. Alternatively, it may be possible to generate a "universal" donor cell line by "knocking out" the genes that are responsible for graft rejection.

Finally, it might be feasible in the future to generate genetically identical nuclear transfer-derived ES cell lines, to provide the patients with autologous grafts.

2. Cell-based delivery system

ES cell-derived progenitor cells may be used as delivery vehicles for the regulated release of drugs or therapeutic proteins, by introducing genetically modified cells that express the therapeutic gene or protein at the site to the damaged tissue. Such a cell based delivery system will permit the production of a therapeutic agent at a steady state level and in consistent physiological concentrations, thus overcoming current limitations caused by incomplete drug accessibility. The use of genetically manipulated stem cells as therapeutic vectors has previously been shown to be feasible in mouse models of genetic disorders[35].

3. Drug screening and toxicology

Human ES cells may have great value in the discovery and the development of pharmaceutical compounds. As these cells can form distinct populations of terminally differentiated cells *in vitro*, they may be used in the discovery of new compounds as well as for the optimization of currently available drugs by carrying out improved screens that are disease oriented. Furthermore, they may be used as cellular assays in the study of drug toxicity and teratogenicity.

4. Model developmental processes

The study of early human development is restricted by ethical constrains on research of human embryos. Human ES cells allow access to study the events occurring during early human development. It has been proposed that expanding EBs mimic, to some extent, early embryonic development, thereby allowing the investigation of processes as complicated and diverse as morphogenesis, differentiation, and apoptosis. It has been demonstrated in the mouse that some temporal and spatial relationships between developmentally regulated genes that exist in the embryo are maintained *in vitro*[36]. Moreover, it should simplify the study of complex processes that occur during early embryonic development by isolating single events such as pre-amniotic cavitation and cell lineage selection.

5. Tool for gene manipulation

One of the great advantages of ES cells over other cell types is their accessibility for genetic manipulation. They can easily undergo genetic modifications while remaining pluripotent, and they can be selectively propagated, allowing the clonal expansion of genetically altered cells. Since the first isolation of ES cells in mice, many effective techniques have been developed for gene delivery and manipulation. These techniques both include transfection and infection protocols, as well as various approaches for inserting, deleting, or changing the expression of genes in the genome. These methods have been extremely useful for monitoring and directing differentiation, discovering unknown genes and studying their function. Similar approaches were recently successfully applied to the genetic modification of human ES cells[37,38].

Genetic manipulation of human ES cells can be applied to the expression of either foreign or cellular genes, allowing the study of gene function as well as the isolation or elimination of specific cell types in culture[39,40]. It may also be used to direct the cell fate, as described earlier in this article[41]. Obviously, the ability of directing *in vitro* differentiation, isolating pure populations of specific cell types, and eliminating undifferentiated cells prior to transplantation, may have great importance in cell-based therapy.

Apart from tagging, selecting, and directing the differentiation of specific cell types, it is possible to inactivate endogenous genes and study their function. This can be achieved by several methods. The most widely used technique for this purpose has been site-directed mutagenesis. This procedure involves the replacement of a specific sequence in the genome of the cell with a mutated copy, through homologous recombination. By targeting both alleles, it is possible to create "loss of function" or so-called "knock out" phenotypes in ES cells that can be used for functional studies of specific genes. This technology has been well practiced in mice, to generate animals that are homozygous for the desired mutation. The creation of human ES cells with a null genotype for specific genes may have great importance in the modeling of human diseases, as recently demonstrated in Lesch-Nyhan syndrome. These *in vitro* models should be most valuable to basic research, but more importantly to the exploration of new therapeutic protocols, specifically to the development of gene therapy-based treatments and to drug discovery.

Conclusion

It has been generally accepted that the derivation of ES cell lines from human embryos has initiated a new era in the fields of reproductive biology, biotechnology, pharmacology, basic scientific research, and regenerative medicine. It is well established that

human ES cell lines can be readily derived in a reproducible manner. However, there still exists a need to increase the number of cell lines that are available to the research community and to generate more lines with a broader genetic and ethnic background. New lines from genetically abnormal embryos are also required, as well as lines suitable for clinical purposes. Much more research and development is required to exploit the remarkable potential of human ES cells. Appropriate public support and adequate legislation are crucial for the realization of the far-reaching applications of human ES cells. Collaboration among clinicians and scientists from diverse fields is also necessary for the development of cell-based therapy and reparative medicine using cells derived form human ES cells.

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แนวทางการใช้ประโยชน์ของเซลล์ต้นกำเนิดจากตัวอ่อนมนุษย์

สุพัชญ์ สีนะวัฒน์

ความก้าวหน้าทางเทคโนโลยีการเจริญพันธุ์ทำให้นักวิทยาศาสตร์สามารถเพาะเลี้ยงตัวอ่อนมนุษย์ในห้องปฏิบัติการได้เป็นผลสำเร็จกว่าสองทศวรรษมาแล้ว ความรู้และประสบการณ์ในการเพาะเลี้ยงตัวอ่อนของสัตว์เลี้ยงลูกด้วยนม ทำให้นักวิทยาศาสตร์สามารถสร้างเซลล์ต้นกำเนิดจากตัวอ่อนของสิ่งมีชีวิตหลายชนิดรวมทั้งของมนุษย์พัฒนาการในการสร้างเซลล์นำสู่ที่พัฒนามาจากเซลล์ต้นกำเนิดของตัวอ่อนมนุษย์ (ES cell lines) ก่อให้เกิดก้าวกระโดดในการสะสมองค์ความรู้ด้านเทคโนโลยีชีวภาพ เภสัชวิทยา วิทยาศาสตร์พื้นฐาน ตลอดจนการรักษาด้วยเซลล์บำบัด ในปัจจุบันนี้มีความก้าวหน้าในการสังเคราะห์ ES cell lines ขึ้นมากแต่ยังขาดความรู้พื้นฐานในการพัฒนาให้เซลล์เหล่านี้เปลี่ยนแปลงไปเป็นเซลล์ที่ต้องการเพื่อใช้ประโยชน์ทางคลินิก ความร่วมมือของแพทย์และนักวิทยาศาสตร์ ตลอดจนความตระหนักในประโยชน์อันเนื่องจากวิทยาการแขนงนี้จากสังคมจะช่วยให้เกิดความต่อเนื่องในการพัฒนาองค์ความรู้ด้านเซลล์ต้นกำเนิดจากตัวอ่อนมนุษย์ซึ่งจะยังผลให้เกิดความก้าวหน้าในการดูแลรักษาด้วยเซลล์บำบัดต่อไป