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Human embryonic stem cells

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The establishment of permanent human embryonic stem cell lines (hESCs) was first reported in 1998. Due to their pluripotent nature and ability to differentiate to all cell types in the body, they have been considered as a cell source for regenerative medicine. Since then, intensive studies have been carried out regarding factors regulating pluripotency and differentiation. hESCs are obtained from supernumerary human IVF (in vitro fertilization) embryos that cannot be used for the couple's infertility treatment. Today, we can establish and expand these cells in animal substance-free conditions, even from single cells biopsied from eight-cell stage embryos. There are satisfactory tests for the demonstration of genetic stability, absence of tumorigenic mutations, functionality, and safety of hESCs. Clinical trials are ongoing for age-related macular degeneration (AMD) and spinal cord injury (SCI). This review focuses on the present state of these techniques.

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Establishment of human embryonic stem cell lines

For quite some time, human embryonic stem cells (hESCs) were derived from the inner cell mass (ICM) of blastocysts, which required destruction of the embryo. This has been considered a major ethical problem in many countries. In particular, many religious groups have adopted the view that cells from a human preimplantation embryo cannot be used for any purpose. Hence, derivation and even culture of hESC are forbidden by law in some European countries [1]. In the USA, there are limitations for making hESCs when human in vitro fertilization (IVF) embryos are destroyed. However, it has recently become possible to effectively derive hESC lines from single biopsied blastomeres of an IVF embryo in xeno-free chemically defined conditions without destroying the embryo [2]. The use of the new human recombinant laminin 521, an adhesive matrix component present in the stem cell niche, which supports ESC growth, has made the procedure easily feasible.

Murine embryonic fibroblasts (MEFs) were initially used as feeder cells, in the presence of fetal bovine serum-containing culture medium. We initiated the use of human neonatal fibroblasts as feeder cells in the derivation of new hESC lines [3], and then serum replacement (SR) instead of bovine serum in order to develop a better-defined culture medium [4], and managed in establishing several new hESC lines in these more defined conditions [5]. The use of human feeder cells was then adopted worldwide. To remove one additional xeno-component from the cultures, we replaced immunosurgery, a method in where a mouse antibody against human trophectoderm and guinea pig complement are used in the isolation of the ICM, by mechanical isolation of the ICM [6].

The impractical use of feeder cells has been replaced in many laboratories by adding conditioned medium from feeder cells instead of having two cell types in the cultures [7,8]. The most commonly used culture substrate was previously Matrigel, a mouse Engelbreth–Holm–Swarm (EHS) sarcoma tumor extract [9]. It is a complex undefined mixture of basement membrane and cellular proteins as well as growth factors. It displays extensive batch-to-batch variation, and it is far from being chemically defined. The International Stem Cell Initiative (ISCI) has carried out studies aiming at standardized cultures using chemically defined media [10]. A major step forward came with a specific cell culture coating of human recombinant laminin-511 (LN-511) [11] that had been originally identified in the early embryo [12] and produced as recombinant protein by Karl Tryggvason's group [13]. The cultures became chemically defined, when a xeno-free chemically defined medium was used in addition to the laminin as growth support. Importantly, this protocol allowed the cells to be cultured as monolayers and passaged as a single cell suspension. Another closely related human laminin, LN-521, an adhesion protein also present in the ICM and other in vivo stem cell niches, was then successfully produced [2]. The result was another robust chemically defined, xeno-free cell culture matrix that now allows the maintenance of highly stable hESC cultures [2]. With this system, it is now possible to derive new hESC lines from a single blastomere biopsied from an eight-cell IVF embryo by culturing it on a mixture of LN-521 and E-cadherin, which provides cell–cell contact inducing signals. The cells grow as a homogeneous monolayer (Fig. 1) such that each cell can be individually inspected. An important aspect is that this procedure does require the destruction of the IVF embryo [2,22]. The blastomere biopsy is similar to that normally carried out to obtain a single cell for preimplantation genetic diagnostics (PGD). In our IVF unit, we regularly get pregnancies and infants from such embryos from which one cell has been removed.

The splitting ratio of the hESC in these cultures is 1:30 instead of 1:3 in the conventional cell clump cultures. This means that we can obtain large numbers of hESCs for regenerative medicine with fewer passages, which is faster and safer.

If the line is family specific, the need for immunosuppression in regenerative medicine is much smaller than when using completely allogenic hESC lines. As the new cell derivation and expansion methods are effective, it has become feasible to establish an hESC bank of clinical quality hESC lines. For such a bank, cell lines of about 150 haplotypes are estimated to be sufficient to provide cell therapy of the majority of the human population [14].

An advantage with hESCs is that they are normal cells existing in the human embryo for 3–6 days after fertilization. In that respect, they differ from the induced pluripotent stem cells (iPSC) that are genetically modified adult cells. The pluripotent stem cells differ from cord blood cells in being capable of dividing without limits. Large amounts of cells for several recipients and treatments can be obtained, and all human cell types for which proper differentiation protocols have been developed can be