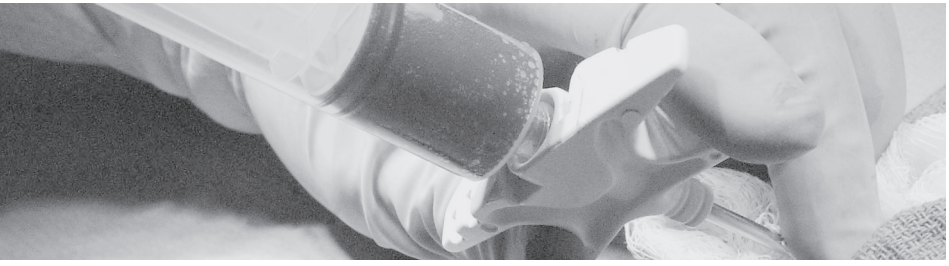
Embryonic Stem Cells



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(ii) blastocoel (a hollow cavity), and

(iii) inner cell mass.

The inner cell mass contains embryonic stem cells which are transferred to a petri dish containing appropriate culture medium to develop into nearly all cell types in the body. This can be achieved by the addition of different growth factors. Human blastocyst can be cultured and embryonic stem cell lines derived. Cheng *et al.* [6] showed that embryo quality on Day 5 is a strong predictor for successful establishment of human embryonic stem cell lines. The emphasis, however, is finding defined conditions to culture human embryonic stem cells to enable their clinical use for cell transplantation because animal-derived biological components in media are no longer acceptable. Recently, Lu and his colleagues [7] developed a simple medium [termed human embryonic stem cells cocktail] containing basic fibroblast growth factor, Wnt3a, April (a proliferation-inducing ligand)/BAFF (B cell-activating factor belonging to TNF), albumin, cholesterol, insulin, and transferrin, which is sufficient for human embryonic stem cells self-renewal and proliferation. Cells were shown to maintained pluripotency. Thus, the cells may be cultured by eliminating animal products in medium, maintaining normal karyotype and harvesting for various clinical applications;

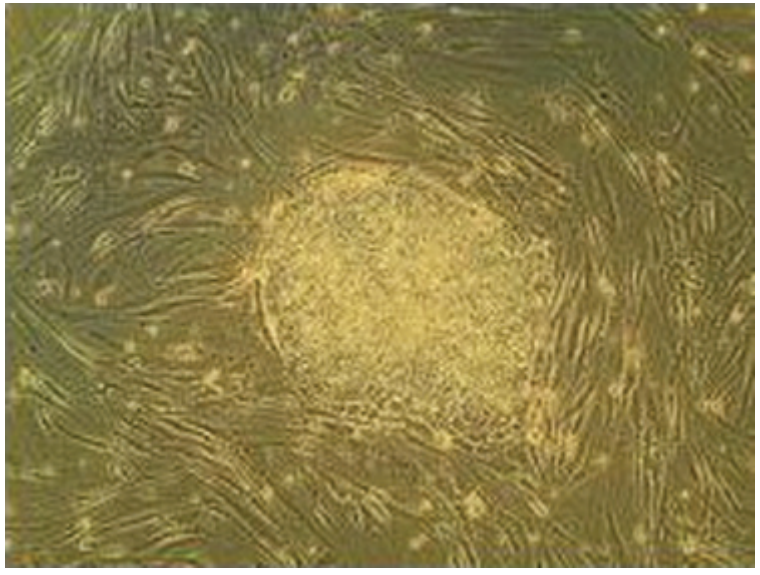

Figure 1. A colony of embryonic stem cells.

however, they may be used in regenerative medicine provided the cell lines established remain stable overtime. In addition, good manufacturing practice (European Medicines Agency and the Food and Drug Administration) is required to produce clinical-grade cells, offering optimal defined quality and safety in cell transplantation [8]. The culture medium should, therefore, be animal substance-free, culture procedures should be well defined, with minimum infection risk due to microbes. Moreover, after generating embryonic stem cell lines it is important to characterize them. Several kinds of tests include growing and subculturing the stem cells for many months, ensuring their capability of long-term self-renewal, determining the presence of cell surface markers found in undifferentiated cells, identifying the presence of octamer-4 (Oct-4; a transcription factor), examining the chromosomes under a microscope, determining if the cells can be subcultured after freezing, thawing, and replating, and testing the pluripotency of human embryonic stem cells. For instance, the human embryonic stem cell lines derived from the inner cell masses of blastocysts are generally characterized by the expression analysis of Oct-4, NANOG, stage-specific embryonic antigen-3 (SSEA3), stage-specific embryonic antigen-4 (SSEA4), tumor rejection antigen-1 (TRA-1-60 and TRA-1-81).

There are many recent developments in the area of embryonic stem cell research. Cybrid human embryos are being developed to understand differentiation, gene expression and genomic compatibility, promoting human cell therapies and searching for new drug targets [9]. Barroso-del Jesus *et al.* [10] first identified, characterized, and functionally validated a human miRNA promoter in stem cells. Tan *et al*. [11] established a human embryonic stem cell line derived from frozen human embryos of Chinese origin. Siti-Ismail *et al.* [12] showed the possibility to maintain human embryonic stem cells in an undifferentiated state, without passaging or embryoid body formation, and without animal contamination. Valamehr *et al.* [13] showed that hydrophobic surfaces serve as a platform to deliver uniform embryoid body populations and may significantly improve the efficiency of embryonic stem cell differentiation. Swijnenburg *et al.* [14] showed that human embryonic stem cells are immunogenic, trigger both cellular and humoral-mediated pathways, and as a result they are rapidly rejected in xenogeneic hosts. This process can be mitigated by a combined immunosuppressive regimen as assessed by molecular imaging approaches. Such developments will revolutionize the fields of regenerative medicine, enabling the application in these cells.

The cell cycle regulation in somatic cells differs from that of embryonic stem cells. The human embryonic stem cells express members of the E2F family and RB-related pocket proteins that are responsible for controlling expression of genes encoding enzymes for both nucleotide metabolism and DNA synthesis; but somatic cells do not express these proteins. G1 checkpoint is present in somatic cells and thus they undergo DNA repair [15]. Conversely, this checkpoint is absent in embryonic stem cells because of altered localization (localized to centrosomes) of checkpoint kinase 2 (Chk2), which is not intranuclear as in somatic cells, and its nonavailability for phosporylation. These cells speed up the molecular mechanisms to proceed to the next phase. Thus embryonic stem cells have abbreviated cell cycle, but are specialized with mechanisms to protect the integrity of their genome. The absence G1 checkpoint is an added advantage for the survival of embryonic stem cells and those with the damaged DNA can be eliminated. Thus, the embryonic stem cells naturally maintain a pure stem cell population. Recently, Card *et al*. [16] showed that Oct-4 and Sox2 bind to a conserved promoter region of miR-302. They also showed miR-302a represses the productive translation of cyclin D1. The transcriptional activation of miR-302 and the translational repression cyclin D1 may provide a link between Oct-4/Sox2 and cell cycle regulation in pluripotent cells.

CELL SIGNALING IN EMBRYONIC STEM CELLS

To date, the factors and signaling pathways controlling stem cell properties have not been fully investigated. There is a necessity to understand how extrinsic factors control human embryonic stem cell self-renewal and differentiation. This will help scientists to culture and differentiate stem cells with higher efficiency. Embryonic stem cells have the ability to self-renew and the underlying molecular mechanisms remain largely unknown. Recent studies indicate that Polycomb group proteins play a dynamic role in maintaining undifferentiated state. Richard Young and his colleagues [17] discovered that Polycomb group proteins silence gene activity through chemical, or epigenetic modifications and are responsible for maintaining developmental genes in an off state. They observed that these proteins preferred to occupy genes for most of the human developmental regulators to repress their activity and these genes encode transcription factors that control development downstream of the embryo. They also found that transcription factors Oct-4, Sox2 and Nanog are key regulators of embryonic stem

cells pluripotency and self-renewal and together they occupy at least 353 genes in human embryonic stem cells. Bernstein *et al.* [18] identified "bivalent domains," consisting of large regions of H3 lysine 27 methylation harboring smaller regions of H3 lysine 4 methylation. They suggested a novel chromatin-based mechanism for maintaining pluripotency. Boyer *et al.* [19] provided new insights into the transcriptional regulation of stem cells and revealed how Oct-4, Sox2, and Nanog contribute to pluripotency and self-renewal.

There exists a similarity between cancer cell and stem cell because both cells process self-renewal ability. It is implicated that the fibroblast growth factor and members of the transforming growth factor-beta superfamily regulates self-renewal capacity of embryonic stem cells. Moreover, fibroblast growth factor 2 pathway holds responsible for cancer cell tumorigenesis. Normal cells in the body divide, mature, and become specialized cells; however, cancer cells carry on cell division, do not respond to the signals from neighboring cells, and fail to become specialized cells.

Embryonic stem cells are quite resistant to DNA damage compared with the adult stem cells. The reason is that despite serial passages they tend to maintain the length of telomere repeats; on the other hand adult stem cells are not able to maintain the length of telomere repeats as embryonic stem cells and thus are sensitive to DNA damage. Recent studies suggested that the DNA damage in embryonic stem cells is eliminated because of tumor suppressor protein p53. This is achieved when p53 induces the differentiation of DNA-damaged embryonic stem cell by directly suppressing the expression of a gene called Nanog. This gene is responsible for unlimited duplication of embryonic stem cells. Thus maintaining genetic stability and suggesting a similar role in suppressing the development of cancer. Hence, understanding the underlying tumor suppressive mechanism may hold key to provide treatment for cancers. Alternatively, Bernie Tuch and his team [20] placed the embryonic stem cells inside alginate microcapsules, prevented the formation of tumors, and transplanted them into laboratory animals.

EMBRYONIC INTRACELLULAR AND CELL SURFACE MARKERS

Human embryonic stem cells have certain key properties including long time self-renewal capacity, pluripotency, basement membrane-related gene expression, potential to generate into cells of the three primary

cells. The protein Rex-1 is a marker for undifferentiated embryonic stem cells and teratocarcinoma cells. Rex-1 promoter is regulated by specific octamer family members in early embryonic cells. In F9 teratocarcinoma cells the promoter elements contributes to the regulation and a region required for Rex-1 promoter activity consists of an octamer motif (ATTTGCAT). Human Rex-1 (hRex-1) is also referred to as zinc-finger protein-42 (Zfp42).

Telomerase

Telomerase is the enzyme (composed of catalytic protein component and an RNA template) which maintains the ends of linear chromosomes in eukaryotic cells and is found at low levels in somatic stem cells; however, embryonic stem cells tend to maintain the length of telomere repeats. Telomere is a repeating sequence of double-stranded DNA and a lengthy telomere is associated with embryonic stem cells and cancer cells. And telomerase is responsible for adding repeated DNA sequences and DNA polymerase helps to complete the complementary DNA strand. Apart from this telomerase plays a main role to protect telomere ends and maintain telomere length during replication. Usually telomerase activity is high in both embryonic stem cells and cancer cells, but low in mature cells. Telomerase activity is controlled by the catalytic core called telomerase reverse transcriptase (Tert). In human beings the telomerase consists of two components human telomerase reverse transcriptase (hTERT) and human telomerase RNA (hTR). The telomerase reverse transcriptase gene (3396 bp) consists of 16 exons and 15 introns. Telomerase adds TTAGGG repeats to the chromosome end and regulates of the replicative lifespan by maintaining the length of telomere.

Surface markers

Stage Specific Embryonic Antigens (SSEAs)

Stage-specific embryonic antigens are cell-surface molecules that are useful markers for identifying embryonic stem cells. These surface markers include SSEA-3 and SSEA-4 (cell surface glycosphingolipids) and are not essential for pluripotency rather they play a role in cellular differentiation. These markers are synthesized during oogenesis and expressed by oocytes, zygotes and human embryonic stem cells. In human morula stage, embryo does not express SSEA-3 and SSEA-4 but they are expressed on the inner cell of mass of human blastocysts

proliferative potential and can differentiate into derivatives of all three embryonic germ layers.

- *The cell cycle regulation in somatic cells differs from that of embryonic stem cells.*
- *Some of the embryonic stem cell markers include Oct-4, Rex-1, SSEA-3 and SSEA-4, TRA-1-60 and TRA-1-81 and alkaline phosphatase.*
- *Research on human embryonic stem cells has resulted in both public interest and moral problems. Although the application of these cells contributes enormously to human welfare, they involve using human embryos for various experiments.*

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