Sem – VI (UG)

CC-13: Developmental Biology

C13T: Unit -5, Implications of Developmental Biology

Prepared by Anindita Das

Stem Cells

What are Stem Cells?

Stem cells are raw cells found within the human body from which all other cells with specialized functions are generated. In proper experimental conditions, these stem cells can be divided to create daughter cells. These daughter cells can either be new stem cells themselves, by the way of self-renewal, or end up being functional cells (i.e. blood cells or brain cells).

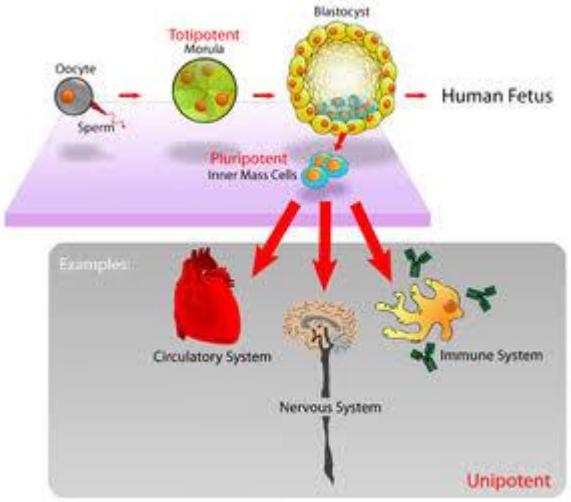
Where do these stem cells come from?

Researchers have discovered several sources of stem cells –

- *Embryonic stem cells:* These stem cells come from embryos that are 3 – 5 days old. They are by far the most versatile and can thus be used for both regenerative and cell replacement purposes.
- *Adult stem cells:* These stem cells are found (in small numbers) in most adult tissues (such as bone marrow or fat). Compared with embryonic stem cells, these have a more limited ability to produce various cells found in the body. These can be versatile, but not to the extent found in embryonic cells.
- *Adult cells altered to have properties of embryonic stem cells:* Scientists have successfully transformed regular adult cells into stem cells using genetic reprogramming. Through altering the genes in the adult cells, researchers can reprogram the cells to act similarly to that of embryonic stem cells.
- *Perinatal stem cells:* Researchers have discovered stem cells in amniotic fluid as well as within umbilical cord blood. These also have the ability to change into specialized cells when required.

Embryonic Stem Cells (ESCs)

ESCs are pluripotent stem cells derived from the inner cell mass of a blastocyst, an early-stage pre-implantation embryo. Human embryos reach the blastocyst stage 4–5 days post fertilization, at which time they consist of 50 – 150 cells. Isolating the embryoblast or inner cell mass (ICM) results in destruction of the blastocyst, a process which raises ethical issues, including whether or not embryos at the pre-implantation stage should have the same moral considerations as embryos in the post-implantation stage of development.



Properties:

Embryonic stem cells (ESCs), derived from the blastocyst stage of early mammalian embryos, are distinguished by their ability to differentiate into any embryonic cell type and by their ability to self-renew. It is these traits that makes them valuable in the scientific and medical fields. ESCs have a normal karyotype, maintain high telomerase activity, and exhibit remarkable long-term proliferative potential.

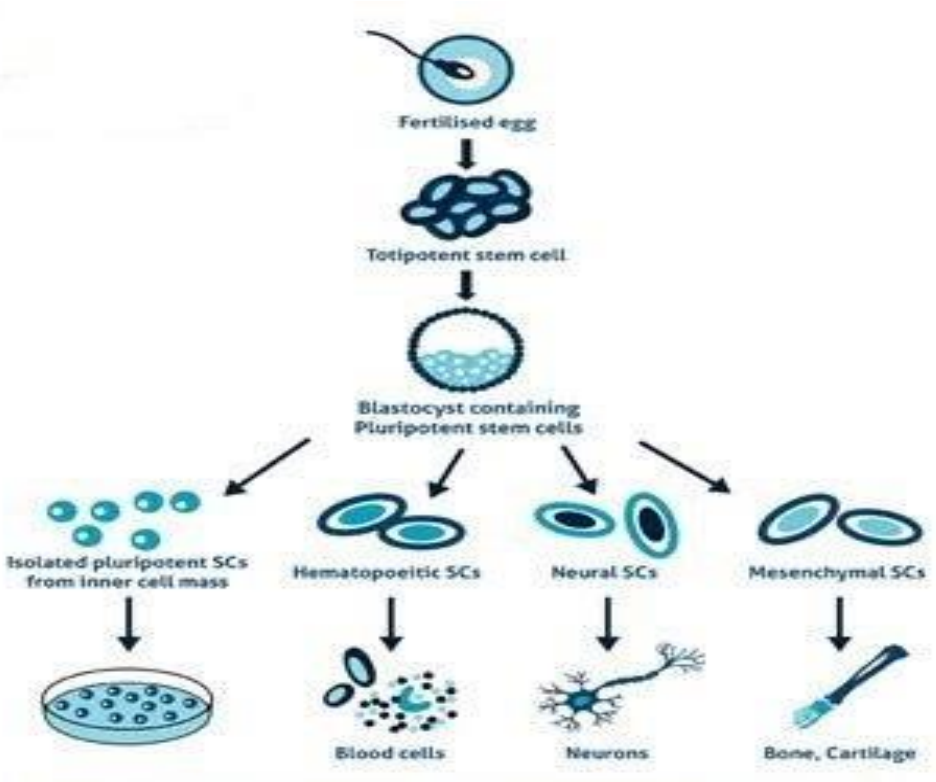
1) *Pluripotent:*
   Embryonic stem cells of the inner cell mass are pluripotent, meaning they are able to differentiate to generate primitive ectoderm, which ultimately differentiates during gastrulation into all derivatives of the three primary germ layers: ectoderm, endoderm and mesoderm. These germ layers generate each of the more than 220 cell types in the adult human body. When provided with the appropriate signals, ESCs initially form precursor cells that in subsequently differentiate into the desired cell types. Pluripotency distinguishes embryonic stem cells from adult stem cells, which are multipotent and can only produce a limited number of cell types.
2) *Self-renewal and repair of structure:*
   Under defined conditions, embryonic stem cells are capable of self-renewing indefinitely in an undifferentiated state. Self-renewal conditions must prevent the cells from clumping and maintain an environment that

supports an unspecialized state. Typically this is done in the lab with media containing serum and leukemia inhibitory factor or serum-free media supplements with two inhibitory drugs ("2i"), the MEK inhibitor PD03259010 and GSK-3 inhibitor CHIR99021.

3) *Growth:*

ESCs divide very frequently due to a shortened G1 phase in their cell cycle. Rapid cell division allows the cells to quickly grow in number, but not size, which is important for early embryo development. In ESCs, cyclin A and cyclin E proteins involved in the G1/S transition are always expressed at high levels. CDK2 that promote cell cycle progression are overactive, in part due to downregulation of their inhibitors. Retinoblastoma (Rb) proteins that inhibit the transcription factor E2F until the cell is ready to enter S phase are hyperphosphorylated and inactivated in ESCs, leading to continual expression of proliferation genes.

These changes result in accelerated cycles of cell division. Although the shortened G1 phase has been linked to maintenance of pluripotency, ESCs grown in serum-free 2i conditions do express hypo-phosphorylated active Rb proteins and have an elongated G1 phase. Pluripotency factors Oct4 and Nanog play a role in transcriptionally regulating the ESC cell cycle.



Uses:

Due to their plasticity and potentially unlimited capacity for self-renewal, embryonic stem cell therapies have been proposed for regenerative medicine and tissue replacement after injury or disease. Pluripotent stem cells have shown promise in treating a number of varying conditions, including but not limited to: spinal cord injuries, age related macular degeneration, diabetes, neurodegenerative disorders (such as Parkinson's disease), AIDS, etc. In addition to their potential in regenerative medicine, ESCs provide a possible alternative source of tissue/organs. ESCs can also be used for research on early human development, certain genetic disease, and in vitro toxicology testing.

Human embryonic stem cells have the potential to differentiate into various cell types, and, thus, may be useful as a source of cells for transplantation or tissue engineering:

- *Cell replacement therapies (CRTs)*–
  Some of the cell types that are differentiating from ESCs, have or are currently being developed include cardiomyocytes (CM), neurons, hepatocytes, bone marrow cells, islet cells and endothelial cells. These are in current research.
- *Clinical potential* –
  - ✓ ESCs have been differentiated to natural killer (NK) cells and bone tissue.
  - ✓ Researchers have differentiated ESCs into dopamine-producing cells with the hope that these neurons could be used in the treatment of Parkinson's disease.
  - ✓ Studies involving ESCs are underway to provide an alternative treatment for diabetes. For example, researchers were able to differentiate ESCs into insulin producing cells to produce large quantities of pancreatic beta cells from ES.
  - ✓ An article describes a translational process of generating human embryonic stem cell-derived cardiac progenitor cells to be used in clinical trials of patients with severe heart failure.
- *Drug discovery* –
  Besides becoming an important alternative to organ transplants, ESCs are also being used in field of toxicology and as cellular screens to uncover new chemical entities (NCEs) that can be developed as small molecule drugs.

Studies have shown that cardiomyocytes derived from ESCs are validated in vitro models to test drug responses and predict toxicity profiles. ES derived cardiomyocytes have been shown to respond to pharmacological stimuli and hence can be used to assess cardiotoxicity. ESC-derived hepatocytes are also useful models that could be used in the preclinical stages of drug discovery.

- *Models of genetic disorder* –
  Several new studies have started to address the concept of modelling genetic disorders with ESCs. Either by genetically manipulating the cells, or more recently, by deriving diseased cell lines identified by prenatal genetic diagnosis (PGD), modelling genetic disorders is something that has been accomplished with stem cells. This approach may very well prove valuable at studying disorders such as Fragile-X syndrome, Cystic fibrosis, and other genetic maladies that have no reliable model system.
  Yury Verlinsky, a Russian-American medical researcher, developed prenatal diagnosis testing methods to determine genetic and chromosomal disorders a month and a half earlier than standard amniocentesis. The techniques are now used by many pregnant women and prospective parents, especially couples who have a history of genetic abnormalities or where the woman is over the age of 35 (when the risk of genetically related disorders is higher).
- *Repair of DNA damage* –
  Differentiated somatic cells and ES cells use different strategies for dealing with DNA damage. For instance, human foreskin fibroblasts, one type of somatic cell, use non-homologous end joining (NHEJ), as the primary pathway for repairing double-strand breaks (DSBs) during all cell cycle stages. Because of its error-prone nature, NHEJ tends to produce mutations in a cell's clonal descendants. ES cells use a different strategy to deal with DSBs. Because ES cells give rise to all of the cell types of an organism including the cells of the germ line, mutations arising in ES cells due to faulty DNA repair are a more serious problem than in differentiated somatic cells. Thus, mouse ES cells predominantly use high fidelity homologous recombinational repair (HRR) to repair DSBs. This type of repair depends on the interaction of the two sister chromosomes formed during S phase and present together during the G2 phase of the cell cycle. HRR can accurately repair DSBs in one sister chromosome by using intact information from the other sister chromosome. Cells in the G1 phase of the cell cycle (i.e. after metaphase/cell division but prior the next round

of replication) have only one copy of each chromosome (i.e. sister chromosomes aren't present). Mouse ES cells lack a G1 checkpoint and do not undergo cell cycle arrest upon acquiring DNA damage. Rather they undergo apoptosis in response to DNA damage. Apoptosis can be used as a fail-safe strategy to remove cells with unrepaired DNA damages in order to avoid mutation and progression to cancer.

- *Clinical trial* –
  Phase I clinical trials for transplantation of oligodendrocytes (a cell type of the brain and spinal cord) derived from human ES cells into spinal cord-injured individuals received approval from the U.S. Food and Drug Administration (FDA), marking it the world's first human ES cell human trial. This first trial was primarily designed to test the safety of these procedures and if everything went well, it was hoped that it would lead to future studies that involve people with more severe disabilities. The makers of the stem cell therapy, Geron Corporation, estimated that it would take several months for the stem cells to replicate. California Institute for Regenerative Medicine (CIRM) is the largest funder of stem cell-related research and development in the world. AST-OPC1 is a population of cells derived from human embryonic stem cells (hESCs) that contains oligodendrocyte progenitor cells (OPCs). OPCs and their mature derivatives called oligodendrocytes provide critical functional support for nerve cells in the spinal cord and brain. Asterias recently presented the results from phase 1 clinical trial testing of a low dose of AST-OPC1 in patients with neurologically-complete thoracic spinal cord injury. The results showed that ASTOPC1 was successfully delivered to the injured spinal cord site. Immune monitoring of subjects through one year post-transplantation showed no evidence of antibody-based or cellular immune responses to AST-OPC1. In four of the five subjects, serial MRI scans performed throughout the 2–3 year follow-up period indicate that reduced spinal cord cavitation may have occurred and that AST-OPC1 may have had some positive effects in reducing spinal cord tissue deterioration.

Concern and controversy:

- *Adverse effects* –
  The major concern with the possible transplantation of ESC into patients as therapies is their ability to form tumours including teratoma. Safety issues prompted the FDA to place a hold on the first ESC clinical trial,

however no tumours were observed. The main strategy to enhance the safety of ESC for potential clinical use is to differentiate the ESC into specific cell types (e.g. neurons, muscle, liver cells) that have reduced or eliminated ability to cause tumours. Following differentiation, the cells are subjected to sorting by flow cytometry for further purification. ESC are predicted to be inherently safer than iPS (induced Plutipotent Stem) cells created with genetically-integrating viral vectors because they are not genetically modified with genes such as c-Myc that are linked to cancer. Nonetheless, ESC express very high levels of the iPS inducing genes and these genes including Myc are essential for ESC selfrenewal and pluripotency, and potential strategies to improve safety by eliminating c-Myc expression. More recent protocols to induce pluripotency bypass these problems completely by using nonintegrating RNA viral vectors such as sendai virus or mRNA transfection.

- *Ethical debate* –
  Since harvesting embryonic stem cells necessitates destroying the embryo from which those cells are obtained, the moral status of the embryo comes into question. Some people argue that the 5-day old mass of cells is too young to achieve personhood or that the embryo, if donated from an IVF clinic (which is where labs typically acquire embryos from), would otherwise go to medical waste anyway. Opponents of ESC research counter that an embryo is a human life, therefore destroying it is murder and the embryo must be protected under the same ethical view as a more developed human being.