CSE 701 SEMINAR
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iPSC - Induced Pluripotent Stem Cells

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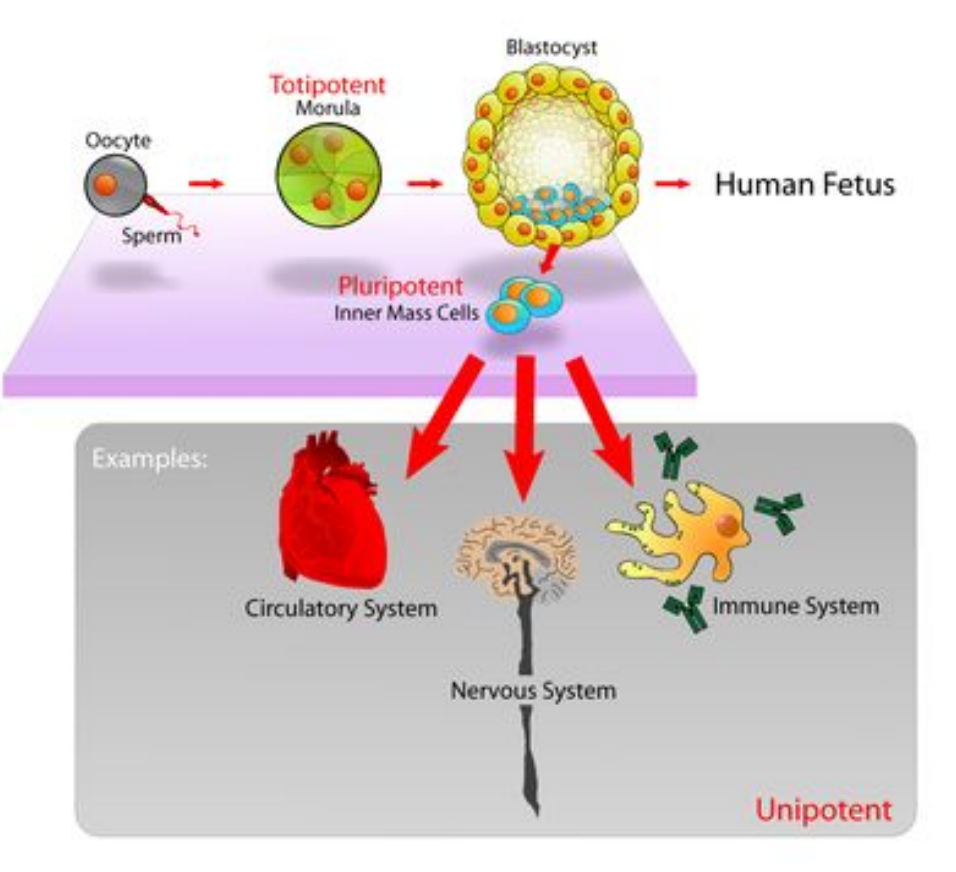
What is a Stem Cell?

- **Stem cells** are undifferentiated biological cells that can differentiate into specialized cells and can divide to produce more stem cells.
- Characterised by - ***Self-renewal*** *and* ***Potency****.*
- In Mammals two broad types
  - Embryonic stem (ESC) cells - contains the pluripotent stem cell.
  - Adult stem cells - act as a repair system for the body, replenishing adult tissues.

Pluripotent Stem Cells

- ***Potency*** specifies the differentiation potential (the potential to differentiate into different cell types) of the stem cell.
  - **Totipotent (a.k.a. omnipotent) SC** can construct a complete, viable organism. These cells are produced from the fusion of an egg and sperm cell.
  - **Pluripotent SC** - descendants of totipotent cells and can differentiate into nearly all cells.
  - **Multipotent SC** - differentiate into a number of closely related cell types.
  - **Oligopotent SC** - differentiate into a few cell types, such as lymphoid stem cells.
  - **Unipotent cells** - produce only one cell type, their own, but have the property of self-renewal.

Pluripotent embryonic stem cells originate as inner cell mass (ICM) cells within a blastocyst.



Need for Induced Pluripotent Stem Cell.

- Nearly all research to date has made use of mouse embryonic stem cells (mES) or human embryonic stem cells (hES).
- By using human embryonic stem cells to produce specialized cells like nerve cells or heart cells in the lab, scientists can gain access to adult human cells without taking tissue from patients.
- They can then study these specialized adult cells in detail to try and catch complications of diseases, or to study cells reactions to potentially new drugs.

- Adult stem cells have limitations with their potency. They are deemed multipotent.
- Reprogramming allows for the creation of pluripotent cells - induced pluripotent stem cells - from adult cells.
- These are not adult stem cells, but adult cells (e.g. epithelial cells) reprogrammed to give rise to cells with pluripotent capabilities.
- Using genetic reprogramming with protein transcription factors, pluripotent stem cells with ESC-like capabilities have been derived.

Induced Pluripotent Stem Cell - iPS cell

- The first demonstration by **Shinya Yamanaka** at Kyoto University. Used the transcription factors Oct3/4, Sox2, c-Myc, and Klf4 to reprogram mouse fibroblast cells into pluripotent cells. *2012 NOBEL PRIZE in Medicine*
- First Human iPSC demonstrated by **James Thomson** at the University of Wisconsin–Madison. Transcription factors Oct4, Sox2, Nanog and Lin28,
- They were able to replicate Yamanaka's finding that inducing pluripotency in human cells was possible.

Challenges to iPS Cell

- Low efficiency
  - Reprogrammed into iPS cells 0.01–0.1%
  - Reflects the need for precise timing, balance, and absolute levels of expression of the reprogramming genes.
- Tumorigenicity
  - Significant risks that limits their use in humans.
  - Major concern with the potential clinical application.
  - iPSCs readily form teratoma.
- Incomplete reprogramming
  - Faces the challenge of completeness.
- iPSCs have not yet yielded any clinical benefits.

Implications of iPS cell

- Create female or male gametes (eggs or sperm cells).
- Act a new tool for treating infertility.
- Helps LGBT couples to have children of their own.
- Using iPSC for creating egg eliminate the need for egg harvesting, which is expensive, unpleasant and risky.
- One hundred embryos seems a more manageable number to work with.
- Sperm harvest has not been as big a problem.
- CONCERNS: Biological, Ethical, and Legal issues. 'parentless' human embryos. Bringing into existence, experimenting upon or destroying human embryos.

CASE STUDIES

http://www.sciencemag.org

CASE STUDY 1: “Eggs Created in Dish Produce Mouse Pups” Oct. 4, 2012

- Sperm - the group started with ES and iPS cells and cultured them in a cocktail of proteins to produce primordial germ cell-like cells.
- Egg cells - they mixed the primordial cells with fetal ovarian cells, forming reconstituted ovaries that they then grafted onto natural ovaries in living mice.
- Four weeks and 4 days later, the primordial germ cell-like cells had developed into oocytes.
- The team removed the ovaries, harvested the oocytes, fertilized them in vitro, and implanted the resulting embryos into surrogate mothers.
- About 3 weeks later, normal mouse pups were born.
- Kyoto University in Japan.

CASE STUDY 2: "Mouse egg cells made entirely in the lab give rise to healthy offspring" Oct. 17, 2016

- Hayashi and his colleague Mitinori Saitou developed ways to derive egg and sperm cells—also called germ cells—from pluripotent stem cells.
- First, used ES and iPS cells to make immature egg precursor cells.
- Then they inserted those precursors into clusters of cells taken from fetal mouse ovaries.
- They carefully cultured those cell clusters for more than a month.
- Lab-based ovaries produced more than 50 mature egg cells.
- More than 75% had the correct number of chromosomes.
- Mixed some of the eggs with mouse sperm, producing more than 300 two-cell embryos, which were implanted into foster mothers.
- 11 of those embryos—or 3%—grew into full-term pups, compared with 62% for eggs taken from adult mice and fertilized in vitro.
- The pups that did survive grew into apparently healthy, fertile adults.

Egg cells derived in the lab from embryonic stem cells.

Mice derived from labmade eggs were normal, fertile adults.

O. Hikabe *et. al.*, *Nature* 538, 7625 (20 October 2016) © MacMillian Publisher Ltd.

Questions???