Course	**: PGPathshala-Biophysics**
Paper 11	**: Cellular and Molecular Biophysics**
Module 8	**: Cellular Differentiation**
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Objectives:

At the end of this module, you should be able to:

1. Explain the term cellular differentiation
2. List various levels of cell potencies
3. Understand clinical aspects of cell potency
4. Explain induced pluripotency
5. Explain trans-differentiation and de-differentiation
6. Explain the importance of cancer stem cells

1. Introduction

Here is a conversation between an evolution sceptic, and Professor JBS Haldane:

Evolution sceptic: "Professor Haldane, even given the billions of years that you say were available for evolution, I simply cannot believe it is possible to go from a single cell to a complicated human body, with its trillions of cells organized into bones and muscles and nerves, a heart that pumps without ceasing for decades, miles and miles of blood vessels and kidney tubules, and a brain capable of thinking and talking and feeling."

JBS Haldane: "But madam, you did it yourself. And it only took you nine months!"

Why is it that a single cell should give rise to different cell types with diverse function? What is the advantage of having multitude of cell types? What are the inadvertent consequences of a precursor cell giving rise to a phenotypically different progeny? All these questions will be addressed in this module on Cellular Differentiation. The mechanisms behind Cellular Differentiation will be discussed in a separate module.

2. What is cellular differentiation?

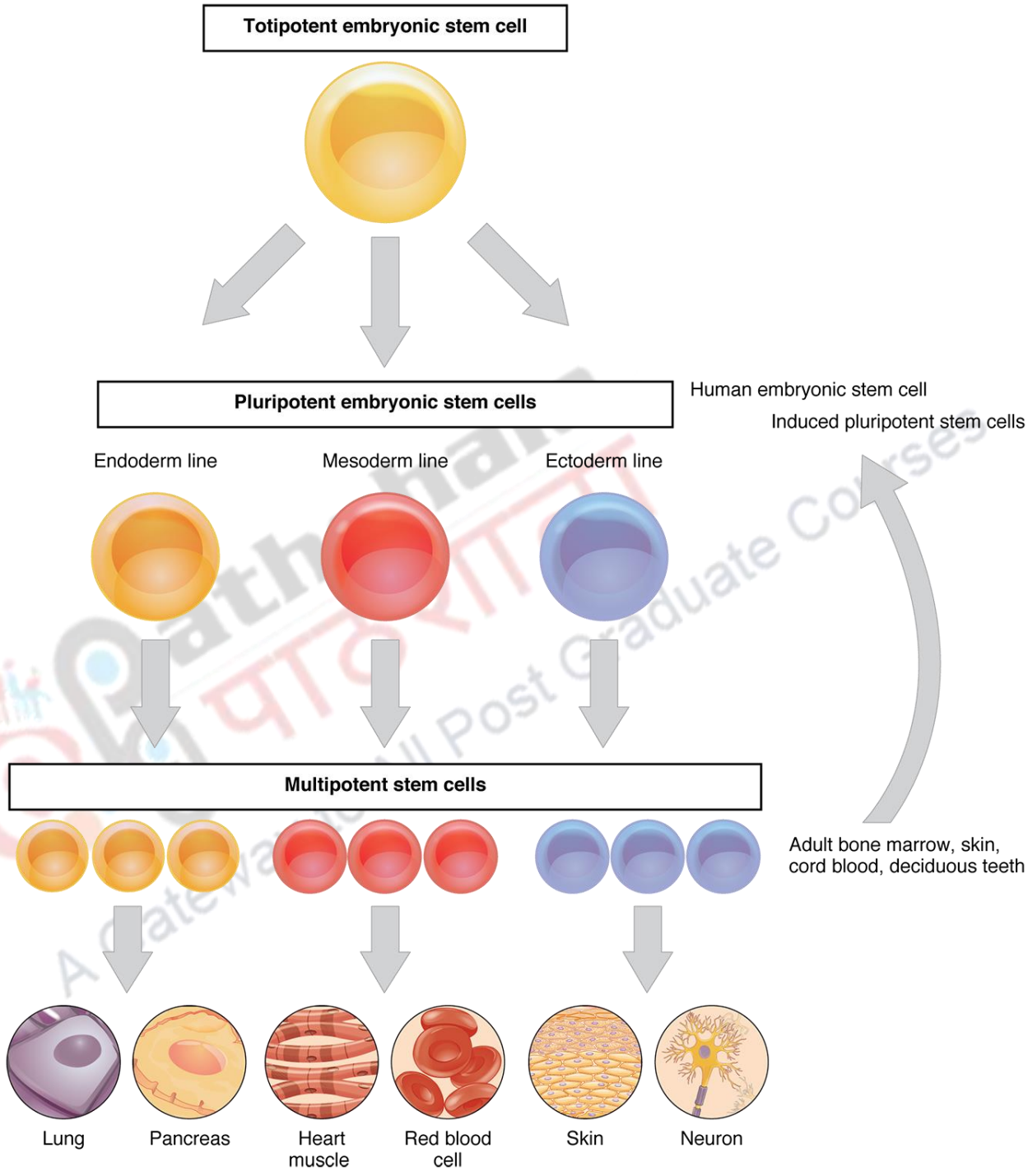
Life originated around 3.7 billion years ago in the form of single cellular organisms. Uni-cellularity is a successful trait, and unicellular organisms still continue to exist, that too in abundant numbers. The first multi-cellular organisms evolved around 600 million years before, much later than the uni-cellular organisms. What is the advantage of multicellularity? Formation of multicellular organisms was a key movement in the course of evolution. The key feature of multi-cellular organisms were co-operativity between various cell types. The individual cell types were phenotypically different. This prevented independent existence of individual cell types in such organisms, necessitating co-operativity. This also meant that a single cell type gave rise to multiple cell types, that are distinct from the parent cell.

Such a process in which a cell changes from one form to another is known as cell differentiation. Differentiation usually provides the cell with new functional capabilities. It is associated with extensive reprogramming at the transcriptional level, thus enabling expression of different sets of proteins at different levels in the differentiated cell compared to the parent cell.

3. Levels of cell potency:

Not all the cells in the body are capable of differentiation, and not all the cells that can differentiate do so at an equal level. The ability of a cell to differentiate into different cell types is known as cell potency. More the ability of a cell to differentiate into different cell types, greater is its cell potency. During

embryonic development, 3 different germ layers are formed- ectoderm, mesoderm and endoderm - different cells in the body arise from one of these germ layers.



a. ***Totipotent cells:*** These cells are capable of generating all the differentiated cells in the organism. Zygote is the classical example of a totipotent cell, and it can generate all the cells that constitute the particular organism.

b. ***Pluripotent cells:*** These cells are capable of generating cells of all the 3 germ layers, i.e., ectoderm, mesoderm and endoderm. An example of pluripotent stem cell is the embryonic stem cells that constitute the inner cell mass of the blastocyst, which is formed at day 5-6 after fertilization.

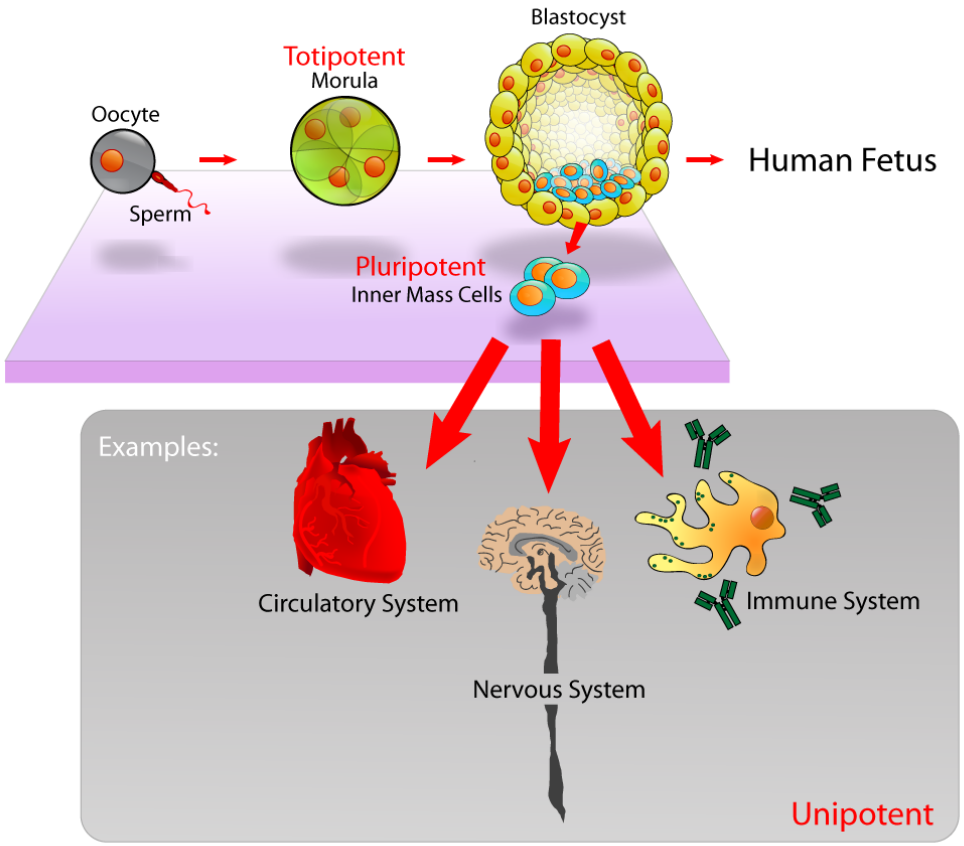
Note: What differentiates totipotent from pluripotent stem cells?

Totipotent cells can give rise to the whole organism and also extra-embryonic tissues (e.g. placenta) whereas are pluripotent cannot form extra-embryonic tissues. Since pluripotent cells cannot from placenta, these cannot develop into a fetus, whereas totipotent cell can.

c. ***Multipotent cells:*** Multipotent cells can give rise to certain discrete differentiated cell types, but are restricted in their cell potency as they cannot differentiate into all cell types. Examples of multipotent cells include mesenchymal stem cells (that can differentiate into various tissues like adipocytes, fibroblasts etc) and hematopoietic stem cells (which can differentiate into various blood cells of different lineages like lymphoid and myeloid lineage)

d. ***Oligopotent cells:*** These cells have a further restricted potency and can generate a few cell types compared to multipotent cells.(1) Eg. Lymphoid progenitor cells and myeloid progenitor cells.

e. ***Unipotent cells:*** These cells can give rise to only one cell type. Unipotent cells can self-renew, a property which is lacked by usual tissue cells.

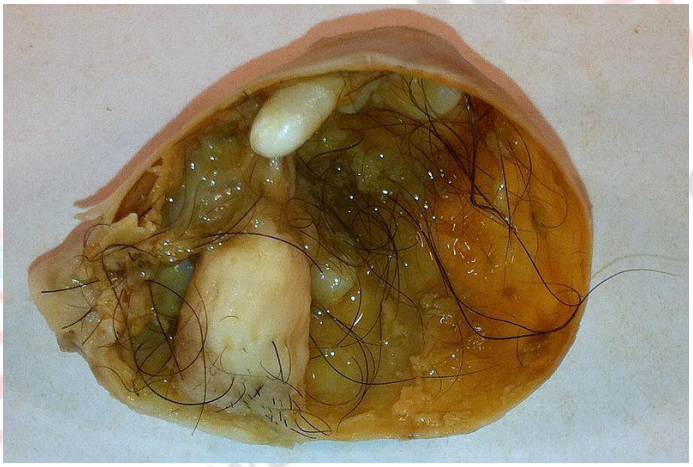


4. Clinical Aspects of Cell Potency:

a. Hematopoietic stem cell transplantation is a treatment modality in various disorders like leukemias, blood disorders and certain genetic disorders. In lysosomal storage disorders, hydrolytic enzymes that are involved in break-down of complex molecules are defective and this leads to dysfunction of lysosomes and eventually other organelles leading to cytotoxicity. The brunt of the attack is bore by macrophages which are the scavenging cells. Treatment for such disorders, till the advent of enzyme replacement therapy, included transplantation of hematopoietic stem cells, which ultimately differentiate into various lineages, including macrophages, so as to restore the normal cellular functions.

b. A similar approach is considered for gene therapy of single gene disorders. Here, the defective gene is corrected/ replaced in a hematopoietic cell which is later introduced back into the affected individual. This should enable a multitude of cells to express the normal protein.

c. Teratomas [Teras (*Greek)*: monster] are tumors that have components of more than one germ layer. These usually arise from pluripotent cells of testis or ovary or aberrantly migrated cells. Such malignant cells have the potential to differentiate into multiple adult type tissues and thus, can give rise to tissues like bone, hair, teeth etc.



d. Tissues are classified based on their proliferative capacity into:

i. Labile tissues
ii. Stable tissues
iii. Permanent tissues

Labile tissues are continuously replenished by a pool of tissue stem cells. Examples include blood cells, intestinal epithelium and skin. Such tissues have a high regenerative capacity after a tissue injury. Stable tissues have a minimal capacity of proliferation, and have a minimal capacity to regenerate following injury. Most of the organ parenchyma is composed of stable tissues. Permanent tissue have almost no proliferative capacity- example: neurons and cardiomyocytes. Thus injury to these tissues cannot be completely recovered, if the injury results in cell death. This emphasizes the concept of "golden hour" in diseases like stroke and myocardial infarction, where an immediate medical intervention can minimize tissue injury and thus prevent irreparable damage.

e. Histological grading of tumors:

Histological grading of tumors is based on the degree of differentiation observed in the tumor tissues microscopically. Tumors are usually classified into well/ moderately/ poorly differentiated or grade I/II/III/IV or low/high grade. Anaplasia refers to the lack of differentiation of tumor cells. Increased anaplasia is usually associated with a high aggressiveness of the tumor and a bad prognosis.

5. Induced pluripotency:

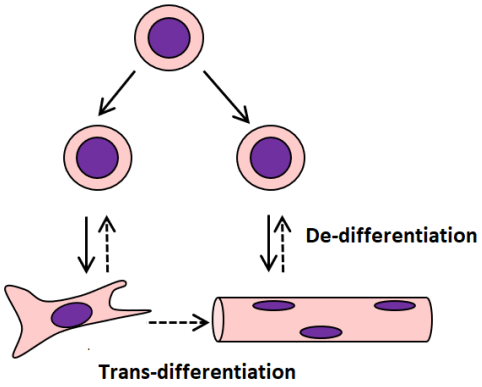
Development of an organism is associated with reversible changes in the epigenome.(2) Studies have shown that it is possible to reprogram adult tissue cells to pluripotent cells by inducing the expression of various transcription factors. At the turn of the millennium, Yamanaka and Takahashi identified 24 transcription factors that are related to pluripotency and screened these factors to eliminate redundant factors. They identified 4 genes that alone can induce pluripotency, the genes being *Oct4* (octamer-binding transcription factor 4), *Sox2* (sex determining region Y-box 2), *Klf4* (Kruppel like factor 4) and *cMyc* (Cellular myelocytomatosis viral oncogene). These are called Yamanaka factors. The induced pluripotent stem cells (iPSCs) thus formed could not be reprogrammed fully to an embryonic stem cell phenotype, and thus were called first generation iPSCs. The first generation iPSCs used a embryonic stem cell specific protein Fbx15 as the marker of pluripotency. In second generation iPSCs, the same set of transcription factors were used, but the selection marker was a different protein called Nanog, another embryonic stem cell specific protein. This lead to generation of cells that almost mimicked embryonic stem cells. Yamanaka was awarded Nobel Prize in Physiology/Medicine in 2012.

There were multiple ethical issues with regard to the usage of embryonic stem cells in research, as certain religious and scientific fronts presume that isolation of embryonic stem cells from human blastocyst involves destruction of life. iPSCs are considered to be an alternative to this. Another potential use of iPSCs could be in autologous transplantations where somatic cells from an individual can be used to generate iPSCs and then differentiated along various tissue lineages as required. Studies are ongoing in this regard.

A major caveat of induced pluripotency is that the transcription factors used in inducing pluripotency are products of oncogenes, and overexpression of their gene products might drive the cells into abnormal proliferation and development of malignancy. Despite such challenges, iPSC technology can have wide clinical applications and this is exemplified by the use of iPSCs to treat retinal degeneration, by developing retinal pigment epithelial cells, in March 2017 in Japan.(3)

6. Trans-differentiation and de-differentiation:

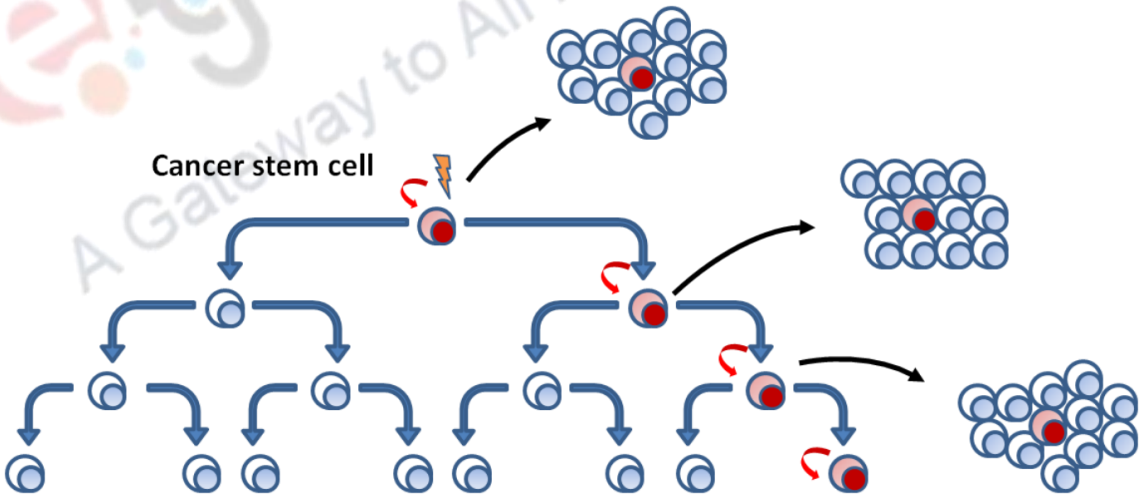
Apart from re-programming a tissue cell to a pluripotent cell using induced pluripotency, other aspects that are especially significant in regenerative medicine are trans-differentiation and de-differentiation. (4)



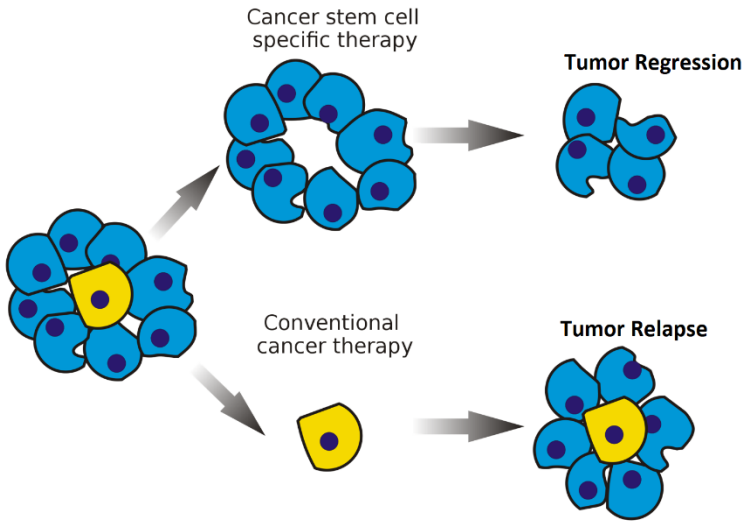
During de-differentiation, a terminally differentiated cell reverts back to a cell primitive in the line of differentiation, and this primitive cell has the potential to proliferate and thus give rise to the cell type these arose from. This is exemplified by Schwann cells present along the neurons. Mature Schwann cells do not proliferate, but once they are detached from the adjoining axon, they de-differentiate and express genes that enable proliferation.

In trans-differentiation, cells switch lineages to form a different cell type. This can be induced using various approaches and can lead to generation of closely related cells from a different lineage (example: macrophages and B-cells). Trans-differentiation should is generally preceded by de-differentiation into an early lineage cell, followed by activation of a new differentiation pathway leading onto generation of a different cell type.

Clinical aspects: Cancer stem cells (CSCs) or Tumor Initiating Cells (TICs) are the cells with stem cell like properties (i.e self renewal and ability to differentiate into tumor cells). The bulk of the tumor tissue is formed by the terminally differentiated tumor cells and Cancer Stem Cells usually comprise only about 1% of the tumor cells. However, due to their proliferation and differentiation capabilities, they can lead to generation of new tumor cells.



Conventional chemotherapy regimens used in the treatment of cancers target the tumor cells and can help in reducing the bulk of the tumor. However, CSCs are resistant to chemotherapy, and unless they are eliminated, new tumor cells will continue to form. Thus, CSCs are considered to be responsible for tumor relapse after treatment, and metastasis.



Two major hypotheses has been put forth to explain the development of Cancer Stem Cells. They could arise from mutations building up in tissue stem cells, which are oligopotent, thus retaining their self-renewal and differentiation capabilities, but driving differentiation along an aberrant pathway, leading to malignancy. The alternate hypothesis suggests that CSCs arise due to a de-differentiation of mature tissue cells, enabling them new capacities of proliferation and trans-differentiation.

Points to ponder:

Chronic myeloid leukemia (CML) was the first malignancy attributed due to a chromosomal abnormality as the molecular defect. It was also the first malignancy in which an abnormal tyrosine kinase (BCR-ABL fusion protein) was identified, and indeed the first malignancy in which a tyrosine kinase inhibitor (eg. Imatinib) was found to be effective. However, such patients require lifelong treatment with imatinib. Any attempt to stop the treatment can result in the relapse of the disease. Can you reason why?

Summary:

1. The process in which a cell changes from one form to another is known as cell differentiation. Differentiation is usually associated with increased functionality and division of labour.
2. The ability of a cell to differentiate into different cell types is known as cell potency. Cells can be totipotent, pluripotent, multipotent, oligopotent or unipotent.
3. Cell potency has various clinical correlations- eg. in stem cell transplant and tumor grading.
4. It is possible to reprogram adult tissue cells to pluripotent cells by inducing the expression of various transcription factors.
5. Sox2, Oct4, c-Myc and Klf4 are known as Yamanaka factors.
6. Trans-differentiation and de-differentiation has a potential application in regenerative medicine.
7. Cancer stem cells can derive from abnormal adult tissue cells and can lead to tumor propagation and chemo-resistance.
