SCRUTINIZING THE PIVOTAL ROLE OF STEM CELLS FOR REGULATORY FRAMEWORK BASED THERAPEUTIC CLONING PRACTICES

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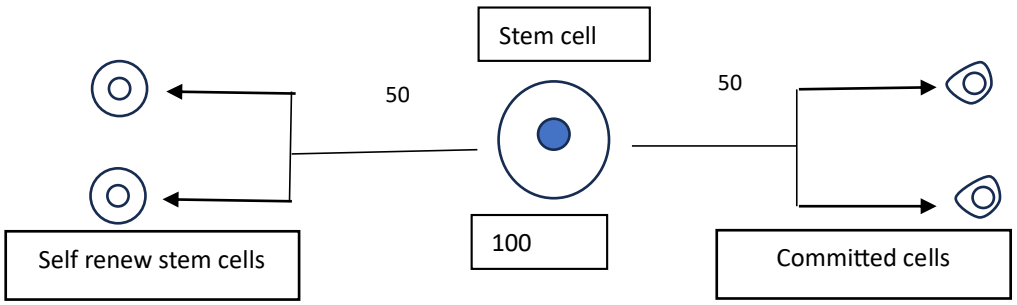
Abstract:- Stem cells- Stem cells are the cells that have capability to self renew, differentiate and have potency to generate multiple cell types (Totipotent, Pluripotent, Multipotent, Progenitor cell type) which play a vital role in development, regeneration and repairment of tissues. Stem cells have tendency to repair damaged tissues and organs, tissue engineering, disease modeling, drug development and therapies can be used in clinical application for treatment planning and diagnosis. In this paper, the study and diagnosis with ongoing research on this topic are also mentioned.

Keywords:- Phosphorylation, Acetylation, Methylation, Epigenetic Regulation, Transcriptional Regulation.

Introduction:- A stem cell play an important role in overall development, repairment of tissue and organs and have self renewing potency. Besides their classic function of repairment and development, it involved in multiple cell function. Followings are the models for Division and self-renewal in stem cells:-

a. Asymmetrical single cell model:- In this asymmetrical division, single cell divide into two types of cells; one becomes stem cell by renewing themself and other becomes committed cell and form differentiated cells.
b. Symmetric commitment division model:- It is also known as symmetrical differential or symmetrical renewing cell. Some stem cells have the ability to divide symmetrically to produce either two stem cells which increases the stem cell pool whereas other divide symmetrically into two committed cells which decreases the stem cell pool.



c. Tissue specific Stem cell lineage:- It developed after birth. It maintains tissue balance. It is present in restricted locations like bone marrow, intestine, skin, liver, muscles, brain etc. it compensate the cell lost by apoptosis, by necrosis or by senescence.

Discussion:- As development proceeds potency get restricted.

- Potency of stem cell- It is the ability of a single cell to form possible type of differentiated cells.

a. Totipotency:- They have the tendency of both the embryonic lineage (that form the somatic cell of the body) and extra embryonic lineage (that form the placenta, yolk sac) e.g. mammalian fertilized egg (zygote), first 4-8 cell stage.
b. Pluripotent:- They have the potency of only embryonic lineage and differentiate into almost any cell type in the body but not extra embryonic tissue like placenta e.g. embryonic stem cell and iPSCs.
   Pluripotency markers- OCT4, SOX2, NANOG
c. Multipotent stem cell:- They have the potency to differentiate into multiple cell types of its residing tissue but have restricted potency e.g. Hematopoietic stem cell- Myeloid and lymphoid progenitor cells.
   Neural stem cell- Adult brain
   Mesenchymal stem cell- Connective tissue like adipocytes, chondrocytes etc.
d. Progenitor stem cell:- These are the cells that have limited self-renewal commitment, specific lineage commitment and have tendency to differentiate into specific cell types. It is considered an intermediate stage between stem cells and fully differentiated cells e.g. Neural progenitor cells, Osteogenic cells.
e. Unipotent stem cell:- They have the ability to differentiate into only one specific cell type. They have self renew, limited differentiation potential property. They help in cell replacement, tissue specific function, tissue homeostasis etc. e.g. Sperm cell

Stem cell niche regulate whether the stem cell is in a state of quiescence (non-dividing), division or differentiation. It regulate stem cell behaviour.

- Extra-cellular mechanism:-

a. Physical mechanism- Mechanical forces due to differences in cell to cell and cell to matrix adhesion as well as cell density.
b. Chemical regulation- Secreted proteins from surrounding cells by endocrine, paracrine, juxtacrine mechanism keeps stem cells in uncommitted stage.

- Intra-cellular mechanism:-

a. Cytoplasmic determinants- Determine cell fate
b. Transcriptional factors- keep stem cell in quiescent or in proliferative stage.
c. Epigenetic modification- affect chromatin accessibility. It include acetylation, methylation, phosphorylation, glycosylation.

Various genes involved in reprogramming:-

i. OCT 3/ 4 & SOX2- Noggin gene expression- maintain pluripotent stem cell
ii. C-Myc- It include growth promoting transcriptional factor, acetylation, methylation, phosphorylation. It help in conversion of heterochromatin to euchromatin.
iii. KIF-4- It inhibit programmed cell death (apoptosis)

Various experiments have to be taken into consideration for stem cell regulation. It plays an important role in regulating the framework based therapeutic cloning.

Level of regulation of gene expression:-

i. Differential gene transcriptional level by RNA polymerase & by presence of activator and repressor- various chromatin modification occur there.
ii. Selective nuclear RNA processing- It include heterogenous RNA formation (HnRNA) it involve intron removal, 5' caping, 3'- poly-A-tail

iii. Transport out of nucleus- It involves various proteins RAN-GTP, Exportin, eIF- 4E binding protein (eukaryotic initiation factor- 4E binding protein).
iv. Selective messenger RNA translation- It involve inhibitory protein, antisense RNA, SiRNA, MiRNA.
v. Differential protein modification by protein folding and later carry out function- It involve glycosylation, phosphorylation, targeting.

Therapeutic cloning involve the transfer nucleus from a patient's somatic cells to an enucleated egg cell to create patient induced pluripotent stem cells. Various 4 recombinant viral gene responsible for this is SOX2, OCT4, KIF4 & c-Myc.

Viral infection to misexpress master regulatory transcription factors. Cultures of differentiated cells in a medium to again transform into pluripotent stem cells. Selection for antibiotic resistance restricts growth to only viral infected cells which result in culture contain iPS'c.

The cell where SOX2 gene expresses, OCT4, KIF4 gene expresses, their shape changes and they are referred to as induced pluripotent stem cells and further by giving intra-cellular and extra-cellular mechanism in order to form desired neuron, pancreatic cells, blood cells etc.

In somatic cell nuclear transfer, nucleus from an adult somatic cell is inoculated into an enucleated egg (oocyte), which is further provoke to divide, allowing for removal of stem cells that can be used for tissue engineering.

Diagnosis:- A technique (therapeutical cloning) is used to create genetically genetically identical stem cells for medical uses, is still in its infancy due to significant challenges. It involve somatic cells nuclear transfer to form new embryonic stem cell that are a genetic match to a patient. In 2025, the stem cell therapies seening increased in various significant areas like in neurodegenerative disorder, cancer treatment, cardio-vascular disease, tissue engineering etc. with the advancement in iPSc technology. Therapeutic cloning could provide personalized stem cells for operating various disease including degenerative condition, genetic disorder etc. with advancement in editing of gene, tissue engineering and nano-technology which may upgrade the capacity of therapeutic cloning and stem cell therapies.

Conclusion:- Research is ongoing to progress various therapies of using SCNT to operate the manufacture of undifferentiated cells for medicinal and curative uses. These cells were genetically modified in-vitro and then transferred back to the same animal to accurate the immunodeficiency. Therapeutic cloning is used to create stem cells from embryos which can help in treating different disease in patients. These cells can be used to model human disease and for develop new treatments, drugs and various cloning therapies.