Stem cell: Transition from Basics to Advanced Technology

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ABSTRACT

The capacity of stem cells to regenerate the damaged/ impaired tissues and subsequent organs has assimilated the focus of research fraternity in recent times. But, on the same note, this research area is plagued with objections and controversies owing to ethical issues of its derivation from human embryos. However, stem cell research enables us to understand the development of an organism from uni-cell as well as the mechanism behind damaged cell repairing. The multipotent functions of stem cell are the outcome of its complex interactome that is governed by diverse protein-protein interaction. Herein, we are eliciting the ancient origin of stem cell. Being the emerging area, the arising discoveries are as closely followed by the new scientific questions and subsequent solutions. One of the major questions raised in stem cell research is the identification of stem cell via specific stem cell marker. This review lists all different types of stem cell marker wheresome of these stem cell markers are also the transcription factors in its interactome like Sox2 and Oct4. The protein interactome of stem cell is mainly governed via transcription factors like Oct4, Sox2, Nanog, Klf4 and c-myc etc. Complex protein-protein interaction is associated with these transcription factors which ultimately plays a critical role in self renewal and differentiation ability of the stem cell. This review illuminates interactome of stem cell for reprogramming and therapeutic purpose. It also focuses on the complex signaling network that creates the interactome of stem cell and governs

the potency of stem cell for different therapeutic purpose.The stem cell markers that have been identified to be unique can be used to solve the identity crisis problem and reprogramming of stem cell.

Keyword: Stem cell, Oct4, Sox2, Nanog, Klf4

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Introduction

The usage of stem cells has garnered as much of excitement as controversies. Objections have been continually raised and pacified throughout the world. Amidst all this, the stem cell research is indeed helping to understand how a stem cell can transform into multitude of specialized functioning cells. This transformation is the core to ample of medical anomalies. Thus, an improvised understanding of this process of cell development shall lead to better solutions to adverse medical conditions. Through this review article, an attempt has been made to cover various aspects of stem cells viz. stem cell marker to solve the identity crisis problem of stem cell and analysis of its interactome. Interactome[1] is a set of molecular interaction in a stem cell that decides its fate.

Two major distinctive properties limited to stem cells make them unique. Firstly, the native stem cells prior to transformation as specialized cells undergo self renewal through mitosis for long period. Secondly, when exposed to some artificial conditions, they stimulate to turn into cells of specialized functioning viz. conversion to insulin producing cells in pancreas or neural cells of the brain. Stem cells hold equal importance for the adults as well as embryos. These cells in bone marrow, nervous tissue and muscles generate specialized cells to replace the damaged cells owing to trauma, disease and periodic wear and tear for adult tissues. While in embryo, stem cells differentiate into vivid specialized cells to make up kidneys, lungs, heart, brain and other tissues. Further studies into this field may lead to innumerous possibilities of using stem cells for drug testing and toxicity research as well as understanding the birth defects

and their causes. For converting these possibilities into effective tools of everyday use, extensive research is required so as to probe how stem cells remain non-specialized and self-renewed for long periods, recognize the signals triggered via specialization of stem cells from native state. To attain the aforesaid purpose, it is altogether necessary to develop an understanding of the basic properties of stem cells viz.embryonic origin of stem cell and its proliferation time *in vitro* as compared to stem cells originating from adults. It is also required to understand the factors that regulate stem cell proliferation and cell division in native undifferentiated state.

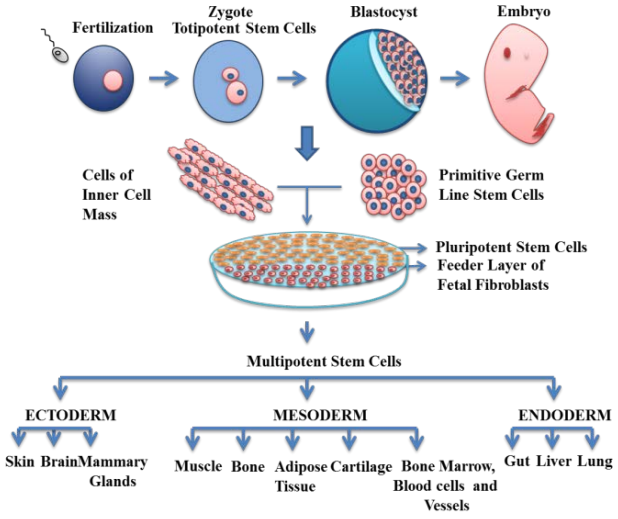

Fig1: Hierarchy of stem cell origin.

The signalling pathway which controls the proliferation and maintains the unspecialized state can embellish the *in vitro* growth and maintenance of unspecialized stem cells for research. Research lobby have just commenced to understand the cell signalling responsible for stem cell differentiation. Intra cellular signalling is found to be triggered by expression of certain genes that are scattered across DNA strands carrying information for structure and functions. Extra cellular signalling on the other hand is dependent upon biochemical molecules secreted byother cells and cell to cell physical contact.Stem cells in adults were observed to be specialized to the specific tissues where they are located. But, at the same time, experiments have revealed a characteristic: plasticity, wherein the stem cells located in a specific tissue are capable to transform into a completely different tissue. For instance, liver stem cells transformation into pancreatic cells. This indicates that the stem cell based therapies are not limited and requires further explorations.

Fertilization of a secondary oocyte by a spermatozoid results in a zygote formation which undergoes mitotic divisions to form blastocyst. Blastocyst further divides into extra embryonic layers and inner cell mass which serves as the originator of all tissues of an adult. Pluripotent stem cells can be isolated from inner cell mass or gonads of developing embryo using a feeder layer of fetal fibroblasts. Pluripotent stem cells further develops into multipotent stem cells that shows multipotency (E.g., Hematopoietic stem cells, Neuronal stem cells and Mesenchymal stem cells) as shown in **Figure 1**.There are two types of stem cell i.e embryonic stem cell and

adult stem cell: ESC can be isolated from embryo, which can self renew and expand in culture and adult stem cells are low in number and can be difficult to obtain. They expand readily in culture. It is important to compare the self renewal and differentiation ability of these two cells to avoid immune rejection.

Despite all the discussion, certain elementary objections need to be addressed before the application like, type of stem cells that migrate to various organs and repair tissues. These stem cells of multiple types or factors/cytokines that decide the migration of stem cell from bone marrow to target organs. A very important question is stem cell self renewal property is driven by exactly which signaling network or protein interactome and identified by which stem cell marker.

Indians have been claiming the origin and development of stem cell from our ancient times. In Mahabharata, Gandhari gave birth to 100 sons and one daughter via ancient sage cloning technology from the flesh of mass piece after miscarriage, as described in one of the chapters of Mahabharata named Adiparva. This indicates that in ancient time, the art of embryo splitting was known and also the development of baby outside mother womb, which is still an illusion for modern science.

In future, the modern stem cell technology needs to understand the process involved in development of babies outside mother's womb. This understanding will also help scientists to understand the technology of organ regeneration in laboratory for organ transplantation. The demand of organs for transplantation is high now and is going to increase over the period of time. Organ failure is a common problem which leads to death and demands the supply of organ regeneration.

Stem Cell Marker:

Each cell type is marked by unique characteristics that help in their isolation and segregation from the mixed cell population. Similarly, stem cells are identified based upon certain distinctive cell surface markers. Irrespective of the chosen tissue, stem cell isolation is a delicate and tedious multistep process. Protocols are tweaked based upon the source of stem cells or organism species.

This may include but not limited to stem cell isolation through gradient separation technique usinghistopaque or trypsin mediated enzymatic digestions. To facilitate a better understanding, isolation of stem cells from most common sources like umbilical cord fluid and bone marrow are elucidated:The expanded cells could then be characterized based on their differentiation markers.

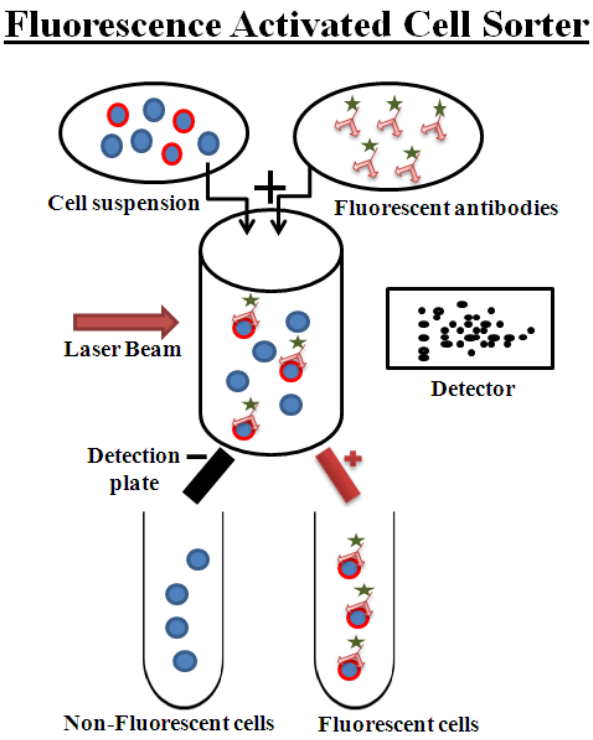

Figure 2: Fluorescence Activated Cell Sorting of stem cell.

The widely used techniques for the characterization of stem cells are FACS (as shown in **Figure 2**) and MACS as they allow the study of cells in viable condition.

Table 1: Stem Cell Marker

Marker Name	Cell Type	Significance	Reference
Nervous System			
CD133	Neural stem cell, HSC	Cell-surface protein Identifies neural stem cell Give rise to neurons & glial cells	[2]
GFAP (Glial fibrillary acidic protein)	Astrocyte	Produced protein	[3]
MAP-2 (Microtubule associated protein-2)	Neuron	Dendrite-specific MAP	[4]
Nestin	Neural progenitor	Intermediate filament structural protein expressed in primitive neural tissue	[5]
Noggin	Neuron	Neuron specific gene	[6]

Pancreas			
CK19 (Cytokeratin 19)	Pancreatic epithelium	Identifies specific pancreatic epithelial cells that are progenitor for islet cells & ductal cells	[7]
Glucagon	Pancreatic islet	Expressed by alpha-islet cell of pancreas	[8]
Insulin	Pancreatic islet	Expressed by beta-islet cell of pancreas	[9]
PDX-1 (Insulin – promoting factor-1)	Pancreatic islet	Transcription factor expressed by beta-islet cell of pancreas	[10]
Nestin	Pancreatic progenitor	Structural filament protein indicative of progenitor cell lines	[11]
Pancreatic polypeptide	Pancreatic islet	Expressed by gamma-islet cell of pancreas	[12]
Blood Vessel			
Flk1 (fetal liver kinase-1)	Endothelial	Cell surface receptor protein, Identifies endothelial cell progenitor, Marker of cell-cell contact	[13]
Vascular endothelial growth factor	Endothelial	Endothelial cell proliferation	[14]
Bone			
BAP (Bone-specific alkaline phosphatase)	Osteoblast	Enzyme expressed in osteoblast, Activity indicates bone formation	[15]
Hydroxyapatite	Osteoblast	Mineralized bone matrix, Provides structural integrity, Marker of bone formation	[16]
(OC) Osteocalcin	Osteoblast	Mineral-binding protein uniquely synthesised by osteoblast, Marker of bone formation	[17]
Cartilage			
Collagen types II & IV	Chondrocyte	Structural proteins produced specifically by chondrocyte	[18]
Keratin	Keratinocyte	Principal protein of skin, Identifies differentiated keratinocyte	[19]
Liver			
Albumin	Hepatocyte	Principal protein, Indicates functioning of maturing & fully differentiated hepatocyte	[20]
B-1 Integrin	Hepatocyte	Cell-adhesion molecule important in cell-cell interaction, Marker expressed during development of liver	[21]
Bone Marrow & Blood			
BMPR (Bone morphogenic protein	Mesenchymal stem	Important for the differentiation of committed mesenchymal cell types	[22]

receptor)	&progenitor cells	from mesenchymal , BMPR identifies early mesenchymal lineages	
CD4 & CD8	White blood cell (WBC)	Cell-surface protein markers specific for mature T lymphocyte (WBC subtype)	[23]
CD34	Hematopoietic stem cell (HSC), Satellite, Endothelial progenitor	Cell-surface protein on bone marrow cell, Indicative of a HSC & Endothelial progenitor, CD34 also identifies muscle satellite	[24]
CD44	Mesenchymal	A type of cell-adhesion molecule used to identify specific types of mesenchymal cells	[25]
Fat			
ALBP (Adipocyte lipid-binding protein)	Adipocyte	Lipid-binding protein	[26]
Skeletal Muscle/Cardiac/Smooth Muscle			
MyoD& Pax7	Myoblast, Myocyte	Transcription factor, Directs differentiation of myoblasts into mature myocytes	[27]
Myosin heavy chain	Cardiomyocyte	A component of structural & contractile protein	[28]
Myosin light chain	Skeletal myocyte	A component of structural & contractile protein	[29]
Pluripotent stem cells			
Alakaline phosphatase	Embryonic stem (ES), Embryonic carcinoma (EC)	Elevated expression leads undifferentiation of pluripotent stem cells	[30]
AFP (Alpha-fetoprotein)	Endoderm	Expressed during development of primitive endoderm, Leads endodermal differentiation of pluripotent stem cell	[31]
CD30	ES, EC	Surface receptor molecule. Found on PSC	[32]
GATA-4 gene	Endoderm	Expression increases as ES differentiates into endoderm	[33]
NCAM (Neuronal cell adhesion molecule)	Ectoderm	Cell-surface molecule, Promotes cell-cell interaction, Indicates primitive neuroectodermformatiom	[34]
OCT4/POU5F1	ES, EC	Transcription factor, Essential for establishment & maintenance of undifferentiated PSCs	[35]

SOX2	ES, EC	Transcription factor, Essential for establishment & maintenance of undifferentiated PSCs	[36]
Pax6	Ectoderm	Transcription factor expressed as ES cell differentiates into neuro epithelium	[37]

The various stem cell markers that help in their identification and characterization are listed in **Table 1.**There are different types of stem cell markers that help in identification of stem cell but still there is controversies regarding stem cell identity. The use of combination stem cell marker and its interactome analysis can help in solving the identity crisis of stem cell. The remarkable ability of this cell to self renew and differentiate is governed and regulated by its transcription factors.

Interactome of Stem Cells

Interactome is the combined interaction of protein within a cell. The protein interaction is quiet unique in specific cells. The governors and regulators of transcriptional regulatory network in stem cells are transcription factors like Sox-2[38, 39], Oct4[40], Nanog[41], Klf4[42] and c-myc[43]. Oct4 (Octamer Binding Transcription Factor-4), Sox2 (SRY Determining Region Y) Box-2, Nanog (Nanog homeobox). These transcription factors interact with a complex signalling network. We have dissected few common interacting partners like, Gli, β-catenin, SUMO1 and SUMO activating enzyme. These set of information indicates critical role of hedgehog and wnt pathway in stem cell self renewal and differentiation**.** SUMO is an acronym for small ubiquitin like modifier (SUMO).

Wnt pathway is a signal transduction pathway made of several proteins like frizzled, dishevelled, β -catenin etc.Wnt pathway plays a critical role in stem cell proliferation. Oct4 and nanog was reported to be novel target of wnt pathway. Gli is a transcription factor, which plays a critical role in activation of hedgehog pathway. The binding of two receptors smoothened and patched leads to inactivation of this pathway. When hedgehog ligand binds to its receptor patched then it leads to activation of this pathway and give signal for cell proliferation. Sox2 is a marker for Shh-dependent medulloblastomas.

Leukemia Inhibitory Factor(LIF) induces the terminal differentiation of leukemic cells. LIF binds to its receptor and leads to activation of JAK-STAT pathway (Janus Kinase and Signal Transducer and activator of Transcription 3). Nanog is a direct downstream effector of LIF-

STAT pathway. Removal of LIF pushes stem cell towards differentiation but they retain their pluripotency. JAK-STAT is essential to mediate the LIF pathway however it is unclear how these signals are linked to Oct4, Sox2 and Nanog.The MAPK/ERK pathway is a chain of protein in the cell that communicate signal from the receptor to the DNA. It activates the transcription factor c-myc, which interacts with the SUMO-activating enzymes and other proteins. It is a family of small proteins that are covalently attached to and detached from other proteins in cells to modify their function. It is a post translational modification which is involved in cellular process like, nuclear-cytosolic transport, transcriptional regulation, apoptosis and protein stability etc. SUMO is a small protein approx. 12 KDa which is attached to its target similar to ubiquitin. A C-terminal peptide is cleaved from SUMO by a protease. SUMO then becomes bound to an E1 enzyme (SAE) which is a heterodimer. SAE are a set of SUMO activating enzyme. Sumoylation is reversible via specific SUMO protease. Sumoylation is a highly dynamic process and their outcomes are diverse.

Wnt, Hh, Lif and mitogen receptor pathway plays a critical role in governing stem cell pluripotency. The exact linkage of these pathway with the governors and regulators of stem cell is not clear but studies suggest a direct role of these pathway in regulating stem cell pluripotency and differentiation. Sulfatase[44,45] is a protein on the cell surface which can modulate the binding of this ligand to its receptor and hence affect the function of these entire pathways in a direct or indirect manner.

Cellular reprogramming describes the process where a fully differentiated cell is induced to transform into a different cell lineage using variety of methods. These methods can be somatic cell nuclear transfer, cell-cell fusion or introduction of certain transcription factors like Oct4, Sox2, Klf4, c-myc. The combination of transcription factors has been drawn in **Figure 3.**

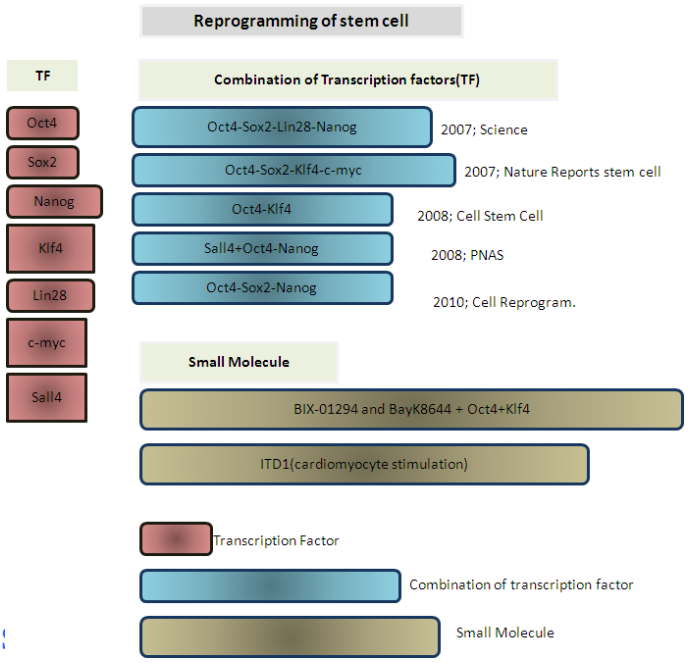

Figure 3: Reprogramming of stem cell.

In 2006, yamanaka lab identified these four factors as these factors caused the fibroblast to revert back to a pluripotent state. A year later these four factors were used to reprogram fibroblast to induced pluripotent stem cell (iPSC). Recently, three chemical factors have been injected into the damaged region

of a heart for cell regeneration. The fibroblasts take the factors and initiate the reprogramming of fibroblast into cardiac muscle cells. These chemical factors are, Gata4, Met2c and Tbx5 collectively known as GMT. These set of chemical factors were used by Dr. Deepak Srivastava at Gladstone institute in California.

Potential Challenge to future researchers: There are potential challenges in the field of stem cell, like identity crisis of stem cell. After solving this problem, there is danger of cell rejection that can be used for cell therapy. The designed stem cell therapy should be effective, less invasive with increased longivity and reduced threat after therapy.

Conclusion

Stem cells because of their two unique characteristics i.e. self-renewal and differentiation capacity are expected to serve as a promising therapy in a field of regenerative medicine to cure many degenerative disorders like Diabetes mellitus, Liver cirrhosis and Parkinson's disease. Stem cell therapy can be a good alternative in curing diseases caused by multi drug resistance infectious agents as it is a natural based therapy with immunomodulatory ability with fewer or no side-effects. This makes stem cells a unicellular miracle. However stem cell therapy poses many challenges and yet is in the experimental stage. One of the major challenges of stem cell is the identity crisis of stem cell. It is difficult to identify stem cell due to lack of specific cell surface markers and interactome analysis. In future as the information explosion occurs from all direction regarding specific stem cell marker and its specific interactome, then identity crisis problem of stem cell can be solved.

Abbreviation: Oct4, Sox2, Nanog

Competing Interest: The authors declare no financial and intellectual competing interest.

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