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# STEM CELLS: A BRIEF PROGRESSIVE OVERVIEW AND FUTURE PROSPECTS

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## ABSTRACT

Cell therapy is a new technology that holds great promise for the treatment of many severe diseases. For the past few decades, researchers have been focusing on special cells used for cell therapy known as stem cells. Stem cells have great potential of proliferation and differentiation and high self renewable capacity. Stem cells are the body's specialized cells from which all other cells with specialized functions are generated. Under the appropriate conditions in the body or in the laboratory, stem cells divide to form more cells called daughter cells which have greater potential either of becoming new stem cells (self-renewal) or specialized cells (differentiation) with a more specific function, such as blood cells, brain cells, heart cells, muscle cells, nerve cells, skin cells, pancreatic cells or bone. Recently successful genetical reprogramming of adult cells have been done to produce induced pluripotent stem cell (iPS) that are very similar to embryonic stem cell.

**KEYWORDS:** **Stem cells, iPS, RNAi, Pluripotency, Niche.**

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# INTRODUCTION

Cell is the basic and the smallest structural unit of living matter and the functional basic unit of life. The human body is composed of 100 trillion cells and each cell is assigned a specialized function to perform. Before becoming specialized some cells have property to form any cell of the body and these cells are called as stem cells. The use of stem cells in the treatment of diseases like Alzheimer's diseases, spinal cord injury, stroke, burns, heart disease, diabetes, osteoarthritis, and rheumatoid arthritis are making these cells more potent. This review mainly focuses on our current understanding about the stem cell, generation of iPS cell and it also highlight present and future applications of stem cells in the field of bio medical science. In this article role of RNAi in maintaining stem cell properties has been discussed.

## *BASICS*

### A) *What are Stem Cells?*

In early 1960s, Ernest A. McCulloch and James E. Till started several experiments leading to the discovery of stem cells. When they injected bone marrow cells into irradiated mice, nodules developed in the spleens. They concluded that each nodule arose from a single marrow cell. Later on, they obtained evidence that these cells were capable of infinite self-renewal, a central characteristic of stem cells[1]. Stem cells are distinguished from other cell types by number of properties like self-renewal and differentiation[2] including the ability to undergo asymmetric cell divisions, exist in a mitotically quiescent form, and clonally regenerate all of the different cell types that constitute the tissue in which they exist [3,4]. There are two main types of stem cells, embryonic and non-embryonic. Embryonic stem cells (ESC) are pluripotent and they can differentiate into all germ layers (ectoderm, mesoderm and endoderm). Non-embryonic stem cells (non-ESC) are multipotent. Their potential to differentiate into different cell types seems to be more limited (Table 1). The capability for potency and the relative ease to isolate and expand these cells are invaluable properties for regenerative medicine.

**Table 1**
***Stem cell related definitions.***

| | |
|---|---|
| **Stem cell** | A cell with the ability for self-renewal and differentiation. |
| **Self-renewal** | Asymmetric cell division which leads to at least one daughter cell which is equal to the mother cell. |
| **Totipotency** | Ability to form whole organism, e.g. zygote |
| **Pluripotency** | Ability to form all germ layers i.e. ectoderm, mesoderm and endoderm, e.g. embryonic stem cells. |
| **Multipotency** | Ability to form multiple cell lineages which form an entire tissue, usually specific to one germ layer (ectoderm,mesoderm,endoderm), e.g. adult stem cells. |
| **Niche** | Cellular microenvironment providing support and stimuli to control stem cell properties. |

### B) *Stem Cell Niche*

Microenvironment within an organism where stem cells reside generally called stem cell niches. It consists of signaling molecule, cell to cell contact, cell to ECM contact, growth factors, pH. This concept was first proposed by Schofield almost 30 years ago[5]. This three-dimensional (3D) microenvironment is thought to control genes and specific properties of stem cell, i.e. its self-renewal and development to committed cells[6]. Stem cells implanted into a totally different niche can potentially differentiate into cell types of the new environment[7]. For example, human neuronal stem cells produced muscle cells when they were implanted in skeletal muscle[8]. Bone marrow cells differentiated into neuronal cells when they were transplanted into a neural environment[9,10].

C) ***Stem cell Types***

Embryonic, adult, and induced pluripotent stem cells are the three major branches of stem cell research (Fig.1). As the name suggests, embryonic stem cells are from embryos and adult cells are from adults. Induced pluripotent are adult stem cells that have been genetically altered by introducing some genes, so that they have many of the properties of embryonic stem cells. Likewise, investigation is underway in the area of cells of amniotic fluid and umbilical cord blood.

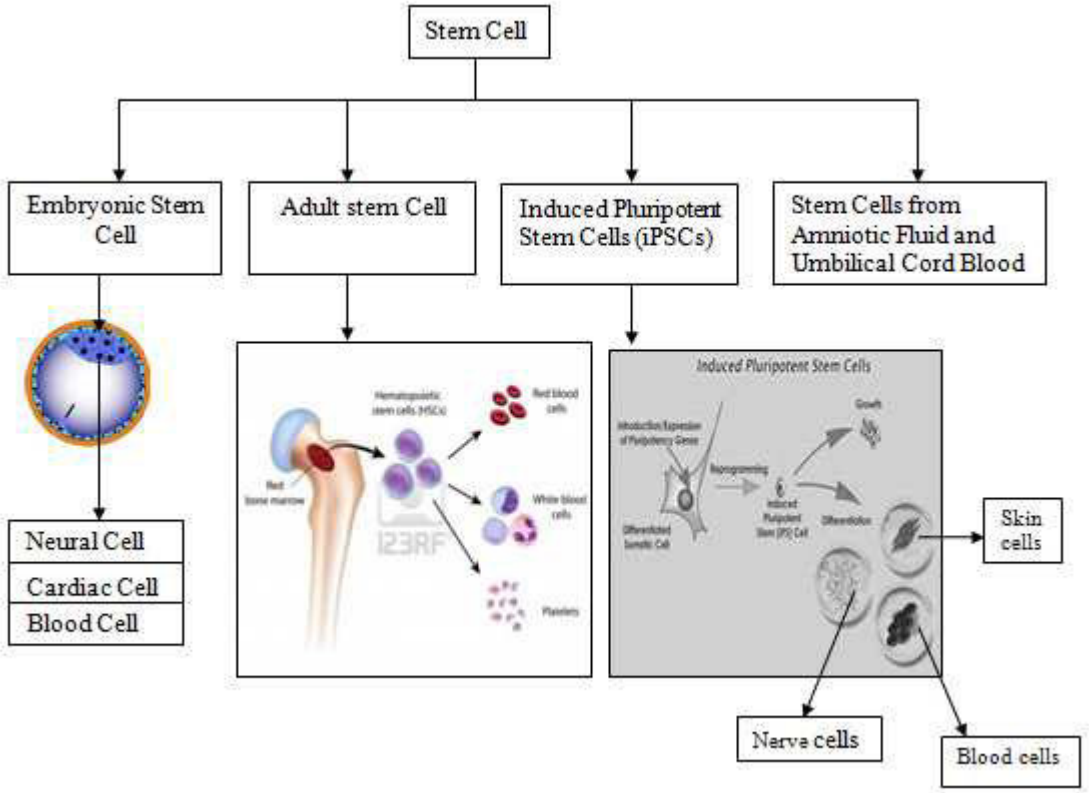


**Figure 1**

***Classification of stem cells and formation of different forms of cells from stem cell.***

***1. Embryonic Stem Cells***

Embryonic stem cells (ES cells) are pluripotent stem cells derived from the inner cell mass of a blastocyst (a structure formed 4-5 days of post fertilization consisting of 50-150 cells) of an early-stage embryo. ES cells have the ability to form any fully differentiated cell of the body and characterized by their self-renewal capacity and the ability to retain their developmental capacity in vivo and in vitro. They have some special requirements to maintain their undifferentiated state such as the presence of feeder cells, serum and the presence of leukemia inhibitory factor (LIF)[11]. Embryonic stem cells (ES cells) differentiate in suspension culture and form spherical structures termed embryoid bodies (EBs). These aggregated structures contain derivatives of all three embryonic germ layers (ectoderm, mesoderm and endoderm)[12].In a study carried out by Maya Schuldineet et al. in 2000 by giving eight growth factors [basic fibroblast growth factor (bFGF), transforming growth factor β1 (TGF-β1), activin-A, bone morphogenic protein 4 (BMP-4), hepatocyte growth factor (HGF), epidermal growth factor (EGF), β nerve growth factor (βNGF), and retinoic acid] to ES cell in vitro showed expression of 24 cell-specific molecular markers covering all embryonic germ layers and 11 different tissues. They divided overall effects of these factors into three categories: a). growth factors (Activin-A and TGFβ1) inducing mainly

the mesodermal cells; b). factors (retinoic acid, EGF, BMP-4, and bFGF) that activate ectodermal and mesodermal markers; and c). factors (NGF and HGF) that allow differentiation into the three embryonic germ layers. These results showed the effect of different cellular factors on the ES cells faith determination and also provide some indication about stem cell niche.

### 2. *Mesenchymal stem cells*

The idea of mesenchymal stem cell was first proposed by Caplan[13]. Mesenchymal stem cells or multipotent stromal cells (MSCs) traditionally isolated from the bone marrow of adult organisms can also be isolated from other tissues including skeletal muscle, adipose tissue, cord blood, peripheral blood, fallopian tube, and fetal liver and lung. MSCs are typically defined as adherent, fibroblastoid-like cells that differentiate to osteoblasts, adipocytes, and chondrocytes in vitro[14]. MSCs can generate the stromal component of neural cells (by increasing cAMP signaling MSC cells)[15–16], bone marrow adherent populations of MSC contain cells that express adhesion molecules[17] and cytokines[18] that regulate aspects of hematopoiesis[19]. MSC can form various cell lineage despite of this, MSC populations commonly express a number of surface receptors like CD29, CD44, CD49a-f, CD51, CD73, CD105, CD106, CD166, and these cells also lack expression of definitive hematopoietic lineage markers including CD11b, CD14, and CD4.

### 3. *Induced Pluripotent Stem Cells (iPS)*

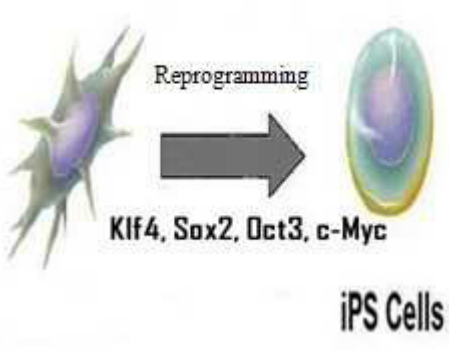


**Figure 2**
***Generation of iPS cells by transferring Oct4, sox2, Klf4 and cMyc.***

Induced pluripotent stem (iPS) cells are generated by reprogramming a differentiated somatic cell into a pluripotent ES cell[20,21]. These iPS cells are identical to human ES cells and have the ability to become every cell type in the human body. Creation of iPS cells has paved a way to reprogram a cell in its somatic state back to its embryonic state. These cells express genes and surface proteins similar to ES cells and are capable of forming teratomas (cell mass) which can develop into all three germ layers. These promising results, suggest that iPS cell may be used to replace certain disease damaged tissues or to study diseased cells towards developing a treatment[22].

***Generation of iPS Cells***

Different methods (viral vectors, plasmid transformation, direct reprogramming Table:2) have been used to transfer transcription factors (Oct4, Sox2, c-myc, Klf4) to induce reprogramming of mouse fibroblasts (Fig.2), neural stem cells, neural progenitor cells, keratinocytes, B lymphocytes and meningeal membrane cells towards pluripotency (Table:3).

Human fibroblasts, neural cells, blood and keratinocytes have also been reprogrammed towards pluripotency. Once a somatic cell is introduced with these factors its phenotype transforms to a partially reprogrammed state[23,24]. Studies have shown that c-myc proteins may loosen chromatin structure of somatic cells, thus rendering them a property similar to pluripotent cells[25]. This structure allows for Oct4 and Sox2 to bind to their target genes and the addition of Klf4 assists them to initiate a key set of ES cell genes in somatic cells. Oct4 and Sox2 then establish an autoregulatory loop which maintains this pluripotent state in somatic cells[26].

**Table 2**
***iPS induction methods in human fibroblast***

| Type of vector | Method | Genomic integration | Efficiency in human fibroblast |
|---|---|---|---|
| **Virus** | Retrovirus | Yes | >0.1% |
| | Lentivirus | Yes | <0.1% |
| | Adenovirus | No | <0.001% |
| | Sendai virus | No | >0.1% |
| **DNA** | Episomal plasmid | No | <0.001% |
| | transposon | No | <0.01% |
| **RNA** | RNA | No | <0.1% |

**Table 3**
***iPS Cell generation: Somatic cell source and reprogramming factors***

| Species | Somatic cell Source | Reprogramming factor |
|---|---|---|
| **Human** | Fibroblast | Oct3/4, Sox2, Klf4, c-Myc, Nanog |
| | Amnion-derived cells | Oct4, Sox2, Nanog |
| | Keratinocytes, Hepatocytes | Oct4, Sox2, Klf4, c-Myc |
| | Neural Stem Cells | Oct4 |
| **Mouse** | Liver cells, Neural progenitor cells, B lymphocytes, Melanocytes | Oct4, Sox2, Klf4, c-Myc |
| | Melanoma cells | Oct4, Klf4, c-Myc |

### *Limitations for iPS clinical applicability*

iPS cells offer enormous clinical potential, although some drawbacks are also asociated like risks of tumor formation. It has been demonstrated in the mouse system that iPS cells-derived chimeras frequently develop tumors resulting from the reactivation of the oncogenes c-Myc and Klf4[27,28]. To avoid these problems, iPS cells have also been generated from mouse and human fibroblasts without oncogenes c-Myc and Klf4[29]. The use of genome integrative methods, such as retroviral/lentiviral vectors, may also cause tumor formation because of integration in to genome. Generation of iPS cells without viral integration has proved possible in mouse hepatocytes using adenovirus[30], however, the frequency of reprogramming was extremely low and a high percentage of clones were tetraploid. iPS cells have also been generated by nucleofection of a polycistronic construct co-expressing Oct4, Sox2, Klf4 and c-Myc with no evidence of integration in the host genome[31]. iPS cells have also been derived from the genomic integration of the four reprogramming factors using plasmids[32], lentiviruses or transposons followed by transgene removal with Cre-mediated excision or the re-expression of transposase[33]. In brief, safer, high efficient and non-integrative methods should be used for generating iPS cells. Moreover, before iPS cells prove suitable for application in regenerative

medicine, the differentiation efficiency of iPS cells into required functional cells should be enhanced by improving differentiation protocols as well as defining the best chemical composition of differentiation factors. Another important aspect to consider is the choice of the reprogramming target cell population. Utilization of somatic cells from easily accessible sources which do not imply invasive methods should be preferred, along with cells that do not produce an immune rejection after transplantation due to the immunological incompatibility between patient and donor cells

### *Micro RNA and Stem Cells*

RNA interference (RNAi) is an apparently ancient defense mechanism against foreign double- stranded RNA (dsRNA). RNAs of just 21-22 nucleotides in length[34], called small interfering RNAs (siRNAs), are snipped from longer dsRNA chains by an enzyme called Dicer. The antisense strand of the siRNA is used by an RNA induced silencing complex (RISC) to guide messenger RNA(mRNA) cleavage, promoting mRNA degradation by binding to its complementary sequence within the 3' untranslated regions (UTRs) of its target mRNAs. They have been implicated in the regulation of many biological processes, including the stem cells self-renewal and pluripotency[35,36.] In ES cells a set of microRNAs miR-302 and miR-17–92 and let7 closely interfere with the key pluripotency factors such as Oct4, Sox2, Nanog and Lin 28[37,38] thereby preventing them from differentiation and controlling their proper self-renewal potential[39]. It has demonstrated miR-145 to control the expression of Oct4, Sox2, and Klf4 and repress self-renewal of human ES cells[40]. On the other hand, c-Myc has been reported to repress miRNAs such as miR-21, let-7a, and miR-29a during reprogramming[41]. Let7 miRNAs have been demonstrated to repress a broad range of targets, including Lin28, cMyc, Sall4, and downstream effectors of the pluripotency genes NANOG, OCT4. miR200, miR34 etc epigenetically regulate cancer stem cell and SOX2 Cells[42] (Table 4). More importantly, microRNAs proved to be effective tools for the iPS generation particularly, inhibition of miR-21, let-7a, or mir-29a has been shown to enhance the reprogramming efficiency.

**Table 4**
***miRNA and their involvement in different type of cancer***

| miRNA | Type of Cancer |
|---|---|
| miR200 | Breast carcinoma |
| Lin28B | Colon Adenocarcinoma |
| miR200, miR200c | Lung Cancer |
| miR34 | Pancreatic Cancer |
| miR200, miR429 | Ovarian cancer |

### *How stem cells can help us*

Current research is focusing on how stem cells may be used to prevent or cure diseases and injuries such as Parkinson's disease, type 1 diabetes, cardiovascular disease (CVD), which includes coronary heart disease, stroke, and congestive heart failure, leukemia, lymphoma, other blood disorders, spinal cord injury, Alzheimer's disease, strokes, burns, osteoarthritis, rheumatoid arthritis, vision, and hearing loss. The term regenerative medicine is used for the power of stem cells and the body's own regenerative capabilities to restore function of damaged cells, tissues and organs. Central nervous system (CNS) does not regenerate itself, and in this case stem cell therapy has become a possible solution. A variety of embryonic and adult stem cells have been implanted in a rat model of spinal cord injury (cultured spinal cord stem cells, bone marrow derived stem cells etc.) and improved locomotory recovery and cure was noticed. Stem cells are also useful in replacing damaged tissue in burn victims. Stem cell therapy is seen

as a highly promising area for type 1diabetes, caused by autoimmune destruction of insulin-producing beta cells in the pancreas. Neural stem cells (NSCs) have been transplanted in different animal models as a tool for the cure of neurodegenerative diseases[43,44]. Transplantation experiments in animal models of brain lesions or neurodegenerations have revealed that NSCs are capable of integrating into the host brain and ameliorate functional defects. Stem cells grown in the laboratory may be useful for testing drugs, chemicals and toxins before they are trialed in people. This may make drug testing safer, cheaper and more ethically acceptable to those who oppose the use of animals in pharmaceutical testing.

***Alzheimer's disease and stem cell***

Alzheimer's disease (AD) is the leading cause of age-related dementia, affecting over 5 million people in the U.S. alone and it is said that by 2050 as many as 115 million people worldwide will have developed symptoms of AD. In this disease patients suffer from progressive neurodegeneration that gradually impairs their memory, ability to learn, and carry out daily activities. Main reasons behind the AD disease is the loss of neurons and synaptic connectivity due to accumulation of β-amyloid (Aβ) peptide which leads to neurofibrillary tangles, neuronal and synaptic loss, and cognitive dysfunction[45,46] and decrease in secretion of neurotrophins[47] like brain derived neurotrophic factor (BDNF) and nerve growth factor (NGF) which play critical functions in synaptic plasticity is also seen in AD patients. By experiment it is seen that neural stem cells (NSCs) can express high levels of neurotrophins including both BDNF and NGF[48-49]. Other stem cell populations including mesenchymal stem cells (MSCs) and embryonic stem cells (ESCs) can also produce several neurotrophins[50,51]. It is also seen that NSC have capability to migrate throughout the adult brain and localize to areas of injury and inflammation due to which they can help to deliver therapeutic proteins like neprilysin (enzymes that degrade Aβ) specifically to those brain regions most affected by disease.

***Stem cell research and ethics***

There are pluses and minuses associated with the research and use of all types of stem cells. The overwhelming objection to stem cell research is due to destruction of fetus or embryo for stem cell isolation and due to this there are many conflicts with religious and moral views held in our society. The *first ethical problem* is: Is it good to produce or use living human embryos for the preparation of ES cells. *Second ethical problem*: Is it right to clone embryo in lab and then destroying them in order to produce ES cells? *Third ethical problem*: Is it right to use ES cells, and the differentiated cells obtained after destroying the embryo, and commercializing to researchers? Possibility of using adult stem cells and iPS cells somewhat address this problem and possibly will help to attain the same goals as would be sought with embryonic stem cells.

## CONCLUSION

This review gives a brief insight about stem cells and how an interesting cell type is the induced pluripotent stem cell (iPSC) cells derived from non pluripotent cells. This review also shows the importance of stem cell to overcome many diseases and to screen effective and safe drugs, as well as to treat patients through the cell transplantation therapy. So hopefully in future stem cells will play an important role in regenerative medicine.

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