

## **Stem Cell: Pluripotent Cell or Reserve cell?**

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**Abstract:** *Stem cells have the remarkable properties of developing into a variety of cell types in the human body. Two basic characteristics of stem pluripotent cells are their capacity for self-renewal and multi lineage differentiation. Since then, some advances have been made towards understanding the basic biology of stemness and their differentiation into different cell lineages, but harnessing of their promised potential to usher in the era of regenerative medicine is still a long way to go. The medical community is now researching and developing the ability to use stem cells in the battle against many types of cancer. It has been shown that the transplant of stem cells into cancerous regions of the body results in the formation of cells that target and attach themselves to the cancerous cells and can trigger cell death, eventually reducing the number of cancer cells and size of the tumor as the process repeats many times. This review is aimed at revisiting classification of stem cell with enlightenment in newer concepts and ongoing related controversies.*

**Keywords:** *Pluripotent stem cell, hematopoietic stem cell, cancer stem cell, ethics, induced pluripotent stem cell.*

### **Introduction**

Stem cell biology has attracted tremendous interest recently. The word “stem” actually originated from old botanical monographs from the same terminology as the stems of plants, where stem cells were demonstrated in the apical root and shoot meristems that were responsible for the regenerative competence of plants.<sup>1</sup>

Several varieties of stem cells have been isolated and identified in vivo and in vitro. Very broadly they comprise of two major classes: embryonic/fetal stem cells and adult stem cells. Some scientists wish to pursue research on embryonic/fetal stem cells because of their versatility and pluripotentiality, while others prefer to pursue research on adult stem cells because of the controversial ethical sensitivities behind embryonic/fetal stem cells. It is their ability to self-renew and differentiate that certain cells are termed stem cells both *in vivo* and *in vitro*. It is very crucial that the correct definition and proof of *stemness* through proper and accepted characterization tests be addressed before a particular cell type is classified as a stem cell. The challenging use of stem cells would come from the directed differentiation or trans-differentiation of stem cells into other cells and tissues to help cure a plethora of incurable diseases.<sup>2</sup> Advances in the understanding as to how embryonic stem cells differentiate should provide answers for re-programming of stem cells from adult tissues.<sup>3</sup>

As stem cells have come to the forefront of medical research, the ethical controversies over embryonic stem cells have become prominent.<sup>4</sup>

Directing stem cell to a goal of treatment or to understand underlying pathogenesis of concerned lesion or disorder had forced us to step in field of stem cell research. Several clinical trials have been carried out using autologous or allogeneic CD34+ve hematopoietic stem cells or mesenchymal stem cells (MSCs) in a variety of clinical indications but most of these have been Phase I or early Phase II trials.<sup>5,6</sup>

## Definition

Stem cells are unspecialized cells in the human body that are capable of becoming specialized cells, each with new specialized cell functions. Basically, a stem cell remains uncommitted until it receives a signal to develop into a specialized cell. When stem cells divide, each new cell has the potential to either remain as a stem cell or become another cell type with new special functions, such as blood cells, brain cells, etc. By definition, a progenitor cell lies in between a stem cell and a terminally differentiated cell. Some vertebrates such as salamanders regenerate lost body parts through the dedifferentiation of specialized cells into precursor cells.<sup>7</sup>

Totipotent stem cells can grow into any other cell type. Only a fertilized egg and the cells produced by the first few divisions of an embryo are totipotent. Pluripotent stem cells can grow into any cell type except for totipotent stem cells. Multipotent stem cells can only grow into cells of a closely related cell family. Unipotent stem cells are similar to normal cells in the body, but unlike a regular cell, they are considered stem cells because they are self-replicating. An example of Unipotent stem cell is epithelial stem cells.<sup>8</sup>

## Classification

Stem cells can be classified into four broad types based on their origin, viz. stem cells from embryos; stem cells from the fetus; stem cells from the umbilical cord; and stem cells from adult. Adult and fetal stem cells evolved from embryonic stem cells and the few stem cells observed in adult organs are the remnants of original embryonic stem cells that gave up in the race to differentiate into developing organs or remained in cell niches in the organs which are called upon for repair during tissue injury.<sup>8</sup>

Adult stem cells are partially undifferentiated cells located among the specialized cells of many organs and tissues. They are found all over the body, in the brain, liver, bone marrow, skeletal muscle, dental pulp, and even fat. These stem cells are also found in children and in umbilical cord blood, so the term *somatic stem cell* is more accurate although less frequently used.<sup>9</sup>

In mammals, the fertilized oocyte, zygote, 2-cell, 4-cell, 8-cell and morula resulting from cleavage of the early embryo are examples of totipotent cells. The fertilized oocyte and blastomeres cannot be termed "stem cells" because the making of them is limited during early cleavage division. They cannot self-renew even though they have the potential to form a complete organism. The inner cell mass (ICM) of the 5- to 6-day old human blastocyst is the source of pluripotent embryonic stem cells (hESCs). Embryonic endoderm cells are rather restricted in their developmental pathways. A small population of multipotent cells, called the definitive endoderm, gives rise to all of the endoderm derived organs in the adult. The definitive endoderm is separated from the pluripotent inner cell mass (ICM) during gastrulation immediately after implantation.<sup>10</sup>

Human embryonic germ cells (hEGCs) which are also stem cells, originate from the primordial germ cells of the gonadal ridge of 5- to 9-week old fetuses. These hEGCs have been successfully isolated and characterized. These stem cells are pluripotent and are able to produce cells of all three germ layers. Fetal stem cells are primitive cell types found in the organs of fetuses. Neural crest stem cells, fetal hematopoietic stem cells and pancreatic islet progenitors have been isolated in abortuses. Fetal neural stem cells found in the fetal brain were shown to differentiate into both neurons and glial cells. Umbilical cord blood contains circulating stem cells and the cellular contents of umbilical cord blood appear to be quite distinct from those of bone marrow and adult peripheral blood. Cord blood shows decreased graft versus host reaction compared with bone marrow, possibly due to high interleukin-10 levels produced by the cells and/or decreased expression of the beta-2-microglobulin.

Wharton's jelly has been a source for isolation of mesenchymal stem cells. These cells express typical stem cell markers, such as c-kit and high telomerase activity; have been propagated for long population doubling times; and can be induced to differentiate *in vitro* into neurons.<sup>11</sup>

Bone marrow possesses stem cells that are hematopoietic and mesenchymal in origin. The hematopoietic stem cell is derived early in embryogenesis from mesoderm and becomes deposited in very specific hematopoietic sites within the embryo. Hematopoietic stem cells can be purified using monoclonal antibodies, and recently, common lymphoid progenitor and myeloid-erythroid progenitor cells have been isolated and characterized. Bone marrow stem cells may be more plastic and versatile than expected because they are multipotent and can be differentiated into many cell types both *in vitro* and *in vivo*. Mesenchymal stem cells (MSCs) are found postnatally in the non-hematopoietic bone marrow stroma. MSCs are multipotent cells that are capable of differentiating into different tissues and cell lines like cartilage, bone, muscle, tendon, ligament and fat. The authors have called this a Multipotent Adult Progenitor Cell.<sup>12,13</sup>

The gastrointestinal epithelial lining undergoes continuous and rapid renewal throughout life. Epithelial renewal is sustained with populations of multipotent stem cells residing in distinct anatomic sites governed by niches. Epithelial cell renewal in the intestine is sustained by multipotent stem cells located in the crypts of Lieberkhun.. Mammals are said to survive surgical removal of at least 75% of the liver by regeneration. Stem cells differentiate into an intermediate cell which is called as "transient amplifying cell" which gives rise to the more differentiated cell types inclusive of the keratinocytes and saebocytes. Stem cells have been recently identified in the adult mouse eye. The adult retinal stem cells were localized to the pigmentary ciliary margin and not to the central and peripheral retinal pigmented epithelium.<sup>14, 15, 16, 17</sup>

### **Controversy Surrounding Stem Cells<sup>18</sup>**

(Like many innovations, stem cell research also involves scientific, ethical and social issues.)

A major advantage of adult stem cells is that they can be taken from a patient, grown in culture, and can be put back into the patient. Thus, the risk of transplant rejection by a patient's immune system is very low. This method avoids many ethical issues associated with using embryonic stem cells. Adult stem cells currently pose many disadvantages as well. They are few in number, difficult to isolate, and sometimes tricky to grow. They may also contain more DNA abnormalities than do embryonic stem cells. Most embryonic stem cells come from donated embryos grown in a clinic. These embryos are the result of *in vitro* fertilization, a process by which eggs are fertilized outside a woman's body. The stem cells are taken from a cluster of undifferentiated cells in the three-to-five-day-old embryo. There exists a widespread controversy over human embryonic stem cell research. The questions at the center of the controversy concern the nature of early human life and the legal status of the human embryo. Embryonic stem cell research often involves removing the inner cell mass from excess blastocysts that are unneeded by couples who have completed their fertility treatment. Although such blastocysts would likely be discarded by the clinics in any case, some believe that this does not make it morally acceptable to use them for research or therapeutic purposes. Some cultures and religious traditions oppose the use of human life as a means to some other end, no matter how noble that end might be. Catholics are against all ES and EG research, while Muslims and Jews feel that these areas of interest can be explored in a morally upright fashion, so long as the research saves human lives. The Islamic faith does not have an official position concerning stem cell research. Buddhists traditionally believe that life begins at conception. They believe that conception requires three things. The first condition is a woman's fertile period. The second condition is it requires sex. The last requirement for conception is a 'being to be born', that is ready for life. Because of these beliefs, Buddhists are often against *in vitro* fertilization. Hindus believe that life begins at conception. They do not believe that an embryo or fetus is growing into a human, but rather that the embryo is a full-fledged human

being. Their religion teaches that abortion is killing, and that it is among the worst of crimes. This belief is contradicted by the cultural preference for male children and the practice of selected abortion of female embryos. This practice is carried out because some feel that the burden of having a female child is greater than the wrongful killing a child. This is not meant to imply that it is considered acceptable, moral behavior, but rather to show hate there is some leeway in the community. This view is also due to the ability of adult stem cells to form such diverse types of cells, rendering the destruction of embryos avoidable in most circumstances.

The main issues with ES and EG research are: when does life begin, is the greater good a valid argument, and how does ES and EG research affects the donors. Laws are currently in place in most countries to regulate stem cell use. Whether the laws are strict or lenient depends on the country. Many traditions emphasize obligations to heal the sick—goals for which embryonic stem cell research holds great potential—and favor embryonic stem cell research for this reason.<sup>19</sup>

### **New Developments in Field Of Stem Cell Research**

Since 2007 there have been several new developments in the field of stem cell research that significantly change the landscape. This includes the development of induced Pluripotent Stem (iPS) cells by introduction of a limited number of genes into adult somatic cells, paving the way for the generation of histocompatible or patient specific pluripotent stem cells.<sup>3</sup>

The progress has been made in growing stem cells without xenogeneic feeder cells; and in well-defined media free from fetal calf serum. However, significant challenges remain with respect to characterizing the cell product for therapy for its purity, safety and potency in an expeditious and cost-effective manner.

Use of stem cells in regenerative medicine holds promise for improving human health by restoring the function of cells and organs damaged due to degeneration or injury. Stem cell biology has potential application in several areas of biomedical research that includes drug development, toxicity testing, developmental biology, disease modelling, tissue engineering etc. However, the potential danger of tumorigenicity of stem cells considering their capacity for unlimited proliferation, possibility of genomic changes arising during *in-vitro* manipulations, and limitations related to immunological tissue incompatibility between individuals are all causes for concern.<sup>1</sup>

Preclinical studies are essential to establish safety and proof-of-principle, prior to conduct of human clinical trials, as per regulatory requirements for any new biological entity (NBE). These studies involve both *in vitro* and/or experiments using animal model systems. According to national stem cell research guideline any stem cell study in experiment animal model should not allowed to be breed incase gonads are affected. Only in specific situations and depending on the nature of the study, large animals and/or non-human primates may be used with prior permission (Clause 10.1.3).

### **Stem Cells for Therapeutic Purposes**

There are no approved indications for stem cell therapy other than the hematopoietic stem cell transplantation (HSCT) for hematological disorders. Accordingly all stem cell therapy other than the above shall be treated as investigational and conducted only in the form of a clinical trial after obtaining necessary regulatory approvals. Stem cell therapies have successfully evolved from one method of treating leukemia, into a more modern and technologically advanced approach to medicine in which stem cells and advanced harvesting techniques are used to combat all types serious medical conditions such as heart disease, diabetes, Multiple Sclerosis, and even in the repair of spinal cord injuries.<sup>1,6</sup> In the blood, HSC are found at a rate of about 1 in 100,000 cells, while in marrow the rate is 1 in 10,000 (Stem Cell Basics- 2005). The higher concentration of stem cells allows for a marrow transplant to be

more effective than a blood transfusion in treating blood disorders or aiding the recovery of the hematopoietic system. The first successful bone marrow transplant that resulted in long term survival of a patient suffering from leukemia was performed by Dr. Donnall Thomas in 1956 (Piccolo, 2006). Dr. Thomas removed bone marrow from a healthy twin sibling which was then transplanted into the sick patient. This procedure caused the leukemia symptoms to recede over time, eventually putting the cancer into remission. Hematopoietic stem cells have also been used to treat immune system disorders, such as Crohn's disease, Behcet disease, and Krabbe disease. Stem cells, from the brain or nasal cavity of mice, have been used to trigger the regrowth of myelin in mice with multiple sclerosis.<sup>8</sup> Adult stem cells (neural) have also been used to treat Parkinson's disease, through transplantation. Diabetes stem cell therapy requires the harvesting and implementation of pancreatic stem cells which are used to grow new islet cells which are destroyed by diabetes. This type of therapy has been shown to have the potential to decrease the need for pancreas (or kidney following pancreatic failure) transplantation in patients suffering from diabetes, as it helps the pancreas to function properly and naturally supply the body with insulin.<sup>5,9</sup>

Many popular cancer treatments involve techniques such as chemotherapy and radiation therapy.<sup>20</sup> These methods have shown to be effective in at least slowing down the cancer or putting it into remission for some time, but both treatment options have negative side effects as radiation and chemotherapy do not exclusively target and kill the cancerous cells, but can cause cell death in healthy cells as well. Aside from the formerly mentioned leukemia, stem cells have been successfully used in the treatment of cancers such as: liposarcoma, Non-Hodgkins and Hodgkins lymphoma, and neuroblastoma to name a few.<sup>21</sup>

Umbilical cord stem cells should not be considered a moral threat. They are isolated from a recently born baby's umbilical cord blood. These cells are harvested without harming the baby, and are cells that would normally be discarded and die. A recent trend is to save and freeze these cells as to provide a source of stem cells in case the child should need them later in life. The umbilical blood cells are used in marrow replacement treatments. These cells are useful not just for the child, but can also be used in marrow replacement for related and unrelated patients. Patients with marrow replacement that uses umbilical cord blood cells, rather than adult marrow, have a higher recovery rate, due to the higher concentrations of progenitor cells and hematopoietic stem cells.<sup>13, 14</sup>

### Tests for Stem Cells

It is not easy to know when you have isolated a stem cell for use, but certain tests are commonly performed. The first test is to culture the cells for several months and make sure they remain undifferentiated. A cell that remains undifferentiated is a cell that has the same characteristics as its parent cell. Differentiated cells tend to be more specialized than their parent cell. This test assures that they have the potential for long term, self-renewal. Another test is to identify certain surface markers that are only present on undifferentiated cells. The "stemness" phenotype and can serve as markers for ES cells. After several decades of investigates, a list of ES-specific markers has been established (The National Institutes of Health resource for stem cell research), such as 5T4, Nanog, ABCG2, Oct-3/4, Alkaline Phosphatase/ALPL, Oct-4A, E-Cadherin, Podocalyxin, CCR4, Rex-1/ZFP42, CD9, SCF R/ckit, CD30/TNFRSF8, sFRP-2, CDX2, Smad2, Chorionic Gonadotropin, lpha Chain (alpha HCG), Smad2/3, Cripto, SOX2, DPPA4, SPARC/Osteonectin, DPPA5/ESG1, SSEA-1, ESGP, SSEA-3, FGF-4, SSEA-4, GCMF/NR6A1, STAT3, GDF-3, SUZ12, Integrin alpha 6/CD49f, TBX2, Integrin alpha 6 beta 4, TBX3, Integrin beta 1/CD29, TBX5, KLF5, TEX19, Lefty, THAP11, Lefty-1, TRA-1-60(R), Lefty-A, TROP-2, LIN-28, UTF1, LIN-41, ZIC3, c-Myc etc.<sup>6,1</sup>

Another test of stem cells is to make sure that they can differentiate into multiple types of cells. For example, for hematopoietic stem cells (HSCs) that form all the cellular components of blood, one such test is to inject them into a mouse that has been irradiated to kill off its own blood cells. If the mouse recovers and replenishes its blood supply, the injected HSCs are

assumed to be stem cells. Human HSCs have been defined with respect to staining for Lin, CD34, CD38, CD43, CD45RO, CD45RA, CD117, CD133, CD166, and HLA DR (human). In addition, metabolic markers/dyes such as rhodamine123 (which stains mitochondria), Hoechst33342 (which identifies MDR type drug efflux activity), Pyronin-Y (which stains RNA), and BAAA (indicative of Aldehyde dehydrogenase enzyme activity) have been described. The positive markers useful for MSC identification are CD106, CD105, CD73, CD29, CD44, and Sca-1. This test shows plasticity, the ability to form several types of cells, and self-renewal.<sup>22, 23</sup>

### Cancer Stem Cells

Cancer stem cells are a sub-group of cancer cells that respond the escaping of cancer chemotherapy and the relapse of tumors. This concept has a great impact on the strategy of cancer chemotherapy and anti-cancer drug design. The existence of cancer stem cells has been debated for many years until the first conclusive evidence was published in 1997 in Nature Medicine. Bonnet and Dick (Bonnet D, 1997) isolated a subpopulation of acute myeloid leukemic cells that express a specific surface antigen CD34, but lacks the antigen CD38. The cancer stem cells are mutants from cancer cells after obtaining the stem cell-like features.<sup>24</sup>

De-differentiation is a reasonable hypothesis, which assumes these cells acquire stem cell like characteristics by reverse-differentiation from cancer cells. This is a potential alternative to any specific cell of origin, as it suggests that any cell might become a cancer stem cell. The tumor hierarchy is another model to propose the origin of cancer stem cells. The main point of this model claims that a tumor is a heterogeneous population of mutant cells with various stages of stem cells. Within the tumor hierarchy model, it would be extremely difficult to pinpoint the cancer stem cell's origin.

They specifically are with the ability to give rise to all cell types found in a cancer sample. Cancer stem cell population consists of only a small portion of tumor mass (around 0.1-1% of total mass) and can be distinguished from the other cells in tumor mass by special cell surface antigens (such as CD34+).<sup>23</sup> Both stem cells and cancer stem cells share the characters of stemness, the capacity of differentiation, the multi-potential differentiation. However, the unique character of cancer stem cells, different from normal stem cells, is the growth out of control.<sup>25</sup> During conventional cancer chemotherapies, the differentiated or differentiating cells are likely to be killed while the cancer stem cells, due to their stemness and inactivity, could remain untouched, therefore to escape from chemotherapies. It is believed they serve as "cancer seeds" and respond to the cancer relapse and metastasis by rising new tumors. Based on the concept of cancer stem cells, it is beneficial to include an induction of the cancer stem cell differentiation during chemotherapies.

### Conclusion

Stem cells are long-lived cells in the body that have the ability to differentiate into a specialized cell to create new tissues. Because of this tissue regenerative capacity, stem cells are currently at the forefront of medical research and development. Stem cells differ according to their source and their malleability. Just as there are many different types of specialized or differentiated cells in the body, there are many different types of stem cells in the body. So far, iPSCs have been regarded as the most promising way to create SCs. However the use of iPSCs has raised concerns. Depending on the methods used, reprogramming of adult cells to obtain iPSCs may pose significant risks that could limit its use in humans. For example, if viruses are used to genomically alter the cells, the expression of cancer-causing genes or oncogenes may potentially be triggered.

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