Adult Stem Cell Plasticity: Dream or Reality?

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Introduction

A stem cell is a primitive cell that can either divide to reproduce itself (undergo self renewal) or give rise to more specialized (differentiated) cells. Stem cells exist in many adult tissues. Stem cell exists in a variety of organs in a variety of phenotypes, in greater plenitude than previously suspected. By contrast, embryonic stem cells are derived from an embryonic cell population at a stage prior to its commitment to form particular tissues of the body. Because of their origin, embryonic stem cells are by nature more versatile than most of their adult counterparts. The first cell commitment event in the mammalian embryo occurs with compaction of the embryo and formation of the outer trophoblast layer. The small cluster of cells inside the blastocyst, the inner cell mass, is destined to give rise to all the tissues of the body. It is at this stage of development that stem cells are isolated. Human embryonic stem cell lines are made by dissecting an early blastocyst- stage embryo and removing its inner cell mass.

The concept of stem cell has changed greatly in recent years. The potential for stem cells to regenerate non-haematopoietic tissues, offers an expanded repertoire of clinical applications for stem cell transplantation.

The presumed ability of tissue specific stem cells to acquire the fate of cell types different from the tissue of origin has been termed adult stem cell plasticity. Since a series of papers heralded the finding of surprising plasticity in a variety of adult stem cells [1, 2], this exciting field has generated almost as much controversy and rhetoric as it has scientific insights.

This brief review will attempt to highlight some of the concepts and concerns regarding adult cell plasticity.

Stem Cell Definition

To define a cell as a stem cell, scientists have used four criteria [3]. Firstly, stem cells undergo multiple,

sequential self- renewing cell divisions. Secondly, single stem cell- derived daughter cells differentiate into more than one cell type. Examples include hematopoietic stem cells (HSC) that give rise to all hematopoietic cells; neural stem cells (NSC) that give rise to neurons, astrocytes and oligodendrocytes [4] and mesenchymal stem cells (MSC) that differentiate into fibroblasts, osteoblasts, chondroblast and adipocytes [5]. However, some adult stem cells may give rise to only a single mature cell type, such as the corneal stem cell. A third criterion is that stem cells functionally re-populate the tissue of origin when transplanted in a damaged recipient, which has been shown extensively for HSC and more recently for liver progenitors [6] and NSC. A final, less well established criterion, is that stem cells contribute differential progeny in vivo even in the absence of tissue damage.

Types of Stem Cells

(a) **Embryonic Stem Cells:** During development, pluripotent cells originate that are capable of giving rise to all somatic and germ- line cells, because the cells are at a point before commitment to any particular tissue [7]. This occurs at the blastocyst stage of the embryo, when the cells from the inner cell mass can be removed and grown in the laboratory on mouse embryonic fibroblast feeder layers, as embryonic stem cells (ESC) [8]. There is no dispute that these pluripotent ESC truly have the capability to regenerate all tissues and to give rise to all cell types.

In the laboratory these cells can then be grown and differentiated in vitro, into all somatic tissue cells, including cardiomyocytes, skeletal and smooth muscle cells, neurons and glial cells, hematopoietic progenitors, adipocytes, pancreatic islet cells and others [9].

Human embryonic stem cell lines are made by dissecting an early blastocyst- stage embryo and

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removing its inner cell mass. The embryo inevitably dies as a result of this procedure. Hence, the question: should we intentionally kill a developing human being at this stage to provide medical benefits to others? The early embryo is entitled to the same moral and ethical rights as you and me. Many Roman Catholics, Evangelical Protestants and some orthodox Jews take this position. Those holding this view do not object to human stem cell research that uses the stem cells found in our bodies, so called adult stem cells. They argue that such research is as promising as human embryonic stem cell research. At the other end of the spectrum are those who believe that the embryo is not yet fully a human being in a moral sense. They tend to hold a developmental view of life's beginning. The controversy that arose from an ethical and religious perspective led to the passage of a bill by the US congress that prohibited both reproductive cloning, in which this nuclear transplantation could theoretically lead to development of a full organism, as well as production of new embryonic stem cell lines. In August 2001 President George W Bush adopted a conservative position that permits one to benefit from acts one morally opposes. Although he stated his belief that it is morally wrong to kill a human embryo, the President permitted the use of existing human embryonic stem cell lines on the grounds that the deaths of these embryos had already occurred. He announced that only ESC lines that had already been established and are now catalogued in the National Institute of Health Embryonic stem cell registry, would be able to be used for federally sponsored research [7]. Use of the established cell lines has demonstrated that ESCs have the potential to regenerate differentiated cells, with applicability to neurological degenerative disorders, [10] diabetes mellitus [11] and heart diseases [12]. The Ethics committee of the American Society for Human Reproductive Medicine has proposed that a consent form be devised that would enable "spare" embryos developed by couples undergoing in vitro fertilization to be donated for research.

Haematopoietic Stem Cells: The HSC is the pluripotent cell capable of both self renewal as well as generation of all the mature blood cells. The only true assay for the presence of HSC is their ability to reconstitute the haematopoietic system of a myeloablated host. This is because haematopoietic reconstitution requires extensive self renewal of the transplanted HSC and their differentiation into every mature blood cell type [13].

There is no single precise surface marker for the HSC. However, certain features can be identified for cells characterized as primitive haematopoietic progenitor cells that correlate with long term

engraftment. These progenitor cells exhibit a lack of expression of lineage markers, a lack of expression of CD38, CD33, CD71 and HLA-DR, extrusion of dyes such as rhodamine 123 and Hoechst 33342 related to expression of an adenosine triphosphate-binding cassette transporter and low expression of Thy 1 (CD 90 surface marker) and CD 45 RA.

Mesenchymal Stem Cells: MSC are derived from the adherent cell layer from bone marrow, therefore they have also been termed marrow stromal cells. These share two features: growth in culture as adherent cells with a finite life span and ability to differentiate into osteoblast, chondroblasts, adipocytes, endothelial cells, cardiac cells, skeletal myocytes and thymic epithelial cells and may contribute to both wound healing and bone healing in response to appropriate stimuli. In a nonhuman primate model, intravenous infusion of retrovirally marked MSC by complementary DNA (cDNA) encoding green fluorescent protein (GFP) revealed widespread engraftment in GIT, kidney, skin, lungs, thymus and liver [7]. MSC have also been shown to contribute to cardiac muscles as demonstrated by engraftment of human bone-marrow derived MSC marked by α -galactosidase.

Multipotent Adult Progenitor Cells: These are cells derived from bone marrow, muscle or brain and are capable of giving rise to an even greater repertoire of cell types, including mesoderm, neuroectoderm and endoderm derived tissues. A population of highly plastic, adult derived bone marrow cells, referred to as multipotent adult progenitor cells (MAPC) can be grown in vitro from the post-natal marrow of mice, rats and humans [14]. Unlike MSC, MAPC can be cultured indefinitely in a relatively nutrient-poor medium. MAPC exhibit many of the features and capabilities of embryonic stem cells [14-24].

Hemangioblast: These are precursor stem cells to hematopoietic blood vessel endothelial cell [25].

Adult Stem Cell Plasticity

Over the past five years, a series of reports have been published suggesting that the previous dogma of tissue specificity associated with adult stem cells may not be correct. The presumed ability of tissue-specific stem cells to acquire the fate of cell types different from the tissue of origin has been termed adult stem cell plasticity. There is however, no official definition of stem cell plasticity. One possible definition though is that tissue-specific adult stem cells, for instance, HSC thought to be committed to a given cell lineage, can under certain micro-environmental conditions, acquire the ability to differentiate into cells of a different tissue type.

Possible Mechanisms for Plasticity

There are various alternative pathways for a stem cell or more committed cell to switch lineages. The first is transdetermination. This is the situation in which a stem cell that is programmed to generate certain lineages switches to another stem cell and gives rise to cell types of that precursor, i.e its potential is redirected. The second pathway is transdifferentiation. In this process a differentiated cell can gain the phenotype of another differentiated cell. In indirect differentiation an HSC changes its gene expression pattern to that of an alternative cell type through a dedifferentiation/redifferentiation pathway whereas in direct differentiation, an HSC may be able to directly change its gene expression pattern. Fusion, an alternative mechanism for plasticity, could be fusion of a bone marrow (BM) derived cell (haematopoietic stem cell) with a non hematopoietic cell to form a 'heterokaryon', thereby converting the gene expression pattern of the original BM cell type to that of the fusion partner. For example myoblast fusion with fibroblast induces expression of muscle proteins in the fibroblasts. This indicates that cytoplasm of myoblasts contain factors that induce muscle differentiation of non-muscle cells. Another example of fusion is between bone marrow stem cells and liver cells to generate hepatocytes in mice. Two recent studies documented that co- culture of adult tissue cells with ESC also leads to cell fusion [26].

Demonstration of Bone Marrow Plasticity

Bone marrow to liver: Bone marrow derived stem cells (BMSC) like haematopoietic stem cells and mesenchymal stromal cells, engraftment as hepatocytes using male-to-female BM transplantation in rats, mice and humans which was first demonstrated in response to liver damage, may promote the BMSC to hepatocyte transition [15]. In perhaps the most exciting demonstration of BMSC plasticity, transplantation of Lin⁻, Kit⁺, sca⁺, thy 1¹⁰ (KTLS) BM cells to irradiated hosts was used to treat an inborn error of hepatic metabolism. Mice lacking the gene for fumarylacetoacetate hydrolase (FAH) develop progressive liver and kidney failure from tyrosinemia unless they are treated with 2- 2- nitro – 4 – trifluoromethyl benzyol (NTBC), a drug that prevents the breakdown of tyrosine to its toxic metabolites. Lethal irradiation followed by transplantation of as few as 50 purified KTLS cells allowed these mice to survive with a nearly normal liver function because of the ability of the engrafted KTLS bone marrow cells to adopt the role of functional hepatocytes.

Bone marrow to heart/cardiac muscle: Therapeutic benefit has been demonstrated in mice with

experimentally induced myocardial infarcts that receive intra cardiac injection of marrow (or kit⁺ bone marrow cells) during the initial period after infarction. In humans, after orthotopic transplantation of female hearts into males, up to 15% of the cardiac myocytes can be donor derived [16]. Two recent phase 1 studies have shown the safety of injecting autologous bone marrow cells into the human heart after infarction. In patients in whom autologous bone marrow cells were injected directly into the damaged myocardium, some improvement in cardiac function was documented based on medication use, quality of life and MRI studies [17]. In a separate study, after autologous AC 133⁺ bone marrow cells were injected into infarction borders following coronary artery bypass grafting (CABG), improved perfusion and cardiac function may have occurred [18-30].

Bone marrow to skeletal muscles: Injection of bone marrow stem cells into damaged muscle leads to development of marrow derived cells with myocyte-specific gene expression. Furthermore, BM transplantation restores low levels of dystrophin expression to the muscles of index mice, which lack the gene for dystrophin. A case report [19] describes a boy with a relatively mild Duchenne Muscular Dystrophy (DMD) diagnosed when he was 12 years old. He had undergone allogeneic BMT for X-linked severe combined immunodeficiency syndrome (SCID) when he was one year old. The report suggests that healthy muscle fibres forming from the donor marrow might have decreased the severity of DMD. At age 14, 13 years after allogeneic BM transplantation, rare donor-derived nuclei expressed normal dystrophin (0.5-0.9%) in the skeletal muscle fibres. The use of stem cells in DMD has been anecdotal and a number of independent studies have failed to reproduce similar results.

Bone marrow to central nervous system: In a mouse model (acid sphingo myelinase null animal), the injection of MSCs that had been genetically modified to express acid-sphingomyelinase into the hippocampus, delayed the onset of neurological defects as seen in Niemann Pick disease. MSC-derived sphingomyelinase-expressing Purkinje cells were identified [28]. Few studies have documented a therapeutic benefit from MSC administration to the spinal cord after injury [20].

Bone marrow to kidney: Two human studies have demonstrated that when female kidneys were transplanted into male recipients, Y chromosome positive epithelial cells develop in the transplanted kidney [21].

Bone marrow to pancreas: BMSC can also differentiate in vivo into islet cells. In a study, streptozotocin – induced diabetes was ameliorated by bone marrow transplantation [22]. This was in a mice model.

Various other examples of bone marrow to skin, GIT and lungs also exist.

Questionable scientific claims

There have been a fair number of scientific claims that are questionable and shortcut methods that have come a cropper. The recent and most shocking is the case of the South Korean scientist Hwang Woo Suk, whose headline grabbing work on cloning, published in *Science and Nature* in 2004 and 2005 unraveled as a case of scientific fraud. Dr Suk, who won world acclaim as the first scientist to clone a human embryo and extract stem cells from it, admitted to faking results. The researcher confirmed that in 2003, two of his researchers donated eggs and a hospital director paid about 20 other women for their eggs [31]. Dr Suk had claimed to have cloned an adult dog. In cloning research, Dr Ian Wilmut and his team at the Roslin Institute in Scotland made the first breakthrough with Dolly, the sheep in 1997. It was followed quickly by Polly, another sheep, and then came Andi, the rhesus monkey, a couple of years later [32].

Stem cell research in India - still in the embryonic stage?

What do all these developments mean for India? Where is stem cell research heading in our country? The Indian National Science Academy, the leading body of scientists, said strict guidelines need to be put in place to put stem cell research on a strong footing [29].

Dr CM Habibullah, a liver specialist who has done considerable work in using foetal stem cells collaborating with the Centre for Cellular and Molecular Biology (CCMB), Hyderabad, cautions against setting up of facilities for cell banks and doctors claiming miracle cures for diseases such as muscular dystrophy. This trend needs to be brought under check [31].

Dr Lalji Singh, Director CCMB, is for therapeutic cloning research in the country, as it could find cures to many diseases [31]. The CCMB is also working with LV Prasad Eye Institute for growing corneal limbal cells which help in repairing the damaged corneal tissues and restoring vision and 200 such procedures have already been performed [31].

All India Institute for Medical Sciences (AIIMS) has claimed reasonable progress in therapeutic stem cell research work. The focus is on treating muscular dystrophy, spinal cord injury, cerebral dysplasia, heart tissue damage, diabetes and motor neuron disease [31].

The Manipal Education Society (Stempeutics) is focusing with renewed vigour on clinical trials using adult human mesenchymal stem cells to treat patients with damaged heart tissue, spinal cord injury, ischemic limb

and optic nerve injury [31]. Stempeutics has developed patented technology for isolation and culture of mesenchymal stem cells derived from the bone marrow which supports long term proliferation of these cells. One batch production of mesenchymal stem cells can easily target 150 patients at one time and hence clinical trials become easy to perform.

Half the battle won

American medical researchers were looking forward to these draft guidelines for eight years, since the US congress banned federal funding of research using human stem cell lines created after 2001. President Barack Obama lifted the ban in March 2009 paving the way for new research.

Stem cell research in armed forces in India

Currently simultaneous research projects are going on in Armed Forces Medical College, Pune and Army Hospital (R & R), New Delhi, which are funded by Department of Biotechnology, New Delhi. The projects are as under:-

- a) Efficacy of stem cell in improvement of left ventricular function in patients with acute myocardial infarction.
- b) Intravenous autologous bone marrow stromal cells (MSC) for patients with acute ischemic stroke.
- c) Efficacy of stem cells in acute limb ischemia.

Conclusion

Multipotent or even pluripotent adult stem cell might be used for therapies of degenerative or genetic disorders of different organs. It is clear that the field of adult stem cell plasticity is not a mere flash in the pan. However, presently their importance, mechanisms and usefulness are in the earliest stages of exploration [23]. India and China are poised to play a key role in the scientific, clinical and commercial development of stem cell research.

Conflicts of Interest

None identified

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