5 *Stem Cells: Current Concepts and Future Directions*

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Abstract: Embryonic stem cells were first derived in 1998. Since then research into embryonic stem cells has been progressing at a rapid pace, especially so in the last 3-5 years. Embryonic stem cells are pluripotent and thereby have the potential to develop into almost any tissue of interest. Considering the wide applicability of stem cells in human disease, expectations are very high, fuelled by intense media attention. We need to be clear on several issues. As of today we do not have any human trials with embryonic stem cells. Hematopoietic stem cell transplant is the only approved modality of treatment in blood disorders and a handful of other conditions. All other research into the use of stem cells, be it cardiology or neurology have utilized autologous bone marrow stem cells. These stem cells are adult stem cells. They are not yet proven to have pluripotency and beneficial effects, if any, are possibly due to their paracrine effects. We have still not understood the factors responsible for controlling pluripotency and commitment of pluripotent stem cells to specific cell lineages. Clinical applications will possibly come about only after these and pertinent ethical issues have been sorted out.

INTRODUCTION

Human embryonic stem cell (hESC) lines were first described in 1998.[1] The ability of hESCs to reproduce limitlessly and differentiate into almost all cell types in the human body has generated a lot of both scientific and common interest. It has raised the possibility of achieving a true understanding of human cellular function and thereby a lasting cure for many human maladies, in addition to a potentially effective source of tissue for organ replacement. Scientific research into hESC has been proliferating steadily in the last 3-5 years.

STEM CELL DEVELOPMENT (Fig. 5.1)

A glossary of commonly used terms in stem cell research is given in Table 5.1.[2] Stem cells are cells with the ability to divide for indefinite periods in culture and to give rise to specialized cells, which gradually lose the ability to proliferate indefinitely.

Cells from the very earliest embryo (up to about the 16 cell stage) are totipotent stem cells. After a few days of development, approximately 5 days, the early embryo forms a hollow ball of cells, called a blastocyst. This blastocyst has not yet implanted in the uterus. The clustered cells within this ball are called the inner cell mass. These cells in the inner cell mass, also known as the embryonic stem cells, are not totipotent. Rather, they are pluripotent. Pluripotent stem cells are more "committed" than totipotent stem cells. These pluripotent stem cells can give rise to the 3 germ layers, ectoderm, mesoderm and endoderm, which in turn give rise to the various tissues in the body. The human body continues to have some stem cells even after the complete anatomical development. These stem cells are called the "Adult" stem cells. For example, hematopoietic stem

cells including cord blood stem cells can form all the blood cells, but no other tissue types. Unlike embryonic stem cells, adult stem cells have a finite proliferative life in tissue culture on account of lower telomerase levels. Thus these stem cells are responsible for the body's ability to repair some but not all tissues. Early papers suggested that these cells have "plasticity", i.e. the ability to form tissues other than the tissue of its origin. As of now there is no proof that adult stem cells derived from bone marrow or cord blood have ability to differentiate into any other tissue. Thus adult stem cells are a distinct entity and should not be confused with embryonic stem cells.

Potential Applications of Pluripotent Embryonic Stem Cells

1. Pluripotent stem cells could help us to understand the complex events that occur during human development. It would help in the identification of the factors involved in the cellular decision-making process that determines cell specialization. An understanding of normal cell processes will allow us to further delineate the fundamental errors that cause illnesses like cancers.
2. Human pluripotent stem cell can be used in research to assess the safety and efficacy of new drugs. Rather than evaluating safety and efficacy of a candidate drug in an animal model of a human disease, these drugs could initially be tested against a human cell line that had been developed to mimic the disease processes.
3. Ultimately embryonic stem cells might generate cells and tissue that could be used for transplantation to treat a myriad of diseases, conditions and disabilities including Parkinson's and Alzheimer's disease, Spinal cord injury, Stroke, Burns, Heart disease, Diabetes and Arthritis.

How Are Pluripotent Stem Cells Produced and Cultured?

Much of the controversy involving ethical issues revolves around the manner in which embryonic stem cells have been acquired for research. These methods have been developed over the past by researchers working with animals. The human embryonic stem cell lines in use today were obtained from inner cell mass of blastocyst stage embryos created in the course of infertility treatment and donated by couples for research to derive stem cells. Figures 5.1 and 5.2 describe the various ways to derive stem cell lines and their culture in the laboratory.

Mouse ES cells and human ES cells, derived from the inner cell mass, are grown on a layer of "feeder cells" along with bovine serum. Feeder cells expose these cell lines to potential risk of animal to human and human-to-human pathogen transfer. Feeder free culture media using high concentrations of basic fibroblast growth factors and recombinant protein components have now been described.[3] Once removed from the influence of feeder cells, ES cells have been shown to differentiate into more mature forms. The factors that govern the growth and differentiation of mouse and human ES cells are still unclear. Besides these factors appear to have differing actions in mouse and human ES cell lines.[4] Mouse ES cells proliferate without differentiation in the presence of certain cytokines which otherwise promote differentiation in human ES cell lines. Furthermore, human ES cells grow more slowly and less efficiently in comparable culture conditions as compared to mouse ES cells. Therefore, it has been difficult to apply the knowledge gained from mouse studies to human ES cell lines.

Stem cells have most widely studied in context of blood, nervous system and cardiology.

Stem Cells in Blood Disorders

A small quantity of hematopoietic stem cells are all that is required to fully restore a functional hematopoietic system after damage to normal hematopoiesis by disease. Work on hematopoietic stem (HS) cells has been seminal in the field of stem-cell research, and has provided reagents and models for other tissue-specific and organ-specific stem cells. The 1990 Nobel Prize awarded to E.

Donnall Thomas who recognized the primacy of such research. Hematopoietic stem cell transplantation for blood disorders is the only approved indication for the use of stem cells in humans. A detailed review of hematopoietic stem cell transplant is beyond the scope of this article and there are excellent recent reviews on this subject.[5] Despite these successes, the wider therapeutic application of HS-cell transplantation to blood disease is limited by the lack of suitable donors, the high rate of mortality associated with the procedure and the recurrence of the underlying diseases. Use of alternative strategies like using haploidentical stem cells are complicated with high transplant related motality which far outweigh potential benefits. The present challenge is to reduce the risk of such transplants and increase the number of patients who can safely access this treatment. In developing countries, such 'one-shot' treatments are highly desirable because chronic treatments are difficult to sustain.

Cord Blood Stem Cells

The presence of hematopoietic stem cells in umbilical cord blood was reported originally in 1974. The first successful cord blood transplant (CBT) was performed by Eliane Gluckman in 1988 in Paris in a child with Fanconi anemia, who is well 18 years later. Cord blood stem cells have distinctive proliferative advantages which include an (a) enriched proportion of immature stem cells, (b) higher clonogenic growth advantage, (c) increased cell cycle rate, (d) autocrine growth factors production, and (e) increased telomere length. The small number and relative immaturity of naïve T cells of cord blood lymphocytes is expected to reduce the risk and severity of graft versus host disease (GVHD).

The main limitation of CBT is the limited number of nucleated cells available in a unit. As compared to bone marrow transplantation, the time for engraftment in a cord blood transplantation is much longer, taking a month for neutrophilic engraftment and more than fifty days for platelet engraftment. There is also a higher incidence of non-engraftment. This leads to a high transplant related mortality. The nucleated cell dose available in a cord blood unit is critical, being 1 log less than in a bone marrow transplant (BMT). The minimum recommended dose for CBT is 2.0 to 2.5 × 10^7 nucleated cells/kg for a successful outcome and at least a 4/6 HLA match. European experience suggests that a cell dose more than 3.7 × 10^7 nucleated cells/kg leads to engraftment in more than 85% cases. The main advantage of CBT is a lower incidence and severity of graft versus host disease. This allows a 1 to 2 HLA antigen mismatch even in unrelated CBT. However the results of HLA identical CBT are superior.[6]

Majority of CBTs have been unrelated CBTs, mainly in children on account of the small infusion quantity. The main indication of CBTs is in those cases where there is no HLA identical sibling donor available, and an unrelated CBT offers similar results as an unrelated HLA identical BMT. CBT can be done earlier and may be preferred where an urgent transplantation is needed, as in acute leukemia.

Recently, a number of private commercial cord blood banking companies have been actively advertising the benefits of autologous cord blood stem cells. Their claims are not supported by scientific fact. The examples cited by these advertisements have actually been studied using embryonic stem cells, which are vastly different form cord blood stem cells. Cord blood stem cells do not have any proven plasticity unlike embryonic stem cells. Table 5.2 enumerates the limitations of autologous cord blood use. Recently a number of professional societies have issued guidelines or opinions on the subject.[7,8] Most professional bodies like the World Marrow Donors Association are against such banks and false advertising.[9,10]

Stem Cells for the Treatment of Neurological Disorders

The nervous system is a complex organ made up of neurons and glial cells, and the loss of any of these cell types may have catastrophic results on brain function. It is hoped that neural stem cells may be able to replenish those that are functionally lost in diseases such as Parkinson's disease,

Huntington's disease, and Amyotrophic lateral sclerosis, as well as from brain and spinal cord injuries that result from stroke or trauma.

The majority of stem cell studies of neurological disease have used rats and mice. There are two possible approaches:

1. Modulate stem cells that are harvested and grown in culture and then transplant these cultured cells into the brain of an animal model and allow the brain's own signals to differentiate the stem cells into neurons or glia. Alternatively, the stem cells can be induced to differentiate into neurons and glia in the culture dish, before being transplanted into the brain.
2. Identify growth (trophic) factors that are normally produced and used by the developing and adult brain. Animal studies have shown that these factors can be used to minimize damage to the nervous tissue and prevent further progression.

Parkinson's disease serves as a good example of work in this field. The NIH in USA funded two large and well-controlled clinical trials in which researchers transplanted tissue from aborted fetuses into the striatum of patients with Parkinson's disease. These studies, performed in Colorado and New York, included controls where patients received "sham" surgery (no tissue was implanted). Unfortunately, both studies showed that the transplants offered little benefit to the patients as a group. Additionally, the New York study showed evidence that some patients' immune systems were attacking the grafts.

It is believed that the more primitive ES cells may be an excellent source of dopamine neurons because ES-cells can be grown indefinitely in a laboratory dish and can differentiate into any cell type. Since ES cells can generate all cell types in the body, unwanted cell types such as muscle or bone could theoretically also be introduced into the brain. There is also the possibility of unwanted synapses occurring and giving rise to distressing symptoms. As a result, a great deal of effort is being currently put into finding the right "recipe" for turning ES cells into dopamine neurons – and only this cell type—to treat Parkinson's disease.

Presently the second approach, that of introducing stem cells for the delivery of trophic factors into the target areas to limit damage that has occurred and preventing disease progression, appears to be a more realistic expectation.[11] Intra-arterial infusion of autologous bone marrow derived stem cells in patients with cerebral palsy and hypoxic ischemic encephalopathy is presently being studied at AIIMS.

Stem Cells and Cardiac Disease

We know now that there is a small pool of cardiac progenitor cells in the heart, which are responsible for continual replacement of apoptotic cardiomyocytes at a low basal level.[12] Their numbers are too small to be utilized for the myocardial repair that is needed after acute myocardial infarction. Human and mouse ES cells are known to differentiate into many lineages which include beating cardiomyocytes. It is not yet possible to induce selective expansion of cardiac progenitor cells after exposure to growth factors and other compounds. It is perhaps not advisable to introduce undifferentiated ES cells directly into the damaged myocardium because of the fear of inducing local teratomas.

Earlier studies, which suggested that bone marrow stem cells could differentiate into cardiac muscle cells, have not been substantiated in recent randomized studies, which studied the effect of intracoronary injection of autologous bone marrow stem cells.[13] At best bone marrow stem cells might confer some beneficial effects by secreting paracrine factors that are cardioprotective or angiogenic. If this be the case then a more realistic short term approach would be to induce adult stem cells to secrete larger quantities of protective paracrine factors which could then be used to protect the myocardium from further injury.[12] Preliminary work in this direction at AIIMS has shown that bone marrow stem cells injected in this manner is safe and potentially beneficial in patients with acute myocardial infarction and dilated cardiomyopathy.

Stem Cells in Other Diseases

Stem cells are being studied in other disease applications as well. Stem cells have been used to develop bone and cartilage tissue. Scientists at AIIMS are studying their potential use in age related macular degeneration and retinitis pigmentosa. Embryonic stem cells have been stimulated to develop insulin producing cells, albeit in clinically insignificant quantities.[14] Alternatively mesenchymal stem cells are now being studied as a source of pluripotent stem cells.[14]

Ethical Considerations

It has been interesting to note that reviews and papers on the ethical and legal aspects of human embryonic stem cell research far exceed original publications describing experimental work on these cells.[15] There are serious objections to the use of live embryos in stem cell research, such research resulting in irreparable damage to the embryos, which have potential to develop into a human being. It is still not possible to extract stem cells from the embryo without damaging it. Stem cells might be derived from dead or non-viable embryos. However, doubts regarding the health of cell lines so derived will persist. Critics of stem cell research also quote favorable articles on adult stem cell plasticity to bolster their arguments against public funding for embryonic stem cell research. The point remains that at the present moment both these varieties of stem cell are not interchangeable. Embryonic stem cells offer the only hope of deriving a truly "plastic" stem cell source that might have wide application. It may also be possible to make human pluripotent stem cells by using somatic cell nuclear transfer also known popularly as cloning.[16] The cloning of mammals showed that the oocyte could reprogram (nuclear reprogramming) an adult stem cell into an embryonic stem cell. The idea is to develop technology that can reprogram an adult stem cell in laboratory conditions without the need of an oocyte. This concept as of now is only a theoretical possibility.

SUMMARY

Ever since we realized the value of organ transplantation in replacing irreversibly damaged human organs, an everlasting source of tissue has been a kind of Holy Grail. Research on embryonic stem cells has brought forth tantalizing visions of this possibility. Owing to extensive media coverage, the lay person is understandably fascinated by the concept and commercial interests have been quick to cash in. We need to realize that clinical applications will come about only after a thorough understanding of the factors controlling pluripotency and commitment of stem cells to particular lineages. We are not yet able to perfectly regulate stem cell differentiation towards definite lineages. This raises the risk of developing teratomas in host tissue. We have still not been able to grow stem cells in clinically meaningful quantities either. Finally, stem cells derived from an allogenic source would still be immunogenic and would require prior immunosuppression if not myeloablation.

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