Human Stem Cells: Is it Possible to Limit Pluripotent Human Stem Cells to Differentiate into a Specific Tissue or Organ?

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Abstract

It would seem that differentiation of stem cells is the remedy of modern times. Yet stem cell research is more complex that the public may recognize and is still in the developmental stage. Recent breakthroughs show promise; various guidelines provide structure to this growing field but it is not ready for gross public application. The objective of this paper is to research various different methods of stem cell production and application. Methods included reviewing articles and studies to evaluate the process in production of affective stem cells. There are many therapies that illustrate this research. Upon completing and explaining this phase, further analysis of research presented risk factors such as negative immune responses, tumor, and teratoma formation are discussed. This paper further examines the different complications that may be triggered by stem cell therapy. The danger analysis is briefed to describe the risks involved in stem cell therapy while explaining the ongoing research and discoveries to assist in limiting these risks. This paper culminated with the success of the most affective form of therapy to date. There are ongoing studies directed to limiting stem cell therapy for safe and risk-free application. Nevertheless, no definite conclusion can be reached as to how stem cells can be limited to the point of remaining simultaneously pluripotent and effective.

Introduction

A young woman walks into her doctor's office complaining of what seems to be a pimple that does not go away. She initially treated it with over counter creams, then a facial and many dermatologist visits. Upon a more extensive examination the specialist realized that this was not the average case of adolescent acne and ordered blood work. The test revealed that this girl has type-one diabetes. Her plans to go to college were placed on hold and her life completely changed. What if diabetes didn't have to be a life altering disease? What if glucose monitoring and insulin injections were replaced with a one-time stem cell therapy that could replace the damaged cells? This example did occur and the girl is being treated with subcutaneous insulin infusion therapy. Stem Cell Research has not reached this breakthrough but the hope is that in the future stem cells will be used to test medical treatments and replace cells lost by devastating diseases that currently have no sustainable cure.

The first several divisions of the mammalian fertilized oocyte give rise to totipotent cells. These cells can form cells in the placenta, embryotic sack, and any cell in the body. Once cells are designated for the body they are termed pluripotent cells. These embryotic stem cells have virtually limitless potential and are an invaluable resource for the biomedical community. Along with the importance of these cells arises a responsibility of using them correctly. The process of understanding how these stem cells work and the services they can provide has not been an easy feat. This battle has been fought in both the courtrooms and laboratories. The legal system bears the responsibility of creating a system in which guidelines are being followed and ethics upheld. This is an ongoing battle, and there is no undermining this work. Regardless of several ongoing debates and some legal verdicts, the biomedical community is addressing stem cell research, cell regeneration, and organ construction from two different perspectives. There is ongoing promising research being done on organ and cell regeneration with the use of somatic stem cells. Specific mature, undifferentiated, adult cells are reprogramed to function as stem cells and referred to as induced pluripotent stem cells (iPSC). These cells, generally found in the epithelial cells of an adult organ, have already been assigned a task and are therefore limited to the regeneration of that particular cell or organ. However, being that they are extracted from the patient, it is assumed, although not tested, that these cells will face less rejection after implantation because of the body's familiarity with the cells. An alternative form of adult stem cells is activation of somatic stem cells (Stem Cell Information, n.d.) within the body. These cells reside within a healthy body and function as a natural maintenance system by regenerating aged or damaged tissue and replacing lost cells. Examples of the somatic stem cells are cord blood and bone marrow. The third of these general stem cell approaches allows for more potential but at a greater risk; pluripotent embryotic stem cells (ESC) can be used to create any cell or organ but at a higher probability for tumor growth. There are several distinct differences between embryotic and adult stem cells. Adult stem cells are rare cells that require extraction, isolation, and then in vivo cell culturing, to multiply. This cell culture process has not fully developed and is a great deal more complicated than the growth of ESCs (Herberts et. al. 2011., Shanthly et. al. 2006).

This paper will explore the potential of regenerating tissue by means of pluripotent stem cells, as well as discuss literature on growth of these cells to build tissues and replace cells. Upon exploring the processes of in vitro and in vivo differentiation, in both induced pluripotent stem cells (iPSC) and human embryonic stem cells (hESC), one can question the technology needed to operate these cells. Is it possible to limit stem cells to differentiate, as they would in utero, to create a select tissue or organ?

Methods

This document was amassed by referencing original research and peer reviewed scholarly articles. US government websites and portions of scholarly articles were accessed to gain knowledge about the background, history, and general methods involved in stem cells therapies. Specific topic information, regarding the research question, was collected, reviewed, and analyzed. Google Scholar, and databases that are made accessible through the Touro College online library, such as Proquest and Ebsco, were used to search and access documents related to the research topic. The following are examples of key words were used: stem cell and apoptosis, c-myc, tumorigenic factors, in vivo growth, nodal signaling. There was no discrimination made regarding the date of publication on articles.

History

Initial stem cell research dates back to 1870, with in vitro fertilization of an oocyte. Any form of promising finding were slow until research intensively propelled in the late nineteen seventies. Notable accomplishments include cloning of mammals in 1997 and development of the first stem cell culture in 1998. The virtually simultaneous discovery of cloning and stem cells were the building block of forthcoming research. Stem cells cultured in today's laboratories are removed from the blastocyst early in differentiation and frozen in their state until they are differentiated into the cells required for a specific medical therapy (Shanthly et. al. 2006). Research is ongoing and promising; the biomedical community has only scratched the surface of the untapped potential of stem cells. There are various issues and setbacks that are limiting stem cell potential (Stem Cell Information, n.d.).

Stem cell research raised sharp scientific, technological, ethical, and political controversies. The United States federal and state governments enforced laws to enhance and restrict actual stem cell research. States such as Florida and New York agree to provide state funding while other states such as Arkansas and Michigan have placed bans on the creation and use of ESC for biotech research (Vestal 2008). Some forms of stem cell research and experimentation are more disputed than others; stem cells derived from oocytes are more controversial than induced adult cells. For example, in 2006 the Senate passed a law allowing federal money to benefit the specific research of iPSC, umbilical blood and bone marrow treatments but not ESC. However, in 2011 President Obama removed all bans that restricted federal funding from being assigned to new lines of embryotic stem cells. Regardless of the origin of the cell, human stem cells research raises ethical sensitivities. The ethical policies are continuously evolving and the biomedical community is attempting to overcome the challenges to ensure that research is carried out in an appropriate manner (Levin et. al. 2013).

In addition to legal constraints the anatomical components that comprise stem cells are intrinsically a hindrance. The same factors, enzymes, and transcription that insure the cell's ability for self-renewal cause biomedical complications (Li et. al. 2015).

Differentiations

Some mammalian tissues, such as the skin and liver, have the ability to regenerate yet others cannot. The capability of bioengineering and sustaining all organs, regardless of their regeneration factor, will be invaluable; this can ultimately circumvent the transplant system ensuring that patients don't die waiting for an organ. Regeneration capability is reliant on stem cells. For the purpose of this paper stem cells are divided into two categories, embryonic stem cells (ESC) and induced pluripotent stem cells (iPSC). In more recent years, researchers have developed methods of directly reprograming differentiated cells to produce iPSC. iPSCs are rare and found in only some differentiated tissues throughout the human body. A common use for iPSC is bone marrow transplants to fight leukemia. Although only one in every one hundred thousand cells is stem cell, advanced techniques allow for harvesting and collecting those cells in concentration to be donated by injecting the donor with colony-stimulating factor 3 (CSF 3). This glycoprotein stimulated the bone marrow to produce more stem cells; a necessary tool to collecting these otherwise dispersed cells. Such techniques can be used to collect adult stem cells but the process is tedious and does not allow for the unlimited potential hESC offer (Shanthly et. al. 2006). Although iPSC involves complications, such as the complexity of collection, it also provides therapeutic potential because these cells are harvested from a patient's own cells. This can alleviate tissue and cell rejection complications.

Since adult stem cells cannot give rise to all cells and tissues in the body, the ideal regenerative cell is embryotic stem cell. There are three different forms of embryonic stem cells used for medical research. The foremost method, true embryotic stem cells, is made from fertilized eggs generally donated by patients undergoing in vitro fertilization. Somatic cell nuclear transfer (ntES cell) is the process of creating an embryotic cell by inserting the nucleus of a general human cell into an egg cell. Although this technology has not been tested on higher mammals, it can be of great use because the nucleus of the cell will be a genetic match to the patient. The third form of ESC, not ready for clinical use, is called parthenogenetic embryonic stem cells (pESC). Through the process of parthenogenesis, an unfertilized egg is stimulated into differentiating as an embryotic cell would (Shanthly et. al. 2006).

Stem Cell Links to Tumorigenic Cells

Regardless of the types of ESC, these stem cells are derived from the core of mammalian blastocysts and can multiply infinitely

and differentiate into three germ layers: mesoderm, endoderm and ectoderm. However, there are stem cells that have been documented to have significantly more genetic mutations as they grow in vitro. Research performed on zebra fish indicates that nodal signaling and pathways are found in both metastasized cancerous cells and embryotic stem cells yet not in healthy skin cells (Topczewska et. al. 2006). Nodal proteins are growth factors important to pattern formations needed for the embryotic differentiation, specifically as it relates to the formation of the mesoderm and endoderm .(Shen 2007). Activation of the nodal pathways is tightly regulated and prompts self-gene inscription with positive and negative feedbacks .(Cheng, Sun, 2014). The similarities between ESC and tumorigenic cells may lead to discoveries that can limit the association between stem cells and cancer. Nodal signaling pathways are not the only unique link between stem cells and metastasized tumor cell; telomerase is an enzyme that facilitates growth and development of DNA in ESC but also found in upwards of fifteen times more often in cancer cells than in healthy cells .(Hiyama, Hiyama, 2007).

Nonsense-mediated mRNA decay (NMD) is been known to clean RNA in order to protect from early termination. At the PTC site, the location where early termination would commence, the NMD complex is assembled. This complex balances and regulates the proteins and is essential for homeostasis and overall survival of not only the embryonic cell, but organisms as a whole. One factor of the NMD complex is Smg5, this factor is involved in telomere and telomerase maintenance. Thus this NMD factor and Telomerase protein are both essential components that regulate cell viability. They may cause cell fatality or paradoxically, cancer (Li et. al. 2015).

Understanding the similarities between these cells help with limiting ESC. This process is called clinical transitions; scientific knowledge is gathered, understood and then turned into medical treatments. Regardless of these setbacks, the utility of these cells should not be undermined; simple laboratory practices such as freezing can assist with excess cell growth. Chemicals have been tested to determine if ESC can be treated to induce certain forms of apoptosis. Apoptosis is the shrinking of a complete cell and its membrane. Apoptosis differs from the generic form of cell death by the fact that it does not leave residue. Excess cell components that did not shrivel; undergo phagocytosis. The purpose of these chemicals would be to generate cell death and on the specific component of the cell that may lead to complications, while leaving the remainder of the stem cell unharmed. Research performed on these compounds and chemical were published in 2012 and these findings claim to identify compounds that altered the viability of ESC (Conesa et. al. 2012).

Research In Vivo/ In Vitro

A ten-year research program beginning in the late 1980's studied soft tissue engineering using totipotent stem cells. The experiment used mouse embryotic stem cells to attempt to build lymphatic tissue, among other structures. Prior to commencement the researchers set out to establish what would be an ideal environment for embryogenesis. Building tissues in vivo requires an environment that mimics all aspects of uterus or in vitro development; components such as nutrient and culture conditions are key. However, the physical creation of such an environment is quixotic, at best (Chen et. al. 1997, Shanthly et. al. 2006). A later study characterizes the complexity of stem cells by explaining the conditions for maintaining undifferentiated status. This study dictates that FGF2 and conditioned medium (CM) support hESC in an undifferentiated state. In 2005 undifferentiated hESC were maintained with either FGF2 or CM. These stem cells remained in undifferentiated colonies apposed to the hESC that were maintained without these factors. Maintenance of the undifferentiated cells is an extremely delicate process, indicating that the steps from that point forward, toward proper differentiation, is that much more complex (Sumi et. al. 2007, Umar et. Al. 2014).

Development of any tissue, regardless of its origin requires three steps: cell differentiation, complex tissue formation, and the building of a three-dimensional, three-layered germ celled organ (Chen et. al. 1997). The most complex portion of complete in vitro embryogenesis is the maintenance of a live culture throughout this process. As it is with a fetus, tissues, and organs can only function if the fetus is alive. Considerable research has been completed, and is ongoing, on many different forms of tissue growth. To better understand the processes of building cells and tissues with stem cells, this paper will only be discussing two different tissues developed in culture, cardiac myocytes and lymphoid tissues. The decision to use these specific examples is based on varied dates of the research and the difference in tissue complexity, due both to function and development of each cell.

Cardiac Myocytes

The differentiation of stem cells to cardiac myocytes is a rather complex. Cardiac muscle is a prime example of a complex tissue due to the various forms of specific muscular tissues and their functions as it relates to an efficiently operating heart. Cardiac tissue is made up of interlocking, single nuclei, striated, muscle fibers. The unique and complex aspect of this tissue includes the intercalated disc, which assist in conduction speed, and autorythmicity, the ability to set its own contractions. Research published in April of 2011 suggests promising potential of differentiation of cardiac myocytes from hESC. By inhibiting Bone Morphogenic Proteins (BMP), a growth factor,

during cardiac differentiation, cardiogenesis is initiated. This step is far from the only one that is needed to allow for ESC to be transplanted for therapeutic uses. Additional research is required such as the study of the regulation of retinoid signaling, the key that defines the difference between ventricular and arterial myocytes (Zhang et. al. 2011, Hartman et. al. 2014).

Table 1

ESC:	iPSC:	SSC:
Derived from inner mass of blastocyst	Derived from somatic cells	Isolated from postnatal adult tissue
Allogenic material	Autologous or Allogenic material	Autologous or Allogenic material
Pluripotent	Pluripotent	Multipotent
Can differentiate into all three germ cells	Can differentiate into all three germ cells	Can differentiate to limited cell types.
Self-renewal	Self-renewal	Limited Self-renewal
Indefinite Growth	Indefinite Growth	Limited Lifespan
Significant Teratoma Risk	Significant Teratoma Risk	No Teratoma Risk
Serious Ethical Issues	Serious Ethical Issues	Serious Ethical Issues
Immuno-priviliged	Not Immuno-priviliged	Unpredictable

(Prendergast 1999)

Lymphatic Tissue

The microenvironment needed for embryogenesis is delicate. Being that lymphoid cells develop toward the end of this process, it was difficult to assume that the microenvironment need for ES cultures could be sustained until that point. Initiation of the research began with mice. Through the use of viral vectors, oncogenes were inserted and developed in vivo to produce B and T cells. Many attempts and adjustment were made. The generation of B and T cells from lymphatic tissue was rare at the time publication of this journal but at the end of the research it was confirmed that lymphoid precursor cells could be derived from ESC. These years of research, experiments, and analysis of data only begins to explain the complexity of building tissues, regardless of their origin (Chen et. al. 1997).

Complications

Regardless of the difficulty of building tissues from stem cells and the improvements to cell cultures explained in cardiomyocytes formation, there are other complications that require attention. In fact, some stem cell therapies such as bone marrow transplants with somatic stem cell, do not involve the intricate complexities that tissue growth requires. Yet even these therapies must be addressed as it relates to the other risk factors involved in such a therapy. Risk factors begin in procurement and linger through the many phases involved, beginning with culturing and injection. Subsequent to any initial success, long-term affects must be analyzed. There is deficient clinical experience in embryonic and adult stem cells. This lack of experience is an inevitable aspect of relatively new, current, and ongoing research and results in a variety of complications, after stem cell injection (Herberts et. al. 2011).

General observations of the three most common forms of stem cells are summarized in Table 1. Improved comprehension of the differences and characteristics of these cells will assist in establishing their risk analysis.

An example of a consequence is tumor formation. This is a known complication that is being investigated from different standpoints. Understanding cell growth and death is crucial to such an analysis. The Myc gene family encompassed both factors. It is for that reason that this paper uses c-Myc as an example of a protein that may help limit tumor genesis. In addition fifty percent of tumor formation found in mouse research of iPSC is attributed to c-Myc.

Tumor Formation

The basic and universal self-renewal factors that assist in feedback and feed forward loops is Oct4, Sox2 and Noanog. However, the Myc gene family is additional self-renewal proteins and factors that affect intrinsic stem cell existence and apoptosis. Research published in 2007 investigated the function of c-Myc in stem cells regulation, differentiation, and apoptosis. Initially the tests were performed on mouse stem cells and later applied to hESC. It was widely known that that c-Myc is required to regulate normal cell development. However, the application of this factor in hESC regulation was confirmed and verified as being expressed as three different factors of the same family (Myc, Mycn, MycL1). Each of these Myc proteins were tested and found in the mRNA of hESC. Furthermore, just as c-Myc was present in regulation of mice cells, it was traced in undifferentiated human stem cells. Testing of this protein suggests that c-Myc is a factor of self-renewal. This research further investigated if c-Myc would also affect apoptosis and differentiation (Sumi et. al. 2007).

The properties of the c-Myc protein are best explained when looking at cancer cells. The protein factor c-Myc is found to be uncontrolled in end-stage cancers. This overexpression in tumorigenic cells helps explain the role it plays in normal cell growth, stem cell proliferation, differentiation, and apoptosis. Even prior to a complete discovery of the potential of c-Myc, it was known that increased levels of c-MYC indicated the initiation of cell death. There are various pathways in which apoptosis is initiated. The first is by the regulator p53 ultimately activating BAX. The second is activation of PI3'K-AKT. The third and final pathway to cell apoptosis is through cell ligations that activate c-Myc. The activation and increase of this protein then caused apoptosis (Prendergast 1999).

Being that this factor plays a role both in the differentiation of stem cells and the death of tumor cells, it must be assumed that investigations on regulation of this protein may be vital to advancements in stem cell therapy. With better control of this protein a clinical laboratory scientist may be able to use it in either furthering the differentiation of cells or leaving them in an undifferentiated state when deemed necessary. The link between the Myc gene family and oncogenic cells will additionally assist with the considerable complications facing stem cell therapy, tumorgenesis.

Aside for the risk of essentially causing tumor formation, stem cell therapy may also affect the growth and formation of existing tumor cells. If, for example, a patient has a dormant tumor of cancerous cells, stem cell therapy may active it. This has not been proven but is under investigation with the use of mouse stem cells.

Immune Response

Any donation requires proper human leukocyte antigen matching (HLA); stem cell therapy is no different. Taking care to ensure that the donor and recipient match decreases the risks of Graft Verse Host Disease (GVHD). As detailed in table one, some forms of stem cell therapy are allogeneic. Therapies that are not recognized by the body risk immune rejection. Even those that are immune privileged (hESC) may be subject to rejection and/or an inflammatory response due to the increase in response stimulus, caused by the actual stem cell therapy induction. For that reason, stem cell therapy may be administered while the patient is on immune suppressants. Placing a patient on immune suppressants runs the risk of adverse drug reactions. There are ongoing studies investigating different methods of stem cells growth in vivo that will limit the risk of rejections and GVHD. Such studies include introduction of mesenchymal stem cells in vivo. Data from such research indicated that these stem cells tranquilize the immune system and regulate its responses (Herberts et. al. 2011).

Teratoma

A teratoma is a benign mass composed of a hazardous mixture of tissues, generally resembling remnants of all three germ layers. It is established in vivo and assumed to be congenital. Being that pluripotent stem cells can create all three layers, this therapy is susceptible to such a risk. In fact the same characteristics that defines stem cell, pluripotency, are the same trait that creates teratomas. Research produced in 2011 details the comparative affects of tumor formation on both hESC and iPSC. The research indicates that because iPSC is a stem cell engineered in vitro and thus a more controlled cell than hESC, iPSC is less susceptible to tumor genesis. In fact the reason that teratomas are found less frequently in iPSC is because the heterogeneity of this cell causes slower growth rate (Zhang et. al. 2012).

In addition to cell type effects on teratomas, injection sites may also influence distinctive increase or decrease levels of formation. Although not enough clinical research and analysis can confidently support this claim, it is reasonable (Zhang et. al. 2012). More research has been performed on mouse stem cells to detect the correlation of the environment in which the stem cells are injected into and malignant tumors. Although tumor growth differs from teratomas, these discoveries may lead to additional exploration into the plausible affect of increased teratomas based off of site administration (Herberts et. al. 2011).

Success

Success is a complicated term to define when associating it with ongoing scientific research. Success can be attributed to a research breakthrough or a clinical accomplishment; it's simply relative. Longevity is fundamental determinate of success. Pluripotent haematopoietic stem-cell transplantation for cancer therapy has made significant stride since initial investigations over sixty years ago. It progressed from research that was plagued with complications and hurdles to a standard form of treatment. This blood forming stem cell is the only therapy approved for standard use because of sufficient understanding of the cells, possible complications, and long term affects. Expansion of this therapy for use other than replacement of blood and immune cells causes similar complications mentioned throughout this document. There are many trials taking place and success may be recognized within each trial but a complete understanding of overcoming challenges and complications can only be determined by continual research and the test of time (Stem Cell Information, n.d.).

Conclusion

Research, along with case studies and accounts show there are far more complications with stem cells than would appear at first glance; they require numerous forms of adjustments to insure an innocuous treatment. Limiting stem cell therapies is a promising prospect, and in some therapies it has been successful. However, there is insufficient research completed at this time to form a definite universal response for limiting any form of stem cell for every therapy application.

Researchers are investigating the factors that cause the risks involved in various therapies and consequently exploring remedies and suggestive solutions. However, stem cell therapies involve a multitude of complexities, some yet to be ascertained. The reason for these uncertainties appears to be based on the varied potential administrations, forms of stem cells (hESC, iPSC, ntES), their associated risks, and the limitless therapies they can

produce. This research is a relatively new and revolutionary field of study. With increase research, funding, and integration of continual findings, selectively listed in this paper, scientists are advancing to the goal of utilizing all that stem cells have to offer. This includes the capability to understand its potential, related limitations, and safely developing the two in an effort to advance medicine. Stem cell therapy is the promise for infinite preventative care and treatment options.

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