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History and Recent Advances of Stem Cell Biology and the Implications for Human Health

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INTRODUCTION

Stem cells are specialized cells that are found in multicellular organisms. The have the unique capability of being able to divide and differentiate into a variety of different kinds of specialized cells. This unique ability allows stem cells to play many roles, one being to contribute as an internal repair system. The ability to divide without limit allows them to help replenish certain cells types and tissues. Since the 1980's when stem cells were first isolated, scientists have been working to understand their behavior and properties in hopes of altering care of individuals with hematologic, oncologic, dermatologic, ophthalmologic, and orthopedic conditions. In recent years, there has been a tremendous increase in our understanding of stem cell biology. Recent studies have shown that adult stem cells can be isolated from a wide variety of tissues, including bone marrow, peripheral blood, muscle, and adipose tissue. The potential clinical applications lead to an extended interest in the use of stem cells in many medical disciplines. The most important role stem cells play in medicine is stem cell therapy: this is when cells that have been lost, destroyed, or altered are replaced. Advances in stem cell research have occurred sporadically since their discovery, however, positive outcomes and benefits are becoming more common with our improved understanding go their properties. Serious ethical concerns have been raised regarding stem cell research. Such concerns for example include and are not limited to issues about disruption of embryos to recover usable stem cell lines. This project reviewed the timeline of development for stem cell research with in depth detail of the significant milestones reached in the development of this important area of biological research.

STEM CELL BIOLOGY

Most of the 300 trillion cells of the body have completely specialized functions. Blood, lung, brain, skin or liver cells are all specialized for what they do. They cannot do anything other than what they were designed for. Stem cells, on the other hand, do not have a specialized function; they are an immature kind of cell that still has the potential to develop into many different kinds of cell. [19]

PROPERTIES OF STEM CELLS

In addition to being unspecialized, all stem cells have two additional properties: self renewal, and potency [16]

Self Renewal: Self-renewal is the ability of cells to proliferate without the loss of differentiation potential and without undergoing senescence (biologic aging). Stem cells are hypothesized to be able to divide symmetrically (in which both daughter cells are either stem cells or differentiated cells) or asymmetrically (yielding one stem cell and one more differentiated cell).

Potency: specifies the potential to differentiate into different cell types of the stem cell. Stem cells can be either totipotent, pluripotent, multipotent, or unipotent.

- Totipotent cells have the capability to produce all cell types of the developing organism, including both embryonic and extraembryonic (ex. placenta) tissues.

- Pluripotent cells can only make cells of the embryo proper, but make all cells of the embryo including germ cells and cells from any of the germ layers. Therefore, they can make any cell of the body.

- Multipotent cells can only make cells within a given germ layer. For example, multipotent stem cells from a mesodermal tissue like the blood can make all the cells of the blood, but cannot make cells of a different germ layer such as neural cells (ectoderm) or liver cells (endoderm).
- Unipotent cells make cells of a single cell type. An example is a germ cell stem cell that makes the cells that mature to become egg or sperm, but not other cell types.

The potency of cells is tied to the time of embryonic development of the organism. Cells that have totipotency arise from the first few cell divisions following fertilization of the egg. Pluripotent cells were thought to be limited to cells derived from either the inner cell mass of the blastocyst or nascent germ cells in the embryo. It is now known that pluripotent cells can arise from other cells types as well. In day 10 to 14 post-fertilization in the human, most stem cells are restricted to be either multipotent or unipotent. [19]

SOURCES OF STEM CELLS

Embryonic Stem Cells (ES)

Embryonic stem cells are derived from the embryo. They are derived 7-10 days after fertilization from the pre-implantation blastocyst. As mentioned above, ES cells are pluripotent. Ethical considerations have prompted research into other stem cell sources because deriving ES cells disrupts the blastocyst. [18]

Adult Stem Cells

Adult stem cells, also known as somatic stem cells, are present in most, but not all tissues. They are mostly multipotent however pluripotent adult stem cells exist in small number. They persist throughout life and possess the role of maintaining and repairing tissue, in which they are found, in response to injury. They have been identified in many tissues including brain, bone marrow, blood vessels, heart, liver, and others. They reside in a specific area of each tissue called a stem cell niche. [18]

Induced Pluripotent Stem Cells (IPS)

In 2006, an experiment was done in which genes that were usually expressed in ES cells were introduces into mature cells. This process called reprogramming, lead a small number of mature cells to revert back to a highly immature cell state that resembled an ES cell. It induced a pluripotent state in a previously differentiated cell, hence the name. [19]

STEM CELL REASEARCH

DISCOVERIES OF THE 20th CENTURY

No one specific scientist or team of scientists discovered stem cells. The discovery of stem cells was an ongoing effort of several decades. The term “stem cell” goes far as back as 1908 when Russian histologist Alexander Maximow developed and introduced a theory of hematopoiesis, a theory upon which our present concept of blood cells' origin and differentiation is based. [10] After that several papers were published regarding hematopoietic stem cells. In 1932, Dr. Florence Sabin published evidence of functional undifferentiated hematopoietic stem cells in the marrow.[14] In the 1950s, Dr. Thomas of the Fred Hutchinson Cancer Research Center began his work on bone marrow transplantation, supporting the existence of hematopoietic stem cells. In the late 1950s he performed the first ever bone marrow transplant that successfully treated leukemia. The transplant involved identical twins one of which had the leukemia.[7] There were no problems with the transplant because both twins shared the same genetic makeup. In the 1960’s, three major discoveries were made. In 1962, in an autoradiographic investigation, Joseph Altamn presented evidence of neurogenesis in ongoing stem cell activity in the brain. In 1963, the presence of self-renewing cells in mouse bone marrow was demonstrated by McCulloch and Till. This discovery was accidental. Dr. McCulloch and Till were studying the effect of over coming radiation injury on animals. They irradiated mice with enough X-rays to kill them within 30 days unless they receive a transplant of fresh, undamaged bone marrow cells. They then injected different amounts of cells to determine how many cells were necessary to keep the animals alive. 10 days later, what they found is nodules in the spleen of the surviving mice. What they then came to the conclusion of is that the cells they were transplanting were a source of the new blood cells that were keeping the animals alive. They showed that a bone marrow-derived cell could replace all the blood elements and rescue a lethally-irradiated animal by simple infusion of donor cells into the blood. [20] It wasn't until 1968, in Minnesota, that the first successful non-twin transplant was performed. By this time, it was known that a key to a successful transplant was a specific type of genetic matching (known as HLA) of the donor to the patient.[7]

The next major discovery was in 1978 when hematopoietic stem cells were discovered in human cord blood.[14] Shortly after that Martin Evans along with Matthew Kaufman, managed to extract mice embryonic stem cells from mice blastocysts. This was the first in vitro stem cell line developed from mice, and the first time embryonic stem cells were discovered in mice. During the same year, Gail R. Martin almost simultaneously illustrated various techniques for extracting mouse embryonic stem cells. She demonstrated the pluripotency of the embryonic stem cells by observing a wide variety of cell types derived from isolated single cells. She is attributed for inventing the "embryonic stem cell". [13] In 1997 two major discoveries occurred. First, Bonnet and Dick showed that Leukemia originated from a hematopoetic stem cell. This was the first direct evidence for cancer stem cells or CSCs.[14] CSCs are cancer cells that are found in hematological cancers or tumors. They possess normal stem cell traits and are able to give rise to all cell types within a particular cancer sample. CSCs are therefore tumorigenic. 1997 also unveiled the first artificial animal cloning of Dolly the sheep. She

was created in a way where a cell nucleus from an adult cell was transferred into an unfertilized oocyte that has had its nucleus removed. This new cell was then electrically shocked and stimulated to divide. Once it developed into a blastocyst it was implanted into a surrogate mother. The production of Dolly showed that genes in the nucleus of a mature differentiated somatic cell are capable of reverting to an embryonic totipotent state, creating a cell that can then go on to develop into any part of an animal.[9]

Major discoveries of the 20th century conclude in 1998, the year the first human embryonic stem cells were derived. Thompson, from the University of Wisconsin, isolated cells from the inner cell mass of early embryos and developed the first embryonic stem cell lines. In this process, fresh or frozen cleavage stage human embryos, produced by in vitro fertilization for clinical purposes, were cultured to the blastocyst stage. 14 inner cell masses were isolated, and five ES cell lines originating from five separate embryos were derived. Then, beginning in 1999, scientists discovered that manipulating adult mouse tissues could produce different cell types. This discovery was exciting for the field of stem cell research. It meant that cells from bone marrow could produce nerve or liver cells and cells in the brain could also yield other cell types. This discovery promised a greater future from stem cell research. [4,9,21]

21ST CENTURY-PRESENT

The beginning of the 20th century saw several experiments regarding adult stem cell plasticity and different sources of adult stem cells. In 2001, the first ever human embryo was cloned at the early stage of 4-6 cells. This was done for the purpose of generating ES cells. So many different studies and information were being released that unfortunately, the beginning of the 21st century also saw different fabricated studies and findings. In 2005, a Korean researcher claimed to have created ES cells from unfertilized human eggs which later shown to be fabricated. Different discoveries followed after that including cord-blood-derived embryonic-like stem cells (CBEs) which have the ability to differentiate into more cell types than adult stem cells, opening up greater possibilities for cell-based therapies.[4,9] 2006 marked the year iPS were discovered, as mentioned above. The ability to induce pluripotency changed the landscape of stem cell research. First of all, it provided an alternative to ES stem cell, the main source of pluripotent cells. Second, it indicated that the state of a differentiation of a cell could be manipulated. Third, a cell taken from an individual can be induced to become a cell type capable of forming any other cell type in that individual's body. Finally, iPS from a given individual represent a highly personalized source of cells.[15] Yet another type of stem cells was discovered in 2007 that was isolated from amniotic fluid. This finding is also important as it could also serve as an alternative to ES cells since they are also pluripotent in nature. In 2010, here in the United States, the first ever human embryonic stem cell trial occurred. In 2002, President George W. Bush banned government funding for embryonic stem cell research due to ethical issues regarding disruption of the embryo. In 2009, however, President Barack Obama lifted this ban and allowed research to continue. The trial was done by Geron Corporation, a biotechnology firm. The company hoped that GRNOPC1, a product derived from human embryonic stem cells, would stimulate nerve growth in patients with debilitating damage to the spinal cord. After investing millions of dollars in the research leading up to this trial, Geron Corporation discontinued the study

in November 2011 to focus on cancer research. Although no official results from the trial have been published, preliminary results from the clinical trial were presented at the American Congress of Rehabilitation Medicine (ACRM) conference in October 2011. None of the participants experienced serious adverse events, although nausea and low magnesium were reported. In addition, no changes to the spinal cord or neurological condition were found. The first patient that enrolled in the trial "begun to get some very slight sensation: He can feel relief when he lifts a bowling ball off his lap and discern discomfort when he pulls on hairs on some parts of his legs. He has also strengthened his abdomen." [2,4,5] The following years up to today included different experiments and clinical trials involving the different types of stem cells which some will be discussed below.

SUCCESSFUL CLINICAL APPLICATIONS

The1963 experiment done by McCulloch and Till showed that damaged tissue can be replaced by bone marrow-derived cell. This led to many clinical testing and applications of hematopoietic stem cell transplantations. Over the next 20 years, hematopoietic stem cell transplantation became the standard way to treat those with hematologic illnesses and bone marrow failures. Overtime, as more types of stem cells were discovered in different tissues, different clinical applications became used. For example in 1975, it was discovered that cultured cells from the skin can generate a large number of cells that can be enough to provide a cutaneous barrier for those with severe burns. Up until that point stem cell replacement was mainly used in hematologic diseases and burns. As time passed, stem cells were discovered in even more areas and so stem cells became used in other areas such as bone grafting in orthopedics and corneal generation in ophthalmology. Over the past two or three years, there has been a rapid surge in clinical trials involving stem cell therapies. Today, there are various preclinical testings of stem cell therapies for other disorders, including those involving muscle, organ, and nerve tissue. The potential use of stem cells to restore missing, lost, or damaged tissues plays a central role in the field of regenerative medicine. Human embryonic stem (ES) cells have been successfully differentiated in vitro into multiple cell types for therapeutic uses, including oligodendrocytes, pancreatic cells, cardiomyocytes, and hematopoietic precursor cells. The therapeutic potential for ES cell-derived somatic cells has been demonstrated in animal models of retinal blindness, Parkinson's disease, Huntington disease, spinal cord injury, myocardial infarction, and type I diabetes mellitus. [1,9,16,21]

RETINAL DISEASES

Human ES cell-derived retinal photoreceptors have been used to improve visual function in blind mice. Following intraocular injection, retinal cells derived from human ES cells migrated into the appropriate retinal layers and expressed markers of differentiated rod and cone photoreceptor cells. Subretinal transplantation of the cells into the subretinal space of mice with Leber's congenital aneurosis restored light responses to the animals. In a later study, ES cell-derived photoreceptors transplanted into the eyes of adult mice were able to integrate and mature into outer segment-bearing photoreceptors. [12] In 2012, the first report of the safety and tolerability of human ES cell use for the treatment of two

patients with retinal disease appeared (one patient with dry age-related macular degeneration, one patient with Stargardt's macular dystrophy). Human ES cell differentiation resulted in greater than 99 percent pure retinal pigment epithelium (RPE), of which 50,000 RPE cells were injected into the subretinal space. The human ES cell-derived RPE proliferation was confined to the subretinal space. Both patients were treated with low-dose immunosuppressants beginning one week before the procedure and continuing for six weeks, followed by six additional weeks with no signs of rejection noted during the follow-up. Improvement in visual acuity was noted in both patients. Results:

- In the patient with macular degeneration, visual acuity improved from 20/500 at baseline to 20/200 at two weeks post-procedure before settling at 20/320 at long-term follow-up. [17]

- In the patient with Stargardt's macular dystrophy, visual acuity improved from seeing hand motions at baseline to counting fingers at two weeks post-procedure with improvement to 20/800 at one, two, and three months follow-up. [17]

This initial report of human ES cell transplantation into the retinae of two patients shows the apparent short-term safety and tolerability of this treatment in humans, with apparent efficacy as well. Additional studies assessing the dosage, efficacy, and longer-term safety are required prior to widespread clinical application of human ES cell transplantation. [17]

Retinitis pigmentosa (RP): comprises a complex group of inherited dystrophies characterized by progressive degeneration and dysfunction of the retina, primarily affecting photoreceptor and pigment epithelial function. Symptoms of retinitis pigmentosa (RP) include night blindness, loss of peripheral vision from progressive loss of photoreceptors, and variably loss of central vision due to cataracts and macular edema. Although there is no cure for RP, new treatments, involving gene therapy, transplantation, and implanted electrical devices, are in development.[16]

The potential for transplanted retinas, retinal sheets, and clumps of retinal neurons to restore vision in mice and humans with RP has been under investigation since the 1980s. Until recently, transplanted tissue or cells have not developed synaptic connections with host cells and thus have not had functional impact. The potential for transplantation of fetal retinal pigment epithelium to rescue abnormal photoreceptors in RP is being investigated. After fetal retinal implantation into six patients with RP, slight improvement in visual acuity was seen in three patients (one patient with bilateral improvement including the eye that did not receive the transplant) although the extent of improvement was not clinically significant. Integration of donor cells into the host retina is dependent upon the stage of cell differentiation at the time of donor harvest. Successful integration of committed fetal progenitor cells that developed into functional rod photoreceptors was demonstrated in a mouse model of RP. Transplanted cells did not integrate into the retina in mice with more active disease and more rapid retinal degeneration. [6]

CARDIAC DISEASES

An increasing number of studies have used hematopoietic stem cells to repopulate the myocardium of patients with an acute myocardial infarction (MI) or ischemic cardiomyopathy. The potential benefit of intramyocardial bone marrow cell injection was studied in 50 patients with chronic angina who were randomly assigned to an intramyocardial injection of either bone marrow-derived cells or placebo
Results:

- After a three-month follow-up, active therapy was associated with a statistically significant, but modest, improvement in myocardial perfusion compared with placebo.
- Further investigation was determined to be necessary to evaluate not only efficacy but also safety concerns. (It should be noted that other studies of intramyocardial cell injections have produced different results and that at the present time there is no scientific rationale for this therapy or any plausible mechanism of action that could explain such a result.) [16]

Several studies have demonstrated that transplantation of human ES-derived cardiomyocytes improves contractile function of the infarcted mouse heart. Using a combination of pro-survival factors that limited cardiomyocyte death after transplantation, one group demonstrated decreased rates of heart failure and partial remuscularization (as evidenced by systolic thickening of the infarction wall) in animals treated with human ES-derived cardiomyocytes when compared to control animals treated with non-cardiac human ES derivatives. [11]

In 2013, a study in the University of Pittsburgh allowed a mouse heart stripped of all its cells to beat again by replacing its cells with human heart precursor cells. For the project, the research team first "decellularized," or removed all the cells, from a mouse heart, a process that takes about 10 hours using a variety of agents. Then, they repopulated the remaining heart framework with multipotential cardiovascular progenitor (MCP) cells. These replacement cells were produced by reverse engineering fibroblast cells from a small skin biopsy to make induced pluripotent stem cells and then treating the iPS cells with special growth factors to further induce differentiation.[3]

"This process makes MCPs, which are precursor cells that can further differentiate into three kinds of cells the heart uses, including cardiomyocytes, endothelial cells and smooth muscle cells," Dr. Yang explained. "Nobody has tried using these MCPs for heart regeneration before. It turns out that the heart's extracellular matrix – the material that is the substrate of heart scaffold – can send signals to guide the MCPs into becoming the specialized cells that are needed for proper heart function." [3]

After a few weeks, the mouse heart had not only been rebuilt with human cells, it also began contracting again, at the rate of 40 to 50 beats per minute, the researchers found. More work must be done to make the heart contract strongly enough to be able to pump blood effectively, and to rebuild the heart's electrical conduction system correctly so that the heart rate speeds up and slows down appropriately. [3]

CONCLUSION

Stem cells have a history that dates back as far as 1908. Research has been going on for more than 100 years in hopes of finding cures for those diseases that have been a great threat for human beings. Certain diseases, which are being cured now and can be cured in the future, include Parkinson's disease, coronary artery disease, cardiomyopathy, congestive heart failure, bone marrow transplants, leukemia, diabetes, and cell replacement therapy in neurological disease. Of course, there are dozens of other diseases on which stem cell research is in progress, including treatments of vision or ocular disease processes like retinitis pigmentosa and corneal regeneration, as well as musculoskeletal disorders like muscular dystrophy. Before widespread application becomes feasible, several complexities must be addressed. These include but are not limited to:

1. Tissue regeneration: The first issue is how transplanted cells will integrate into surrounding tissue to achieve a physiologically beneficial effect. This is important where coordination of complex networks of cells is essential, such as in the heart and brain where abnormal circuits can result in serious adverse events.[16]
2. Oncogenesis: A second concern is the potential for transplanted cells to form tumors. This is of particular importance when using pluripotent cells, since these are characterized by the ability to form teratomas in animal models [16]
3. Directed differentiation: A third concern is the ability to direct the differentiation state of the cells to be used. Generating the proper cell type from pluripotent cells remains a significant challenge for some cell types.[16]

However, even with the vast amount of research that is yet to be done, stem cells hold a future in regenerative medicine that cannot be denied in the 21st and the upcoming centuries.

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