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Munazza Gull
Biochemistry Department, Faculty of Sciences, King Abdul Aziz University, Jeddah, Saudi Arabia

Lujain Abdulaziz Alsaleh
Biochemistry Department, Faculty of Sciences, King Abdul Aziz University, Jeddah, Saudi Arabia

Muhammad Aamer Mehmood
Department of Bioinformatics & Biotechnology, GC University Faisalabad, Pakistan

Abida Kausar
Department of Botany, GC Women University Faisalabad, Pakistan

Taha Abdullah Kumosani
Biochemistry Department, Faculty of Sciences, King Abdul Aziz University, Jeddah, Saudi Arabia

Correspondence
Munazza Gull
Biochemistry Department, Faculty of Sciences, King Abdul Aziz University, Jeddah, Saudi Arabia

Growth, origin and applied potential of embryonic stem cells: A recent fact sheet about stem cell therapies as a promising option for incurable diseases in humans

Munazza Gull, Lujain Abdulaziz Alsaleh, Muhammad Aamer Mehmood, Abida Kausar, Taha Abdullah Kumosani

Abstract
The undifferentiated inner mass cells of a human embryo represents the major source of embryonic stem cells. These embryonic stem cells are pluripotent in nature showing that they can differentiate into all the derivatives of three primary germ layers which are ectoderm, endoderm and mesoderm. They have a potential to develop into each of the more than 200 cell types of an adult body when they are instructed or specified to do so. Embryonic stem cells have two unique properties: (1) they replicate to create many more stem cells and (2) they can grow into different types of cells throughout the body like liver, muscle, bone, nerve, etc. In fact, certain types of adult stem cells could replicate for several months outside of the body in the laboratory, creating more stem cells that are used in different medical treatments. Scientists are trying to develop new medical solutions capable of enhancing the capacity and potential for growth and healing available in embryos and very useful in later stages of adult life. These novel capabilities and strategies present in embryonic stem cells could regenerate or replenish tissues or specialized cells damaged by Alzheimer's, cancer and other chronic and fatal diseases and which can leads towards death. By utilization of embryonic stem cells as a tool, more knowledge about body development and maturity could be explored and utilized in therapeutics. In medical science, the cutting edge technology of stem cells and its discoveries about hidden potential within the cells of our own bodies could make wonders. Conclusively, this review summarizes that the use of human embryonic stem cells as therapy with respect to in vitro propagation and differentiation as well as their use in basic cell and developmental biology particularly for various tissue regeneration and transplantation is a profitable investment in future for ourselves and for coming generations.

Keywords: incurable diseases, embryonic stem cells, induced pluripotent stem cells, human embryo

Introduction
Stem cells are natural units of embryonic development and regeneration of tissues. Particularly, embryonic stem (ES) cells possess a nearly unlimited self-renewal potential and developmental capacity to differentiate into virtually any cell type of an organism. These stem cells with pluripotent property can proliferate in the human body to all the different types of cells but do not contain the genetic information to make a placenta. These stem cells are primarily found in the human embryo during its earliest stages. Pluripotent stem cells are typically what people are referring to when they generically refer to stem cell [1]. Embryonic stem cells are derived from a four- or five-day-old human embryo that is in the blastocyst phase of development. The embryos are usually extras that have been created in IVF (*in vitro* fertilization) clinics where several eggs are fertilized in a test tube, but only one is implanted into a woman.

Sexual reproduction begins when a male's sperm fertilizes a female's ovum (egg) to form a single cell called a zygote. The single zygote cell then begins a series of divisions, forming 2, 4, 8, 16 cells, etc. After four to six days - before implantation in the uterus - this mass of cells is called a blastocyst. The blastocyst consists of an inner cell mass (embryoblast) and an outer cell mass (trophoblast). The outer cell mass becomes part of the placenta, and the inner cell mass is the group of cells that will differentiate to become all the structures of an adult organism. This latter mass is the source of embryonic stem cells - totipotent cells (cells with total potential to develop into any cell in the body) [2].

The first successful derivation of human embryonic stem cells
Thomson's Lab was the first to report the successful isolation of human embryonic stem cells. On November 6, 1998, Science published this research in an article titled "Embryonic Stem

Cell Lines Derived from Human Blastocysts", and later featured in its "Scientific Breakthrough of the Year" [3]. In this experiment researchers isolated the inner cell mass (ICM) plated onto mitotically inactivated mouse embryonic fibroblasts (MEF) and expansion of outgrowth into established embryonic stem (EM) cell lines. Recently rapid progress has been achieved and many researchers have indicated the derivation of new human embryonic stem (hes) cell lines and its growth potential.

In vitro growth of the embryonic stem cells

Cell culture represents the growth of cells in the laboratory under controlled conditions. The human embryonic stem cells (hes) lines are cultivated from a preimplantation-stage embryo cells by transferring into a plastic laboratory culture dish having nutrient broth medium. The cells proliferate and spread over the surface of the dish. In the original method, specially treated mouse embryonic skin cells which are unable to divide was coated on the inner surface of the culture dish. This coating layer of cells is called a feeder layer. The mouse cells in the bottom of the culture dish provide the cells a sticky surface to which they can attach. Nutrients are released into the culture medium by these feeder cells. Scientists have now established the protocols for successful growth of embryonic stem cells without mouse feeder cells. This shows an emerging and eminent scientific achievement to avoid the risk of transfer of viruses or other macromolecules from the mouse cells to the human cells.

Experiment of generating an embryonic stem cell line is not always successful each time when cells from the preimplantation-stage embryo are placed into a culture dish. But if the plated cells survive, multiply and proliferate enough to crowd the dish, they are gently separated and re-plated into different other fresh culture dishes with nutrient medium. For months, the process of sub culturing of cells is repeated and each cycle of sub culturing is referred as a passage. After establishment of cell lines, the millions of embryonic stem cells could be yielded from original cells. Embryonic stem cells that have proliferated in cell culture for a prolonged period of time without differentiating, and are pluripotent are referred to as an embryonic stem cell line.

At any stage in the process, batches of cells can be frozen and shipped to other laboratories for further culture and experimentation [4]. It is well known now that success rate in deriving human embryonic stem cell lines is highly dependent on the quality of recovered blastocysts, isolation conditions and experience of the group. However, a common factor in nearly all studies which describe successful derivation of hES cells is that the blastocysts used for immunosurgery are recovered in sequential two-step culture system using G1 and G2 media. This system is designed not only to allow for changes in nutrient requirements and metabolism as development of early human embryos proceeds but also to facilitate the development of highly viable blastocysts.

The Origin of Embryonic Stem Cells

Embryonic stem cells are generated from cells found in the few days old embryo. At this stage the embryo is a ball of about 100 cells in humans, mice and other mammals. It is called as blastocyst and it has two halves: 1. trophectoderm which is an outer layer of cells and form placenta that provides support to the growth of embryo within the uterus.2. the inner cell mass (ICM) which is an inner clump of cells looks like a ball of 10-20 undifferentiated cells. These cells further specialized after multiplication and extensively differentiated to make the several types of cells required to form the tissues, organs and finally the entire animal body [5].

Treatments of some diseases by embryonic stem cells

Embryonic stem cells for diabetes cure

Diabetes mellitus is one of the major causes of death in advanced countries, and has been shown to adversely affect health and quality of life. It is associated with various severe or fatal complications, including blindness, kidney failure, heart disease, stroke, neuropathy, and amputations. Type I diabetes, or insulin-dependent diabetes, results from the cellular-mediated autoimmune destruction of pancreatic islet cells that are known to produce insulin. Type I diabetic patients experience high blood glucose levels as a result of insulin deficiency. There is no cure for this form of diabetes to date. Several approaches have been used in attempts to reverse the disease process for type I diabetes, including whole organ pancreas transplants and islet transplants [6, 7]. In addition, options such as the potential use of pancreatic stem and progenitor cells are being investigated [8, 9]. Currently, the only clinically approved treatment for type I diabetes, with the exception of insulin injection, is islet cell transplantation in combination with immunosuppressive therapy [10]. Unfortunately, this option is only available to a very limited number of patients because of a severe shortage of donor tissue sources. This shortage has focused interest in developing renewable sources of insulin-producing cells appropriate for transplant.

Embryonic stem (ES) cells have been proposed as a potential source of pancreatic β cells because they are self-renewing elements that can generate the many cell types of the body [11-17]. Recent studies suggest that mouse ES cells can be manipulated to express and secrete insulin [18-21]. However, insulin-producing grafts derived from ES cells in these initial reports have a high degree of cellular heterogeneity and proliferation, uncharacterized growth and tumor-forming potential, as well as low insulin levels compared to pancreatic islets. Additionally, some researchers claim that the insulin-positive cells derived from ES cells may not be real insulin-producing β-like cells. In one study, contrary to previous reports, no message for insulin was detectable in culture, which suggested that the cells may be concentrating the hormone from the medium rather than producing[22,23]. Another study showed that the main producers of insulin in culture were neurons and neuronal precursors and a reporter gene under the control of the insulin I promoter was activated in cells with a neuronal pheno-type [23]. Therefore, it is now a matter of controversy whether true pancreatic β cells can be derived from ES cells with the protocols so far developed. The issue whether ES cells can be used clinically for the treatment of diabetes also needs to be addressed.

The original protocol adapted a strategy used to generate neurons to derive endocrine pancreatic cells from ES cells [22]. It involves sequential *in vitro* differentiation steps during which cultures were highly enriched in cells expressing nestin, an intermediate filament present in neural stem cells and possible islet precursors. A reproduced and modified version of the original protocol for the differentiation of islet-like structures and further characterization of the system and its potential suitability for the amelioration of a diabetic condition has been developed providing a new hope in treatment of diabetic patients [24-25].

Embryonic stem cells for Parkinson's disease cure

Parkinson's patients always suffer due to shortage of dopamine chemical that controls messages sent to the brain and thus regulate the process of movements and thinking. The kill effect of this disease happens directly on dopamine-producing nerve cells and neurons. It attacks the part of the brain called the substantia nigra. However this disease does not affect other nerve cells of brain whose damage may cause the other forms of Parkinson's disease like insomnia, motivation resistance and thinking constraints. Parkinson's may also caused due to the formation of clumps in the brain by the abnormal alpha-synuclein protein generally called Lewy bodies.

Advancement in technology helps the scientists to know that which cells and areas of the brain are involved in development of Parkinson's disease. However the underlying cause of Parkinson's is yet to be unfolded. Researchers are aiming to grow the dopamine-producing nerve cells in the lab using stem cells which will help to understand the causes and advance therapy of the disease particularly if it is the case of genetic disorder. Parkinson's treatment could be possible by replacing the lost nerve cells with healthy new ones because a single, well-defined type of cell is affected. Researchers are expecting breakthrough results by introducing young cells into the brain might be able to cure Parkinson's disease. The big limitation in the further proceedings is that not enough fetal tissue available to treat the large numbers of people with Parkinson's disease. The use of fetuses also raises ethical questions and poses a hindrance in next level treatment.

Stem cells as an alternative source of new dopamine cells for Parkinson's patients comes as a ray of hope in the successful treatment of this disease. Transplantation of embryonic stem (ES) cells that can make dopamine-producing neurons into patients could be current possible solution to this disease. Both mouse and human embryonic stem cells in the laboratory are being used for making dopamine-producing neurons. The human cells have recently been shown to have similar effects as the fetal cells in a rat model of Parkinson's disease. Induced pluripotent stem (iPS) cells could be made from a patient's adult skin cells in the lab, and then used to make dopamine-producing neurons. American scientists successfully treated rats with neurons made from human skin cells using iPS techniques in 2010. The transplanted neurons in these rats gave promising results and showed improved features of Parkinson's disease. But mice and rats need less number of neurons than humans requirement and it is not yet to conclude that this approach might successful in human Parkinson's patients. Large scale studies are also needed to confirm that these cells are safe and would not cause brain tumours, and also do not show the symptoms and pathology of Parkinson's given they are derived from the patient's own skin [26].

Embryonic stem cells for cancer cure

Stem cell researchers at the University of Minnesota have persuaded human embryonic stem cells to develop cancer-killing cells in the laboratory for the first time and showed an optimistic approach for future cure of various types of cancers and tumors. Researchers are able to generate the "natural killer" cells from the human embryonic stem cells. Natural killer cells are normally present in the blood stream as a part of the immune system and are play a role in defending the body against infection and against some cancers by production of interferons. This research show the ability to make cells from human embryonic stem cells that are able to treat and fight cancer, especially leukaemia's and lymphomas. These results also depicted the potential of stem cells to treat conditions such as Parkinson's disease, diabetes, and Alzheimer's disease. This research suggests it is possible that we could use human embryonic stem cells as a source for immune cells that could better target and destroy cancer cells and potentially treat infections. The results also provided the researchers with a model of how the immune system develops. The possible use of human embryonic stem cell-derived natural killer cells can target cancer cells in animal models are being explored. Two federally approved embryonic stem cell lines were used in this research, however, new lines and advance research methods would have to be developed for use of these cells as successful treatment in people with cancer diseases.

Embryonic stem cells for eye diseases cure

For patients with degenerative eye diseases, a clinical trial in Korea was conducted for the first time to test the safety of an embryonic stem cell therapy for Asian descent people. Four individuals were followed for a year after they were treated with embryonic stem cell-derived retinal pigment epithelial cells for macular degeneration. This treatment showed no serious side effects like tumor growth or other unexpected effects. The goal of this safety study was to prevent the progress of disease in eye patients and to improve the visual perspicacity in the patients. More wider studies with broader case groups needed to conclude the role of embryonic stem cell therapy and positive responses from the eye patients need to be interpreted cautiously.

In 2014, a clinical trial in the United States emphasized that embryonic stem cells could be used safely for patients with degenerative eye diseases but most of the patients were with Caucasian origin. The patients in both case trials either suffer with age-related macular degeneration or Stargardt's macular dystrophy. These are the leading forms of adult and juvenile blindness in the developed world and both are incurable eye diseases currently. An attractive option to treat these diseases is embryonic stem cell-derived retinal cell therapy because these can be used to regrow the retina cells that are lost in both diseases. However, it is important that the clinical trials on wide scales should be conducted in a safe and responsible mode to increase the understanding of the underlying pathophysiology of embryonic stem cell-derived retinal cell therapy [28].

Embryonic stem cells for heart diseases cure

Until a few years ago, scientists thought that it was impossible to repair a damaged heart. The discovery of possible cardiac (heart) stem cells at the beginning of this century opened up new possibilities to use stem cells to repair hearts that have been injured through heart attacks (acute myocardial infarction) or chronic disease (chronic coronary artery disease). Several early studies in animals suggested that transplanting bone marrow stem cells into injured hearts would indeed partially repair these hearts. Later studies have shown that transplanted bone marrow cells do not produce new heart muscle cells. Research is ongoing to understand exactly what affect the bone marrow cells do have on the heart.

There are many ongoing clinical trials of bone marrow transplants to treat heart disease, particularly heart attacks (acute myocardial infarction). Generally speaking, in these

trials patients who have suffered a heart attack are given preparations of their own bone marrow stem cells- these are called autologous transplants. These clinical trials have demonstrated that this treatment is safe and some have recorded small improvements in heart function, but it has not been proven that bone marrow cells have a significant enough positive effect to improve on existing medical approaches. Many scientists feel that the findings of the different studies are not consistent and a lot of questions remain about their clinical relevance and long-term effects of the transplants. Today a continued laboratory research is needed, using both animal models and cells grown in the laboratory, in order to progress the development of potential new therapies. Some of the questions scientists are trying to answer include what source of cells can be used to obtain replacement heart muscle cells. For example, researchers are investigating the possibility of using heart muscle cells grown from embryonic stem cells, or made by 'reprogramming' adult specialised cells. Both techniques produce a mixture of types of cells so it is also critical to develop methods for obtaining pure heart muscle cells from these sources [29].

Embryonic stem (ES) cell based therapies

Currently, no ES cell-based therapies are enduring in humans. Only allogeneic or matched donor-derived adult stem cells have been employed in human cell-grafting therapies, the best examples of which are bone marrow transplantations for the treatment of leukemia after myeloablative therapies. The availability of human ES cells, however, represents an extraordinary opportunity for cell transplantation that may be applicable to a wide range of human ailments. Three properties make ES cells relative to adult stem cells very attractive for replacement therapies. 1) Human ES cells can be grown indefinitely in culture. 2) ES cells can be genetically manipulated, and loss of function genes can theoretically be repaired by the introduction of transgenes into ES cells either by random transgenesis or through gene targeting. Importantly, homologous recombination could be used to correct specific genetic mutations that would not lead to random mutations in tumor-promoting genes. 3) Numerous differentiation protocols have already been established that permit the generation of almost any cell type, either through the use of established culture conditions or when coupled with genetic manipulations. In theory, hES cells could be applied to a wide range of human ailments, but the proof of principle has largely come from the use of mouse ES cells.

Ethical issues in embryonic stem cell research

Human embryonic stem cells research provide strong grounds in favor of the research considering its potential therapeutic benefits. Health benefits achieved by this therapy overshadow the loss of embryos involved. Opposite views about this research claim that the constraints against killing innocent persons to promote social efficacy apply to human embryos also. The vital argument supporting the claim that it is unethical to destroy human embryos intentionally for therapeutic purpose. The human embryo is an innocent human being itself, therefore it is unethical to kill the human embryo which might develop into one normal complete body as individual person. The positive answer to this question is that most investigators engaged in human embryonic stem cells research do not participate in the derivation of human embryonic stem cells but instead use cell lines that researchers who performed the derivation have made available [30]. The socio-ethical concerns and legal aspects of stem cell research and related therapies usually arise are

1. Respect for human life requires that one should show respect for human embryos. People in opposition believe that embryonic stem cell research violates this principle, as an embryo is destroyed during the process of stem cell line derivation. Persons in favor argue that the potential benefits of stem cell research (e.g. alleviating human suffering) represents a way of showing respect for human life. A permanent fear about the creation of embryos for research purposes and the derivation of stem cell lines showed the potential dangerous use of human body/ organs for potentially uncontrolled commercialization.
2. The concept of human. Many people recognize the idea of human dignity as our indispensable civilization, what makes us human. So this thought is closely connected to belief regarding the honorable status of the human embryo.
3. Status of the human embryo is a very noteworthy debate. Stem cell researchers consider embryos as cell masses having no more value than any other biological cell or tissue. But some consider the human embryo complete personhood rank, should be treated as a human being that has been born.
4. Lastly, many people present a gradualist vision. According to this row, as the embryo proceeds through stages of development, it gradually establish ethical worth. The embryo's prospective to become a human being claim a "special honor, esteem and aspiration [31].

Conclusion

Embryonic stem (ES) cells possess a nearly unlimited self-renewal capacity and developmental potential to differentiate into virtually any cell type of an organism. Human ES cell lines have recently been derived, may serve as an unlimited source of cells for regenerative medicine. Before therapeutic applications can be realized, important problems must be resolved. Ethical issues surround the isolation of cells from in vitro fertilized human embryos. Recent molecular and cellular advances with human embryonic stem cells (hES) have demonstrated an enormous potential for generating tissues of therapeutic value. The application of human embryonic stem cells for therapeutic purposes is still in primary stages but the promising results in research during the last few years demonstrate rapid progress and the increased importance of human ES cells in both basic research and the long-term future of modern medicine for human incurable diseases.

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