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ES Cells: A Revolutionary Approach for Medicine

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Synopsis

ES cells are a population of undifferentiated cells characterized by the ability to extensively proliferate, usually arise from a single cell and differentiate into different types of cells and tissue (potent). In recent years, ES cell therapy has become a very promising and advanced scientific research topic. The development of treatment methods has evoked great expectations. This paper is a viewing on the discovery of ES cells and the potential therapies based on these cells. A wide variety of possibilities makes this cutting edge therapy a turning point in modern medicine, providing hope for untreatable diseases.

Keywords

- **Cell biology**
- **Cell Therapy**
- **Differentiation**
- **Utilization**
- **Vesicle based therapy**
- **Uses**
- **Rejuvenation**
- **Harvesting**
- **Obstacles**

Preface

Cells in the body have specific purposes, but ES cells are cells that do not yet have a specific role and can become almost any cell that is required. Stem cells are undifferentiated cells that can turn into specific cells, as the body needs them. Stem cells offer great promise for new medical treatments. Through stem-cell research, scientists hope to discover cures for disease that are currently incurable. ES cells are useful not only as potential therapies but also for research purpose.

Procedure

Knowledge gained through analysis of various electronic, print, social, online media data/broadcast program/article and various national and international scientific periodicals/journals/brochures is one of

the main bases of this article. Various aspects of ES cells and stem cell therapy, their usefulness in future medical fields have been highlighted.

ES cell Biology

A blastocyst is formed after the fusion of sperm and ovum fertilization. Its inner wall is lined with short lived stem cells, namely, embryonic stem cells. Blastocysts are composed of two distinct cell types: the inner cell mass (ICM), which develops into epiblasts and induces the development of a foetus, and the trophectoderm (TE). Blastocysts are responsible for the regulation of the ICM microenvironment. The TE continues to develop and forms the extra embryonic support structures needed for the successful origin of the embryo, such as the placenta. As the TE begins to form a specialized support structure, the ICM cells remain undifferentiated, fully pluripotent and proliferative. The pluripotency of stem cells allows them to form any cell of the organism.

Sources of ES cells

From the very earliest stage of pregnancy, after the sperm fertilizes the egg, an embryo forms.

Around 3-5 days after a sperm fertilizes an egg, the embryo takes the form of a blastocyst or ball of cells.

Embryonic stem cells come from a blastocyst that is 4-5 days old.

When scientists take stem cells from embryos, these are usually extra embryos that result from in vitro fertilization (IVF)

iPS cells with their theoretically unlimited propagation and differentiation abilities are attractive for the present and future sciences. They can be stored in a tissue bank to be an essential source of human tissue used for medical examination.

The umbilical cord is known to be rich in mesenchymal stem cells. Due to its cryopreservation immediately after birth, its stem cells can be successfully stored and used in therapies to prevent the future life threatening diseases of a given patient.

What is ES cell therapy?

ES cell therapy, also known as regenerative medicine, promotes the repair response of diseased, dysfunctional or injured tissue using stem cells or their derivatives.

Turning point in ES cell therapy

The turning point in stem cell therapy appeared in 2006, when scientists Shinya Yamanaka, together with Kazutoshi Takahashi, discovered that it is possible to reprogram multipotent adult stem cells to the pluripotent state. This process avoided endangering the foetus life in the process. Retrovirus-mediated transduction of mouse fibroblasts with four transcription factors (Oct-3/4, Sox2, KLF4, and c-Myc) that are mainly expressed in embryonic stem cells could induce the fibroblasts to become pluripotent. This new form of stem cells was named iPSCs. One year later, the experiment also succeeded with human cells. After this success, the method opened a new field in stem cell research with a generation of iPSC lines that can be customized and biocompatible with the patient. Recently, studies have focused on reducing carcinogenesis and improving the conduction system.

iPSCs

Although pluripotency can occur naturally only in embryonic stem cells, it is possible to induce terminally differentiated cells to become pluripotent again. The process of direct reprogramming converts differentiated somatic cells into iPSC lines that can form all cell types of an organism. Reprogramming focuses on the expression of oncogenes such as Myc and Klf4 (Krupper-like factor 4). This process is enhanced by a downregulation of genes promoting genome stability, such as p53.Additionally, cell reprogramming involves histone alteration. All these processes can cause potential mutagenic risk and later lead to an increased number of mutations. Quinlan et al. checked fully pluripotent mouse iPSCs using whole genome DNA sequencing and structural variation detection algorithms. Based on those studies, it was confirmed that although there were single mutations in the non-genic region, there were non-retrotransposon insertions. This led to the conclusion that current reprogramming methods can produce fully pluripotent iPSCs without severe genomic alterations.

During the course of development from pluripotent hESCs to differentiated somatic cells, crucial changes appear in the epigenetic structure of these cells. There is a restriction or permission of the transcription of genes relevant to each cell type. When somatic cells are being reprogrammed using transcription factors, all the epigenetic architecture has to be reconditioned to achieve iPSCs with pluripotency. However, cells of each tissue undergo specific somatic genomic methylation. This influences transcription, which can further cause alterations in induced pluripotency.

Sources of iPSCs

Because pluripotent cells can propagate indefinitely and differentiate into any kind of cell, they can be an unlimited source, either for replacing lost or diseased tissues. iPSCs bypass the need for embryos in stem cell therapy. Because they are made from the patient's own cells, they are autologous and no longer generate any risk of immune rejection.

At first, fibroblasts were used as a source of iPSCs. Because a biopsy was needed to achieve these types of cells, the technique underwent further research. Researchers investigated whether more accessible cells could be used in the method. Further, other cells were used in the process: peripheral blood cells, keratinocytes, and renal epithelial cells found in urine. An alternative strategy to stem cell transplantation can be stimulating a patient's endogenous stem cells to divide or differentiate, occurring naturally when skin wounds are healing. In 2008, pancreatic exocrine cells were shown to be reprogrammed to functional, insulin-producing beta cells.

The best stem cell source appears to be the fibroblasts, which is more tempting in the case of logistics since its stimulation can be fast and better controlled.

Directed differentiation

Differentiation of ESCs is crucial because undifferentiated ESCs can cause teratoma formation in vivo. Understanding and using signalling pathways for differentiation is an important method in successful regenerative medicine. In directed differentiation, it is likely to mimic signals that are received by cells when they undergo successive stages of development. The extracellular microenvironment plays a significant role in controlling cell behaviour. By manipulating the culture conditions, it is possible to restrict specific differentiation pathways and generate cultures that are enriched in certain precursors in vitro.

Teratoma formation Test

The self-renewal and differentiation capabilities of iPSCs have gained significant interest and attention in regenerative medicine sciences. To study their abilities, a quality-control assay is needed, of which one of the most important is the teratoma formation assay. Teratomas are benign tunours. Teratomas are capable of rapid growth in vivo and are characterstic because of their ability to develop into tissues of all three germ layers simultaneously. Because of the high pluripotency of teratomas, this formation assay is considered an assessment of iPSC's abilities.

Teratoma formation assays are considered the gold standard for demonstrating the pluripotency of human iPSCs, demonstrating their possibilities under physiological conditions. Due to their actual tissue formation, they could be used for the characterization of many cell lineages.

iPSCs quality control and recognition by morphological differences

The comparability of stem cell lines from different individuals is needed for iPSCs lines to be used in therapeutics. Among critical quality procedures, the following can be distinguished:

- Short tandem repeat analysis
- Identity analysis
- Residual vector testing
- Karyotype
- Viral testing
- Bacteriology
- Single nucleotide polymorphism arrays
- Flow cytometry
- Phenotypic pluripotency assays
- Histone modification and DNA methylation

hESC derivation and media

hESCs can be derived using a variety of methods, from classic culturing to laser-assisted methodologies or microsurgery. hESC differentiation must be specified to avoid teratoma formation.

hESCs spontaneously differentiate into embryonic bodies(EBs). EBs can be studied instead of embryos or animals to predict their effects on early human development. There are many different methods for acquiring EBs, such as bioreactor culture, hanging drop culture, or microwell technology. These methods allow specific precursors to form in vitro.

Utilization and their manufacturing standards and culture systems

The European Medicines Agency and the Food and Drug Administration have set Good Manufacturing Practice (GMP) guidelines for safe and appropriate stem cell transplantation. In the past, protocols used for stem cell transplantation required animal-derived products.

The risk of introducing animal antigens or pathogens caused a restriction in their use. Due to such limitations, the technique required an obvious update. Now, it is essential to use xeno-free equivalents when establishing cell lines that are derived from fresh embryos and cultured from human feeder cell lines. In this method, it is crucial to replace any non-human materials with xeno-free equivalents.

E`xtracellular vesicle-based therapies

Extracellular vesicles (EVs) can be released by virtually every cell of an organism, including stem cells, and are involved in intercellular communication through the delivery of their mRNAs, lipids, and proteins. AS Oh et al. prove, stem cells, together with their paracrine factors-exosomes-can become potential therapeutics in the treatment of, e. g. skin ageing. Exosomes are small membrane vesicles secreted by most cells (30-120nm in diameter). When endosomes fuse with the plasma membrane, they become exosomes that have messenger RNAs (mRNAs) and microRNAs (miRNAs), some classes of non-coding RNAs (IncRNAs) and several proteins that originate from the host cell. IncRNAs can bind to specific loci and create epigenetic regulators, which leads to the formation of epigenetic modifications in recipient cells. Because of this feature, exosomes are believed to be implicated in cell to cell communication and the progression of diseases such as cancer. Recently, many studies have also shown the therapeutic use of exosomes derived from stem cells, e. g. skin damage and renal or lung injuries.

Uses:

Tissue regeneration: Tissue regeneration is probably the most important use of it.

Until now, a person who needed a new kidney, for example, had to wait for a donor and then undergo a transplant.

There is a shortage of donor organs but, by instructing stem cells to differentiate in a certain way, scientists could use them to grow a specific tissue type or organ.

AS an example, doctors have already used stem cells from just beneath the skin's surface to make new skin tissue. They can then repair a severe burn or another injury by grafting this tissue onto the damaged skin, and new skin will grow back.

Cardiovascular disease treatment: In 2013, a team of researchers from Massachusetts General Hospital reported in PNAS (The Proceedings of the National Academy of Sciences) Early Edition that they had created blood vessels in laboratory mice, using human stem cells.

Brain disease treatment:

In Parkinson's, for example, damage to brain cells leads to uncontrolled muscle movements. Scientists could use stem cells to replenish the damaged brain tissue. This could bring back the specialized brain cells that stop the uncontrolled muscle movements.

Cell deficiency therapy: Scientists hope one day to be able to develop healthy heart cells in a laboratory that they can transplant into people with heart disease.

Similarly, people with type 1 diabetes could receive pancreatic cells to replace the insulin-producing cells that their own immune systems have lost or destroyed.

Blood disease treatments: Doctors now routinely use adult hematopoietic stem cells to treat diseases, such as leukemia, sickle cell anemia, and other immunodeficiency problems.

Use ES cells in medicine

ES cells have great potential to become one of the most important aspects of medicine. In addition to the fact that they play a large role in developing restorative medicine, their study reveals much information about the complex events that happen during human development.

The difference between a stem cell and a differentiated cell is reflected in the cells' DNA. In the former cell, DNA is arranged loosely with working genes. When signals enter the cell and the differentiation process begins, genes that are no longer needed are shut down, but genes required for the specialized function will remain active. This process can be reversed, and it is known that such pluripotency can be achieved by interaction in gene sequences. Takahashi and Yamanaka and Loe et al. discovered that octamer-binding transcription factor 3 and 4(Oct3/4), sex determining region Y(SRY)-box2 and Nanog genes function as core transcription factors in maintaining pluripotency. Among them, Oct3/4 and Sox2 are essential for the generation of iPSCs.

Rejuvenation by cell programming

Ageing is a reversible epigenetic process. The first cell rejuvenation study was published in 2011. Cells from aged individuals have different transcriptional signatures, high levels of oxidative stress, dysfunctional mitochondria, and shorter telomeres than in young cells. There is a hypothesis that when human or mouse adult somatic cells are reprogrammed to iPSCs, their epigenetic age is virtually reset to zero. This was based on an epigenetic model, which explains that at the time of fertilization, all marks of parenteral ageing are erased from the zygote's genome and its ageing clock is reset to zero.

Harvesting

Donations can come from the following sources:

Umbilical cord blood: cells can be harvested from the umbilical cord after delivery, with no harm to the baby.

This harvesting of stem cells can be expensive, but the advantages for future needs include:

- The cells are easily accessible
- Less chance of transplanted tissue being rejected if it comes from the recipient's own body.

Obstacles in the future

Currently, there are several challenges concerning ES cells. First, the most important one is about fully understanding the mechanism by which stem cells function first in animal models. This step cannot be avoided. For the widespread, global acceptance of the procedure, fear of the unknown is the greatest challenge to overcome.

The efficiency of stem cell-directed differentiation must be improved to make stem cells more reliable and trustworthy for a regular patient. The scale of the procedure is another challenge. Future ES cell therapies may be a significant obstacle. Transplanting new, fully functional organs made by stem cell therapy would require the creation of millions of working and biologically accurate cooperating cells. Bringing such complicated procedures into general, widespread regenerative medicine will require interdisciplinary and international collaboration.

The identification and proper isolation of stem cells from a patient's tissues is another challenge. Immunological rejection is a major barrier to successful stem cell transplantation. With certain types of stem cells and procedures, the immune system may recognize transplanted cells as foreign bodies, triggering an immune reaction resulting in transplant or cell rejection.

Stem cell therapy is already available for treating several diseases and conditions. Their impact on future medicine appears to be significant.

Is ES cell therapy available in Bangladesh?

Bangladesh has started the journey of hematopoietic stem cell transplantation (HSCT) with successful autologous stem cell transplantation for multiple myeloma and lymphoma patients in Dhaka Medical College Hospital (DMCH).

Closure

After several decades of experiments, ES cell therapy is becoming a revolutionary game changer for medicine. With each experiment, the capabilities of ES cells are growing, although there are still many obstacles to overcome. Regardless, the influence of stem cells in regenerative medicine and transplantology is immense.

Abbreviations

c-MYc: Cellular Myelocytomatosis (a transcription factor)
ESCs: Embryonic stem cells
GO: Graphene Oxide
hESCs: Human embryonic stem cells
ICM: Inner cell mass
iPSCs: Induced pluripotent stem cells
IVF: In vitro fertilization
KLF4: Krupple-like factor 4(a transcription factor)
MSCs: Mesenchymal stem cells
Oct3/4: Octamer-binding transcription factor 3 and 4
SRY: Sex determining region Y
Sox2: Sex determining region Y-box2
TE: Trophectoderm

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