International Journal of Anatomy and Research,
Int J Anat Res 2014, Vol 2(3):571-76. ISSN 2321- 4287

Review Article

HUMAN EMBRYONIC STEM CELLS AND THEIR CLINICAL RELEVANCE

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ABSTRACT

Human embryonic stem cells are derived from the inner cell mass of the blastocyst at the pre implantation stage of the embryo. The present review is done to describe the derivation, culture method, directed differentiation of the human embryonic stem cells with particular emphasis on their clinical applications, and their role in the field of research and regenerative medicine.

KEYWORDS: Embryonic Stem cells, Regenerative Medicine, Stem cell therapy, Tissue Engineering.

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Access this Article online

Quick Response code

Web site: International Journal of Anatomy and Research
ISSN 2321-4287
www.ijmhr.org/ijar.htm

Received: 21 Aug 2014
Peer Review: 21 Aug 2014 Published (O):30 Sep 2014
Accepted: 09 Sep 2014 Published (P):30 Sep 2014

INTRODUCTION

Embryonic stem cells are derived from totipotent cells of the early human embryo and are capable of unlimited, undifferentiated proliferation in vitro [1]. The term "Embryonic stem cell" was introduced to distinguish these embryo-derived pluripotent cells from teratocarcinoma-derived pluripotent embryonic carcinoma cells [2]. Thus embryonic stem cell is defined as a pre implantation embryo derived cell that is pluripotent, capable of indefinite self renewal and has the capacity to differentiate into extra embryonic tissue and tissues representive of all three embryonic germ layers i.e. the ectoderm, the endoderm and the mesoderm [3].

DERIVATION OF EMBRYONIC STEM CELLS

The term embryonic stem cells originated from the isolation in 1981 of pluripotent stem cell cultures from mouse blastocyst by Evans and Kaufman and independently by Martin [1,2]. Normally, the inner cell mass cells of the blasto-cyst are pluripotent but after implantation they are quickly depleted as they differentiate to other cell types with more limited developmental potential. However, if the inner cell mass cell is removed from its normal embryonic environment and cultured under appropriate conditions, these cells can continue to proliferate and replicate themselves indefinitely and still maintain the developmental potential, to form any cell type of the body. These pluripotent, inner cell mass derived cells are the embryonic stem cells [4].

The origin of human embryonic stem cells is from the pre implantation embryo. These cell lines are derived from the inner cell mass cells of human blastocyst, produced by in-vitro fertilization for clinical purposes and donated by individuals after informed consent. In this process, the outer trophectoderm layer of the blastocyst was selectively removed using specific antibodies (immune surgery), and the inner cell mass cells

were isolated and plated on a mitotically inactive mouse embryonic fibroblast feeder layer. Cells from the periphery of the colonies that formed were mechanically isolated and replated in the same fashion until homogenous colonies appeared. These colonies were selected, passed and expanded for the creation of embryonic stem cell lines [5]. The human embryonic stem cell derived clones retained all the properties of the parental line, including prolonged undifferentiated proliferation with a stable karyotype, expression of high levels of telomerase and the ability to generate teratomas after in vivo transplantation to immune deficient mice [5]. Several groups have attempted to define growth factors that sustain human embryonic stem cells and have attempted to identify culture conditions that reduce the exposure of human embryonic stem cells to non human animal products. One important growth factor bFGF, (basic fibroblast growth factor) allows the use of serum replacement to sustain human embryonic stem cell in the presence of fibroblasts, and this medium allowed the clonal growth of human embryonic stem cell [6].

A feeder free human embryonic stem cell culture system has been developed, in which human embryonic stem cells are grown on a protein matrix (Mouse Matrigel or Laminin) in a bFGF – Containing medium that is previously "conditioned'' by co-culture with fibroblasts [7]. However, the possibility of pathogen transfer from human to human in these culture system still remain. Sato et al reported that activation of Wnt pathway by 6- bromo indirubin 3'- oxime promotes the self renewal of embryonic stem cells in the presence of bFGF, matrigel and a proprietary serum replacement product [8]. Amit et al reported that bFGF, TGFß and Leukemia inhibitory factor could support some human embryonic cell lines in the absence of feeders [9]. The transcription factor oct4 has been used as a key marker for embryonic stem cells and for the pluripotent cells of intact embryo and its expression must be maintained at a critical level for embryonic stem cells to remain undifferentiated. The oct-4 protein itself however is insufficient to maintain embryonic stem cells in the undifferentiated state [10].

The human embryonic stem cell lines expressed high levels of telomerase activity. Telomerase is a ribo nucleic protein that adds telomere repeats to chromosome ends and is involved in maintaining telomere length, which plays an important role in replicative life span [11, 12]. Telomerase expression is highly correlated with immortality in human cell lines and reintroduction of telomerase activity into some diploid human somatic cell lines extends replicative life span [13]. The high level of telomerase activity expressed by the human embryonic stem cell lines therefore suggests that their replicative life span will exceed that of somatic cells.

DIRECTED DIFFERENTIATION OF EMBRYONIC STEM CELLS AND THEIR ROLE IN REGENERATIVE MEDICINE

One of the most important aspects of embryonic stem cells lines is their ability to differentiate in vitro, via precursor cells into terminally differentiated somatic cells of all tissue types including cardiac tissue, neuronal tissue, ß islet pancreatic cells, hematopoietic progenitors and endothelial cells [14,15,16].

The most common method used for in-vitro differentiation is to remove the embryonic stem cells from the feeder layer and to cultivate them into 3D cell aggregates, termed embryoid bodies. The human embryonic stem cells, when removed from the mouse embryonic fibroblast feeder layer and cultivated in suspension, begin to differentiate by forming embryoid bodies [17]. These bodies displayed regional expression of embryonic markers specific to different lineages of ectodermal, mesodermal and endodermal origin [18].

Directing the differentiation of human embryonic stem cells toward definitive endoderm would help generate specific cell types such as islet cells or hepatocytes which could be used towards the treatment of diseases such as diabetes or liver diseases respectively. Amongst the most successful examples to date is the generation of pancreatic islet progenitors devised by kroon et al [19], accomplished through the sequential exposure of human embryonic stem cells to activin A and Wnt 3A, followed by the addition of keratinocyte growth

factor or fibroblast growth factor 7 to induce the formation of primitive gut tubes. Subsequently retinoic acid, cyclopamine and noggin are added to inhibit hedgehog and TGF ß signaling, and thus induce the differentiation of posterior foregut cells; the source of pancreatic cell progenitors. These are cultured further to generate pancreatic endoderm cells. When these cells are engrafted in immuunodeficient mice, they display the histological and structural characteristics of pancreatic islet cells, and are able to sustain insulin production for atleast 100 days.

Similarly a robust population of functional hepatocytes was generated with the sequential addition of low serum medium, collagen 1 matrix, and hepatic differentiation factors that include FGF, BMP4, hepacyte growth factor, oncostatin M, and dexamethasone. These cells expressed known markers of mature hepatic cells when injected into mice with liver injury [20].

Directing the differentiation of human embryonic stem cells to mesoderm requires the activation of the TGFß signaling pathway and can be accomplished through the step wise and dosage-dependent addition of activin A, BMP4, and growth factors, VEGF and bFGF [21]. Robust differentiation of human embryonic stem cells into hematopoietic lineage cells, which give rise to all blood cell types and components of the immune system, has been achieved under serum-free condition through spin human embryoid body formation [22]. Hematopoietic progenitor cells that give rise to functional T and natural killer cells capable of targeting human tumor cells both in vitro and in vivo have also been derived from human embryonic stem cells co-cultured with stromal cells[23]. Thus, the ability to differentiate human embryonic stem cells into hematopoietic lineage cells promises to be useful in improving existing therapies that require induction of the immune response in an antigen specific manner [24].

The dominant differentiation pathway in human embryonic stem cell culture leads to the formation of ectoderm which makes up cells of the nervous system and the epidermis. Human embryonic stem cells derived neural progenitor cells are characterized by rosette-like neural structures that form in the presence of growth factors, FGF2 or EGF, through either spontaneous differentiation from an over growth of human embryonic stem cells or after human embryoid bodies are plated onto the adherent substrates. These neural rosettes have become the signature of human embryonic stem cell derived neural progenitors capable of differentiation into a broad range of neural cells in response to appropriate developmental signals like oligodendrocytes which are injected into the spinal cord following acute spinal cord injuries [25,26].

ROLE OF HUMAN EMBRYONIC STEM CELLS IN TISSUE ENGINEERING

Tissue engineering and regeneration Utilize biological substitutes to restore and maintain tissue function. Human embryonic stem cells provide much promise in tissue engineering and regeneration since these cells can act as an inexhaustible in vitro source of differentiated cell types. The potential use of human embryonic stem cells in tissue engineering include organ substitutes, vascularization, and ex vivo cartilage / bone construction.

Basal keratinocyte, the cells that make up the pluri stratified epidermal layer of skin, have successfully differentiated from human embryonic stem cells. These cells can be used as a source of allograft for patients requiring skin restoration [27].

The use of human embryonic stem cells to treat lung injury has also been an area of active investigation. Wang et al reported directed differentiation of lung specific cells, in which genetically modified human embryonic stem cells carrying lung specific reporters under the control of promoters from tissue-specific genes such as surfactant protein C, aquaporin 5, and T1α, resulted in the purification of type I and type II alveolar epithelial cells. When engrafted into mice suffering from acute lung injury, these cells terminally differentiated in vivo into type I and type II alveolar epithelial cells and exhibited functional properties that include the capacity for gas exchange and histological amelioration of lung injury [28].

Human embryonic stem cells are a valuable sou-

-rce of cells suitable for connective tissue replacement therapy for a number of bone and joint diseases, such as osteoarthritis ant other diseases. Human embryonic stem cells co-cultured with primary chondrocytes, or in the presence of osteogenic supplements and polymeric scaffolds, yield cartilagenous-or osteogenic –like cells [29].

More recently, feeder free 3D culture systems have successfully derived multipotent connective tissue progenitors from human embryonic stem cells yielding tendon-like structures. The engraftment of these in vitro differentiated tendon structures in injured immuno suppressed mice restored ankle joint movements that rely on an intact Achille's tendon [30].

Human embryonic stem cells should offer insights into development events that cannot be studied directly in the intact human embryo but that have important consequences in clinical areas, including birth defects, infertility and pregnancy loss [31]. Recently, human embryonic stem cells have also been observed to differentiate into cells expressing genes characteristics of germ cells. Thus it may be possible to derive oocytes and sperms from human embryonic stem cells, allowing the detailed study of human Gamatogenesis for the first time [32]. Human embryonic stem cells will be particularly valuable for the study of development and function of tissues that differ between mice and human embryonic stem cells to specific lineages could identify gene targets for new drugs, genes that could be used for tissue regeneration therapies and teratogenic or toxic compounds [33].

Homologous recombination, a method in which a specific gene inside the embryonic stem cells is modified with an artificially introduced DNA molecule, is an even more precise method for genetic engineering that can modify a gene in a defined way at a specific locus. This technology has recently been successfully developed, thus opening new doors for using embryonic stem cells as vehicles for gene therapy and for creating in vitro models of human genetic disorders such as Lesch – Nyhan disease [34]. Another method, to test the function of a gene is to use RNA interference to decrease the expression of a gene of interest and this technology has also been applied to human embryonic stem cells [35].

Human embryonic stem cells are used as a source of retinal pigment epithelium for the treatment of retinal degenerative diseases such as macular degeneration. Since primary retinal epithelial tissue can't be obtained in large enough quantities for wide scale clinical use [36].

The ability of human embryonic stem cells to provide in vitro cardiomyocyte tissue for long term assessment may also prove invaluable for drug discovery, drug screening and toxicity testing [37]. Embryonic stem cells can potentially provide an unlimited number of cells for transplantation. Furthermore, specialized sub types of cardiomyocytes with different phenotypes (e.g.atrial, ventricular) can be differentiated in vitro from embryonic stem cells and can be tailored to specific procedures. The embryonic stem cells cultures could end themselves to extensive characterization and genetic engineering to promote desirable characteristics such as resistance to ischemia, apoptosis and improved contractile function [38].

CONCLUSION

Research on human embryonic stem cells has progressed significantly since their first derivation in 1998. Human embryonic stem cells may be useful in future in cardio vascular research, including human developmental biology, functional genomics, pharmacological testing, tissue engineering, gene and cell-based therapies.

Acknowledgment:

Authors acknowledge the immense help received from the scholars whose articles are cited and included in references of this manuscript. The authors are also grateful to author/ editors/ publishers of all those articles, journals and books from where the literature for this article has been reviewed and discussed.

Conflicts of Interests: None

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How to cite this article:

Takkallapalli Anitha, Sanjay H Kalbande. HUMAN EMBRYONIC STEM CELLS AND THEIR CLINICAL RELEVANCE: A REVIEW. Int J Anat Res 2014; 2(3): 571-76.