Thai Journal of Obstetrics and Gynaecology
April 2013, Vol. 21, pp. 50-55

SPECIAL ARTICLE

Human Embryonic Stem Cells : From Reproductive Technology to Regenerative Medicine

Ruttachuk Rungsiwiwut DVM, PhD,
Pranee Numchaisrika BSc,
Pramuan Virutamasen MD, MSc,
Kamthorn Pruksananonda MD.

Reproductive Medicine Unit, Department of Obstetrics and Gynecology, Faculty of Medicine, Chulalongkorn University and King Chulalongkorn Memorial Hospital, Bangkok 10330, Thailand

ABSTRACT

Stem cells are the cells that have the capability to renew themselves and differentiate to the specific cell types. The successful of derivation of human embryonic stem cells (ESCs) in 1998, together with the discovery of reprogramming the human somatic cells into embryonic-like stage, so called "human induced pluripotent stemcells (iPSCs)" in 2007, dramatically increase the attention of the scientists and the public about the useof human ESCs or iPSCsforboth research and application. Although stem cells can be isolated and identified from several tissue of the human body such as limbal tissue, bone marrow or skin, the powerful of differentiation ability of human ESCs make these cell types a good candidates for cell replacement therapy. Recently, the successful of clinical trials using human ESCs for treating the patients who suffered from macular degeneration, underline the possibility of application of human ESCs for therapeutic purposes. In this review, the general biology of stem cells, source of human ESCs and the potential application of human ESCs were discussed.

Keywords: stem cells, reproductive tissue, embryo, regenerative medicine

Introduction

Stem cells are the cells that have two major characteristics: prolonged self devision and differentiate into more than one specialized cell types. The characteristics of stem cells posses the enormous potential application for cell base therapy. In general, by using the tissue origin, stem cells can be identified into i) embryonic stem cells (ESCs) which referred to the cells that isolated from pre-implantation embryos, ii) adult stem cells which referred to the cells that isolated from the specific tissue or organ and iii) induced pluripotent stem cells (iPSCs) which referred to the somatic cells or adult stem cells that are reverted to the embryonic-like stage and exhibit the similarities of characteristics and molecular biology to ESCs[1, 2].

The adult stem cells was described in the tissues

that have relatively limited regenerative capacity and typically can differentiate to a limited number of cell types[3]. The adult stem cells can be found and isolated from several part of human body for example, bone marrow[3], limbal tissue[4] or dental tissue[5]. In contrast to adult stem cells, ESCs can be isolated from the pre-implantation embryos, showed the abilities of unlimited cell devision and differentiated into all kind of cells in human body. Thus, ESCs represent a good source for using as the starting material for cell replacement therapy. However, the major obstacle of generation and application of ESCs are ethical debates from the public and ESCs are not a patient-specific stem cells. In many countries, isolation of ESCs from embryos is considerred as destruction of human life, thus it is prohibited to use ESCs for cell therapy. As ESCs is not patient-specific stem cells, transplant of ESCs will provoke immunorejection that responded by the patients. To overcome the limitation of ES cells, the researchers discovered and successfully converted the somatic cells or adult stem cells into the embryonic-like stage so called induced pluripotent stemcells (iPSCs). Generation of human iPSCs involve the process of overexpression of transcription factors that related to the pluripotency stage in the somatic cells. This process is also known as "reprogramming" and resulting to the iPSCs[1, 7].

Human Embryonic Stem cells: Stem Cells from The Embryo

In 1981, the term of ESCs was originated for the first time after the successful of derivation of ESCs from mouse blastocysts. The ESCs were capable to renew themselves and prolonged their proliferation in cultured[8,9]. Mouse ESCs were intensively used for gene targeting study, differentiation of specific cell type as well as a model for derivation of ESCs from other species including human.

At the ESC laboratory of Department of Obstetrics and Gynecology, Faculty of Medicine, Chulalongkorn University, our group were successfully derived mouse ESCs and those ESCs have been proved to be able to maintained their proliferation and differentiation both in vitro culture and in vivo condition by showing the germ-line chimera transmission[10].

In human, ESCs exhibit normal karyotype, proliferates in vitro under undifferentiated morphology and differentiate into the various cell types both in vitro and in vivo[11, 12]. Human ESCscan be established from various stages of pre-implantation embryos. Originally, human ESCs were derived from the inner cell mass (ICM) of blastocyst-stage embryo[11, 12]. The ICM was isolated, grown with- or without feeder layer and under ESC conditions until the permanent human ESCs can be established. Besides generation of human ESC from the ICM, human ESC lines can be generated from single blastomere[13, 14] or arrested embryos[15] of the fertilized zygotes. In addition, human ESC lines can be derived from blastocyst developed from non-fertilization including parthenogenetic activation[16, 17] and nuclear transfer- or cloning technique[18].

The human ESCs represent unique characteristics including capable of self-renewal, differentiating into multiple cell lineages of human body. Their characteristics provide new sources for cell transplantation, drug screening test as well as study of developmental biology.

Human Embryonic Stem Cells from Fertilized Embryo

After the fertilization, the zygote developed through the cell division process until reaching the blastocyst stage. Cells in the blastocyst stage embryos consisted of two types of cells, ICM and trophectoderm (TE). The ICM located inside the ball-like structure, surrounded by the TE (Fig 1A). The ICM will be later develop into the three embryonic germ layer including, ectoderm, mesoderm and endoderm. When the ICM was separated from the TE and plated onto the culture dish in the proper conditions, it will give rise to the pluripotent, ESCs (Fig 1B). The ESCs can be derived from fresh- or cryopreserved embryos[19]. Interestingly, the embryo that had been frozen for 18 years remains their viability, developed to blastocyst stage and gave rise to human ESC line[19].

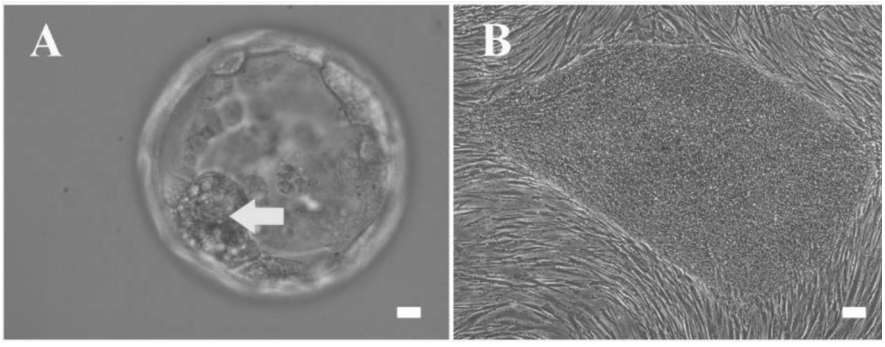

Fig 1. Morphology of human blastocyst-stage embryo and embryonic stem cells.

At day 5-7 after fertilization, the embryo developed to blastocyst stage (A). The inner cell mass (ICM; white arrow) is surrounded by the trophectoderm (TE). Human embryonic stem cells (B) can be derived by culture of the ICM under the human embryonic stem cell culture condition, resulting to the pluripotent cells. Scale bar = 100 μm.

The fertilized embryos are widely used as the starting material for derivation of human ESCs. In the present, more than 100 human ESC lines were generated in the laboratories around the world[20]. In Thailand, there are three human ESC lines generated from cryopreserved- and fresh embryos[19]. These lines are progressively studied in differentiation ability as well as the effect of culture conditions on the maintenance of their pluripotentcy.

Human Embryonic Stem Cells from Parthenogenetic Embryo

The parthenogenetic (PG) embryo is the embryo that develop and growth without the fertilization process. PG embryos can be generated by activation of haploid or diploid oocytes with the electrical stimuli or chemical agents[16, 17, 21]. Similar to the fertilized embryos, PG embryos enable to develop to blastocyst stage,which consisted ofthe ICM and TE. Several reports demonstrated that human ESC lines could be derived from PG embryos and known as human PGESC[16, 17]. Thus, human PG ESCs are derived from the ICM of blastocyst-stage embryos display the same properties of both self renewal and differentiation ability as the human ESCs derived from fertilized embryos[16, 17]. Therefore, human PGESCs possibly are the candidate cells from cell base therapy in the future due to its genetically match to the recipient.

Human Embryonic Stem Cells from Nuclear transferred-generated embryos

Dolly the sheep, the first mammal that was generated by the nuclear transfer (NT) technique, was the evidence that proved the ability of reprogramming of somatic cells to the embryonic stage[22]. By using the NT technique and subsequent to isolation of the ICM from NT-generated embryo, ESCs from several species including mice and cow can be derived[23, 24]. NT-generated ESCs or NT-ESCs is considered as the allogeneic ESCs due to the NT-embryos were generated from the somatic cells of the patients. Hence, there is no risk of stimulationtothe immune response by the patients after transplantation of NT-ESCs.

Very recently, it has been reported for the first time of generation of human ESCs from NT-generated embryos[18]. These human NT-ESCs exhibit the phenotypic, genotypic especially in the molecular level similar to those human ESCs generated from fertilized embryos. Although this breakthrough is considered as an alternative method to generate a patient specific ESCs, as the somatic cells from the patient is used for generation of NT-generated embryos,the limitation of this method are the ethical debates of using human oocytes, the technical difficulties as well as the discovery of generation of human iPSCs.

Differentiation ability of Human Embryonic Stem Cells

Due tothe outstanding potential of human ESCs including an inexhaustible source of specific cell populations and the ability to differentiate into lineage-restricted progenitors, human ESCs promise to be an important candidate for clinical applications of treating aging-associated diseases[25]. The protocol of differentiation of human ESCs into specific progenitor cells involve with the exposure of human ESCs to growth factors or cytokines that mimic differentiation pathways of specific lineages.

The endodermal derivative cells can be initially generated by induced differentiation of human ESCs into definitive endoderm. The definitive endoderm can populate to the specific progenitor cells such as hepatocytes[26, 27], alveolar epithelial cells[28] or pancreatic islet β cells[29] that can be applied for treatment of liver disease, lung disease or diabetes in the future. In the previous reports, human ESCs were differentiated into hepatocytes after the exposure to fibroblast growth factor (FGF), bone morphogenetic protein (BMP)-4, hepatocyte growth factors, oncostain M and dexamethasone[26]. In case of pancreatic islet β cells, Activin A, Wnt3, keratinocyte growth factor, fibroblast growth factor (FGF) 7, retinoic acid, cyclopamine and Noggin are widely used for differentiation of human ESCs[29].

To generate the mesoderm derivative cells, the activation of the transforming growth factor (TGF) signalling pathway by the addition of Activin A, BMP-4, vascular endothelial growth factor (VEGF) and FGF2 into the culture of human ESCs[30]. One of the therapeutically important mesoderm derivative cells is cardiomyocytes that have been successfully generated using several methods[31]. Although the clinical trial of cardiomyocytes in human has not yet started, the results obtained from the animal models are promising and useful for cardiomyocytes transplantation in the human in the future[32].

For the ectodermal derivative cells, the induction of neural progenitor cells by culture of human ESCs in the presence of FGF and epidermal growth factor (EGF) give rise to the structure called neural-like rosette. Subsequently, culture of neural-like rosette without of FGF and EGF with the addition of some small molecules lead to the differentiation of neural rosette to the cells of the nervous system and epidermis[25]. This differentiation ability of human ESCs into neuronal subtype generated much interest for drug screening test and cell replacement therapy for neurodegenerative diseases.

Clinical Application of Human Embryonic Stem Cells

The clinical application of stem cells began and successfully applied for a decade. The stem cells isolated from bone marrow, bone marrow-derived hematopoietic stem cells (HSCs) were transplanted for treatment of leukemia and blood-related diseases[33, 34]. Despite of HSCs, multiple mesenchymal stem cells (MSCs) are studied for application on the treatment of central nervous system disorder[35].

For the clinical application of human ESCs, the phase I clinical trial using human ESC-derived oligodendrocytes was studied by Geron company. This was the first clinical trial approved by the United States Food and Drug Administration (FDA). The aim of the trial was to use oligodendrocytes-derived human ESCs for treatment of spinal cord injury to the patient within two week after injury[36]. However, this clinical trial was terminated before the end of the trial. In addition, another clinical application of human ESCs is the second and third FDA-approved clinical trial of using human ESC-derived RPE for treatment of the Stargard's macular dystrophy and Dry aged-related macular degeneration, which are sponsored by Advance Cell Technology. RPE cells involve with the process of phagocytosis and generation the photoreceptor of rhodopsin, thus RPE cells play an important role in the function of retina. RPE cells were transplanted into the area of degeneration of retina to rescue the visual acuity. Interestingly, the preliminary results of these trails are very promising and underline the possibility of clinical application of human ESCs for regenerative medicine[37].

Acknowledgments

This work was financially supported by grants from The Thai Government Research Budget 2007-2011 (Grant No. GRB_APS_05_54_30_01) and The National Research University Project of CHE (HR1166I).

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