Turkish Journal of Biology

Volume 40 | Number 5 Article 8

1-1-2016

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TÜBİTAK

Turkish Journal of Biology

http://journals.tubitak.gov.tr/biology/

Review Article

Turk J Biol
(2016) 40: 1033-1039

doi:10.3906/biy-1508-15

Pluripotent stem cells and their use in hearing loss

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Received: 06.08.2015 • **Accepted/Published Online:** 21.12.2015 • **Final Version:** 08.11.2016

Abstract: Throughout its half a century of development, stem cell research has included two main fields: embryonic stem (ES) cell research and the reprogramming of body somatic cells. In the present review we focused on stem cell reprogramming and its relation with otolaryngology. The human body somatic cells are transformed into pluripotent cells by three basic methods: the somatic nuclear transfer method, the somatic cell fusion method (getting cellular pluripotent capacity in cellular reprogramming), and by transcription factors influencing the body somatic cells to generate reprogrammed induced pluripotent stem (iPS) cells. ES cells and iPS cells have pluripotency and differentiate into cells originating from the three germ layers; they are preferred for cellular treatment, drug development, and disease modeling research. Because of ethical restrictions in obtaining ES cells, iPS cells are an alternative pluripotent cell source and patient-specific autologous pluripotent cells are obtained by this method. Cellular treatment and regenerative medicine with pluripotent cells are currently developing and we aimed to raise awareness about this topic in our paper on using iPS cell technology in the biological treatment of hearing loss, which is an important area of research in otolaryngology.

Key words: Stem cell, sensory hair cell, iPS cell

1. Introduction

Throughout its half a century of development, stem cell research has included two main fields: embryonic stem (ES) cell research and the reprogramming of body somatic cells (Yamanaka, 2012). In the present review we focused on stem cell reprogramming and its relation with otolaryngology. The human body somatic cells are transformed into pluripotent cells by three basic methods: 1) the somatic nuclear transfer method was developed by Gurdon et al. (1958), who transferred a frog intestine epithelial cell to the ovum and succeeded in getting a frog embryo; Wilmut et al. (1997) cloned a mammalian epithelial cell by a similar method and reported the birth of Dolly; 2) somatic cell fusion is another method in cellular reprogramming for getting cellular pluripotent capacity (Miyazaki et al., 2012); and 3) the third method involves transcription factors influencing body somatic cells to generate reprogrammed induced pluripotent stem (iPS) cells (Takahashi and Yamanaka, 2006). Currently iPS cell technology is quite advanced and it is possible to take a blood or skin sample from a patient and convert the cells into iPS cells. Scientists are working on the experimental phase to solve hearing loss and in the near future it might be possible to forward differentiate the patient iPS cells into inner ear cell types such as sensory hair cells (Jansson et al., 2015).

Stem cells have three basic functional properties: 1) they have self-renewal capability (it is highest at the early embryonic phase and can be transferred to next generations, but decreases with advanced phases of differentiation); 2) they cannot perform specialized functions and do not have specific tissue characteristics; and 3) they have plasticity and can differentiate into many special tissues such as heart, nerve, muscle, blood, and cartilage.

Stem cells are grouped into five categories in terms of their plasticity capacity (Ilic and Polak, 2011; Kolios and Moodley, 2013): 1) Totipotent stem cells are undifferentiated stem cells of the earliest embryonic phase from zygote formation to the blastocyst phase. 2) Pluripotent stem cells are the embryoblasts from the inner cell mass on day 5 or 6 of the embryo. They can transform into all germ cells (except for placenta) with endo, meso or ectoderm character and form all tissues and organs; they have infinite proliferation capacity, are important for regenerative medicine, and are also known as ES cells

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(Kolios and Moodley, 2013). 3) Multipotent stem cells are adult stem cells that have the capacity of self-renewal during the developing phases of the embryo; they are programmed to differentiate within a single germ layer. They are somatic stem cells and can be found in the bone marrow, muscles, nerves, liver, and skin. Bone marrow stem cells, mesenchymal stem cells, and hematopoietic stem cells forming all blood cells are multipotent stem cells; they are undifferentiated cells and they replace dead or damaged cells; they can divide and have self-renewal property. Mesenchymal stem cells have the capacity of transforming into chondrocytes, myocytes, adipocytes, neurons, and pancreatic beta cells. 4) Oligopotent stem cells can differentiate into two or more cell lines in a special tissue or organ. They have self-renewal capacity. Hematopoietic stem cells and cells that differentiate into myeloid and lymphoid cells are examples of these cells. 5) Unipotent stem cells are the least potent cells and can only differentiate along a single cell line. Muscle stem cells are one example of this category of cells (Ilic and Polak, 2011; Kolios and Moodley, 2013).

2. ES cells

ES cells are derived from the inner cell mass, one of the two layers forming the blastocyst, on day 5 or 6 of fertilization. The external cellular layer, known as the trophoblast, forms the placenta and the amniotic membrane. The inner cell mass forms the ectoderm, endoderm, and mesodermal tissue cells. ES cells were derived for the first time from the embryos of mice by two different groups of scientists (Evans and Kaufman, 1981; Martin, 1981). The first human stem cells with pluripotent and self-renewal capacity were found by Thompson et al. (1998).

Today ES cells are obtained from three sources: 1) ES cells obtained from unused embryos in in vitro fertilization units; they are called real ES cells or fertile ES cells. 2) ES cells obtained by somatic nuclear transfer; the ovum nucleus is removed by a fine needle and replaced with a body somatic nucleus. The reprogrammed ovum cell is activated and left to duplicate. These cells are called nuclear transfer ES cell. 3) ES cells obtained by parthenogenesis of an unfertilized ovum; they are called parthenogenetic ES (pgES) cells. Under certain circumstances the embryo is called a parthenote (Paffoni et al., 2008).

After obtaining the ES cells, they are stored in special cultures containing growth factors or they are kept frozen. ES cells should be kept in special cultures to maintain their undifferentiated state. Mice embryonic fibroblast cells are used in the feeder layer for this purpose. An antidifferentiation cytokine (leukemia inhibitor factor) is also added. ES cells lose pluripotency and get differentiated in the case of culture change or growth factor addition (Takahashi and Yamanaka, 2006). Because the blastocyst is interrupted for ES cell research, ethical, religious, or political issues arise.

The pgES cells are obtained through parthenogenesis, unisexual reproduction that is usually encountered in plants, bumble bees, amphibians, and reptiles, but is not found in mammals. The human oocyte occasionally gets activated and forms a teratoma. The unfertilized mammalian oocyte may form a nonembryonic blastocyst with parthenogenetic activation by the way of mechanical and chemical factors. Pluripotent pgES cells can be obtained from the inner cell mass of the blastocyst. These cells show self-renewal and clonogenic capacity in vitro. This embryo shows no normal or extra embryonic development (Paffoni et al., 2008). The pgES cell was first isolated from a mouse and later from a rabbit, a monkey, and a human by Kaufman et al. (1983). The pgES cells show similarities with sperm-fertilized embryonic cells, express outside stem cell markers, form an embryoid body, and can differentiate into all cells across the three germ layers (Mai et al., 2007). Ethical issues, teratoma formation, and immune reactions in experiments with ES cells limit the clinical trials with those cells. Moreover, the difficulties in gaining iPS cells, the high cost, and the length of time involved in such research forced researchers to seek alternative pluripotent stem cells. The ethical debate ceases with the pgES cells as there is no intervention to the blastocyst. Moreover, pgES cells are easy to obtain at a low cost and they are human leukocyte antigen (HLA) compatible; these properties make them an alternative stem cell resource (Horii and Hatada, 2011; Liu et al., 2013).

3. iPS cells

The functions of iPS cells are similar to those of ES cells. iPS cells are obtained by the reprogramming of body somatic cells with some gene transcription factors. They were first produced from mice dermal fibroblast cells by the retroviral gene transfer method with Oct3/4, Sox2, Klf4, and cMyc (OSKM) factors, also known as the Yamanaka factors (Figure) (Takahashi and Yamanaka, 2006; Bayart and Cohen-Haguenauer, 2013). iPS cells were obtained from human dermal fibroblasts a year later by the same authors (Takahashi et al., 2007). It was found that they carry the same properties as ES cells. Different authors obtained iPS cells by using gene transcription factors Sox2, Oct3/4, Nanog, and Lin28 (Yu et al., 2007). Since then different groups with different transcription factors by different gene transfer techniques were able to obtain iPS cells (Gonzalez et al., 2011).

3.1. Sources of iPS cells

iPS cells are obtained by the reprogramming of many cells with transcription factors: human fibroblasts, peripheral blood cells, neuronal stem cells, pancreas cells, and cord

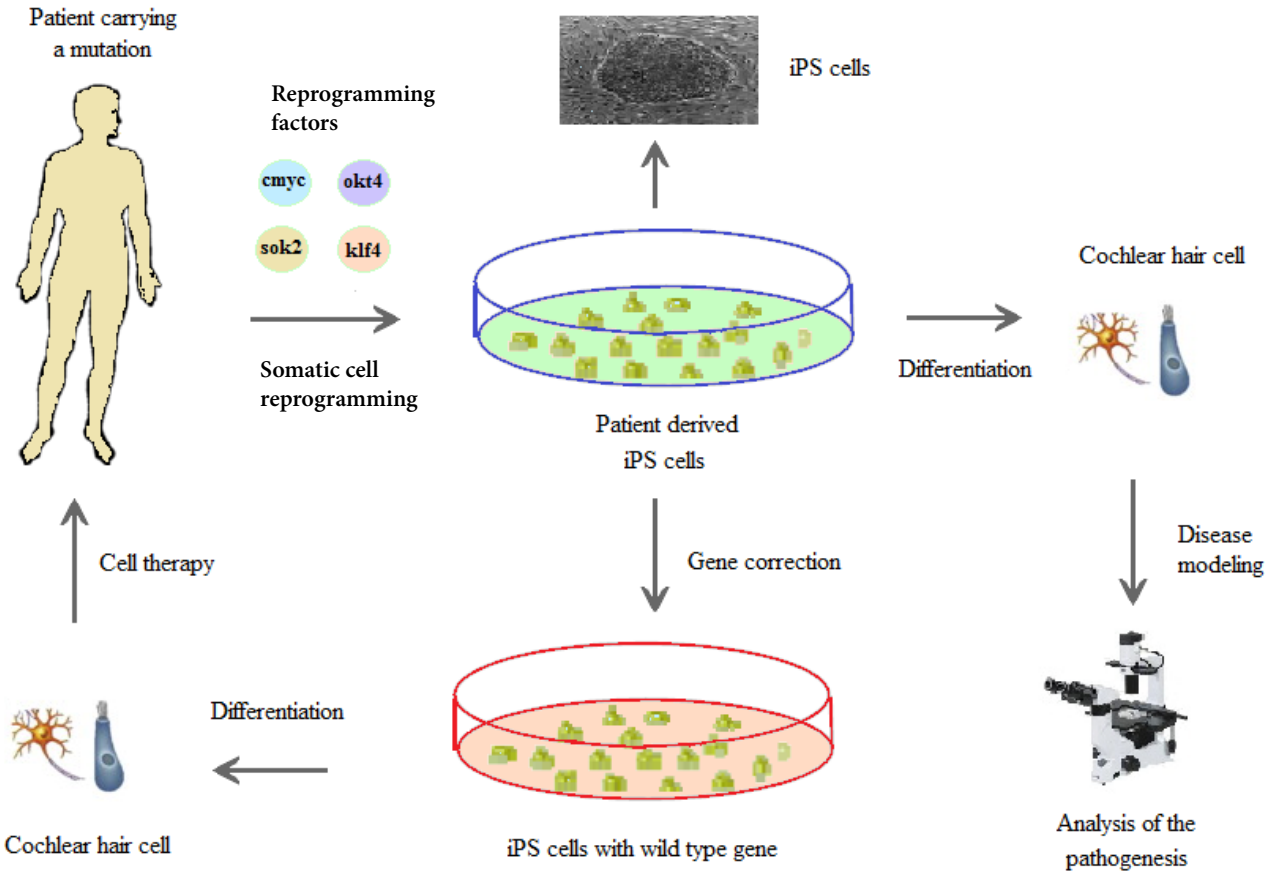

Figure. The scheme of how iPS cells are produced and can be applied in disease modeling and gene correction for the treatment of hearing loss.

blood cells are all sources of iPS cells (Gonzalez et al., 2011). Successful iPS cell harvest depends on: cell culture, the mature cell type and characteristic (lymphocyte, fibroblast, etc.), the gene transfer method (viral or nonviral), and the differentiation level of the cell, which determines the time and rate of the derived iPS cells (Takahashi and Yamanaka, 2006). iPS cells are obtained with retroviral gene transfer in 20–25 days from dermal fibroblasts of humans; iPS cell are also obtained from CD133 + stem cells from the cord blood using only Oct4 and Sox2 factors for the reprogramming (Takahashi et al., 2007).

Obtaining patient-specific iPS cells is the most important step in cellular treatments. Since dermal cells have a high potential to generate iPS cells, they are the first choice for a cellular source. The main drawbacks are the need for skin biopsy, the risk of infection, mutations due to UV radiation exposure, and the long time (3 to 4 weeks) needed for the primer cell culture (Zhang, 2013).

3.2. Gene transfer methods in the reprogramming of iPS cells

Different gene transfer methods are used for the reprogramming of iPS cells. There are basically two types of methods, genome integrated methods and nongenome integrated methods, and each type has a viral and a nonviral approach. In the genome integrated methods high iPS cell ratios in vitro are possible with the retroviral and lentiviral gene transfer method because of their high integration with the host genome. The lentiviral gene transfer produces high cellular infection rates. Lentiviruses can infect either dividing or nondividing cells, and have higher reprogrammed cell ratios (Bayart and Cohen-Haguenauer, 2013). In the nongenome integrated methods adenoviruses (Stadtfeld et al., 2008) and Sendai viruses are used for this purpose. Episomal RNA, proteins, and small molecules are some of the nonviral methods of gene transfer. Synthetic mRNA is used for obtaining iPS cells from human somatic cells in the RNA transfer method (Stadtfeld et al., 2008).

3.3. Generation steps of iPS cells

Characterized patient-specific iPS cell lines can be obtained from skin biopsy material within 2–3 months; the time depends on the experiment's starting phase. The first steps are the generation of dermal fibroblasts and lentiviruses (Ohnuki et al., 2009). Donor forearm skin

is used in dermal fibroblast biopsy and the specimen is divided into 1 mm parts; cells are obtained after 3–4 weeks of dermal fibroblast culture. Lentiviruses are derived in 4–5 days with transfection by the three-plasmid system. Newly harvested fibroblasts are used in the reprogramming experiment. They are cultivated at 37 °C with 5% CO_2 24 h prior to the experiment. The next day the lentivirus-infected solution with transcription factors is added to the fibroblast culture plaques and fibroblasts are transduced. Incubation goes on until iPS cell colonies begin to collect on the plaques. Starting from week 4, the iPS cell colonies start to appear and the colonies are cut and harvested using an invert microscope. Quality control and pluripotency proofing characterization tests should be carried out. Morphologic examination, karyotyping, alkaline phosphatase and fluorescent staining tests, immunochemical pluripotency marker examination, and differentiation and microbiological tests should be carried out (Marti et al., 2013).

3.4. Primary utilization sites for iPS cells

iPS cells are utilized in human disease modeling, regenerative medicine, and drug research experiments (Sevim and Gürpınar, 2012). In recent years, significant advances have been made by iPS cell technology in the research of inherited monogenic diseases, opening an avenue to generate patient-specific pluripotent stem cells. iPS cell technology has a broad application in blood disorders, neurodegenerative diseases, and retinal degenerative diseases, but little is known about its application in hereditary hearing loss (Wang et al., 2012; Peng et al., 2014; Ross and Akimov, 2014; Wiley et al., 2015).

3.4.1. Patient-specific disease modeling

Many countries have strict ethical rules for obtaining human experimental material to be used in genetic disease research. Enough amount of material at the desired time is one of the hardest issues for researchers. Human iPS cells are the way to overcome all these problems. They are very useful in the understanding of disease mechanisms. Patient- and disease-specific iPS cells were produced, cellular lines for experiments were obtained, and thus iPS cell models for many diseases were established. For example, these models have helped to research the pathogenesis and drug development for the treatment of spinal muscular dystrophy, Parkinson disease, thalassemia, Gaucher disease, Duchenne muscular dystrophy, hearing loss, and type 1 diabetes (Park et al., 2008; Wang et al., 2012; Ross and Akimov, 2014).

3.4.2. Drug development research

A new molecule should be tested for activity and side effects during drug development; rats, dogs, or pigs are usually used for this purpose. Such tests are expensive and hard to standardize because of biological and physiological differences with humans. iPS cell experiments prior to animal tests help to have better focus and prevent unnecessary duplication of animal tests. The developed disease- and patient-specific iPS cells make the drug screening tests easier and accelerate pharmacological and toxicological research.

3.4.3. Regenerative medicine

iPS cells and their cousins, ES cells, are very suitable for the generation of "replacement parts" for damaged or sick organs. Immune rejection is one of the main problems in organ transplantation and cellular treatment. Since patient-specific iPS cells are autologous and have the same HLA tissue antigen, tissue rejection is not encountered due to HLA compatibility. The main goal in regenerative medicine is to provide self-identical cells and tissues to be transplanted by using iPS cells. There are still many limitations. First of all, safe and dependable iPS cell-obtaining methods are not fully developed. Merling et al. (2015) obtained iPS cell lines for chronic granulomatous disease (CGD) by peripheral blood CD34+ cell reprogramming and the molecular mutation was repaired by using the zinc finger nuclease technique in cellular lines. Therefore, gene therapy is an available therapeutic option for the correction of genetic mutations in iPS cells of patients with CGD and other immunodeficiencies (Flynn et al., 2015; Merling et al., 2015).

4. The use of iPS cell technology in otolaryngology and the treatment of hearing loss

4.1. Cartilage tissue production with iPS cells for tissue repair

One of the stem cell research areas in otolaryngology is cartilage tissue research, and particularly research on tissue repair in the ear and nose. A typical example of cartilage regenerative medicine is autologous chondrocyte implantation, in which chondrocytes isolated from the patient's cartilage or derived from mesenchymal stem cells are cultured and injected into the cartilage defects in liquid or gel form. The mesenchymal stem cells inhibit the release of proinflammatory cytokines and are helpful in cartilage repair and osteoarthritis (Diekman et al., 2012). The ear and nose cartilage tissue has high regeneration capacity and future therapy with iPS may not be needed for these problems. However, the capacity of articular cartilage for repair and regeneration is extremely poor. iPS cells have the potential to provide an abundant cell source for tissue engineering and the generation of patient-matched in vitro models to study genetic and environmental factors in cartilage repair (Diekman et al., 2012).

4.2. Olfactory sensory neuron production from iPS cells

The human olfactory stem cells are multipotent cells, generating oligodendrocytes and neurons, and breeding for many generations. The olfactory epithelium has olfactory sensory neurons and within the epithelium there are basal stem cells that can restore this tissue following damage; moreover, within the lamina propria there are other stem cells with both neural and mesenchymal characteristics. The cells obtained from human olfactory mucosa generate neurospheres that are multipotent in in vitro conditions and cloned neurosphere cells show this multipotency and self-replication (Roisen et al., 2001; Murrell et al., 2005). Recently, neurospheres from olfactory mucosal biopsy have been generated from patients with schizophrenia, Parkinson disease, and familial dysautonomia; these results demonstrate the existence of multipotent cells in the olfactory mucosa useful for cellular studies of brain diseases (Roisen et al., 2001; Murrell et al., 2005). In disease modeling studies multipotent stem cells like ES cells or iPS cells give results that include targeted cells, without the contamination of other cells (Mackay-Sim, 2013). Therefore, iPS cells are useful in the modeling of numerous brain diseases because of their ability to be differentiated into specific cells of interest, e.g., sensory neurons (Grskovic et al., 2011; Mackay-Sim, 2012).

4.3. Sensory epithelial hair cell production from iPS cells

The inner ear contains sensory epithelia that have a role in detecting impulses from head movements, gravity, and sound. Currently investigations are underway on how to develop these sensory epithelia from pluripotent stem cells, a process that will be critical for modeling inner ear disorders or developing cell-based therapies for underlining hearing loss and balance disorders (Brigande and Heller, 2009; Bermingham-McDonogh and Reh, 2011). The restoration of auditory function is an important subject of iPS cell studies and hearing loss is one of the most common disabilities, affecting approximately 10% of the population at different levels. Humans are born with 30,000 cochlear and vestibular hair cells per ear. Hair cells and spiral ganglion neurons are usually damaged in most cases of hearing loss. The inability of the mammalian inner ear to regenerate lost hair cells is the major reason for the permanence of hearing loss and certain balance disorders. Recent developments in stem cell technology provide new insights for the treatment of deafness and iPS cells are virtually bringing a biological approach to replace damaged hearing cells (Hu and Ulfendahl, 2013; Peng et al., 2014). Two major strategies have been investigated: one is the differentiation of endogenous stem cells into new hair cells; and the other is the introduction of exogenous cells into the inner ear to substitute injured hearing neurons. Although there are still animal experiments in stem cell-based replacement in deafness, there is a probability of utilizing personalized stem cells in a novel intervention for patients in future clinical research trials (Figure) (Hu and Ulfendahl, 2013; Peng et al., 2014).

4.4. The role of iPS cells as potential cures of hearing loss in the future

It will be possible to generate inner ear cell types such as sensory hair cells from iPS cells; iPS cell technology is at an early stage and it will be possible to recreate the causes of hearing loss in a test tube (Koehler et al., 2013). iPS cells from patients can be used to conduct research on the causes of age-related hearing loss and its relation with environmental threats. If such research is combined with genomic studies, it could lead to a very patient-specific prediction of whether a hearing loss is expected in a particular patient. Recently mouse studies have shown that the generation of inner ear sensory epithelia from pluripotent stem cells in 3D culture is possible and a protocol for obtaining hair cell-like cells with stereociliary bundles from iPS cells was announced (Oshima et al., 2010; Koehler et al., 2013).

Additionally, if a patient has a genetic disease-caused hearing loss, an iPS cell-based transplantation therapy would require another crucial step in the repair of the mutation with gene therapy before the iPS cells go back into the patient (Merling et al., 2015).

5. Conclusion

ES cells and iPS cells have pluripotency and differentiate into cells originating from the three germ layers; they are preferred in cellular treatment, drug development, and disease modeling research. Because of ethical restrictions in obtaining ES cell, iPS cells are an alternative pluripotent cell source and the embryo is not damaged by this method. Thus, ethical and political disadvantages are solved with iPS cells and patient-specific autologous pluripotent cells can be obtained by this method. Cellular treatment and regenerative medicine progress with pluripotent cells is currently underway and we tried to raise awareness about this topic in our paper on the biological treatment alternative of hearing loss, which is an important area of research in otolaryngology.

Acknowledgment

This study was supported by the Scientific and Technological Research Council of Turkey (TÜBİTAK), project 110S252.

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