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Induced Pluripotent Stem Cells: Acquirement, Characteristics and Medicinal Applications

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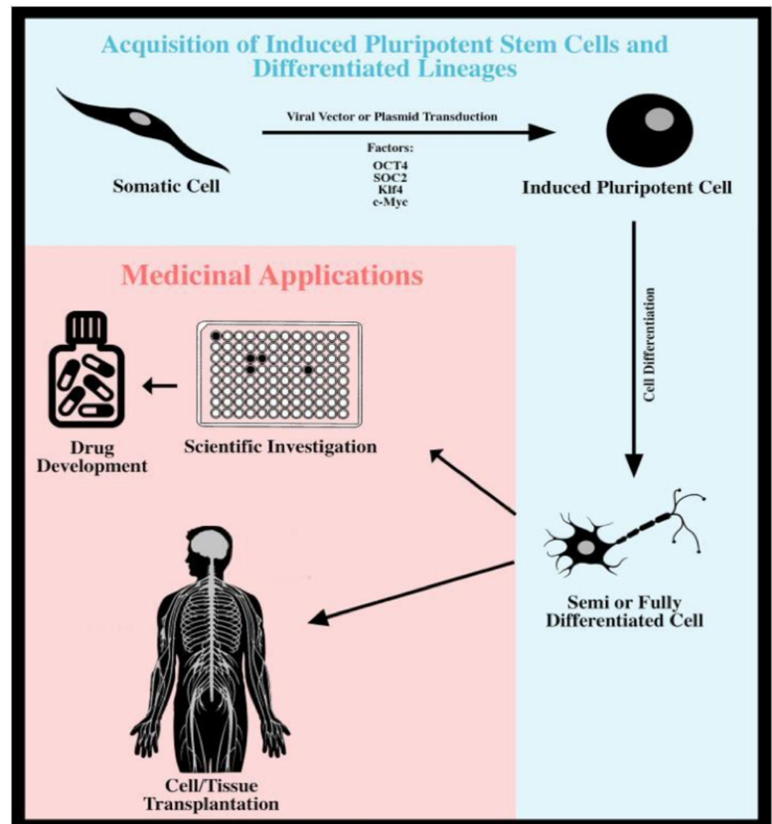

GRAPHICAL ABSTRACT

iPS: Induced pluripotent stem - ES: Embryonic stem - ICM: Inner cell mass
ALS: Amyotrophic Lateral Sclerosis - LQTS: Long QT Syndrome

ABSTRACT

The isolation of human embryonic stem cells in 1998 has since fueled the ideology that stem cells may eventually be used for human disease therapies as well as the regeneration of tissues and organs. The transformation of somatic cells to a pluripotent state via somatic nuclear transfer and embryonic stem cell fusion brought the scientific community nearer to understanding the molecular mechanisms that govern cellular pluripotency. In 2006, the first induced pluripotent stem (iPS) cell was reported, where a mouse somatic cell was successfully converted to a pluripotent state via transduction of four essential factors. This cellular breakthrough has allowed for robust scientific investigations of human diseases that were once extremely difficult to study. Scientists and pharmaceuticals now use iPS cells as means for disease investigations, drug development and cell or tissue transplantation. There is little doubt that scientific progress on iPS cells will change many aspects of medicine in the next couple of decades.

Keywords: Pluripotent, stem, cells, genetics, iPS.

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INTRODUCTION

Induced pluripotent stem cells are relatively novel in the scientific literature, however, their use in clinical science has been immense. The first iPS cells were developed from murine organisms in 2006[1] and later in humans in 2007[2]. These pluripotent cells are created from an organisms' somatic cells by transducing four essential factors required for pluripotency. Once iPS cells are yielded, they can differentiate into different cell lineages for scientific investigations or potential organismal transplantation. The benefit of employing iPS cells is the avoidance of host immune rejection after transplantation, as the newly produced cells carry the same genome and characteristics of an individual's regular somatic cells. Current research focuses on using iPS cells to create novel therapeutics for common human diseases such as Amyotrophic Lateral Sclerosis and Congenital Long QT Syndrome. Using similar techniques, stem cells similar to iPS cells have also shown therapeutic potential for treating human cancers. The purpose of this review is to depict the acquisition, characteristics, and medicinal application of iPS cells. Specifically, this paper will discuss the following:

1. Stem Cells
2. Acquisition and Characteristics of iPS cells
3. iPS Cell Medical Therapeutics
4. iPS-Cell-Alike Medical Therapeutics

STEM CELLS

Stem cells have three characteristics that define their identity. First, stem cells are capable of self-renewal that entails the capability of symmetrical divisions, where both daughter cells have stem cell characteristics. This usually occurs during development, after a stem cell transplantation, or after an insult to an existing stem cell pool[3]. The second characteristic of stem cells is that they are able to yield differentiated progeny. Depending on the potency of the cells, differentiated descendants may be from one, or a combination, of the ectoderm, mesoderm or endoderm germ layers. Third, stem cells must be capable of populating tissue in vivo[3]. This phenomenon, however, is still under scientific investigation.

All mammals are derived from a totipotent stem cell that can give rise to embryonic and extraembryonic tissue. During the process of embryogenesis, descendent cells- usually the cells of the inner cell mass (ICM)- lose their potency and become pluripotent, giving rise to only embryonic tissue[3]. Eventually, cells of the ICM differentiate to become constituents of different tissue types and stem cells for different tissues. These tissue stem cells are deemed multipotent or unipotent and may only be differentiated as one particular cell type. Whether all tissues have resident stem cells is a topic still under debate in the scientific literature. For instance, it remains unresolved whether true stem cells reside in adult heart muscle in postnatal life, or in the pancreas, among other tissues[3].

INDUCED PLURIPOTENT STEM CELLS

Pluripotent stem cells have the capability of differentiating into all three germ layers: ectoderm, mesoderm and endoderm. In 2006, Takahashi and Yamanaka discovered a method for yielding pluripotent cells from organismal somatic cells (Refer to section 4.1). This was first created using murine animals, but one year later, in 2007, Yu and colleagues identified key factors for inducing pluripotent cells in human somatic cells. This finding is immense as it allows scientists to obtain easily accessible somatic cells from an organismal model or patient, transform it to a pluripotent state and thereafter differentiate it to another cell type. Examples of this include transforming blood cells to peripheral neural sensory cells, which are nearly impossible to obtain from human patients due to potential neuronal damage and ethical reasons[4].

ACQUISITION OF PLURIPOTENT STEM CELLS

Somatic Nuclear Transfer

The somatic cell nuclear transfer experiment was the first to show that differentiated cells are capable of becoming pluripotent. In 1960, it was demonstrated that implanting an albino *Xenapus laevis* nucleus into a brown *Xenapus laevis* enucleated oocyte was able to generate a clone of the donor brown frog[5]. Hence, a nucleus of a differentiated cell is capable of undergoing changes that entails a pluripotent state to eventually give rise to a fully developed organism. The reprogramming of the cell occurs 24-48 hours after the implantation of the nucleus into the enucleated oocyte. A similar experiment has also been conducted with sheep somatic nuclear transfer giving rise to a clone famously known as Dolly[6].

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Fusion of Somatic Cells with Embryonic Stem Cells

Another method of transforming somatic cells to a pluripotent state is to fuse differentiated cells with embryonic stem (ES) cells[7]. Similar to the somatic cell nuclear transfer experiment, molecular changes occur to allow differentiated cells to become pluripotent. Studies have found that ES cell fusion with differentiated cells activated OCT4 expression in somatic cells[8], which is a gene essential for pluripotency. The exact mechanism of this transformation, however, is not fully understood.

INDUCED PLURIPOTENT STEM CELL GENOME EXPRESSION

Factors Needed for Murine Models

Studies have identified certain genes highly expressed in embryonic stem cells of murine organisms. A total of 24 factors were identified including: b-catenin, c-Myc, STAT3, S33Y-b-catenin[9], T58A-c-Myc[10], STAT3-C[11], and Grb2DSH2[12]. When these 24 factors were transduced in differentiated somatic cells, they effectively transformed the cells into undifferentiated murine stem cells. However, further investigation proved that not all 24 factors are needed and only genes c-Myc, Klf4, SOX2, and OCT3/4[1] are necessary for the induction of induced pluripotent stem cells from differentiated mouse fibroblast cells. iPS cell colonies did not form when either OCT3/4 or Klf4 were removed. The removal of SOX2 resulted in only a few cell colonies, and the removal of c-Myc caused cell colonies to emerge but without ES-cell-like morphology[1]. Therefore, if any one of these 4 factors are removed, cells fail to transform into iPS cells, hence all four gene factors are deemed necessary.

Factors Needed for Humans

There are differences between mouse fibroblast cells and human cells. In mice, c-Myc promotes pluripotency, however, in human cells c-Myc causes death and differentiation of ES cells[13]. Hence, a different combination is needed to initiate the transformation of somatic cells to iPS cells. These factors are OCT4, SOX2, NANOG, and LIN28[2]. Removal of OCT4 and SOX2 eliminates the formation of iPS cell colonies[2]. Meanwhile, overexpression of NANOG shows a 200-fold increase in reprogramming efficiency, and LIN28 has a consistent but modest effect on the reprogramming of cells[2].

GENOME MODIFICATION TECHNIQUES

The first method used to transform somatic cells into iPS cells was performed using viral transduction with the reprogramming factors. Viruses commonly used include retrovirus and lentivirus. This method poses risks as it may lead to insertional mutagenesis, tumour formation and unpredictable genetic dysfunction[14]. Instead, recent studies have shown that short-term overexpression of the transcription factors required for the 4 factors using plasmids, or using transposons that are subsequently removed by a Cre-Lox system, are adequate to transform differentiated cells to pluripotent cells[15]. Specifically, studies have demonstrated that repeated transfection of two expression plasmids, one containing the complementary DNAs of OCT3/4, SOX[2], and Klf4 and the other containing the c-Myc cDNA, into mouse embryonic fibroblasts resulted in iPS cells without evidence of plasmid integration[14]. Also, these cells produced teratomas when transplanted into mice and contributed to adult chimeras, thus proving their pluripotent cell state[14].

Furthermore, cell penetrating peptides (CPP) may be used to overcome the cell membrane barrier during the cellular reprogramming process. CPPs contain a high concentration of arginine and lysine amino acids that allow associated peptides to surpass the cell membrane[16]. Studies have shown that red fluorescent protein fused to 9 arginine (a form of CPP), injected into COS7 cells and human newborn fibroblasts successfully surpassed the cell membrane and entered the cytoplasm. Therefore, CPP provides a plausible mechanism to deliver factors for transcription of the four essential genes into cells[17]. Other studies have also concluded that chemical agents, BIX-01294 and BayK8644, cause histone modifications, particularly methylation and acetylation modulation of the OCT4 and Klf4 genes to improve the transformation of somatic cells to pluripotent cells[18]. Therefore, many safe and secure methods exist for the formation of induced pluripotent stem cells from somatic cells, which yields great advantages for clinical applications.

ASSAY FOR STEM CELL CULTIVATION

To determine if the cultivated cells are indeed pluripotent, an assay is employed. A potent example of this in

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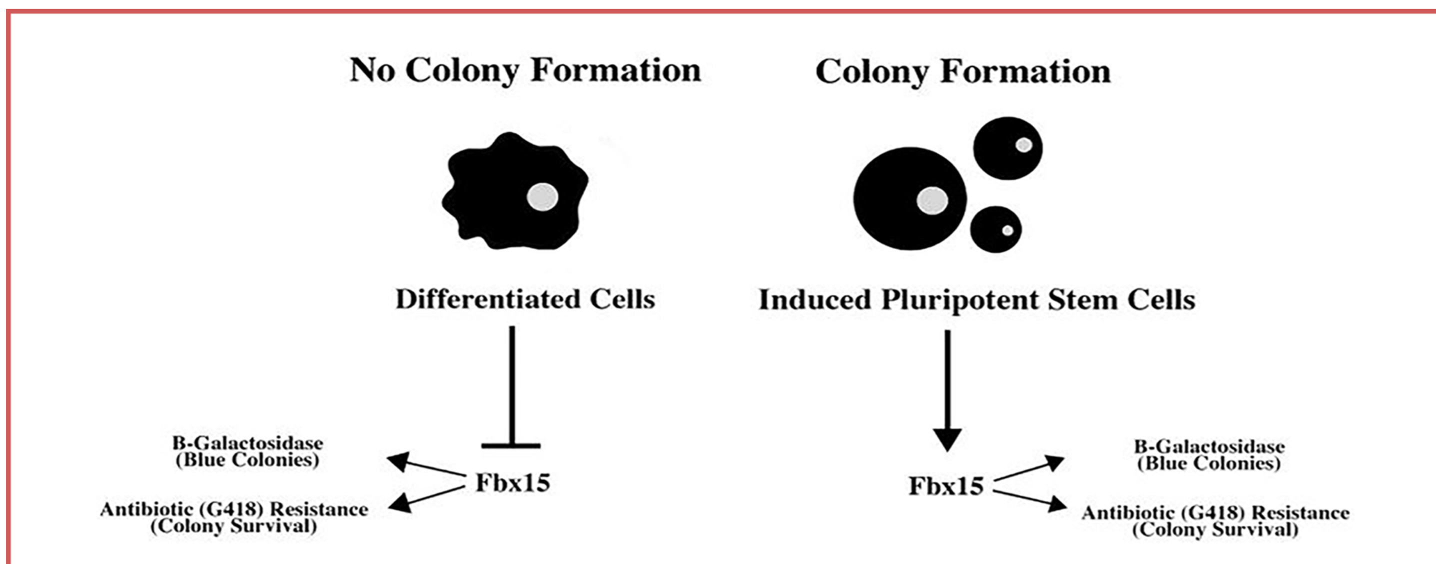

Figure 1 – Formation of colonies occurs only for pluripotent stem cells as they transcribe Fbx15 with the ßgeo cassette, a fusion including the ß-galactosidase and neomycin resistance genes. Pluripotent stem cell colonies appear blue and have embryonic stem cell-like characteristics.

-cludes an antibiotic resistance assay. ßgeo cassette of a fusion of ß-galactosidase and neomycin resistance genes is inserted into the Fbx15 gene by homologous recombination[1]. Hence, by cultivating cell cultures in G418 antibiotic conditions, only stem cells will survive and will be illuminated blue in colour[1] (due to the ß -galactosidase insertion into the Fbx15 gene) (Figure 1.0). Stem cells homozygous for the ßgeo knock-in construct ($Fbx15^{bgeo/bgeo}$) were resistant to extremely high concentrations of G418 (up to 12 mg/ml), whereas somatic cells derived from $Fbx15^{bgeo/bgeo}$ mice were sensitive to normal concentrations of G418 (0.3 mg/ml) as these differentiated cells do not express the Fbx15 gene[1].

COMPARISON TO EMBRYONIC STEM CELLS

Doubling Time and Genetic Markers

Most cultivated iPS cells demonstrate morphologies similar to that of embryonic stem cells. These characteristics include round cellular shape, large nuclei and a scant cytoplasm[1]. The doubling time of these cultivated pluripotent stem cells was also similar to those of ES cells at approximately 17.0 hours[1]. Reverse transcription PCR demonstrated further similarities of iPS cells and ES cells. Particularly, iPS cells were found to transcribe OCT3/4, NANOG, E-Ras, Cripto, Dax1, Zfp296[19], Fgf4[20], Myb, Kit, Gdf3, and Zic3 which are deemed gene markers and highly expressed among ES cells[1]. Overall, these characteristics confirm that iPS cells are similar but not identical to ES cells.

Teratoma Formation

Another test that is employed to determine if the obtained iPS cells are pluripotent involves the implantation of iPS cells into immunosuppressant mice and the subsequent observation for teratoma formation. Several of the implanted iPS cell colonies proved that the injected cells formed benign tumours of all three germ layers including: neural tissues, cartilage, and columnar epithelium[1]. However, some teratomas only differentiated to the endoderm and ectoderm layers, and other tumours did not differentiate at all[1]. These observations effectively establish that most, but not all, of the colonies transform into a pluripotent state.

Embryoid Bodies and Differentiation

Cell cultures can also be tested for the formation of embryoid bodies and differentiation. When grown in tissue culture dishes, embryoid bodies from iPS cells containing the 4 factors promoting pluripotency effectively attached to the bottom of the dish and began differentiation[1]. Immunostaining tested positive for the formation of the ectoderm (using βIII tubulin), mesoderm (using α-smooth muscle actin) and endoderm (using α-fetoprotein) germ layers[1]. However, cells without the 4 necessary factors failed to form embryoid bodies and did not differentiate[1].

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MEDICINAL AND THERAPEUTIC APPLICATIONS OF INDUCED PLURIPOTENT STEM CELLS

Applications for Autologous Transplantations

Implantation of ES cells into patients for tissue replacement or repair poses significant consequences in terms of bodily immune responses. Patients would require lifelong immunosuppressive therapy[21]. However, if patient somatic cells are used to derive tissue-specific stem cells, immunosuppression would not be needed as the inserted cells would be the patients' own cells. One drawback to this method of disease resolution is that autologous iPS cells would have the potential genetic mutations that underlie the existing disease. This, however, can be overcome with in situ repairs of the defected genome using homologous recombination[21]. Another concern regarding the implantations of iPS cells into patients is the potential for the iPS cells to form teratomas in the body. A plausible solution to this is to inject differentiated and descendent multipotent or unipotent stem cells into patients, in order to prevent tumour development.

Applications for Amyotrophic Lateral Sclerosis

Amyotrophic Lateral Sclerosis (ALS) is a neurodegenerative disorder where motor neuron loss in the spinal cord and motor cortex leads to paralysis and death[22]. Glia cells from ALS animal models have shown to produce factors that are toxic to motor neurons[23]. A study conducted by Dimos and colleagues, 2008, demonstrated that somatic fibroblast cells of elderly ALS patients may be transformed into iPS cells for pathology investigation. Specifically, an 82-year-old patient was used for this study. This patient was heterozygous for the L144F dominant allele of the superoxide dismutase gene resulting in a slowly progressing form of ALS[25]. Primary skin cells isolated by biopsy from the patient had factors KLF4, SOX2, OCT4, and c-MYC transduced into it by means of vesicular stomatitis virus glycoprotein pseudotyped Moloney-based retroviruses[24]. Some of these differentiated skin cells transformed into iPS cells and demonstrated nearly complete silencing of viral SOX2 and KLF4. Nevertheless, some expression of viral OCT4 and c-MYC persisted[24]. The embryoid bodies formed from these iPS cells were treated with an agonist of the sonic hedgehog signalling pathway and retinoic acid to induce neuronal differentiation[24]. This experiment was successful and motor neuron differentiation took place with the patient's exact genotype[24]. These cells may then be used to study the physiology and anatomy of the dysfunctional cells or alter the genome to make it functional and transplant the cells into the body for repair. However, some limitations exist for this protocol. First, non-viral techniques (as discussed in Genome Modification Techniques) must be employed for clinical trials to begin, as the use of viral vectors poses health risks. Second, patient genome defects must be understood, or successfully mitigated, for successful therapeutic applications[24].

Congenital Long QT Syndrome

Congenital long QT syndrome (LQTS) is a disease classified into 12 subtypes, all in which share a common feature of delayed repolarization, a prolonged QT interval in the electrocardiogram, and a life-threatening polymorphic ventricular tachycardia known as torsade de pointes[26]. There is currently a lack of in vitro sources for human cardiomyocytes, thus an inability to model the disease in humans[27]. However, by utilizing iPS cells, LQTS may now be modelled in vitro to better understand the disease and potentially derive therapeutics. Recently, scientists have isolated dermal fibroblasts from a 28-year-old woman with a diagnosis of familial type-2 LQTS due to a missense mutation in exon 9 of the KCNH2 gene that encodes the pore-forming region of potassium channels, hence leading to a significant reduction of the delayed-rectifier potassium current[27]. From these dermal cells, transduction of SOX2, Klf4 and OCT4 was committed with retroviral vectors. All of the iPS cells showed ES cell-like morphologies and expressed the pluripotency gene markers: NANOG, SSEA4, OCT4 and TRA-1-60[27]. These iPS cells are then differentiated into the cardiac lineage, confirmed by the presence of cardiac-specific transcription factors: NKX2-5 and genes MLC2V, MYH6 and MYH7[27]. From these differentiated cells, it was found and modelled that action-potential duration prolongation occurred in LQTS differentiated cardiomyocytes relative to control cells[28]. Therefore, iPS cells have provided a foundation for understanding human pathologies specific to the cardiac system. These studies have proven that if cells are not easily obtainable or isolated, iPS cells may be used to induce the creation of one cell type from another, in order to better understand diseases and to potentially develop therapeutics.

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CELL DIFFERENTIATION WITHOUT USE OF INDUCED PLURIPOTENT STEM CELLS

Although common transformations of one cell identity-type to the other (e.g. blood cells to neurons) makes use of the iPS cell state, there are methods that only achieve multipotent intermediate cells rather than pluripotent. In certain contexts, this situation is favoured as it limits the potential for cells to differentiate into undesired or malignant cell lineages. The method of transformation, however, is similar to that of the pluripotent state discussed previously. In this conversion process, OCT4 is preferentially expressed but levels of SOX2 and NANOG expression remain relatively low[29]. This method will transform the donor cell to a stem-cell state, however, pluripotency will not be achieved.

CONCLUSION

Overall, the scientific community has expanded its knowledge of stem cells and their applications over the past few decades. There is little doubt that this field of research will continue to grow, and develop into new avenues that will ultimately advance human health. The identification of the four factors necessary to induce somatic cells to a pluripotent state was a scientific breakthrough that led the authors to acquire a Nobel Prize in 2006. However, there is more work to be conducted. Perhaps further research on this topic may lead researchers to find ways to create human organs in the lab for autologous transplantation. Likewise, stem cell investigations may uncover an underlying malignant pathway that is necessary for tumour formation, allowing us to intercept the tumours before they form. Although the advances in stem cell research have been vast thus far, there is still much more to learn in this field of science.

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