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Biomedical Applications of Induced Pluripotent Stem Cells

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Abstract

Induced Pluripotent Stem Cells (iPSCs) which are efficiently produced from somatic cells by the introduction of four transcription factors (Oct4, Sox2, Klf4, and c-Myc) in fibroblasts could circumvent the restrictions of multipotent stem cells that obligated to differentiate into only several lineage cells and also, the ethical argument about ESCs that causes oocytes and embryo destruction. In addition, iPSCs are powerful tools applicable in biomedicine, cell therapy, pharmacology and toxicology. Therefore, the use of iPSCs in stem cell therapy has immense prospects and offer remarkable applications in regenerative medicine. This review aimed to summarize the most recent findings on iPSCs and focus on their biomedical applications.

Introduction

Stem cells are unspecialized cells in the human body that possesses two prominent properties: a capability for self-renewal and potency, which is the efficiency of proliferation and differentiation to various cellular lineages under suitable conditions. Essentially, stem cell stays uncommitted until it is signaled to change into a specialized cell. Stem cells have the special properties of developing into an expansion of different cells in the human body. They function as a repair system by being able to divide without restriction to replenish other cells [1].

Self Renewal of Stem Cells

Stem cells fate is decided by their communication with the surrounding niche or microenvironment. The stem cell niche is made up of several stromal cells, Extracellular Matrix (ECM) and signaling factors, which together with the intrinsic properties of the stem cells outline its characteristics and capabilities [2]. Quiescent fate stem cells remain without dividing or differentiating, hence, preserving the stem cell pool. Second fate is symmetric self-renewal. Stem cell divides and gives rise to two daughter stem cells, copies of parent cell. No differentiated progeny is generated but the stem cells pool expands with subsequent division, specialized cells can be developed. Third fate, the asymmetric self-renewal, stem cell divides and yields two daughter cells, one a duplicate of the parent, while the other a differentiated cell, termed a somatic or progenitor cell. This type of self-renewal generates specialized progeny essential for developing and regenerating the natural tissue and at the same time preserving the stem cell pool. Fourth fate stem cells produce two differentiated daughter cells both different from the parent cell. This increases the specialized progeny with a net loss in the stem cell pool [3].

Potency of Stem Cells

The stem cell Potency indicates the differentiation capability which means the ability to specialize into various types of cell:

Totipotent stem cells

Can differentiate into embryonic and extraembryonic cell types. The fertilized egg and the cells generated by the first few divisions of the fertilized egg are totipotent [4].

Pluripotent stem cells

Are considered the progeny of totipotent cells and can specialize into almost all cells including cells derived from any of the three germ layers. Pluripotent cells are distinguished by self-renewal and a differentiation capability to all cell types of the adult organism [4]. Embryonic Stem Cells are categorized under this group [5].

Multipotent stem cells

Can produce several types of cells, but limited to those of closely related types. For example,

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hematopoietic (adult) stem cells are multipotent stem cells that can generate all blood cells [6].

Oligopotent stem cells

Can specialize into fewer cells, such as lymphoid or myeloid stem cells. The squamous epithelium in the cornea is considered Oligopotent [7].

Unipotent cells

Can differentiate into only their own type of cells, but possess the capability to renew it, which distinguish them from non-stem cells as muscle stem cells [8].

Types of Stem Cells

Stem cells are classified depending on their origin into adult stem cells and embryonic stem cells [9].

Adult stem cells

They are unspecialized tissue-specific stem cells possessing high capability for self renewal, and have the ability to differentiate into mature cells related to their own origin. The main function of adult stem cells is to preserve and/or renew the cells of injured tissues [10]. Adult stem cells are basically classified as multipotent or unipotent stem cells as they can only proliferate into progeny limited to the tissue of origin. Mesenchymal Stem Cells (MSC), Hematopoietic Stem Cells (HSC) and hair follicle stem cells are multipotent stem cells; these cells can give rise to several cell types of a single tissue. However, epidermal stem cells, myosatellite cells of muscle, and endothelial progenitor cells are considered unipotent stem cells; they can differentiate only into single specialized cell type [11].

Currently, there are several restrictions about the use of adult stem cells. Despite there are various kinds of identified multipotent stem. However adult stem cells that could proliferate into all cell and tissue types have not yet been discovered. As the cells start to differentiate into other cell lineages, they lose their self renewing potential owing to several epigenetic variations which decreases their pluripotency [12]. Adult stem cells cannot be isolated and purified easily as they are often present in only minute [13]. Studies demonstrated that they do not possess the same multiplication capacity as embryonic stem cells. Finally, adult stem cells could include more DNA abnormalities-caused by sunlight, toxins, and mistakes during DNA replication over long time. This possible deficiency might restrict the advantages of adult stem cells [14].

Embryonic stem cells

Embryonic Stem Cells (ESCs) are the cells present in the Inner Cell Mass (ICM) of a blastocyst, an embryo in the early stage. The blastocyst stage is reached by human embryos in 4-5 days after fertilization, at that time they are composed of 50 to 150 cells. ESCs are pluripotent and develop to produce all cells derived from the three primary germ layers: ectoderm, endoderm and mesoderm [9]. They are characterized by immortality in culture and could be preserved for several hundred passages in the unspecialized condition. ESCs exhibit a normal karyotype, preserves telomerase activity, and their proliferative potential is remarkably long [15]. ESCs are known to express several genes responsible for pluripotency, as Oct-4, Rex-1, SOX-2, Nanog, LIN28, Thy-1, and SSEA-3 and -4.7. Also they express elevated telomerase levels which justify their immortality in culture [16].

Owing to their plasticity and unrestricted potential for self-renewal, ESCs therapeutic potentials have been candidate as a remarkable tool for regenerative medicine and tissue replacement following damage or disease. Pluripotent stem cells have exhibited great prospects in curing many conditions, among which: age related macular degeneration, diabetes, spinal cord injuries, neurodegenerative disorders and AIDS [17]. Besides, their use in regenerative medicine, ESCs offers a replacement source for tissue/organs to circumvent donor shortage problem. Moreover tissue/organs obtained from ESCs can be made immunocompatible to match the recipient. Also, ESCs can be used to study early human development, investigate genetic disease and function as *in vitro* systems for toxicology assay [18].

Embryonic stem cell research has faced a lot of controversial opinions and arguments. As harvesting embryonic stem cells obligate destroying the embryo to obtain these cells, the ethical status regarding the embryo becomes questionable. Opponents of ESC research defy that as any embryo has the capability to turn into human, hence it is considered a murder to destroy it and the embryo must be protected under the same moral view as a developed human being. Owing ethical controversy, many nations currently have moratoria or imposed restrictions on either human ES cell research or production [19].

Another essential restriction on ESCs is that upon transplantation the cells may be immunorejected due to incompatibility between the donor and the recipient cells. To overcome this problem, a comprehensive storage for many of HLA typed stem cell lines for compatible match with specific patients is being developed. However, this solution has also been hindered [20].

To circumvent these limitations, biologically similar alternatives that can overcome the moral debate surrounding stem cells are very important. Numerous studies and researches in this regard have led to the generation of non-embryonic sources of pluripotent stem cells.

Induced pluripotent stem cell

Induced Pluripotent Stem Cells (iPSCs) are adult cells that have been genetically reprogrammed by ectopic introduction of transcriptional factor genes into an embryonic stem cell-like status essential for preserving the characteristics of ESCs [20].

Takahashi and Yamanaka in 2006, discovered that somatic mouse fibroblasts could be reprogrammed into ESC-like colonies by the introduction of four transcription factors: Oct 3/4, Sox2, Klf4 and c-Myc which are called "OSKM factors or Yamanaka cocktail" have been selected from 24 factors screened. The cells produced are termed induced Pluripotent Stem (iPS) cells [21]. These OSKM factors are pluripotency-linked genes which are expressed during the early development of the embryo hence, considered the essence of pluripotency circuitry. The transcription factors functions mainly to preserve pluripotency and self-renewal. In 2007, Takahashi et al. [22] declared human iPS cell generation. After additional investigations to improve the technique, Nanog and Oct4 were introduced, these modulations enhanced human iPS cells to exhibit similar characteristics to ESCs concerning multi-lineage, *in vitro* specialization, teratoma formation, and generation of entire animals [23].

Oct4, Sox2, and Nanog are crucial for regulating embryonic development and essential for pluripotency preservation [24]. They are expressed in ESCs and ICM of blastocyte. Klf4 is involved in several processes in the cell, including development, proliferation,

differentiation, and apoptosis [25]. Klf4 interacts with Oct4-Sox2 complexes and plays an important role iPSCs generation due to its tumor suppressor activity [26]. c-Myc is a powerful oncogene linked with apoptosis, cell proliferation, and cell cycle regulation [27].

Moreover, the studies explore that Nanog, Sox2 and Oct4, interact with each other to form an autoregulatory loop to enhance the promoters of their own genes and each other [25]. The three factors work together to sustain their expression, thereby promoting pluripotency gene expression stability. In addition, these factors target transcriptionally active and inactive cascade genes [28]. The targeted actively transcribed genes are responsible for maintaining pluripotency and self-renewal. They enclose several ESC transcription factors and chromatin modifying enzymes. However, the inactive genes are associated with development which is silent in ESCs, because their expression is linked with cellular differentiation and lineage formation [27]. Oct4, Sox2, and Nanog regulate pluripotency by promoting pluripotency genes transcription, conversely silencing genes linked to development and differentiation [28].

Also c-Myc and Klf4 together play an integrating role in maintaining pluripotency. c-Myc can cause an elevation in p53 levels which is tumor suppressor proteins which has an inhibitory effect on iPSC production by stimulating senescence, apoptosis and cell cycle inhibition, and Klf4 can inhibit the apoptotic effect induced by c-Myc by restraining p53 levels [29]. Moreover, Klf4 can depress proliferation by stimulating p21 (a cyclin-dependent kinase inhibitor). Conversely, c-Myc can block this anti-proliferative effect of Klf4 by curbing p21 [30]. Hence, c-Myc and Klf4 roles are reciprocally complementary and balancing between their expressions is essential for efficient reprogramming.

Reprogramming factors are introduced into somatic cells by various delivery methods. Which can be assigned into two systems: integrative systems and non-integrative systems? The integrative delivery methods are applied using viral vectors (retrovirus), and non-viral vectors (linear/plasmid DNA fragments and transposons). While, the non-integrative delivery methods utilize viral vectors (adenovirus) and non-viral vectors (mRNA, and proteins) [28]. Although the reprogramming efficiency of the integrative delivery methods was higher than the non integrating methods but the former are considered risky due to the hazard of insertional mutagenesis as it could lead to the integration of various viruses into the genomes of iPS cells, causing tumorigenesis [27].

The produced iPSCs are approved to resemble embryonic stem cells in terms of their morphology, cell behavior, gene expression, epigenetic status and differentiation potential both in culture and *in vivo* [24].

Recently the advancement in the area of iPSC generation has been increased. Initially iPSCs have been reprogrammed from mouse fibroblasts, now different types of somatic cell, mainly melanocytes, mesenchymal cells, peripheral blood cells, and adipose stem cells, have been successfully reprogrammed [28]. Also different reprogramming protocols have been documented. Recent studies reported Oct4 is considered to be an indispensible transcription factor and the other components could be substituted by the endogenous expression by initial cell types [29]. Also, studies revealed that reprogramming using genome-integrating viral vectors may result alteration in gene expression [30], and associated with possible hazards of reactivation of viral transgenes. Consequently, such iPS cells are considered inappropriate for therapeutic applications as the phenotypes of the derived cells may be affected. As a result, recent techniques have substituted the use of genome-integrating viral vectors by other methods that do not require them [29].

Biomedical Applications of iPSCs

iPSCs technology is swiftly developing. These stem cells have significantly proven their usefulness in regenerative medicine, disease modeling, drug development and discovery, gene therapy, different models of cell treatments and blood components synthesis [31].

Disease modeling

iPSC lines are considered as infinite cell source for patients suffering from diseases of definite or indefinite etiologies. *In vitro* differentiation of these cells into the afflicted cell types mimics the "disease in a Petri dish" model. This approach offers the chance to deeply investigate the underlying mechanism of the disease and enable to utilize these cells to discover new disease-specific drugs to correct the disorder [28].

Ebert et al. [32] could successfully generate iPS cells from a child afflicted with a genetic spinal muscular atrophy. These cells were able to maintain the disease genotype, and capable of differentiating into motor neurons that revealed specific defects in survival of motor neuron protein aggregates, and exhibited the exact phenotype described in motor neuron disease. Moreover, Agarwal et al. [33] illustrated that generating iPS from patient with dyskeratosis congenita could offer new approaching into the disease pathology. Patients suffering from dyskeratosis congenita experience degeneration in several tissues due to disordered telomere preservation. Reprogramming somatic cells in these patients resulted in telomere elongation and following rectification of the malfunctioning telomerase in this disorder. The reverse of the defective telomerase RNA component may suggest a prospective new remedial hope for patients suffering from dyskeratosis congenita. Further studies showed that iPSCs can offer a novel vaccination opportunity. Where, iPSCs derived from somatic cells of patients were enhanced to an immune cell fate to generate memory B cells that produce functional antibodies to diverse pathogens. The produced cells were then re- implanted into the patient [34].

The main objective behind modeling the disease is to comprehend its underlying molecular mechanism, and eventually creating suitable drugs for their treatment [35].

Drug screening for toxicity, drug development and drug discovery

Cells differentiated from disease and patient specific iPSCs are critical option for investigating potential therapeutic compounds. The use of iPSCs in drug discovery or determination of toxicity is considered an efficient substitute for using animal models. Animals or *in vitro* animal derived cells are utilized as testing systems but are restricted by their failure to imitate the "accurate" human physiological conditions and connected phenotypic attributions [36]. Although genetically modified rodents and animal models have afforded valuable data in investigating safety and efficacy of drugs, the treatment response to drugs in animal models cannot be relied on to predict effectiveness in humans [37]. For instance, treating transgenic mouse model of amyotrophic lateral sclerosis due to over-expression of mutant superoxide dismutase with creatine was found to be very efficient in changing the disease characteristics. Nevertheless, no

clinical improvements have been detected in human clinical trials [36].

The iPS cells technology can offer a novel advance in pharmacological and toxicological screening for the following reasons [38]: a) iPS cell permit the production of human disease-specific cell types (neurons, cardiomyocytes and hepatocytes) to facilitate better testing of therapeutic response and toxicology. b) a library of various iPS cell lines for the same disease can be produced and grant a close look into the genetic and possible epigenetic variation of a wide-ranging sector of the population. c) The variant remedial effect of a possible drug can be investigated at an individual level. Which enables applying Personalized medicine (selecting suitable and best possible therapies based on the context of a patient's genetic content or other molecular or cellular analysis) [39].

Cardio and hepatotoxicity are a main reason of drug failure during pre-clinical and clinical investigation. The absence of an *in vitro* model to identify pro-arrhythmic effects of drugs on human heart cells has delayed the progress of many therapeutic agents. On the other hand, iPS cell-derived cardiomyocytes can supply a valuable cell source to test drug efficacy and safety preceding clinical testing [39].

The iPS cell technology can significantly reduce the number of animals sacrificed during drug testing, permit the detection of early human toxicity in preclinical trials, and diminish the dangers and cost linked to clinical trials [40].

Gene therapy

Gene therapy indicates the introduction of genetic material into specific cells or tissues for therapeutic objectives; it was developed especially to be utilized in gene correction for mutation in severe monogenic diseases [41]. Strategies have been planned to merge iPSC with gene therapy for treatment of human diseases [42]. These strategies are divided into gene targeting therapy and gene augmentation therapy.

In gene targeting therapy, Zinc Finger Nucleases (ZFN) is one of the most convenient approaches [43]. ZFN are created by binding a zinc finger DNA binding domain to the nuclease domain of the endonuclease to particularly cut a mutated gene for correction [44]. By the introduction of these ZFN into iPSC along with a transposon conveying the correct gene sequence, the mutation can then be reversed and the phenotype could be permanently corrected. The utilization of ZFN for precise targeting of gene for correction was denoted to be notably very efficient [45]. iPSC was applied for gene correction of α1-Antitrypsin (A1AT) deficiency was lately stated [46]. A1AT gene mutation is most frequently connected with liver disease leading to cirrhosis. iPSCs were produced from patients with A1AT deficiency. Consequently, two ZFN were designed to particularly attach to upstream and downstream of the mutated gene. The DNA bordering the mutation were then cleaved by the nucleases and substituted with transposon conveying the accurate sequence. The produced patient-derived iPSC with the corrected A1AT sequence were differentiated into hepatocytes. And upon transplantation of these iPSC derived hepatocytes into immunodeficient mice they were capable of generating albumin and the corrected A1AT protein [47].

Gene augmentation therapy includes the supply of exogenous duplicated wild type allele to cells to rectify the phenotype by expressing functional proteins. This method is appropriate for application in monogenic diseases resulting from recessive mutations in a single gene [48]. Hemophilia A is an inherited hereditary disease linked to the X chromosome identified by the deficiency of the factor VIII protein. This often affects men and could cause excessive bleeding that would result in disability or death [49]. In the iPSC model, fibroblasts from the tip of the rat's tail were isolated and reprogrammed into iPSC, thus differentiated into endothelial cells and their progenitors. These cells derived from iPSC express the specific factor VIII. After transplanting these cells into mice with hemophilia A, the latter survived the tail-clip bleeding test for more than 3 months and their plasma factor VIII levels increased to 8% to 12% [50]. Yadav et al. [51] investigated transdifferentiation of iPSC-derived endothelial progenitor cells into hepatocytes (primary cells of FVIII synthesis). These cells were transplanted into the hepatic parenchyma where they were functionally incorporated and corrected the hemophilic phenotype. Elevated levels of FVIII mRNA have been observed in the tissues of the spleen, heart and kidneys of treated animals without evidence of tumor development or any other long-term adverse event.

Cancer therapy

Modeling cancer pathogenesis & drug screening: IPSCs offer the opportunity to understand the molecular mechanism involved in malignant transformation, the interconnection of oncogenic mutations with different types of tissue and the way these mutations determine the fate of malignant cell [52]. Furthermore, drug screening and development could be analyzed by reprogramming cancer-cell specific into iPSCs. The principle behind this approach is to inspect different groups of drugs that particularly destroy cells that carry oncogenic mutations. Because chemotherapy drugs have adverse side effects, screening assays of cytotoxic drugs should be developed to selectively target cancerous cells and have attenuated side effects [53].

Cancer immunotherapy: Immunotherapy refers to the generation of tumor-specific immune cells that, at the time of transfer, activate a cytotoxic response against the tumor. IPSCs technology could offer the opportunity to clone and expand specific tumor T cells. Further studies have stated that iPSCs-derived T cells could be effectively used for adoptive immunotherapy utilizing Rag-deficient mice model [54]. Also, Natural Killer (NK) cells, another participant of the innate immune system, play essential role in the generation of the antitumor response through the secretion of Th1 cytokines [55]. NK cells were also efficiently differentiated from human iPS cells. NK cells derived from iPSC have the capacity to dynamically slow down human tumor cells and generate high levels of interferon-γ [56]. Recently, studies have revealed that NKT cells can also be derived from iPS cells. These cells could offer a vital role in cancer immune therapy, since they include NK and T cells properties [57].

Gene correction in iPSCs and autologous transplantation: Cancer is the result of a mutation in a specific gene; Genome editing tools can be employed to correct the mutated gene of patient cells. Rectified patient cells can be transformed into iPSC and differentiated into a specific cell lineage. And it can also be used in autologous therapeutic transplantation [58]. For example, the mammary gland is the main target of breast cancer following mutations in BRCA1/BRCA2 genes [59]. Breast cancer therapy generally includes the incorporation of radiation therapy and chemotherapy that frequently causes the destruction of the mammary glands. Regeneration of the damaged mammary gland is the only way to restore its function. Li et al., have effectively generated iPSCs derived from mouse mammary epithelial cells (ME-iPSCs) then re-differentiated them into mammary

epithelial cells (D-ME-iPSCs) [60]. Further transplantation of these D-ME-iPSCs into the fat pads of the mammary glands of nude mice induced the production of mammary tree-like structures *in vivo* [53]. Hence, Parallel use of iPSCs-derived tissue with gene editing tools could generate genetically corrected BRCA1/BRCA2 mammary cells [52].

Regenerative medicine

Diabetes mellitus: Diabetes is assigned to two correlated but different diseases with different etiologies. Type 1 diabetes is caused by autoimmune damage of insulin-generating β-cells in the pancreas. Type 2 diabetes is usually linked with obesity, occurs when the demand for insulin due to the constant high level of blood sugar exceeds the ability of beta cells to secrete enough insulin to curb hyperglycemia. In Type 2 diabetes, peripheral tissues, such as muscle and fat, also develop resistance to insulin effects. Persistent high demand for beta cells often causes beta cell malfunction, dedifferentiation and death [61]. Jeon et al. [62] investigated the functionality of iPSC-derived insulin generating cells produced from pancreas-derived epithelial cells in non-obese diabetic mice. These insulin-secreting cells express several pancreatic β cell markers and produce insulin upon glucose stimulation. Implantation of these cells into non-obese diabetic mice (a model of autoimmune type 1 DM) successfully responded to glucose stimulation and subsequently normalized blood glucose levels. Further study mentioned the production of iPSCs from keratinocytes of aged patients with type 2 DM [63]. These cells were reprogrammed and consequently specialized into insulin-generating islet-like cells. Reprogrammed keratinocytes efficiently produced iPSCs, and successfully transformed into insulin-producing islet-like cells, this approach allows the generation of a resourceful strategy to model the disease as well as a highly developed therapy to treat it.

Neurodegenerative diseases: The therapeutic prospective of iPSC in regenerative medicine has been confirmed in neuronal cell replacement study for Parkinson's disease [64]. Parkinson's disease is a motor system disorder caused by the loss of dopamine secreting brain cells. Wernig and colleagues effectively differentiated neuronal progenitor cells from iPSC that that could form glial cells and neuronal cells in culture. Transplantation of these cells into fetal mouse brain exhibited functional incorporation with differentiation to glia and neurons. Significantly, these cells also differentiated to dopamine neurons which improved behavior in the rat model of Parkinson's disease [47].

Blood: Recent studies discussed the utilization of iPSC for the development of *ex vivo* blood of different blood components. These cells can produce Red Blood Cells (RBCs) that could be used to produce blood, which is essential throughout the world for the treatment of various injuries or diseases, as they are crucial for oxygen delivery and hemostasis. There are many techniques that allow ESC/iPSCs to be used to generate RBCs [65]. Derivation of these blood elements from induced Pluripotent Stem cells (iPS) has the ability to create products independent of blood donors and prone to gene manipulation to complement or replace the recent transfusion banking, which also reduces the risk of alloimmunization [66].

iPSCs derived from adult somatic cells have been used for *in vitro* generation of hematopoietic stem cells. The produced HSCs were transplanted for treatment of hematopoietic disorders. Further differentiation of HSC in a cytokine-defined culture system could produce mature blood cell types [66].

Emerging technologies

- The implantation of rat pluripotent stem cells into blastocysts of Pdx1 deficient mice-which are incapable of forming a pancreas-resulted in an effectively functioning rat pancreatic tissue [67].

- Priming the immune system with induced pluripotent stem cells to inhibit or hinder the progression of cancer in mice. iPSCs express tumor-linked antigen and can exhibit anti-tumor responses in cancer vaccines. iPSC vaccine improves an antigen-specific anti-tumor T cell response [68].

- An initial human iPSC clinical trial was started for treating macular degeneration by transplanting human iPSC-derived Retinal Pigment Epithelial (RPE) cells .Macular degeneration was restrained in patient, with enhanced vision [69-72].

Advantages of iPSCS Technology

1. The utilization of iPSCs has resolved the ethical debate concerning the use of ESCs in research and overcome the imposed restrictions on either human ES cell research or production.

2. Minimized immunorejection options. iPSCs are produced from the somatic cells of the same individual, therefore there is no hazard of immunorejection of these autologous cells [73].

3. Detection and study of toxicity/therapeutic reactions of recently produced drugs [74].

4. Reduce the total cost and risk of clinical trials. iPSCs can provide information on drug intoxication through various cytotoxicity analyzes and reduce the budget associated with the use of animal models, ultimately reducing the cost of clinical trials.

5. The application of a personalized approach to drug delivery and individual modeling of disease by iPSCs allows the screening of disease development and pharmacological agents to offer the appropriate choice for each individual.

6. Gene targeting and Correction Techniques (Gene Therapy). Reprogramming of somatic cells with genetic mutation to iPS cells enabled the production of cell lines that have mutations that cause disease. The ability to alter specific sites in a genome to modify specific mutated genes is very important here.

Limitations and Challenges for iPS Cell Technology

The iPS cell application faces several obstacles that are mainly related to the current reprogramming methodology. The viral vectors used in the delivery of genes could lead to the integration of various viruses into the genomes of iPS cells, causing tumorigenesis due to genetic abnormalities in the cells. In addition, the reprogramming of human fibroblast iPS cells efficiency is very low, almost less than 0.02%. The use of the Myc gene as a transcription factor of reprogramming and/or the reactivation of a silenced c-Myc gene in iPS cells could lead to cancer cells.

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