DOI: 10.5937/halo26-27861
UDC: 602.9

Rančić N, i sar. Induced pluripotent stem cells. Halo 194. 2020; 26(3):153-161.

REVIEW ARTICLE

INDUCED PLURIPOTENT STEM CELLS: WHERE WE ARE CURRENTLY?

Nemanja RANČIĆ[1,2], Sanja RAŠČANIN*[3], Milijana MILJKOVIĆ[1,2], Mirjana JOVANOVIĆ[3,4]*

*Nemanja Rančić and Sanja Raščanin share first authorship.

[1]Center for Clinical Pharmacology, Military Medical Academy, Belgrade, Serbia; [2]Faculty of Medicine of the Military Medical Academy, University of Defence, Belgrade, Serbia; [3]Faculty of Medical Sciences, University of Kragujevac, Kragujevac, Serbia; [4]Psychiatric Clinic, Clinical Center "Kragujevac", Kragujevac, Serbia

Rad primljen: 10.08.2020.

Prihvaćen: 01.12.2020.

Correspondence:

Nemanja Rančić,
Center for Clinical Pharmacology
Military Medical Academy
11000 Belgrade
Serbia
Tel: 063-8524443
E-mail:
nece84@hotmail.com

ABSTRACT

Induced Pluripotent Stem Cells (iPSCs) are a type of pluripotent stem cells generated by reprogramming an adult somatic cell genome to the stage of a pluripotent stem cell *in vitro* by inducing a forced expression of specific transcription factors that are important for the maintenance of pluripotency. The iPSCs seem to be very similar to Embryonic Stem Cells (ESCs) in terms of morphology, cell surface markers and gene expression levels, but recent studies have demonstrated some differences between the two cell types. However, iPSCs might have potential application in regenerative medicine, transplantation, drug testing, disease modelling, and avoidance of tissue rejection and with less ethical concern than ESCs. This paper aims to present the most important characteristics of iPSCs which have therapeutic significance.

Keywords: induced pluripotent stem cells, therapy

INTRODUCTION

Induced Pluripotent Stem Cells (iPSCs) are a type of pluripotent stem cells generated by reprogramming an adult somatic (terminally differentiated) cells' genome to the stage of a pluripotent stem cell *in vitro* by inducing a forced expression of specific transcription factors that are important for the maintenance of pluripotency such as Sex-determining region Y HMG box 2 (Sox2), Octamer-binding transcription factor 3/4 (Oct3/4), v-myc myelocytomatosis viral oncogene homolog (c-Myc), Krüppel-like factor 4 (Klf4), or using the similar set of four transcription factors Octamer-binding transcription factor 4 (Oct4), Sox2, RNA binding protein - RBP (Lin28) and Homeobox protein Nanog (Nanog). These stem cells seem to have similar features as Embryonic Stem Cells (ESCs) in terms of morphology, surface antigen expression, proliferation, gene expression, telomerase activity, doubling time, embryoid body formation (EB), teratoma formation (a tumor containing tissues of all three germ layers), viable chimaera formation, potency and differentiability and can also differentiate into cells of all three germ layers. Recent papers have demonstrated significant differences between ESCs and iPSCs on a transcriptional level, in gene expression, DNA methylation patterns, differential activation of promoters by pluripotency, culture conditions, delivery methods, exogenous factor combinations. Further experiments will show if these differences will have an impact on the potential therapeutic utility of iPSCs. iPSCs have been derived from various somatic cells, dermal fibroblasts, pancreatic beta cells, keratinocytes, hepatocytes, neural cells, bone marrow cells, peripheral blood, gastric epithelial cells, retinal-pigmented epithelial (RPE) cells[1-9].

The iPSCs technology was first developed by *Shinya Yamanaka* with his team of associates in the Kyoto Laboratory in Japan in 2006 [10]. It has been shown that by retroviral transduction, four specific transcription factors Oct3/4, Sox2, Klf4 and c-Myc can convert an adult non-pluripotent cell into a pluripotent stem cell. He was awarded a Nobel Prize in 2012, together with *John Gurdon*[11]. iPSCs were first created from adult mouse fibroblasts. Only a year later, the same team (*Yamanaka and associates*) produced human iPSCs from human adult fibroblasts [12]. During the same year, *James Thomson* with his team at the University of Wisconsin also described a method for the production of iPSCs from human skin cells and identified a different combination of factors for the generation of human iPSC, using Oct4, Sox2, Nanog and Lin28 [13].

MORPHOLOGICAL AND FUNCTIONAL CHARACTERISTICS OF INDUCED PLURIPOTENT STEM CELLS

iPSCs are similar to ESCs in many aspects, but these two cell types are different. However, it is interesting to note that the differences in DNA methylation patterns between iPSC and ESC have no significant effect on their ability to produce functional differentiated cell types. iPSCs are small, round, thickly compacted cells that grow in flat, clearly limited monolayer colonies with well-defined, smooth edges.

They are characterized by a large nucleus with one or more prominent nucleoli and scant cytoplasm (high nucleus to cytoplasm ratio). iPSCs express the same specific surface antigen as ESCs: including stage-specific embryonic antigen 3,4 (SSEA-3, SSEA-4) and tumor rejection antigen (TRA-1-60, TRA-1-81). Reverse transcription-polymerase chain reaction (RT-PCR), immunocytochemistry and microarray analysis make it possible to determine whether iPSCs express ESCs specific transcription factors such as Nanog, growth and differentiation factor 3 (GDF3), Oct4, Sox2, reduced expression 1 (REX1), fibroblast growth factor 4 (FGF4), embryonic cell-specific gene 1 (ESG1), Developmental Pluripotency-Associated 2 (DPPA2), DPPA4, and telomerase reverse transcriptase (TERT). The fundamental characteristic of iPSCs is the ability to differentiate into cells of all three germ layers, which can be proven in several ways. Perhaps the most reliable test of pluripotency is the formation of chimeric mice, but this test is not suitable for human cells. The formation of embryonic bodies *in vitro* and the formation of teratomas after injection into immunocompromised mice are currently used to measure pluripotency in a human system[1,12, 14].

YAMANAKA FACTORS - Only four transcription factors, the so-called *Yamanaka factors* (Oct3/4, Sox2, Klf4, and c-Myc), from the 24 initial genes important for the maintenance of ESC pluripotency were sufficient to transform mouse fibroblasts into pluripotent cells similar to ESC [10, 15, 16]. Oct3/4 is one of the first identified transcription factors to be a significant pluripotency regulator. It belongs to the family of Octamer binding proteins, which modulate the expression of the gene by binding to the 8 base pairs of the sequence -ATGCAAAT- in the regulatory region of genes involved in self-renewal and differentiation. Precise expression of Oct4 in combination with other reprogramming factors is important for efficiently obtaining iPSC. It has been shown that a three times higher expression of Oct4 than Sox2, Klf4 and c-Myc improves the production of iPSC. Some studies have reached a major finding showing that Nr5a2, also known as the liver receptor homolog-1 (LRH-1), can replace Oct4 and improve the reprogramming efficiency for iPSC [17]. LRH-1 is an orphan nuclear receptor that activates and maintains Oct4 expression in the embryonic development epiblast stage. Orphan nuclear receptor-estrogen-related receptor beta (ESRRB) plays a major role in activating and regulating Oct4 as well as in maintaining self-renewal and pluripotency modulating promoter activity over its DNA binding domain that also reacts with Nanog. Sox2 is an important transcription factor of pluripotent stem cells, known as the precursor cells of the nervous system. It is expressed in the internal cell mass, epiblast, and the extraembryonic ectodermal cells during the development of the mouse embryo. Sox2 expression is reflected in nerve stem cells and excessive Sox2 expression favours neural differentiation in mESCs. It is known that Sox2 interacts with several factors, including Oct4 in maintaining pluripotency. Decreased Sox2 expression in mESCs and hESCs results in loss of pluripotency and differentiation towards the trophectodermal cell line. Sox2 and Oct4 remain the two basic reprogramming factors that have wide application in various protocols for generating iPSCs. Sox2 expression was observed during the development of the central nervous system, inner ear in mammals, foreskin, eyes, pituitary gland, stomach, lungs, and in the adult neural nervous system. KLF4 and members of its family have emerged as important regulators for the maintenance of pluripotency. KLF4 can act as an oncogene or tumor suppressor gene depending on the physiological context, usually expressed in adult tissues that have a certain degree of regenerative ability, including intestines, testicles and skin. It has been proven that excessive expression of KLF4 prevents the differentiation of mESCs into erythroid progenitors. In combination with Oct4, Sox2 and c-Myc, KLF4 was one of the first factors used to generate iPSCs. The current view is that KLF4 acts only as a secondary factor for improving reprogramming of somatic cells since iPSCs can also be generated without KLF4 using different combinations of reprogramming factors. C-Myc is a proto-oncogene needed for cell growth and proliferation. It was found that c-Myc is not necessary for the iPSC induction, but greatly increases the efficiency and speed of the reprogramming process. However, the use of the Myc family for iPSC induction is a problem because animals derived from c-Myc transduced iPSCs have developed tumors [1].

THOMPSON'S MODIFICATION - *Thomson and associates* have proven that four factors such as Oct4, Sox2, Nanog and Lin28 are sufficient to reprogram human somatic cells into pluripotent stem cells that exhibit the basic characteristics of hESCs. These hiPSCs had a normal karyotype, superficial cell markers, telomerase activity expression, and hESC's specific genes that maintain the development potential to differentiate into derivates of all three germ layers. Nanog is a homeodomain transcription factor expressed in pluripotent cell lines *in vitro* and *in vivo*, plays a crucial role in the development process and is considered a major regulator of stem cell pluripotency. A reduction in Nanog expression causes the ESCs to gradually begin to lose their pluripotency and start to differentiate into cells of the extra-embryonic endoderm and trophectoderm. Nanog co-expression with Oct4 was recently found to augment tumor genesis by promoting cancer-stem like properties. The expression of Nanog itself is regulated by Oct4-Sox2 binding to its promoter region. LIN28 encodes a cytoplasmic RNA binding protein that acts as a translator enhancer and, used in combination with Nanog, Oct4 and Sox2, generates human iPSC. It was shown that LIN28 can block the processing of let7 microRNA family members that also act as tumor suppressors. Downregulation of let7 by LIN28 could increase cell proliferation and drive cellular transformation. A recent study on mice has shown that LIN28 accelerates reprogramming kinetics by enhancing cell proliferation. It remains speculative that LIN28 may be able to selectively degrade certain pro-differentiation mRNA to support stem cell pluripotency [1].

METHODS FOR GENERATING INDUCED PLURIPOTENT STEM CELLS

iPSCs have been derived from various types of somatic cells such as dermal fibroblasts[2-4], pancreatic beta cells [5], neural cells [6], hepatocytes [7], keratinocytes [7], bone marrow cells, peripheral blood [8], and retinal pigmented epithelial (RPE) cells [9].

After the discovery of the iPSC reprogramming method by the expression of four transcription factors Oct4, Sox2, cMyc, and Klf4 by *Yamanaka and his associates*, or Oct4, Sox2, Nanog and Lin28 by *Thomson and his associates*, significant progress has been made in identifying new strategies for improving the reprogramming efficiency and new methods for improving the clinical application of iPSC. The reprogramming methods could be grouped into two categories: integrative and non-integrative. The integrative methods are based on involving the integration of exogenous genetic material into the host genome; these delivery methods include the use of viral vectors such as retrovirus and lentivirus, but also include the use non-viral vectors such as linear/plasmid DNA fragments, transposons. The non-integrative methods are based on involving no integration of genetic material into the host genome; these delivery methods include the use of viral vectors such as adenovirus and Sendai virus, and non-viral vectors such as episomal DNA vectors, mRNA and proteins. The problem with the use of retroviral and lentiviral methods is the uncontrolled integration of foreign transgenes in the host chromosome with the consequence of cell damage and tumour formations which limits the clinical application of these modified cells. Scientists have focused on the development of non-integrated methods for obtaining iPSC, including reprogramming by Sendai virus, adenovirus, episomal vectors, piggyBac transposons, mRNA transfection, excessive protein expression [18].

VIRAL INTEGRATIVE VECTORS: *Yamanaka and his associates (2006)* generated human iPSCs by retroviral transduction, at the same time *Yu and his associates (2007)* also had success with the implementation of human iPSCs using a lentiviral method. Retroviruses are effective genetic delivery vectors widely applied to different types of cells. They can infect only dividing cells and integrate randomly into the host genome leading to an increased risk of insertional mutagenesis, unexpected genetic mutations within the genome and aberrant transgenic expression. Lentiviruses are subclasses of retroviruses; they offer the capability of high efficacy of infecting dividing and non-dividing cells with stable expression of the transgene and low immunogenicity. Lentiviruses are integrated randomly in the host genome, similar to other retroviruses, which can complicate the clinical use of generated iPSCs. Minimizing the number of viral integrations reduces the risk of tumour genesis and genomic instability. Reprogramming factors introduced into somatic cells by the retrovirus and the lentivirus are silenced during the reprogramming process because the expression of those factors can induce differentiation and cell death. The creation of polycistronic viral vectors for the retroviral and lentiviral introduction of the excisable (Cre/loxP) vector system and inducible (tetracycline/doxycycline-inducible) vector system has enabled better control of transgene expression thus reducing the effects of inefficient silencing and transgene reactivation [18,19].

NONVIRAL INTEGRATIVE VECTORS: iPSCs successfully generated from mouse fibroblasts with the non-viral polycistronic vector combined with an excisable Cre/loxP system for deleting the reprogramming construct. An alternative to viral vectors is the transfection of DNA (plasmid/linear) into cells using liposomes or electroporation. Cre-mediated recombination can be used to remove transgenes from the iPS cell genome, but the long terminal repeats sequence (LTR) remains in the genome [20].

The piggyBac (PB) transposon-based delivery system is a mobile genetic element which includes an enzyme PB transposase (that mediates gene transfer by insertion and excision). Once the reprogramming is achieved, the enzyme can precisely delete the transgenes without any genetic damage thus avoiding the risk of insertional mutagenesis. The advantage of the piggyBac transposon system is that it can be completely removed from the host genome without changing the DNA sequences at the integration sites. However, transgenic excision may still lead to gene DNA microdeletion, which could interfere with the clinical application of iPSC obtained by this method [19].

VIRAL NONINTEGRATIVE VECTORS: Reprogramming somatic cells with adenoviral vectors was first reported by Hochedlinger and colleagues who reprogrammed mouse tail-tip fibroblasts to iPSCs. Using adenoviral vectors, mice and human iPSCs were generated using *Yamanaka factors* in different cell types, although with low reprocessing efficiency compared to the integration of viral vectors. Adenoviral vectors have no mechanism to integrate into the host genome, which makes them suitable for reprogramming somatic cells. This allows for a high level of expression of exogenous genes and a low risk of the integration of viral transgenes into the host genome [19].

Sendai virus (SeV) is an RNA virus that replicates as single-stranded RNA in the cytoplasm of infected cells. It also allows expression of transgenes without the risk of modification of the host genome and the efficiency of iPSC generation is significantly higher than by other methods. SeV stays in the infected cell through several passages and can easily be removed by antibody-mediated negative selection. Some products are sold with viral extracts that can be used for reprogramming experiments. Eventually, this method results in viral-free genetically intact iPSCs that carry the same genome DNA as the original cells [19].

NONINTEGRATIVE NONVIRAL VECTORS: Another nonintegrative method consists of the use of conventional plasmid. The results are a significant number of iPSC colonies that contain integral transgenes, but the efficacy is significantly lower than in those achieved by retroviral vectors. With the use of conventional plasmids, the expression of transient transgenes gradually decreases after each cell division. Minicircle vectors are circular vectors in which the plasmid backbone is released leaving only the eukaryotic promoter and DNA. Mini-circular DNA offers a higher rate of transfection than conventional plasmids but it is still significantly lower than the integrative viral methods. The use of synthetic RNAs for reprogramming was reported by *Warren et al. (2010)* with surprisingly high reprogramming efficiency [21].

Episomal plasmids are another nonintegrative method used to reprogram somatic cells in iPSCs. They can be stabilized in many types of cells according to the choice of drug. All methods based on the use of plasmids have lower reprogramming efficiency compared to integrative viral methods. Further research will combine advanced factors, such as small molecules which can help to improve the efficiency of reprogramming based on the use of plasmids [19].

An alternative approach to reprogramming somatic cells without genetic modification is the use of protein transduction. The protein labelled with the C-terminal polygamine domain provides efficient protein transduction through the cell membrane. *Zhou and associates (2009)* were the first to use recombinant reprogramming factors labelled poly-arginine to generate iPSC muscle. This virus-free and DNA-free method, with iPSC-free genetic modification, is suitable for clinical applications. This is a promising cell reprogramming technology but special technical skills for the synthesis of bioactive proteins are needed. Still, there are no published studies on the efficacy of this method in reprogramming other cell types, rather than neonatal fibroblasts [19].

Comparing all mentioned reprogramming methods, we can conclude that the ability of episomal plasmids and Sendai viruses to generate iPSCs with good efficacy and without integration into the genome, might be the best choice for projects with the translational application. Other methods without integration either have an unacceptably low level of efficacy, such as adenovirus and proteins or have not shown to be effective in reprogramming somatic cells other than fibroblasts (minicircles and synthetic mRNA/miRNA)[19].

CULTIVATION OF PLURIPOTENT STEM CELLS

Multiple matrix proteins, such as laminin, vitronectin and fibronectin, support the growth of hESCs. Synthetic surfaces are also developed for ESCs. Vitronectin supports the initial binding and survival of hESCs in the E8 medium (eight components, including the DMEM / F12) Depending on the protocol, other cells, the "feeder" layer and other media can be used. After the preparation of the flasks with the "feeder" layer, the fibroblast isolation is reached. First, mechanically take a sample of the skin with the peeled dermis, which is milled and then incubated for half an hour with a mixture of enzymes (collagenase, hyaluronidase) at room temperature. Then, the cells should be separated by centrifugation and washed twice with a fibroblast medium, then seeded in a bottle containing vitronectin with a fibroblast medium or mitotic-inactivated human embryonic fibroblasts. After sowing, human fibroblasts are incubated in an incubator with sterile conditions, an optimum temperature of 37°C and 5% CO2, 95% of O2. After 3 to 7 days, fibroblasts appear and proliferate. When the cell achieves 70% flushing confluence, a passage is done (about 15 passages). This way, fibroblasts are multiplied to the required amount, which will later be exposed to a viral vector that should perform the insertion of four Yamanaka factors (Oct3/4, Sox2, Klf4, c-Myc). Then the fibroblasts are exposed to viral or non-viral reprogramming vectors, which results in their transformation from fibroblasts to iPSCs. When rescheduling is performed, it is followed by the isolation of the obtained iPSCs [22].

WHY iPSC?

iPSCs have common features with Embryonic Stem Cells (*ESCs*) in term of morphology, surface antigen expression, proliferation, gene expression, telomerase activity, and teratoma formation. They can also be differentiated into cells of all three germ layers. Based on extensively reviewed literature we found some advantages of iPSCs. These cells are readily available, they can be used for modelling human genetic diseases *in vitro* or drug development and screening, individualized regenerative cell therapy, to repair organs and tissues in regenerative medicine, they are less of an ethical issue than ESCs, and they can be isolated from patients themselves and have some genetic background so rejection of the transplant is low. The established iPSCs have a wider variety of differentiation ability and gene expression than ESCs, but a small proportion of these stem cells sometimes show spontaneous differentiation during serial passage [23, 24].

THERAPEUTIC POTENTIAL AND BARRIERS TO THE SAFE USE OF iPSC

In addition to the potential use of iPSCs in regenerative medicine, transplantation, disease modelling, gene therapy, to improve knowledge on the development of the human body and numerous genetic mechanisms and signalling pathways, to study the effects of drug testing and toxicological screening, it should be noted that there are many barriers to overcome.

Some of them are low efficacy and induction kinetics, activation of muted ectopic transgenes used to induce iPSC, high risk of insertion mutagenesis for methods based on genetic modification and subsequent tumour formation. Future efforts in the induction of iPSC should be directed to finding non-reactive induction methods that produce iPSC without carcinogenic potential. New techniques are an important step towards creating iPS specialized cells that will be safer for patient application. To realize the full potential for iPSCs in cell therapy and drug discovery, it is necessary to monitor the status of these stem cells and to define their exact stage during processes of growth and/or differentiation. More research is needed to fully understand the reprogramming system and how iPSC can be controlled to produce a sufficient number of cells with high quality and safety requirements for use in therapy. It is especially important to resolve the problems associated with using retroviruses and oncogenes for reprogramming before iPSCs can be considered for human therapy. iPSCs can be used as disease models *in vitro* and appear to be more attractive candidates for the study of cancer initiation and progression. iPSCs from somatic cells could also be used to study carcinogenesis via overexpression or silencing of oncogenes and tumour suppressor genes, tracking the cellular changes and behaviours during cancer initiation and progression [25]. Organs-on-chips are microfluidic cell culture systems seeded with patient-specific iPSC-derived phenotypic cells that serve as functional units of human organs on *in vitro* models with a controlled, dynamic condition that recapitulates the complex tissue architecture and the physio-chemical microenvironment of tissues in the human body. These chips are increasingly used as physiologically relevant pre-clinical disease models such as Barth syndrome-associated cardiomyopathy, drug-induced kidney glomerular injury, dilated cardiomyopathy; brain disease [26-29]. iPSCs in regenerative therapy could be used to promote endogenous regenerative repair or to replace injured tissues after cellular transplantation or to improve the function of degenerated organs due to ageing such as age-related macular degeneration, genetic predisposition, injury, chemotherapy. *Hanna and associates (2007)*[30] show that mice can be rescued after transplantation with hematopoietic progenitors obtained *in vitro* from autologous iPSCs by using a humanized sickle cell anaemia (SCA) mouse model, which was achieved after correction of the human sickle haemoglobin allele by gene-specific targeting. This resulted in the rescue and correction of the disease phenotype. *Wernig and colleagues (2008)*[31] showed that iPSCs differentiated into dopamine neurons and were able to improve behaviour in a rat model of Parkinson's disease upon transplantation into the adult brain. The first clinical trial using human iPSCs was initiated by *Masayo Takahashi*, an ophthalmologist at the RIKEN Center for Developmental Biology (CDB) in Kobe, Japan. Her team made iPSCs from the skin cells of two people with age-related macular degeneration and used them to create sheets of retinal pigment epithelium cells (RPE) for a clinical trial. In 2014, doctors implanted the first RPE sheets into the right eye of a woman in her seventies to treat macular degeneration. *Takahashi* said that the therapy halted the woman's macular degeneration and brightened her vision. But, not long after that, *Yamanaka's team* identified two small genetic changes in both the patient's iPSCs and the RPE cells derived from them and advised *Takahashi* and her team to put the trial on hold [12]. *Kimbrel and Lanza (2015)*[32] had also performed several iPSC-based therapies including glaucoma and macular degeneration. Ocular diseases dominate these first-in-man trials, and Phase I and II results showed promising safety data as well as possible efficacy. The Phases I and II clinical trials are testing the safety and therapeutic benefits of iPSC derivatives in five major areas of clinical need: Spinal cord injury [33]; Diabetes [34]; Neurodegeneration: Huntington disease, Parkinson disease [35], Amyotrophic lateral sclerosis (ALS); Loss of vision: wet and dry age-related macular degeneration (AMD)[33], Stargardt disease, myopic macular degeneration; Heart disease [36, 37]. iPSCs offer more advantages over animal models and clinical testing because animal models cannot perfectly mirror the true human disease phenotype. Aside from that, iPSCs as toxicity models are less expensive and save time when compared with conventional testing systems, also due to species differences that cause a different response to drug toxicity in animals and humans [38]. When protocols were first described for the derivation of induced pluripotent stem cells (iPSC) from adult somatic cells it was suggested that such cells would be ideal for patient-specific cell therapy. While clinical-grade autologous cells could be produced, this seems unlikely to occur on a large scale because of the costs and time that are involved, at least with present protocols. Rather, it has been suggested that a useful partial match should be obtained by establishing banks of cells from individuals homozygous at the major HLA antigens [39]. Although clinical trials are now evolving for iPSCs, it will take time before they are available for patients. It is possible for clinicians to obtain approval for compassionate use that bypasses Phase II and III proof of concept, and also bypasses efficacy trials, in situations where no other life-saving treatment is available. A new regulatory system was established in Japan that enables therapeutic cell products to enter the marketplace with provisional approval, therefore bypassing Phase II and III clinical trials, but this system will require the eventual collection of data to demonstrate the efficacy of the product [39].

Although clinical trials are now evolving for iPSCs, it will take time before they are available for patients. It is possible for clinicians to obtain approval for compassionate use that bypasses Phase II and III proof of concept, and also bypasses efficacy trials, in situations where no other life-saving treatment is available. A new regulatory system was established in Japan that enables therapeutic cell products to enter the marketplace with provisional approval, therefore bypassing Phase II and III clinical trials, but this system will require the eventual collection of data to demonstrate the efficacy of the product [39]. Many stem cell researchers who are trying to introduce cell therapy are confronted with significant challenges that involve a long and expensive process. The Food and Drug Administration (FDA) and other agencies have substantial regulatory requirements [40] that stem cell therapies must fulfil, such as carrying out biodistribution, tumorigenicity, immunogenicity, dose toxicity, and pharmacodynamic proof-of-concept studies. To fully realize the tremendous potential of stem cell therapy, careful planning and proper resources must be devoted to meeting regulatory and scientific requirements alike that will demonstrate their safety and efficacy [41].

Although iPSC technology is rather costly [42], the use of iPSCs could lead to a significant reduction of medical costs for the treatment of many disorders, which are very important in medicine nowadays [43-45]. The iPSCs can provide guidance and assist healthcare decision planners and policymakers in allocating resources optimally.

CONCLUSION

In a future perspective, the iPSCs possibly will substitute the ESCs because they offer the opportunity to generate disease-specific and patient-specific iPSCs for modelling human diseases, drug development and screening, individualized regenerative cell therapy in drug testing and regenerative medicine, but they are still at their infant stage of development, especially the iPSC–based cell therapy. The impact of iPSC technology on basic research has been monumental in such a short timeframe. To realize the iPSC full potential, it is necessary to demonstrate its safety and efficacy. This can be achieved only by careful planning and with adequate resources in a regulatory-friendly environment. Fast-track breakthrough therapy designation with accelerated approval and priority review are the other ways to hasten patient access to new cell therapies. Although iPSC techniques have yet to receive FDA approval, in the future they could be appropriately developed, scaled, and employed in an allogeneic iPSC bank to offset costs, whereas autologous production would require individualized screening.

Conflict of interest: None declared.

REFERENCES

1. Lanza R, Atala A. Essentials of Stem Cell Biology, 3rd ed. Elsevier Inc, 2014. DOI: https://doi.org/10.1016/C2012-0-06957-8.
2. Tai L, Teoh HK, Cheong SK. Reprogramming human dermal fibroblast into induced pluripotent stem cells using nonintegrative Sendai virus for transduction. Malays J Pathol. 2018; 40(3): 325-329. PMID: 30580364.
3. Castro-Viñuelas R, Sanjurjo-Rodríguez C, Piñeiro-Ramil M, Hermida-Gómez T, Rodríguez-Fernández S, Oreiro N, et al. Generation and characterization of human induced pluripotent stem cells (iPSCs) from hand osteoarthritis patient-derived fibroblasts. Sci Rep. 2020; 10(1): 4272. PMID: 32144293. doi: 10.1038/s41598-020-61071-6.
4. Belviso I, Sacco AM, Romano V, Schonauer F, Nurzynska D, Montagnani S, et al. Isolation of Adult Human Dermal Fibroblasts from Abdominal Skin and Generation of Induced Pluripotent Stem Cells Using a Non-Integrating Method. J Vis Exp. 2020; (155). PMID: 32009646. doi: 10.3791/60629.
5. Bar-Nur O, Russ HA, Efrat S, Benvenisty N. Epigenetic memory and preferential lineage-specific differentiation in induced pluripotent stem cells derived from human pancreatic islet beta cells. Cell Stem Cell. 2011; 9(1): 17-23. doi: 10.1016/j.stem.2011.06.007. Erratum in: Cell Stem Cell. 2012; 11(6): 854. PMID: 21726830.
6. Kim J, Lengner CJ, Kirak O, Hanna J, Cassady JP, Lodato MA, et al. Reprogramming of postnatal neurons into induced pluripotent stem cells by defined factors. Stem Cells. 2011; 29(6): 992-1000. PMID: 21563275. doi: 10.1002/stem.641.
7. Lim SJ, Ho SC, Mok PL, Tan KL, Ong AH, Gan SC. Induced pluripotent stem cells from human hair follicle keratinocytes as a potential source for *in vitro* hair follicle cloning. PeerJ. 2016; 4: e2695. PMID: 27867768. doi: 10.7717/peerj.2695.
8. Gu H, Huang X, Xu J, Song L, Liu S, Zhang XB, et al. Optimizing the method for generation of integration-free induced pluripotent stem cells from human peripheral blood. Stem Cell Res Ther. 2018; 9(1): 163. PMID: 29907164. doi: 10.1186/s13287-018-0908-z.
9. Hu Q, Friedrich AM, Johnson LV, Clegg DO. Memory in induced pluripotent stem cells: reprogrammed human retinal-pigmented epithelial cells show tendency for spontaneous redifferentiation. Stem cells. 2010; 28: 1981-1991. PMID: 20882530. doi: 10.1002/stem.531.
10. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell. 2006;

126(4): 663-676. PMID: 16904174. doi: 10.1016/j.cell.2006.07.024.
11. Gurdon JB. From nuclear transfer to nuclear reprogramming: the reversal of cell differentiation. Annu Rev Cell Dev Biol. 2006; 22: 1-22. PMID: 16704337.doi: 10.1146/annurev.cellbio.22.090805.140144.
12. Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, et al. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell. 2007; 131(5): 861-872. PMID: 18035408. doi: 10.1016/j.cell.2007.11.019.
13. Yu J, Vodyanik MA, Smuga-Otto K, Antosiewicz-Bourget J, Frane JL, Tian S, et al. Induced pluripotent stem cell lines derived from human somatic cells. Science. 2007; 318(5858): 1917-1920. PMID: 18029452. doi: 10.1126/science.1151526.
14. Wakui T, Matsumoto T, Matsubara K, Kawasaki T, Yamaguchi H, Akutsu H. Method for evaluation of human induced pluripotent stem cell quality using image analysis based on the biological morphology of cells. J Med Imaging (Bellingham). 2017; 4(4): 044003. PMID: 29134187. doi: 10.1117/1.JMI.4.4.044003.
15. Yamanaka S. Induction of pluripotent stem cells from mouse fibroblasts by four transcription factors. Cell Prolif. 2008; 41 Suppl 1(Suppl 1): 51-6. PMID: 16904174. doi: 10.1111/j.1365-2184.2008.00493.x.
16. Zakrzewski W, Dobrzyński M, Szymonowicz M, Rybak Z. Stem cells: past, present, and future. Stem Cell Res Ther. 2019; 10(1): 68. PMID: 30808416. doi: 10.1186/s13287-019-1165-5.
17. Heng JC, Feng B, Han J, Jiang J, Kraus P, Ng JH, et al. The nuclear receptor Nr5a2 can replace Oct4 in the reprogramming of murine somatic cells to pluripotent cells. Cell Stem Cell. 2010;6(2):167-174. PMID: 20096661.doi: 10.1016/j.stem.2009.12.009.
18. Omole AE, Fakoya AOJ. Ten years of progress and promise of induced pluripotent stem cells: historical origins, characteristics, mechanisms, limitations, and potential applications. PeerJ. 2018; 6: e4370. PMID: 29770269. doi: 10.7717/peerj.4370.
19. Rao MS, Malik N. Assessing iPSC reprogramming methods for their suitability in translational medicine. J Cell Biochem. 2012; 113(10): 3061-3068. PMID: 22573568. doi: 10.1002/jcb.24183.
20. Woltjen K, Michael IP, Mohseni P, Desai R, Mileikovsky M, Hämäläinen R, et al. PiggyBac transposition reprograms fibroblasts to induced pluripotent stem cells. Nature. 2009; 458(7239): 766-770. PMID: 19252478. doi: 10.1038/nature07863.
21. Warren L, Manos PD, Ahfeldt T, Loh YH, Li H, Lau F, et al. Highly efficient reprogramming to pluripotency and directed differentiation of human cells with synthetic modified mRNA. Cell Stem Cell. 2010; 7(5): 618-30. PMID: 20888316. doi: 10.1016/j.stem.2010.08.012.
22. Chen G, Gulbranson DR, Hou Z, Bolin JM, Ruotti V, Probasco MD, et al. Chemically defined conditions for human iPSC derivation and culture. Nat Methods. 2011; 8(5): 424-429. PMID: 21478862.doi: 10.1038/nmeth.1593.
23. Raščanin S, Rančić N, Dragović S, JovanovićM. Embryonic Stem Cells: Where Do We Stand At The Moment?Acta Medica Medianae. 2019; 58(3): 138-146. doi: 10.5633/amm.2019.0320.
24. Volarevic V, Markovic BS, Gazdic M, Volarevic A, Jovicic N, Arsenijevic N, et al. Ethical and Safety Issues of Stem Cell-Based Therapy. Int J Med Sci. 2018; 15(1): 36-45. PMID: 29333086. doi: 10.7150/ijms.21666.
25. Crespo M, Vilar E, Tsai SY, Chang K, Amin S, Srinivasan T, et al. Colonic organoids derived from human induced pluripotent stem cells for modeling colorectal cancer and drug testing. Nat Med. 2017; 23(7): 878-884. PMID: 28628110. doi: 10.1038/nm.4355.
26. Esch EW, Bahinski A, Huh D. Organs-on-chips at the frontiers of drug discovery. Nat Rev Drug Discov. 2015; 14(4): 248-260. PMID: 25792263. doi: 10.1038/nrd4539.
27. van den Berg A, Mummery CL, Passier R, van der Meer AD. Personalised organs-on-chips: functional testing for precision medicine. Lab Chip. 2019; 19(2): 198-205. PMID: 30506070. doi: 10.1039/c8lc00827b.
28. Atchison L, Zhang H, Cao K, Truskey GA. A Tissue Engineered Blood Vessel Model of Hutchinson-Gilford Progeria Syndrome Using Human iPSC-derived Smooth Muscle Cells. Sci Rep. 2017; 7(1): 8168. PMID: 28811655. doi: 10.1038/s41598-017-08632-4.
29. McKinney CE. Using induced pluripotent stem cells derived neurons to model brain diseases. Neural Regen Res. 2017; 12(7): 1062-1067. PMID: 28852383. doi: 10.4103/1673-5374.211180.
30. Hanna J, Wernig M, Markoulaki S, Sun CW, Meissner A, Cassady JP, et al. Treatment of sickle cell anemia mouse model with iPS cells generated from autologous skin. Science. 2007; 318(5858): 1920-1923. PMID: 18063756. doi: 10.1126/science.1152092.
31. Wernig M, Zhao JP, Pruszak J, Hedlund E, Fu D, Soldner F, et al. Neurons derived from reprogrammed fibroblasts functionally integrate into the fetal brain and improve symptoms of rats with Parkinson's disease. Proc Natl AcadSci USA. 2008; 105: 5856-5861. PMID: 18391196. doi: 10.1073/pnas.0801677105.
32. Kimbrel EA, Lanza R. Current status of pluripotent stem cells: moving the first therapies to the clinic. Nat Rev Drug Discov. 2015; 14(10): 681-692. PMID: 26391880. doi: 10.1038/nrd4738.

33. Lu P, Woodruff G, Wang Y, Graham L, Hunt M, Wu D, et al. Long-distance axonal growth from human induced pluripotent stem cells after spinal cord injury. Neuron. 2014; 83(4): 789-796. PMID: 25123310. doi: 10.1016/j.neuron.2014.07.014.
34. Wang Q, Donelan W, Ye H, Jin Y, Lin Y, Wu X, et al. Real-time observation of pancreatic beta cell differentiation from human induced pluripotent stem cells. Am J Transl Res. 2019; 11(6): 3490-3504. PMID: 31312361.
35. Kikuchi T, Morizane A, Doi D, Magotani H, Onoe H, Hayashi T, et al. Human iPS cell-derived dopaminergic neurons function in a primate Parkinson's disease model. Nature. 2017; 548(7669): 592-596. PMID: 28858313. doi: 10.1038/nature23664.
36. Cyranoski D. 'Reprogrammed' stem cells approved to mend human hearts for the first time. Nature. 2018; 557(7707): 619-620. PMID: 29844563. doi: 10.1038/d41586-018-05278-8.
37. Trounson A, DeWitt ND. Pluripotent stem cells progressing to the clinic. Nat Rev Mol Cell Biol. 2016; 17(3): 194-200. PMID: 26908143. doi: 10.1038/nrm.2016.10.
38. Vanneaux V. Induced pluripotent stem cells for clinical use. In: Kallos MS (ed). Embryonic Stem Cells – Diferentation and Pluripotent Alternatives. London: Intech Open. 2019. DOI:10.5772/1789.
39. Neofytou E, O'Brien CG, Couture LA, Wu JC. Hurdles to clinical translation of human induced pluripotent stem cells. J Clin Invest. 2015; 125(7): 2551-2557. PMID: 26132109. doi: 10.1172/JCI80575.
40. Dyer O. Stem cell treatment: FDA court victory opens way to regulation in US. BMJ. 2019; 365: l4128. PMID: 31175090. doi: 10.1136/bmj.l4128.
41. Ortuño-Costela MDC, Cerrada V, García-López M, Gallardo ME. The Challenge of Bringing iPSCs to the Patient. Int J Mol Sci. 2019; 20(24): 6305. PMID: 31847153. doi: 10.3390/ijms20246305.
42. Vekic B, Dragojevic-Simic V, Jakovljevic M, Kalezic M, Zagorac Z, Dragovic S, et al. A Correlation Study of the Colorectal Cancer Statistics and Economic Indicators in Selected Balkan Countries. Front Public Health. 2020; 8: 29. PMID: 32133335. doi: 10.3389/fpubh.2020.00029.
43. Rancic N, Mladenovic K, Ilic NV, Dragojevic-Simic V, Karanikolas M, Ilic TV, et al. Patient-Controlled Intravenous Morphine Analgesia Combined with Transcranial Direct Current Stimulation for Post-Thoracotomy Pain: A Cost-Effectiveness Study and A Feasibility For Its Future Implementation. Int J Environ Res Public Health. 2020; 17(3): 816. PMID: 32012977. doi: 10.3390/ijerph17030816.
44. Rancic N, Dragojevic-Simic V, Vavic N, Kovacevic A, Segrt Z, Djordjevic N. Economic Evaluation of Pharmacogenetic Tests in Patients Subjected to Renal Transplantation: A Review of Literature. Front Public Health. 2016;4:189. PMID: 27630984. doi: 10.3389/fpubh.2016.00189.
45. Vekic B, Dragojevic-Simic V, Jakovljevic M, Pilipovic F, Simic R, Zivic R, et al. Medical Cost of Colorectal Cancer Services in Serbia Between 2014 and 2017: National Data Report. Front Pharmacol. 2019; 10: 526. PMID: 31156439. doi: 10.3389/fphar.2019.00526.

PREGLEDNI RAD

INDUKOVANE PLURIPOTENTNE MATIČNE ĆELIJE: GDE SMO TRENUTNO?

Nemanja RANČIĆ[*1,2], *Sanja RAŠČANIN*[*3], *Milijana MILJKOVIĆ*[1,2], *Mirjana JOVANOVIĆ*[3,4]
*Nemanja Rančić i Sanja Raščanin dele prvo autorstvo.

[1]Centar za kliničku farmakologiju, Vojnomedicinska akedimija, Beograd, Srbija; [2]Fakultet medicinskih nauka Univerziteta u Kragujevcu, Kragujevac, Srbija; [3]Fakultet medicinskih nauka Univerziteta u Kragujevcu, Kragujevac, Srbija; [4]Psihijatrijska klinika Kliničkog centra Kragujevac, Kragujevac, Srbija

SAŽETAK

Indukovane pluripotentne matične ćelije (iPSC), su vrsta pluripotentnih matičnih ćelija, koje nastaju reprogramiranjem genoma zrele (diferentovane) somatske ćelije, u stadijum pluripotentne matične ćelije, *in vitro* indukcijom prisilne ekspresije specifičnih faktora transkripcije, koji su važni za održavanje pluripotencije. Iako postoje sličnosti između iPSC i embrionalnih matičnih ćelija (ESC) u pogledu morfologije, markera na površini ćelije i nivoa ekspresije gena, nedavne studije su ipak pokazale razlike između ova dva tipa ćelija. iPSC mogu imati potencijalnu primenu u regenerativnoj medicini, transplantaciji, testiranju lekova, modeliranju bolesti i izbegavanju odbacivanja tkiva, a njihova upotreba je udružena sa manjim etičkim dilemama u poređenju sa ESC. Cilj ovo grada je da predstavi najvažnije karakteristike iPSC-a koje mogu biti primenjene u lečenju.

Ključne reči: Indukovane pluripotentne matične ćelije; terapija, etičke dileme