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INDUCED PLURIPOTENT STEM CELLS FOR DRUG DISCOVERY AND DISEASE TREATMENT

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Abstract: Modern innovative studies show that it is now possible to transform somatic cells into induced pluripotent stem cells that closely look like embryonicStem cells. These induced pluripotent stem (iPS) cells can be created without using human embryos or oocytes, thus avoidingcertain of the ethical matters that have inadequate the use of human embryonic stems (hES) cells. Furthermore, they can be derived from the patient to be treated, thus overcoming difficulties of immunological rejection connected with the use of allogeneic hES cell derived progenitors. At the same time as these patient specific iPS cells have pronounced clinical application, their instantusefulness is likely to be in drug screening and for understanding the disease development.

Keywords: Embryonic stem cells, induced pluripotent stem cell.

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INTRODUCTION

Human world is increasing and similarly there has been a persistent progression of diseases and disease agent. Hence, it is essential to improve methods to cure and fight the most intimidating diseases. One important foot step in this way has been the discovery of stem cells, shadowed by the isolation of human embryonic stem (ES) cells. In recent times, the induced pluri potent stem (iPS) cell technology developed the world of scientific research. Development of iPS cells through reprogramming of somatic cells was a breakthrough innovation in the field of medical sciences, meanwhile it made it possible to create cell lines from adult tissues and most significantly, from patient- derived tissues, which have the likely for use in cell-based drug discovery.[1] Stem cells are categorized into numerous groups, depending on their differentiating ability and developmental stage at which they are obtained. Adult stem cells are multipotent cells isolated from adult or fetal tissues; these cells have the capability to renew themselves and distinguish into the specialized cell type of the originating tissue. They are principally used for exchanging damaged and injured tissue, and to date, they have been derived from the brain, bone marrow, spinal cord, skin, liver, retina and many other tissues.[2] Then, it is challenging to identify, isolate and culture the adult stem cells due to their rareness in adult tissues. ES cells, on the other hand, are derived from the embryo and are capable of forming stable cell lines, which retain the pluripotency to differentiate into cells from all three germ layers (Ectoderm, mesoderm, and endoderm).[3] Owing to the widespread developmental potential of ES cells, scientists can manipulate ES cells *in vitro* to form various cell types.[4] Even though numerous studies have reported on ES cells in animal models, applications of the findings in humans are still not predictive and need further extensive studies. The use of human ES cells can overcome many obstacles that occur in conducting studies using animal models, but it also announces two important issues. A disadvantage of using ES cells is the destruction of embryos, something that has raised moral and ethical issues amongst the public. In addition, an ES cell transplant carries a risk of immunogenic reactions, since ES cells from a random embryo donor are likely to face immune rejection after transplantation. Generation of iPS cells from somatic cells by retrovirus- mediated transduction of four transcription factors.[5] Provide innovative platform for stem cell use in cell-based drug screening and disease therapy. The iPS cells are similar to ES cells in their morphology, gene expression, epigenetic status of pluripotent cell-specific genes and pluripotency (i.e. differentiating into all three germ layers.[6] essentially, iPS cells provide an alternative to the use of human embryos, overcoming ethical issues. In addition, iPS cell technology permits the use of patient'sspecific somatic cells to generate therapeutic iPS cells, thus overcoming the potential for immune rejection. The recent improvements made with iPS cells hold potential for creating patient-specific disease model iPS cell lines that can be used for disease mechanism studies and drug discovery.

HISTORY AND PROGRESS OF iPS CELLTECHNOLOGY:

Prior work in research ranging from nuclear transfer and cloning to the establishment of immortal pluripotent cell lines influenced and lead to the present status of iPS cell technology. In mammals, embryonic development is characterized by a gradual restriction in the developmental potential of the cells that constitute the embryo. The zygote and blastomeres of the early morula stage are totipotent and further divide to form the blastocyst the inner cell mass of the blastocyst is pluripotent and self-renewing, while the outer cells of the embryo develop into the placenta. Adult stem cells, derived from various adult tissues, are multipotent and also capable of self-renewal.[3, 4] To understand the developmental potential of Nuclei, a cloning technique was established by transplanting isolated nuclei into enucleated oocytes.[7] This was later followed by the generation of a cloned frog and a sheep. [8, 9] This further supported the finding that fully specialized cells remain genetically totipotent

In 1964, researchers established the pluripotent teratocarcinoma cell line which replicated and grew in culture as stem cells called embryonic carcinoma (EC) cells. Even though EC cells provide a model structure for the study of cellular commitment and differentiation, they are tumor cells and typically an euploidy. These disadvantages associated with the use of EC cells led to the establishment of ES cells. [10] In 1981, Evan and Martin first derived pluripotent ES cells from a blastocyst stage mouse embryo.[11] The discovery of lineage-associated transcription factors, which help maintain cellular identity during development, contributed to the discovery of iPS cells. These transcription factors were first shown in fibroblast cell lines transduced with retroviral vectors. [12] Subsequently, transcription factors responsible for expression of cell-type specificgenes that are important for pluripotency were observed. In the study to identify transcriptional regulators responsible for reprogramming somatic cells into pluripotent cells, Takahashi and Yamanaka first successfully produced iPS cells from mouse fibroblasts using four retro virally transduced transcription factors, *c-Myc*, *Oct3/4*, *Sox2*, and *Klf4*. [5] Human iPS cells were derived in 2007 by the transduction of either the same set of transcription factors (*c-Myc*, *Oct3/4*, *Sox2*, and *Klf4*) or another set of transcription factors (*Oct3/4*, *Sox2*, *Nanog*, and *Lin28*) into human somatic cells. [6] Initially, iPS cells were derived from somatic cells by retroviral or lentiviral transduction of transcription factors. However, such a method of establishing iPS cells with carcinogenic *c-Myc* and retrovirus may induce tumor formation and affect the efficiency of somatic cells to produce iPS cells thus hampering the application of iPS technology in disease therapy. [13] To avoid genetic modification and to improve the efficiency of iPS cell generation and differentiation, iPS cell production technology was advanced by techniques that avoided stable integration of foreign genetic material into the host genome. For the first time used an adenovirus carrying *Oct4*, *Sox2*, *c-Myc,* and *Klf4* to generate iPS cells from mouse hepatocytes, but this method did not receive much clinical application due to low efficiency. Vector

integration-free mouse iPS cells have been derived from embryonic fibroblasts by repeated plasmid transfections however, the practical approaches of this method is limited by the associated low reprogramming frequency. Human iPS cells have also been derived using non-integrating episomal vectors and vectors based on Sendai virus Further research, using the Piggy Bac transposon in both mouse and human fibroblast cells, has led to the Generation of iPS cells without vector integration In 2008, generated a transducible version of transcription factors, OCT4 and SOX2, and used a vector system to fuse it with a TAT sequence. The recombinant OCT4 and SOX2 proteins displayed DNA binding properties, exhibited cellular entry, and maintained pluripotency in mouse stem cells. Moreover, in 2009, DNA-free iPS cells were generated from human and mouse. [14, 15] Fibroblasts by direct delivery of four reprogramming factors (OCT4, SOX2, KLF4, and c-MYC). These DNA-free iPS cells were similar to ES cells, in terms of morphology, proliferation, and gene expression. They were capable of differentiating into three germ layers and led to teratoma formation, but the efficiency was significantly lower than virus-based methods the addition of small molecules was successfully able to increase the reprogramming efficiency. Interestingly, reported that reprogramming of human skin cells can be achieved using only micro RNAs (miRNAs), specifically by exogenous expression of the miR-302 cluster. More recently, miR-93 and its family members were found toenhance iPS cell generation in mouse, demonstrating that iPS cell induction efficiency can be greatly enhanced by modulating miRNA levels in cells.[16] The role of miRNAs involved the regulation of multiple signaling networks and has been reported to have a similar role in iPS cell generation. [17] The iPS cells can also be established by various growth factors and chemical agents. For instance, lithium, a drug used to treat mood disorders, greatly enhanced the efficiency of iPS cell generation in mouse embryonic fibroblasts and human umbilical vein endothelial cells by enhancing the transcriptional activity of Nanog. [18] Another set of compounds, including rapamycin and curcumin were found to enhance the efficiency of somatic cell reprogramming to iPS cells.[19] In a recent report, iPS cells were generated from adult human adipose tissues using non-viral mini-circle DNA vectors containing the reprogramming genes, *Oct4*, *Sox2*,*Nanog*, and *Lin28*. [20]

In the experiment, they used plasmid vector to yield a mini-circle vector separated from the parental plasmid vector containing the bacterial elements. An advantage of this technique is that iPS cells are derived with no viral sequence or *c-Myc* oncogene, overcoming possible safety concerns. In a slightly different approach, magnet-based nanofection was shown to be an efficient method of transfection in various cell types, including ES cells which could avoid the harmful genome-integrating effect of viral DNAs. On the basis of this study, successfully performed the nanofection method in mouse embryonic fibroblast (MEF) cells and could generate iPS cells with the ES-like properties of self-renewal and pluripotency.[21] In the study, a magnetic-nanoparticle coated with a biodegradable cationic polymer was employed to

transfect the iPS factors into mouse fibroblasts to generate iPS cells without using a viral system. The reprogramming efficiency of this method for generating iPS cells was comparatively higher than other non-viral systems. As the aforementioned history and progress in iPS cell research indicates, the reprogramming of somatic cells into pluripotent cells has opened a new avenue for generating patient- and disease-specific pluripotent stem cells. Although further work is needed to increase the efficiency of reprogramming and to overcome the carcinogenic effects, application of iPS cell technology for the treatment of human disease will help toenhance our understanding of disease mechanisms; further, this technology may have applications in drugscreening, toxicity screening and regenerative medicine.

Therapeutic application of ips cell for Transplantation:

Starting with bypassing ethical concerns regarding the use of human embryos.In addition, this technology enables autologous transplant, which is considered as the "gold standard" method. With allogeneic ES cell transplantation, patients must receive immunosuppressive therapy for at least 4 months and need to be monitored for signs of rejection for 15 years shortly after iPS cells were introduced; several cases of transplantation using iPS cell technology were reported. [22] The therapeutic effect of iPS cells was first described in a Parkinson's disease (PD)model by Wernig et al. Dopaminergic neuronal death in the midbrain is known as the main cause of PD. successfully reprogrammed fibroblasts into iPS cells and differentiated them into neural precursor cells before finally differentiating them into dopaminergic neurons. When these functional neurons were transplanted into a rat model of PD, normalized dopamine activity was reported and alleviation of PD symptoms was observed. [23] iPS cell therapy was also used to treat an inherited genetic disease, Hemophilia A is caused by a mutation in factor VIII (FVIII), which is involved in blood clotting; thus, hemophilia A patients suffer from internal or external bleeding. In the study, iPS cells, reprogrammed from mouse fibroblasts, were differentiated into endothelial progenitor cells (EPC) and endothelial cells, and FVIII protein release from these cells was measured. Following transplantation of FVIII producing EPC/endothelial cells, mice with hemophilia A survived from a death-inducing bleeding assay, demonstrating pathologic phenotype correction. [24] iPS cells have also been differentiated into functional cardiac myocytes in an attempt to treat cardiac infarcts.[25] Additionally, iPS cells generated from type I diabetes patients were differentiated into insulin-producing pancreatic progenitor cells.[26] Finally and most importantly, there is a repairing chance of disease-causing mutations by iPS cell transplantation, which was unsuccessfully attempted several times using adult stem cells. Mutation repair can be employed for diseases caused by genetic disorders. After fibroblast cells are reprogrammed to iPS cells, disease causing mutations are corrected with the traditional gene therapy techniques of insertion, alteration, or removal of genes. Briefly, iPS cell therapeutic approaches follow four steps: (1) iPS cell generation from mutant donor somatic

cells, (2) genetic correction of mutant genes with gene therapy, (3) *in vitro* differentiation of corrected iPS cells into mature cells, and (4) transplantation. To date, two such studies have been reported Hanna et al. applied this method to the humanized sickle cell anemia mouse model. First, iPS cells generated from fibroblasts were used to correct the human sickle hemoglobin allele by gene specific targeting. These corrected iPS cells were then differentiated into hematopoietic stem cells *in vitro*, followed by transplantation. Eight weeks later, the corrected iPS cells had reconstituted the hematopoietic system of sickle mice and restored hemoglobin functions.[27] additionally; Raya et al. generated iPS cells from a patient with Fanconi's anemia (FA), a common bone marrow failure syndrome that results from 13 gene mutations in the FA pathway. The iPS cells derived from the FA patient were genetically corrected by reprogramming and differentiated into hematopoietic progenitor cells. *In vitro*, the corrected iPS cells showed functional FA pathway reestablishment.[28]

Application of iPS cells for drug discovery:

To launch a novel drug into the market, a pharmaceutical company goes through the following steps: drug discovery, preclinical development with animal models and phase I, II, and III clinical trials. Depending on the success rate of treating the disease in a disease model, the drugs may progress into clinical trials in humans. However, current drug discovery procedures have many limitations. First, disease-specific cells are not available for many diseases, especially many degenerative diseases, including Alzheimer's disease (AD), PD and amyotrophic lateral sclerosis (ALS), in which it is difficult to obtain cells or tissues with pathological phenotypes. Moreover, it is difficult to prepare relevant cells for diseases occurring in sporadic forms and those affected by complicated unknown genetic aspects, such as autism spectrum disorders and type I diabetes. The current iPS technology may resolve these issues by using its "disease modeling" potential. When iPS cells derived from a patient's fibroblasts are differentiated, mature cells express disease phenotypes and show disease progression. Therefore, disease modeling aids in both the understanding of disease mechanisms and the discovery of novel therapeutic compounds**.** [29] Another limitation in current drug discovery is that while drugs may show a significant activity in cell culture and animal models during the discovery steps, they might not show any activity during clinical trials. [30] These clinical failures may result from differences in disease mechanisms and developmental in addition to physiological aspects between humans and animals. Moreover, toxic effects of compounds that are specific to humans may not be detected in cell lines and animal models. These discrepancies can be resolved with iPS cell technology, which reprograms a patient's fibroblasts and differentiates them into affected cells. In addition, iPS cell technology opens the door for personalized medicine.

In drug screening and cell therapy, iPS cells have many advantages over ES cells; however, because the consistency of pluripotency and the tendency of these cells to develop into the cell types desired for therapeutic application are still under investigation.

Challenges involved in the application of iPS cell technology.

1. The first major concern is to identify the best reprogramming method to achieve high iPS cell generation efficiency and less genetic alteration in the generated iPS cell lines.
2. Another challenge is overcoming the heterogeneity of iPS cells in culture, which makes it important to optimize the protocols for derivation of target cell types without inducing large undifferentiated cell (which may lead to tumor formation).
3. Heterogeneity of iPS cells in culture may also distort the high-throughput screening of compounds, as homogeneity of cells is essential for drug screening.[31]

The application of iPS cells to study diseases such as AD and PD, which take a long time for the first onset of disease symptoms, poses another challenge. A possible alternative could be the application of external stress to speed up the onset of disease in culture. Finally, for drug screening, cell-to-cell interactions are the important criteria to consider, other than the cell's autonomous program. In this regard, the use of tissue engineering to generate a desired disease-relevant phenotype in iPS cells may provide a more in-depth understanding of particular drug's effects.

SUMMARY

In summary, the concept that mature, differentiated cells can be reprogrammed to a pluripotent stem cell state is a paradigm shifting discovery. This insight has influenced essentially all areas of medicine or physiology.

CONCLUSION

Current advances in iPS cell technology has advised scientists to study diseases *in vitro*, looking for disease phenotypes, applying micro-environmental stresses, and testing new drugs. Although the reprogramming process for efficient generation of iPS cells faces many Challenges, many groundbreaking approaches have helped to overcome some of the challenges. iPS cell technology provides a innovatory method for drug discovery, which bridges genetics, cell biology and physiology.

REFERENCES:

1. Amabile G, Meissner A, Induced pluripotent stem cells current progress and potential for regenerative medicine *Trends Mol. Med* 2009, 15: 59-68.

2. Pittenger M. F, Mackay A. M, Beck S. C, Jaiswal R. K, Douglas, R, Mosca, J. D, Moorman M. A, Simonetti D. W, Craig, S, and Marshak D. R; Multilineage potential of adult human mesenchymal stem cells. *Science* 1999, 284:143-147.

3. Thomson J. A, Itskovitz-Eldor J, Shapiro S. S, Waknitz M. A, Swiergiel J. J, Marshall V. S and Jones, J. M; Embryonic stem cell lines derived from human blastocysts. *Science* 1998, 282: 1145-1147.

4. Keller G, Embryonic stem cell differentiation: emergence of a new era in biology and medicine. *Genes Dev*2005, 19: 1129-1155.

5. Takahashi K. and Yamanaka S; Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell*, 2006. 126, 663-676.

6. Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K and Yamanaka S; Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell* 2007, 131: 861-872.

7. Briggs R. and King T. J; Transplantation of living nuclei from blastula cells into enucleated frogs' eggs. *Proc. Natl. Acad. Sci. U. S. A*. 1952, 38, 455-463.

8. Gurdon J. B; The developmental capacity of nuclei taken from intestinal epithelium cells of feeding tadpoles." *J. Embryol. Exp. Morphol.* 1962, 10: 622-640.

9. Wilmut I, Schnieke A. E, McWhir J, Kind A J, and Campbell K. H; Viable offspring derived from fetal and adult mammalian cells. *Nature* 1997,385, 810-813.

10. Kleinsmith L. J. and Pierce G. B. Jr; Multipotentiality of single embryonal carcinoma cells. *Cancer Res.* 1964, 24, 1544-1551.

11. Evans M. J. and Kaufman M. H; Establishment in culture of pluripotential cells from mouse embryos. *Nature* 1981,292, 154-156.

12. Davis R. L, Weintraub H. and Lassar A; Expression of a single transfected cDNA converts fibroblasts to myoblasts. *Cell* 1987, 51, 987-1000.

13. Okita, K., Ichisaka, T., and Yamanaka, S., Generation of germline-competent induced pluripotent stem cells. *Nature* 2007, 448, 313-317.

14. Kim D, Kim C. H, Moon J. I, Chung Y. G, Chang M. Y, Han B. S, Ko S, Yang E, Cha K. Y, Lanza R. and Kim K. S; Generation of human induced pluripotent stem cells by direct delivery of reprogramming proteins. *Cell Stem Cell* 2009, 4, 472-476.

15. Zhou Q, Brown J, Kanarek A, Rajagopal J. and Melton, D. A; In *vivo* reprogramming of adult pancreatic exocrine cells to beta-cells. *Nature* 2008, 455, 627-632.

16. Li Z, Yang C. S, Nakashima, K. and Rana T. M, Small RNA-mediated regulation of iPS cell generation. *EMBOJ* 2011,30, 823-834.

17. Yang C. S, Li Z. and Rana T. M; microRNAs modulate iPS cell generation. *RNA* 2011, 17, 1451-14.

18. Wang Q, et al. Lithium, an anti-psychotic drug, greatly enhances the generation of induced pluripotent stem cells. *Cell Res.* 2011,21, 1424-1435.

19. Chen T. et al. Rapamycin and other longevity-promoting compounds enhance the generation of mouse induced pluripotent stem cells. *Aging Cell* 2011, 10, 908-911.

20. Jia, F. et al. A nonviralminicircle vector for deriving human iPS cells. *Nat. Methods*, 2010, 7, 197- 199.

21. Gersting S.W. et al. Gene delivery to respiratory epithelial cells by magnetofection. *J. Gene Med.* 2004, 6, 913-922.

22. Charron D, Suberbielle-Boissel C. and Al-Daccak R; Immunogenicity and allogenicity: a challenge of stem cell therapy. *J. Cardiovasc. Transl. Res*, 2009, 2, 130-138.

23. Wernig, M. et al. Neurons derived from reprogrammed fibroblasts functionally integrate into the fetal brain and improve symptoms of rats with Parkinson's disease. *Proc.Natl. Acad. Sci. U. S. A.* 2008, 105, 5856-5861.

24. Xu D. et al. Phenotypic correction of murine hemophilia A using an iPS cell-based therapy. *Proc. Natl. Acad.Sci. U. S. A.* 2009, 106, 808-813.

25. Mauritz C, et al. Generation of functional murine cardiac myocytes from induced pluripotent stem cells. *Circulation* 2008, 118, 507-517.

26. Maehr R. et al. Generation of pluripotent stem cells from patients with type 1 diabetes. *Proc. Nat. Acad. Sci. U. S. A.* 2009, 106, 15768-15773.

27. Hanna J. et al. Treatment of sickle cell anemia mouse model with iPS cells generated from autologous skin. *Science* 2007, 318, 1920-1923.

28. Raya et al. Disease-corrected haematopoietic progenitors from Fanconi anaemia induced pluripotent stem cells. *Nature* 2009, 460, 53-59.

29. Paul S. M. et al. How to improve R&D productivity: the pharmaceutical industry's grand challenge. *Nat. Rev. Drug Discov.* 2010, 9, 203-214.

30. Di Bernardo A. B. and Cudkowicz M. E, Translating preclinical insights into effective human trials in ALS. *Biochim. Biophys. Acta* 2006, 1762, 1139-1149.

31. Ellis J. and Bhatia M; iPS technology: platform for drug discovery. Point. *Clin. Pharmacol. Ther*. 2011, 89, 639-641.