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Advanced Discoveries and Applications of Induced Pluripotent Stem Cell Technology

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Abstract

The induced pluripotent stem cell (iPSC) technique allows indefinite self-renewal of cells and differentiation of somatic cells into any cell type of the body using different (reprogramming) transcription factors. The need for a type of treatment able to provide rapid response and therapy has been found necessary considering patient heterogeneity in variety of disease pathogenesis. Induced pluripotent stem cells continue to show potential in personalized diagnosis and treatment of diverse disease. Many findings have shown that iPSC can be of tremendous importance in the treatment of some presently non-curable diseases like diabetes, cancer and many others. In essence, iPSC has been demonstrated as a tool that can be used to analyze mechanisms of different diseases and for the production of impaired body substances and parts such as; insulin and malfunctional vasculature respectively. iPSC technique has the potential of identifying the best drug for any individual patient. Despite the progress made in this diagnostic and treatment approach, an evidence-based iPSC treatment approach is rare and remains a dire unmet need.

Keywords: Diagnosis, Induced pluripotent stem cell, Treatment.

1. Introduction

Stem cells are (young) cells capable of developing into almost any type of cell of the body part. The production of pluripotent stem cells from non-pluripotent resource is referred as induced pluripotency. Induced pluripotent stem cells (iPSCs) are the cells which are reprogrammed from somatic cells using different (reprogramming) transcription factors under specific conditions. The major characteristic properties of iPSCs include their capability to differentiate into any type of cells found in the body and their indefinite self-renewal. The main technique behind iPSC generation is targeted on the ectopic expression of master reprogramming factors and epigenetic reactivation of endogenous pluripotency genes. iPSCs are specie- and individual-specific, hence disease-causing mutations can be studied in the genomic context of the diseased patient. iPSC models of diseases allow for direct observation of genetic variation on phenotype and minimize the influences of environment and lifestyle (Sun *et al*., 2012).

1.1. Tools that Facilitate iPSC Techniques

Molecular scissors are the powerful gene editing tools to conduct precise-genome editing including the correction of disease-associated mutations. Synergistically with iPSCs these tools enhance phenotyping of targeted alterations of DNA in live cells, via introduction or deletion of a DNA variant in a healthy or mutated cell. These tools make it possible for the respective monitoring of a disease and healthy phenotypes upon differentiation (Yusa, Rashid & Strick-Marchand, 2011). They include:

Zinc finger nucleases (ZFNs), these are synthetic DNA-binding proteins: They cleave a domain known as the FokI restriction endonuclease in DNA at a user-specified location, thereby facilitating genome editing by creating double stranded break. This double-stranded break stimulates the cell's natural DNA-repair processes and thus by using well-established protocols, these cellular processes can be harnessed to generate precisely targeted *in vitro* or *in vivo* genomic edits with targeted gene deletions (Knockouts), integrations, or modifications (Urnov, Rebar, Holmes, Zhang & Gregory, 2010).

Transcription activator like-effector nuclease (TALEN): By assembling arrays of transcription activator like proteins (TALs) and fusing them to a FokI nuclease, specific cutting of the genome can be achieved. When two TALENs bind and meet, the FokI domains induce a double-strand break which can inactivate a gene, or can be used to insert DNA of interest. TALENs are more specific and don't have the cross-reactivity problem that are encountered in the use of ZFN (Deng, Yan, Wu, Pan & Yan, 2014).

Clustered regularly interspaced short palindromic repeats (CRISPR): This generates RNA-guided nucleases, such as Cas9, with customizable specificities. These systems will influence to perform targeted and highly efficient alterations of genome sequence which will certainly alter biological research program towards development of novel molecular therapeutics for human disease (Sander & Joung, 2014).

2. Applications of iPSCs

2.1. Disease Modelling.

iPSCs can be successfully applied to better understand the mechanisms of disease pathogenesis so as to develop new drug treatments (Fong, Wang, Knoferle, Walker, Balestra, & Tong, 2013; Randolph, Jiang & Lian, 2017; DiStefano *et al*., 2018; Ho, Pek, Boon-SengSoh, 2018). This can help to address the difficulties in discerning true disease-causing genetic mutations, complementing standard *in silico* predictions and epidemiologic studies.

2.2. Drug screening for toxicity, drug development and drug discovery.

High throughput screens of pharmaceuticals against cells differentiated from disease iPSCs have allowed for rapid assessment of efficacy and toxicity (Liang *et al*., 2013). Furthermore, some drugs seem to be ineffective after administration, due to unpredictable patient response and heterogeneity underlying many common diseases. However, iPSC disease models also present an opportunity to tailor therapies to the disease-causing DNA mutation, which can be transplanted to the site of injury or the site of degeneration in any given tissue.

2.3. Cell based therapy

The discovery of iPSCs have ushered in personalized gene therapy, with the exclusion of ethical concern while simultaneously addressing the issue of transplantation without the fear of graft rejection in the case of autologous gene. Evidence of increased T-cell proliferation or antigen-specific secondary immune response is eliminated, taking care of immune-rejection risk. The synthesis of blood components has been generated from iPSCs, typical examples include red blood cells, platelets (Teoh & Cheong, 2012).

2.4. Streamlined research and development.

iPSCs have elicited interest in both pharmaceutical industry and academia. Disease specific iPSC lines will help in the identification of drug candidates and speed up the screening of toxic and off target effects (De Souza Fernandez, De Souza Fernandez, & LuizMencalha, 2013).

3. Indicated Findings on iPSC-derived cells

Researches have shown that successful in vitro generation of iPSCs is possible as outlined in the table below. However, Tateishi, He, Taranova, Liang, D'Alessio & Zhang (2008) indicated that differentiated insulin-secreting pancreatic beta cells showed limited response to glucose due to their lack of Nkx6.1 and MAFA expression; leading to the production of what they regarded as immature non-functional pancreatic beta cells. Raikwar, Kim, Sivitz, Allamargot, Thedens & Zavazava (2015) demonstrated stabilization of serum glucose levels, after transplantation of iPSCs in type 1 diabetes mellitus (T1DM) mice model. Additionally, Jeon *et al.,* (2012) and Gerace *et al*., (2015) have further shown that transplantation of pancreatic progenitor via a macro-encapsulation device, led to efficient differentiation of the cells into functional mature insulin-secreting pancreatic beta cells. Mouse wild type pluripotent stem cells was injected into the pancreatogenesis-disabled mouse ($Pdx1^{-/-}$) blastocyst, production of interspecific chimera between mouse and rat with injection of mouse or rat PSCs into embryos from the other species were confirmed (Kobayashi *et al*., 2010). Again, the injected PSCs were also confirmed to be distributed all over the other body parts and normal function was observed. According to Ohmine (2012), it is now possible to generate a diabetic-derived iPSCs (DiPSCs) using an elderly patient's keratocytes. In line with the development, Stepniewski *et al*., (2015) and his research group were able to produced DiPSCs from murine model ($lep^{db/db}$ (db/db) mice) as well as human model from patients with MODY (maturity onset diabetes of the young) 3 (HNF1A MODY). After evaluating the various DiPSCs, there was an indication of an impaired differentiation in the db/db mice towards endothelial progenitor-like cells. Whereas there is no evidence of any teratomer formation in the HNF (hepatocyte nuclear factor) 1α MODY of the human-derived DiPSCs even after induction in their various patient specificities.

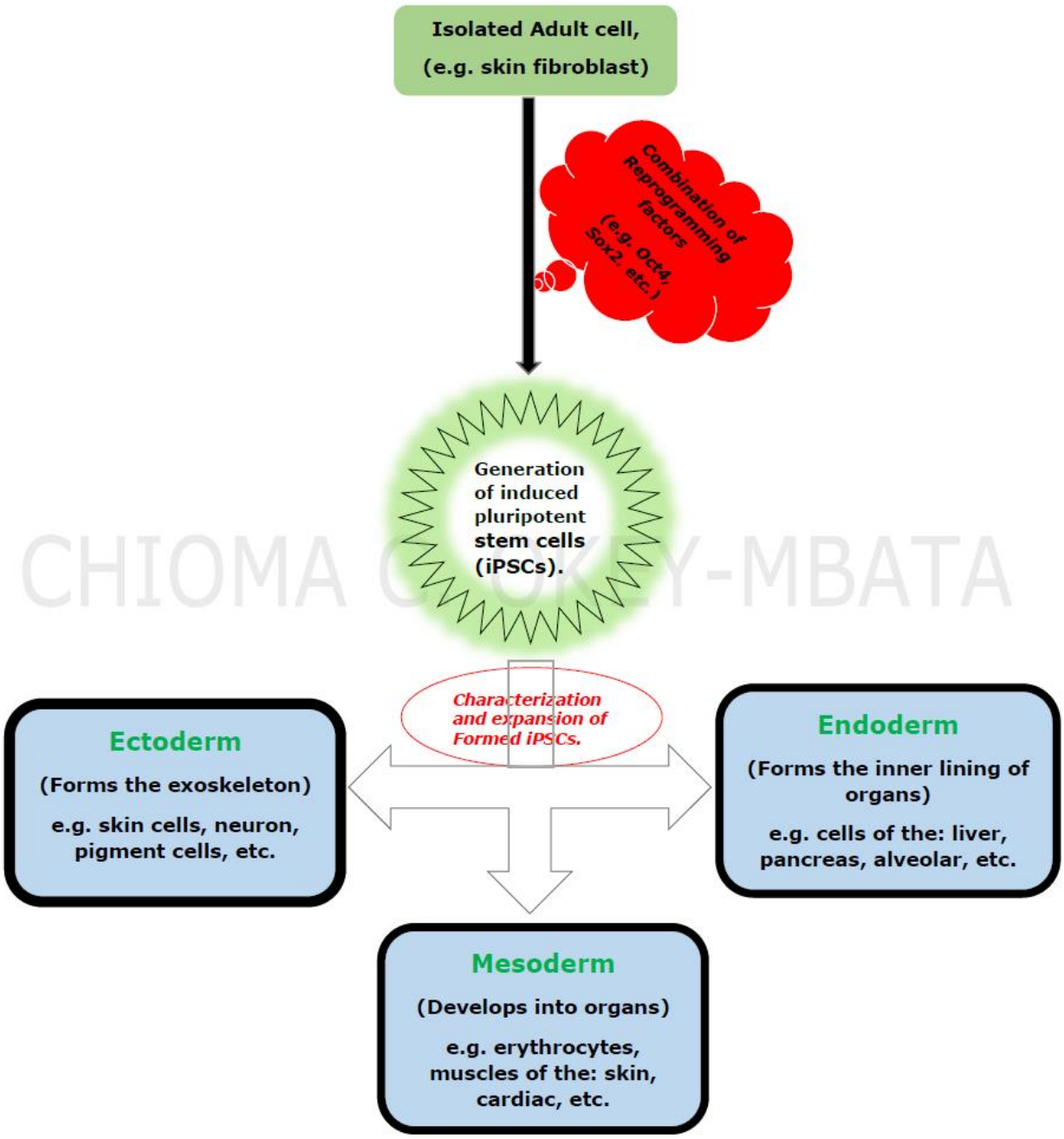

Fig (1.0): Schematic illustration of generation and differentiated parts of iPSCs and their various applications.

4. Improvements in the iPSC Technique

4.1. Reprogramming transcription factor.

Some factors and/or chemicals have been found to possess the potentials to replace the Yamanaka factors used for reprogramming somatic cells. Examples: NANOG together with Lin 28 are able to replace K and M (Yu, Vodyanik, Smuga-Otto, Antosiewicz-Bourget, Frane & Tian, 2007), BIX-01294: an inhibitor of G9a histone methyltransferase is able to replace S and O (Shi, Do, Desponts, Hahm, Scholer, & Ding, 2008), Esrrb: an orphan nuclear receptor capable of replacing only K (Feng, Jiang, Kraus, Ng, Heng & Chan, 2009), STAP: stimulus triggered acquisition of pluripotency (Obokata *et al*., 2014a, b).

4.2. Delivery methods for transcription factor transfer.

The reprogramming transcription transfer methods initially employed in iPSC technique has been enhanced from integrating methods such as retroviral transduction (Takahashi & Yamanaka 2006), lentviral (Yu *et al*.,2007), inducible lentviral (Maherali, Ahfeldt, Rigamonti, Utikal, Cowan, & Hochedlinger, 2008), to mention but a few into non-integrating methods such as plasmid DNA transfer (Okita, Nakagawa, Hyenjong, Ichisaka, & Yamanaka, 2008), polyarginine tagged polypeptide (Kim, Kim, Moon, Chung, Chang, Han, 2009), RNA modified synthetic mRNA (Warren, Manos, Ahfeldt, Loh, Li, & Lau, 2010), non-integrating reprogramming method (Ban *et al*., 2011) and many others.

5. Challenges Facing iPSC Technology

Regardless of the fact that iPSC technique has been shown to have some relieving factors in research and development, it has been found to possess some challenges which include:

- Lack of quality control methods for comprehensive routine high throughput characterization of human iPSCs and their cellular derivatives. Ethical reflections on cell reprogramming (Aznar & Martinez, 2012).
- Low rate of DNA repair (Zhang, Shang, Yang, Cao, Pan & Zhou, 2012),
- Sensitive to ionizing radiation, which is due to the characteristics of iPSCs (Zhang, Yang, Liu & Sun, 2013).
- Cases of insertional mutagenesis, tumorigenesis, considering the type of technology involved in iPSCs development. It has the potential for acquired somatic mutations and chromosomal rearrangements during the induction of pluripotency, risk associated with the use of lentiviral or retroviral vectors resulting in genomic integration and insertion mutagenesis (Baum 2007; Okita, Ichisaka & Yamanaka, 2007; Park, Gum, Kakar, Kwon, Deng & Kim, 2008).
- It is often time consuming and resource intensive. No sufficient reduction in time and labor to justify wide-scale routine implementation, particularly in clinical settings (Hanna *et al*., 2009).
- Cases of teratoma development and immunogenicity have been reported in allogeneic iPSC (Gutierrez-Aranda, Ramos-Mejia, Bueno, Munoz-Lopez & Real, 2010; Zhao, Zhang, Rong & Xu, 2011; Fu 2014).

6. Overcoming some of Challenges of iPSC Technique

I. Developing a very simple and effective differentiation protocols that is capable of exclusively generating particular and distinctive mature cells of interest which are necessary for modeling various diseases and cellular therapeutic purposes.

II. Research towards development of differentiation techniques which can be very specific and competent to avoid the production of undifferentiated cells, this will invariably eliminate the formation of teratoma in any applicable situation.

III. Further studies on the elucidation of molecular mechanisms concerning effective differentiation protocols and production of high-density cultures of any desired cell is really of great importance.

IV. Encapsulation of the iPSCs intended for transplantation. This will prevent both *in vivo* overgrowth and undesired cell growth. Furthermore, encapsulation could ensure that no undifferentiated cell(s) contaminates iPSCs after differentiation.
V. Identification of defects in transcription factors in every stage of iPSC differentiation using genome-transcriptional analysis should be carried out.
VI. Recognition of differences in gene expression typically by comparing in vivo development of cells and in vitro differentiation of iPSCs.
VII. Development of diverse differentiation protocols by employing the various signaling pathways that control the in vitro differentiation processes.

Table 1: Selected procedures where iPSC techniques have been found useful.

Condition(s)	Body part	References
Congenital brain malformations, Encephalopathy	Brain	Kelava & Lancaster (2016); Raja *et al*., (2016); Dang et al., (2017); Monzel *et al*., (2017)
Cystic fibrosis	Liver	Guan *et al*., (2017)
Diabetes mellitus, Pancreatic ductal adenocarcinoma	Pancreas	Baker, Tiriac, Clevers & Tuveson (2016), Kim *et al*., (2016); Hohwieler *et al*., (2017).
Colorectal cancer, Host microbial interactions	Intestine	Van de Wetering *et al*., (2015); Ettayebi *etal*., (2016).
Ovarian cancer	Kidney	Yucer *et al*., (2017).
Fibrotic lung disease	Lung	Barkauskas, Chung, Fioret, Gao, Katsura & Hogan (2017); Chen *et al*., (2017)
Cardiomyopathy	Heart	Lan *et al*., (2013); Sun *et al*., (2012); Tzatzalos, Abilez, Shukla & Wu (2016); Wu *et al*., (2015); Yazawa *et al*., (2011).
Severe anaemia (such as sickle cell disease, β- Thalassemia, hemophilia)	Blood	Kimbrel & Lu (2011); Lengerke & Daley (2010); Daley (2014).
Transfusion of blood	Blood	Shah, Huang & Cheng (2014).
Genetic polymorphism	Genome	Ebert *et al*., (2015).
Macular degeneration	Retina	Di Stefano *et al*., (2018).

7. Conclusion

iPSCs as a novel approach to model human body part (like organs) development and disease. It might be interesting to know/ and bear in mind that, in the coming decades, data gained from iPSCs constitute one of the most promising primary building blocks of precision medicine. There is need for establishment of quality control measures that will take care of any contamination case which may result in the production/utilization of iPSCs for clinical purposes. Though there may be no much need for a large bank for autologous cell but for the sake of initial reprogramming variability which may occur, autologous cell bank is necessary for identification and variability minimization. However, the bank required for

allogeneic cell is necessary to ascertain consistency of the starting cell material. Furthermore, humanized platform for (organ-specific and patient-specific) drug screening and testing of therapeutic options in vitro and in vivo can be made readily available. Again, there is need to provide imaging procedure for iPSC technique to facilitate translation of information or results obtained from reprogrammed cells. Additionally, more advances on how to key-in and explore this technique in more comprehensive manner should not be neglected.

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