iPSC for modeling neurodegenerative disorders

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Abstract

Neurodegenerative disorders such as Parkinson’s and Alzheimer’s disease, are fundamental health concerns all around the world. The development of novel treatments and new techniques to address these disorders, are being actively studied by researchers and medical personnel. In the present review we will discuss the application of induced Pluripotent Stem Cells (iPSCs) for cell-therapy replacement and disease modelling. The aim of iPSCs is to restore the functionality of the damaged tissue by replacing the impaired cells with competitive ones. To achieve this objective, iPSCs can be properly differentiated into virtually any cell fate and can be strongly translated into human health via in vitro and in vivo disease modeling for the development of new therapies, the discovery of biomarkers for several disorders, the elaboration and testing of new drugs as novel treatments, and as a tool for personalized medicine.

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1. Introduction

Throughout history, stem cells (SCs) have been widely employed, even before knowing their mechanisms. The first successful therapeutic application of stem cells was the bone marrow transplantation in 1968 [1], from that date to the present the development technology has preside over multiple advances on identification, understanding, and applications; the best example, is the breakthrough development of induced pluripotent stem cells (iPSCs) from somatic cells, establishing a watershed in science progress [2–4]. Cell reprogramming mediated by the Yamanaka factors expanded the view around stem cells, which already showed a very promising picture.

Since the isolation of human embryonic stem cells (hESC), stem cells were proposed as a platform of study of human developmental biology, disease modeling, drug screening, and cell therapy [5,6]. Recent trends in regenerative and personalized medicine aim for the development of specific disease models, where iPSCs cells can offer an additional advantage to achieve a more representative approach [7–10]. In this review, we will recapitulate the origins, obtainment methods, novel research, applications, and future perspectives of iPSC for the modeling of Parkinson’s (PD) and Alzheimer’s diseases (AD), since these pathologies are of major concern due to their incidence worldwide.

2. What are iPSCs & how are they obtained?

In the early 2000’s a great discovery was achieved by Yamanaka, S. and Takahashi, K., in which they successfully generated new kinds of stem cells similar to ESCs from mouse embryonic and adult fibroblasts cultures, these were called induced pluripotent stem cells (iPSCs) (Fig. 1). They were able to reprogram the gene expression of the fibroblasts genome by adding four transcription factors to the culture media: OCT4, SOX2, KLF4 and C-MYC, known as the Yamanaka factors, their role is to help to induce and maintain the pluripotent state via integrating viral vectors, in particular retroviral and lentiviral transduction [2,11]. iPSCs have been widely used for disease modelling, drug screening of neuroprotective compounds, medications and potential cell therapy research [12,13].

Furthermore, the combination of iPSCs with organoids and gene editing, such as CRISPR-Cas9, turns these cells into a highly versatile tool for regenerative medicine and drug screening to evaluate compounds with the potential to treat several diseases, resulting in clinical candidates being identified and some approved for their application [11,14]. However, it is important to acknowledge their inconveniences, such as: the high cost, the amount of time needed to be developed, the requirement to downregulate the MHC (Major Histocompatibility Complex) in the host cells if the iPSCs-derived cells will be transplanted in order to limit the recognition by the immune system, the specific culture conditions, reprogramming and differentiation processes contribution to karyotype abnormalities by genetic instability, which is difficult to standardize, turning these cells inadequate for their clinical application at the moment [15,16].

To overcome the issues associated to genome instability and reduce risks corresponding with vectors, non-integrating methods have been developed in the later years, including: episomal DNAs, synthetic mRNAs, Sendai virus, amongst others [11]. Additionally, genome editing technologies provide the possibility to introduce genetic changes into iPSC in a site-specific manner, generating isogenic iPSCs lines, distinctly important in sporadic and polygenic diseases [17].

iPSCs can be obtained, technically, from any tissue of the body, nevertheless the most employed sources are fibroblasts and peripheral blood mononuclear cells due to their easy access [18,19]. The process of generation of iPSCs initiate by the recollection of somatic cells from the patient or animal model, which then are transduced with a virus containing the reprogramming factors, once the reprogramming is achieved, morphological changes can be appreciated in the cultured cells forming ESCs-like colonies, consequently collected for further expansion for several passages to ensure the conservation of their characteristic morphology [20] (Fig. 2). These cells should express ESCs antigens, such as SSEA-4 and TRA-1-80 to ensure their similarity, additional steps that might be performed include the chromosomal analysis for a normal karyotype or identify possible translocations [21].

To verify the iPSCs pluripotency potential, it is necessary to test their capacity to form specialized tissue from the three germ layers. When cultured in suspension, the ESCs-like reprogrammed cells...
start to transform into embryoid bodies and after two weeks approximately the embryoid bodies must have differentiated, in a spontaneous manner, to the three germ layers. This differentiation is analyzed by immunostaining of the corresponding markers of each germ layer, the most commonly used antibodies are: alpha-fetoprotein (AFP) for the endoderm layer, beta-III-tubulin (B-III-TUB) for the ectodermal layer, and smooth-muscle antibody (SMA) for the mesodermal layer [22]. Another protocol used to examine the differentiation capacity is by the in vivo teratoma formation test [23]. This test consists in administrating the obtained iPSCs into a animal model, commonly mouse, until a teratoma is formed around two to three months later, expressing markers from the three germ layers [24,25].

Before applying iPSCs technology to the clinic for therapies, it is necessary to prove their safety using animal models [26]. A new approach for regenerative therapy is the chimera formation, which consists of the development of interspecies blastocyst complementation, dependent on the matching of the species origin of trophectoderm derivatives and maternal uterus, or targeted organ complementation restricting the differentiation into a specific organ [27]. Due to the similarity of pigs to humans in the aspects of organ size, physiology, and anatomy they represent an excellent model, since experimentation in non-human primates is highly regulated [28]. The main objective in the clinic of this technology is the development of human organs in pigs for transplantation [26]. However, competent iPSCs from pigs to create viable chimeras are still not available and there is a public discomfort about the unnaturalness of chimeras [29].

A novel tool to process and analyze transcriptome sequencing data is bioinformatics, allowing us to investigate how the neural system develops using genetic markers, single cell transcriptomics, and fate mapping [30]. With this technology we are able to create de novo whole genome sequencing (WGS) and sequencing libraries, after isolating DNA from a sample, using equipment such as illumina HiSeq or MiSeq [31]. Posterior data analysis can be achieved by digital platforms such as Fast Length Adjustment of Short Reads (FLASH), a software tool that helps to find the correct overlap between paired-end reads and extend the reads by stitching them together [32]; UCHIME, a program that detects chimeric sequences after PCR amplification either comparing chimera-free sequences from a database or detecting them de novo by analyzing the data provided [33]; IDRMutPred, a prediction tool for disease-associated germline nonsynonymous single nucleotide variants (nsSNVs) in intrinsically disordered regions of proteins [34]. The newest and most popular technology nowadays is the clustered regularly interspaced short palindromic repeats (CRISPR)-Cas9, a genome editing system mediated by guide RNA (gRNA) that directs the Cas9 protein to the target DNA in the genome, followed by its catalytic subunit generating a double-strand break in order to edit the DNA, activate or repress a gene, generate epigenomic modifications, etc [35]. All these equipments and bioinformatic softwares, are tools to research the sequence-structure-function protein paradigm [36], allowing us to understand disease mechanisms of genetic variants, generate preclinical disease models, and develop structure-based drug discovery [37].

3. Current iPSCs models

Complex iPSC-based differentiation systems are currently being developed to conscientiously recapitulate human tissue-level and organ-level dysfunction, incorporated into disease modelling, drug screening and the study of host–pathogen interactions [38].
Currently, the available models are in vitro culture and animal models. Animal models have provided several tools for modelling human diseases, contributing to the identification of pathological molecular mechanisms in an in vivo setting. However, species differences obstruct the possibility to capture the complete vision of the human disease, generating an urgent need to establish human disease modelling platforms for biomedical research [11,39]. Disease modelling applying iPSCs, requires cells harboring the disease-causing mutation or damage, to differentiate them into the predisposed cell type needed to analyze the disease and identify the pathological mechanisms involved in its etiology. The phenotype of cells differentiated from iPSCs provide a good model for diseases with an early onset rather than late onset due their fetal-like properties [40]. If necessary, ageing in cells could be induced by treating them with cellular stressors, which target mitochondrial function or protein degradation pathways [41].

To improve disease modelling, it has been developed a coculture method of more than one cell type, in order to study their interaction [11]. Yet, the interaction between different cell types could be better employing iPSCs-derived 3D organoids, due their resemblance to endogenous cell organization and organ structure, enabling a cellular context that mimics physiology and development, with the potential to model drug response in a spatiotemporal context [42]. Nonetheless, 3D cultures still need to be improved with a more standardized culture medium and extracellular matrix in order to correctly mimic the human physiological environment, with greater vascularization, growth, maturation, and nutrient supply.

4. Neurodegenerative disorders: Parkinson’s disease & Alzheimer’s disease

Neurodegenerative disorders are a prominent class of neurological diseases, they are characterized by an inexorable loss of a specific type of neuron according to the disease. The two most conditions are Alzheimer’s disease and Parkinson’s disease, respectively, which will be discussed in the present review [43–45] (Fig. 3).

4.1. Parkinson’s disease

The core feature of PD involves selective loss of A9-type dopaminergic neurons that project from the substantia nigra pars compacta (SNpc) in the midbrain to the dorsal striatum [46]. Dopamine dyshomeostasis is highly related to the metabolite 3,4-dihydroxyphenylacetaldehyde (DOPAL), resulting in the alteration of functional protein residues, protein aggregation, oxidative stress, and finally cell death. These fatal results are due to the DOPAL triggering of α-synuclein oligomerization leading to synapse impairment, including dopamine leakage from synaptic vesicles [47]. Therefore, α-synuclein metabolism plays an important role in the development of PD, some factors that promote this protein aggregation are mitochondrial dysfunction, oxidative stress, neurotoxins, and oxidation, trafficking or mutation of α-synuclein [48,49].

PD can be categorized in two types: genetic or familial and sporadic or idiopathic. The genetic form only represents the 10% of the total population diagnosed with this disease, while the other 90% represents a sporadic form. A great discovery achieved with iPSCs models is the neuronal death induced by mitochondrial dysfunction due to oxidative stress, playing an important role in the sporadic PD research [50,51].

The first gene that was linked to PD was the SNCA gene, which encodes the protein α-synuclein, although the function is not well understood. PD-iPSCs models have shown that SNCA is associated with α-synuclein aggregation and Lewy body-like deposition in dopaminergic neurons. A second gene that has been correlated with α-synuclein protein, elevated expression of key oxidative stress-response genes, and mitochondrial dysfunction in dopaminergic neurons that were derived from PD-iPSCs [52]. Another related gene is PARK2 or Parkin, this is an E3 ubiquitin ligase that targets mitochondria, mutations in PARK2 are associated with an autosomal recessive early-onset familial PD and are correlated to loss of tyrosine hydroxylase-positive dopaminergic neurons [53]. One event that results in the loss of Parkin expression is exon deletion, leading to increased oxidative stress, reduced dopamine (DA) uptake and increased spontaneous DA release in dopaminergic neurons derived from PD-iPSCs. These observations suggest that Parkin is involved in controlling DA neurotransmission and suppressing DA oxidation in human midbrain dopaminergic neurons [54]. In the case of sporadic PD, GBA1 encodes a lysosomal enzyme, β-glucocerebrosidase, involved in glycolipid metabolism, mutations in this gene were correlated to lowered β-glucocerebrosidase activity and α-synuclein accumulation in dopaminergic neurons derived from PD-iPSCs [55].

One alternative to study Parkinson’s disease is through the recently developed method to generate brain organoids: these may help to create complex 3D models of midbrain tissue from iPSCs [56]. Propagation and differentiation of patient-derived iPSCs into specific neuronal subtypes are now enhancing the understanding of the cellular progression concerning neurodegeneration in vitro. Thus, leading researchers to discover new methods to manipulate iPSC-derived neurons, revealing novel disease phenotypes and ultimately identifying novel clinical interventions [57] (Fig. 4).

Models of PD today focus on the two principal characteristics of the disease: loss of functionality of dopaminergic neurons and α-synuclein protein aggregates [58]. One of the most used models

Fig. 3. Cellular neurodegenerative process. The neurodegenerative process is initiated by metabolic disruption events in the neurons, such as protein overexpression or misfolding. These events activate microglial cells and the Blood Brain Barrier (BBB), sending signals (cytokines) which induce inflammation in the brain. Inflammation leads to neuronal damage and finally neurodegeneration. Created with BioRender.
are the SH-SY5Y neuroblastoma cell line and the PC12 cell line, because they are able to produce catecholamines and develop neuron like properties, since differentiation of these lines is difficult they are used for pharmacological interventions [59]. A third line used for modeling PD is the Lund human mesencephalic cell line (LUHMES), providing a more stable dopaminergic phenotype, since they show neuronal markers, long processes and electrical properties similar to dopaminergic neurons [60].

Another approach is the use of iPSCs from patients to generate more accurate models of the disease, providing a better tool for understanding pathogenic mechanisms of neurological disorders, developing personalized therapeutic strategies, and studying early pathogenic mechanisms. Likewise, a model generated from iPSCs of young-onset PD from patients with the disorder, confirmed the implication of lysosomal degradation pathways, mitochondrial dysfunction and impaired mitophagy in its pathogenesis. In addition, they tested two neuroprotective compounds, phorbol esters, in their iPSCs-PD models, resulting in reduced α-synuclein aggregation, increased tyrosine hydroxylase (TH) positive neurons in mesencephalic dopaminergic neuron cultures [61]. It is important to mention that the creation of libraries of iPSCs from patients are becoming an important source of preclinical models of PD for future developments [62].

4.2. Alzheimer’s disease

AD is characterized by a brain volume reduction and hippocampal degeneration, along its pathological hallmarks: extracellular Aβ plaques and aggregation of hyperphosphorylated tau protein in neurofibrillary tangles, which tend to aggregate, causing neuronal dysfunction and cell death [63,64]. Researchers found that iPSC-derived neurons from different patients showed different pathophysiology of AD, especially in the accumulation of Aβ oligomers [65]. Additional signs of AD include gliosis, inflammation, abnormalities of the Blood–Brain Barrier (BBB), altered pathways of cellular and endocytic degradation, and elevated DNA damage [66,67].

There are some investigations relating impaired mitochondrial energy metabolism as the defining characteristic of several cases of AD, due a compensatory mechanism of energy production to maintain challenged neuronal cells [68]. An increase of molecular disorder in mitochondrial metabolism in neurons and energy production in astrocytes generate higher thermodynamic entropy, thus inducing detrimental effects in molecular fidelity, such as, conformational changes and covalent modifications in structure, resulting in protein misfolding and amyloid formation [69,70]. Regularly, mature neurons do not enter the cell cycle, nor complete mitosis, therefore, if there is DNA damage it is due to oxidative stress, leading to cell cycle entry, activation of DNA repair mechanisms, and if repair is not successful, apoptotic pathways will be triggered and cause neuronal loss [71].

Apolipoprotein E4 (APOE4) is a strong genetic risk factor for Alzheimer’s disease [72,73]. Several studies are being carried out for AD that use brain cells derived from iPSC to generate neuron degeneration in mutant APOE4 neurons that demonstrates high expression in Aβ, p-tau, and GABAergic. Complementary, APOE3 and APOE4 mutations manifest impaired Aβ clearance and increased cholesterol content of APOE4 astrocytes. For microglia the isogenic APOE3 and APOE4 mutations have demonstrated a reduced Aβ uptake from media and organoids [74]. The generation of neural precursors from iPSC has also been extensively studied, in the production of astrocytes, the mutation in PSEN1 increased Aβ production and oxidative stress, altered cytokine release and Ca²⁺ homeostasis, thus reducing neuronal support function in PSEN1 astrocytes [75]. Kondo et al. and Israel et al. report the discovery that Aβ-oligomers accumulating neurons from individual iPSC cell damage are associated with increased reactive-oxygen-species (ROS) [65,72]. For that, AD patient iPSC-derived neurons might be a competent therapeutic model and testing ground to find the patient’s optimal pharmacological regimen and with the use of 3D in vitro cultures, explore the pathophysiology of Alzheimer’s disease (Fig. 5).

5. Future applications

Massive progress has been done investigating disease mechanisms and potential treatments through patient-derived iPSCs in combination with gene editing technologies, such as CRISPR-Cas9 and bioinformatic softwares making available new tools for understanding biological mechanisms underlying iPSC pluripotency, maintenance and differentiation.

Autologous iPSCs might avoid the serious side effects associated with lifelong immunosuppression required for allogeneic cell transplantation, representing a major advantage in the use of these cells for clinical purposes in the future [76]. The clinical utility of iPSCs-derived products relies on the state of the art of directed differentiation, cell state conversion and tissue engineering [77]. In addition to pathological mechanisms of diseases investigated via
in vitro. 2D modeling and future 3D models represent a better overview to comprehend pathophysiological conditions, enabling the spatiotemporal cellular interactions analysis, although there are several limitations in the present status of this technology that must be overcome in order to exploit the potential of the novel tools and techniques [78].

6. Limitations

Even though iPSCs are a well-established technology, there are still some obstacles associated with iPSC-based therapy before enabling it for clinical applications. One issue is the risk of tumorigenicity due to their pluripotency state, besides, they can accumulate karyotypic abnormalities if maintained in culture for long periods of time. If these barriers are overcome, and cells have been correctly delivered, patients should be monitored for potential tumors and the immune system reactivity [79]. Because of these concerns, iPSCs-derived products need to be completely characterized and screened for the absence of potential genetic alterations, ensure their quality and innocuousness. There are still challenges to overcome for iPSCs regarding the mechanisms of pluripotency, reprogramming and the derivation of some functional mature cells, nevertheless several research groups are looking behind these fundamental processes and it will be a hotspot for stem cell research in the upcoming years.

7. Conclusions

The iPSCs models can be induced from somatic cells and can be differentiated into specific cell types to mimic many worldwide known diseases. Novel studies generate new opportunities for medical treatments, but they also face many challenges such as the improvement of differentiation protocols, in addition to prioritizing the safety and effectiveness of transplanted cells. We must keep in mind that stem cells may provide an important tool for SC therapy, but it also results in some ethical concerns, the great advantage is that derivation of human-induced pluripotent stem cells from somatic cells avoid the destruction of human embryos and their manipulation for research, thus bypassing ethical problems arising from ESCs obtainment and manipulation. iPSCs models of neurodegenerative diseases such as PD and AD are just the beginning of a new era of medical advancements and technology that will be available for clinical treatments, disease prevention, finding novel cures for diseases and more in a not so far future.

Declaration of competing interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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