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Review Article

Stepping back to move forward: a current review of iPSCs in the fight against Alzheimer's disease

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Abstract: The successful generation of the first iPSCs about ten years ago has provided deeper insight into previously unknown disease mechanisms and therapeutic opportunities for many diseases. In particular, iPSCs are becoming an important tool in advancing modeling and therapeutic intervention for Alzheimer's disease. In this manuscript, we assess the research climate surrounding the application of iPSCs to familial and sporadic Alzheimer's disease, including the generation and isolation of individualized neural stem cells, the introduction of neural stem cell transplants using iPSCs, and an estimation of the potential use of iPSCs as research models for Alzheimer's treatments and therapies. The clinical application of stem cells in the treatment of Alzheimer's disease appears promising, but much of the recent experimentation has been conducted using animal models or embryonic stem cells. As induced pluripotent stem cell research advances, iPSCs will likely provide investigators with a more applicable tool to progress advances in research and treatment for Alzheimer's and other neurodegenerative diseases.

Keywords: IPSC, Alzheimer's disease, induced pluripotent stem cell

Introduction

Alzheimer's disease (AD) is the 6th leading cause of death in the United States and typically presents after six decades of life. There are over twenty-four million individuals afflicted with dementia worldwide and over 60% of those are clinically diagnosed with AD [15]. AD is a spectrum disease that exists on a continuum between genetic (familial) and sporadic, both of which have early and late onset forms [25]. Since the current understanding of AD's pathogenesis is limited and the incidence of AD has risen 68% from 2000 to 2010, the need for better scientific understanding is more critical now than ever [25]. Perhaps even more concerning is that along with an increase in life expectancy and the maturation of the 'baby boomer' generation, expectations for AD incidence do not foresee a decline any time soon barring a significant medical advance.

Encouragingly, the gradual introduction of induced pluripotent stem cell (iPSC) methodology to the modeling and research for new therapies to treat neurodegenerative diseases is producing promising early results. In fact, researchers have already demonstrated the viability of dopaminergic neurons, derived entirely from iPSCs using a CORIN cell signature, as a potential "replacement therapy for Parkinson's disease" [4]. Additionally, iPSCs have been successfully differentiated into the sort of motor neuron that degenerates in a familial form of Amyotrophic Lateral Sclerosis (ALS) [3]. Procedures for efficiently isolating and differentiating astrocytes, oligodendroglia, and other neural stem cells from a heterogeneous population of iPSC-derived neural cells using immunophenotyping screens have been established [33]. While certain challenges must be addressed in this field, iPSCs are emerging to revolutionize both AD research modeling strategies and clinical interventions.

A history of the induced-pluripotent stem cell

The most acknowledged first concept of generating pluripotent stem cells from somatic cells came to fruition in 2006, when it was demonstrated that retrovirus-mediated transfection of transcription factors into mouse embryonic

fibroblasts (MEFs) resulted in the development of what are now known as iPSCs [27]. From twenty-four original transcription factors, Takahashi and Yamanaka's team identified four transcription factors both necessary and sufficient to sustain pluripotency in their mouse model: octamer-binding protein 3/4 (Oct3/4), SRY-related HMG-box gene 2 (Sox2), Krüppel-like factor 4 (Klf4), and c-Myc, commonly abbreviated as the OSKM transcription factors. These iPSCs demonstrated similar morphologies and differentiation patterns to embryonic stem cells (ESCs), illustrated by the iPSCs' subsequent differentiation into endodermal, mesodermal, and ectodermal derivatives. These new types of stem cells demonstrated considerable promise as an extraordinary addition to, or potentially a replacement for, human ESCs.

Researchers then aimed to generate iPSCs using human somatic cells as a substrate. The transcription factor 'cocktail' was further tested with adult human dermal fibroblasts (HDFs), derived from the facial dermis of a thirty-six-year-old Caucasian female [26]. The human iPSCs resulting from this experiment demonstrated efficient reprogramming without the need for continuous expression of the transgenes.

These initial discoveries suggested a promising alternative to the more controversial human ESCs for potential cell-based therapies for human diseases. While human iPSCs effectively bypass the ethical issues regarding the usage of human embryos and human ESCs, it is important to note that the science surrounding the development of iPSCs is quite young, and there is much more to be learned about IPSCs prior to the introduction of the science into human applications. Early work with retroviral integration for all four of the OSKM transcription factors drastically increased the risk of tumorigenesis [26]. Nonetheless, this seemingly insurmountable obstacle was overcome, when, Sendai viral vectors (SeV vectors) were used as a means of bypassing the issue of retroviral integration and impending tumorigenesis in iPSCs created through retroviral transfection. The introduction of a SeV vector containing the OSKM cocktail resulted in iPSC expression of the transgenes without modification of the host genome, a markedly increased efficiency of iPSC generation. Then, after treatment with siRNAs, viral genome-depleted iPSCs were virtually identical to the original cell [6, 34]. This demonstrates that, though there are genetic hurdles and still much progress remains before iPSCs fully gain clinical use, great strides have already been made towards developing safe methods to create useful IPSCs.

iPSCs and Alzheimer's disease

Since the successful generation of the first iPSCs ten years ago, their addition to the researcher's toolbox has provided deeper insight into previously unknown disease mechanisms and therapeutic opportunities. A primary area of this focus has been the investigation of both familial and sporadic AD. Considering the regenerative and modeling potential of the iPSC, the neurodegenerative pathology of AD presents an ideal candidate for this research.

Similar to some other neurodegenerative diseases (NDs), AD is marked by the progressive loss of cognitive ability [25]. To complicate therapy, the central nervous system is largely unable to repair itself following destruction. Cerebral atrophy with loss of cells and synapses, hallmarks of AD, translates to an inability to retain new information as well as access previously stored information [15]. Prior to iPSCs' arrival, extensive research into the pathophysiology of AD primarily addressed ways of reversing or at least slowing disease progression. Most research into AD prior to having IPSC tools suggested that specific proteins aggregates induced gradual neuronal cell neurodegeneration and/or apoptosis [17]. Specifically, AD presents with the concordant accumulation of certain deleterious proteins, extracellular amyloid-β (Aβ) and intracellular tau proteins, which may be responsible for this neuronal cell death [17].

A similar association is noted between the aggregation of these harmful proteins and the subsequent activation of degradation pathways. In particular, Aβ plaques are found to induce the activation of both the ubiquitin-proteasome system and macroautophagy, both of which contribute to neuronal cell death [19]. For familial AD, further progress has been achieved by isolating mutations in specific genes-namely, the *E5-1* gene on chromosome 1 and the *S182* gene on chromosome 14-that are found to possibly be involved in disease onset [18]. In addition to this gene mutation

discovery, significant data suggested another gene, the *e4* variant of the *APOE* gene, also plays a major role in the early onset of familial AD. Increasing the risk of AD, the *ApoE4* gene has been identified as a pivotal factor in the accumulation of Aβ and tau proteins [15].

While all of the aforementioned associations appear to have isolated key factors, correlation does not imply causation, and the pathogenesis of AD remains largely a mystery. Coupling these enigmatic disease mechanisms with the inherent inability of the CNS to regenerate, the progress in clinical trials to identify an effective treatment for AD has been discouraging.

While the development of iPSC-derived neural stem cells (NSCs) effectively bypasses many barriers such as tissue rejection and ethical considerations, the precise mechanistic process of synthesizing NSCs presents some discrete challenges. Two primary issues include the ability to adequately generate definitive neural stems cells (dNSCs) and to avoid recollecting primitive neural stems cells (pNSCs), the latter of which, if implanted, have a high likelihood of teratoma formation [19]. In other words, the initial lack of a process to efficiently generating highly pure dNSCs posed a barrier to their clinical use. In order to overcome these barriers, a suitable and efficient environment needed to be determined to maximize the creation of dNSCs.

Currently, the accepted model of neuralization argues that the ectodermal germ cell's default differentiation is to indeed become a pNSC [22]. Also, considering that both neuronal and epidermal cells derive from the ectoderm, a simple epidermal cell with all external cues excised to ensure a default state could go on to create a dNSC [19, 22]. To first achieve this neuralization, any ectodermally derived cell is added to a serum-free solution containing leukemia inhibiting factor (LIF), which synthesizes pNSCs [19]. Following a subsequent purification from LIF and replication, the addition of Fibroblast Growth Factor 2 (FGF-2) creates the desired end-product of dNSCs [19]. While this process yielded the successful generation of dNSCs, research has been ongoing on how to improve the efficacy of this process.

In particular, there are two factors that could help improve the production rate of dNSCs. Firstly, through the addition of bone morphogenic protein (BMP), a transforming growth factor, there is a marked improvement of non-neural fate differentiation [22]. This finding was confirmed through the addition of the BMP inhibitor Noggin, resulting in an increase in pNSCs, as opposed to the desired dNSCs [19]. Secondly, and seemingly more promising, the NOTCH pathway was identified as an indispensable component of neurodevelopment [24]. When the NOTCH pathway was disrupted, significant decrease in NSC production ensued [22]. Furthermore, primary ligands Delta-like ligand 4 (DLL4) and Jagged were established as fundamental components of the NOTCH pathway [24]. Armed with this information, a process of exogenously influencing the signaling pathways with the objective of efficiently synthesizing iPSC-dNSCs was possible. In murine studies, activating the NOTCH signaling pathway through DLL4 successfully improved synthesis of dNSCs, decisively introducing iPSCs as a utilizable tool for NDs [19].

Sporadic and familial Alzheimer's disease models

Though both sporadic and familial AD possess distinct characteristics, they also share cellular pathologies including axonal transport defects, synapse loss, and selective neuronal death [11]. The primary causes of early-onset familial AD are dominantly inherited mutations of the Presenilin 1 or 2 genes (*PSEN1*, *PSEN2*) that encode proteins PS1 and PS2, as well as their substrate amyloid precursor protein (APP). This accounts for less than 5% of all AD cases [7]. On the other hand, epidemiology and population genetics suggest that sporadic AD results from a combination of genetic and environmental risk factors, and accounts for more than 95% of AD cases [23].

Through conventional disease models, it has been difficult to determine the pathogenesis of sporadic AD due in part to the lack of appropriate experimental models, that is, until the development of iPSC-derived neuron models. Multiple studies revealed that numerous other genes can become vulnerable in the sporadic form of AD, investigated through the use of iPSC-derived neuron models that could not have been possible through conventional disease models [31]. One such gene is the aforementioned *APOE4* gene, which suggests that

lipid metabolism plays a role in the etiology of AD [31]. Studies have also found a significant correlation between increased expression of the sporadic AD associated *SORL1* haplotype (sortilin-related receptor L) and the brain-derived neurotrophic factor (*BDNF*) gene through human iPSC-derived neurons [32]. In addition, a significant reduction of Aβ peptides is consistent with the observation of reduced expression of SORL1 [21].

Understanding the etiology and pathogenesis of AD is currently limited due to both the marked difficulty associated with obtaining and culturing live human neurons and the inability to precisely model AD's great variability. However, the past ten years have seen improvements in the quality of iPSCs, thus progressing this research and filling in the gaps where other stem cell models were insufficient.

Using the OSKM transcription factor cocktail, human somatic cells have been reprogrammed into both human iPSCs and some iPSCs expressing the Enhanced Green Fluorescent Protein (EGPF) [12]. Typically most iPSCs are obtained by taking a small skin biopsy from a patient, expanding the biopsy into primary fibroblasts, then transducing the cells with retroviruses that encode the cocktail of transcription factors mentioned above [8]. One study performed the transduction modifying the c-MYC transcription factor, known to be linked to an increased risk of uncontrolled proliferation and tumor formation, resulting in decreased reprogramming efficiency [8]. Therefore, to combat the potential for uncontrolled proliferation, a recombinant pTAT-mcMYC protein was used as a substitute [8]. All iPSC lines generated with pTAT-mcMYC maintained ESC-like morphology and expressed the ability to differentiate into cells of all three primary germ lineages in vitro, providing potential for further research on modifying the expression of c-MYC while still taking advantage of its necessary role in the efficacy of iPSCs formation [8].

While it was established that familial AD presents itself with elevated or altered secretion of Aβ peptides by fibroblasts [12], it was not known if fibroblasts from sporadic AD patients also exhibited this elevated production of Aβ. One study observed significantly increased levels of three major biochemical markers of AD: Aβ, aGSK-3B, and p-tau in iPSC-derived neurons [12]. iPSC-derived neurons from one sporadic AD patient exhibited significantly higher levels of Aβ compared to a control and another sporadic AD patient, which indicates the variability of genetic expression even within the same category of AD [12]. In the same study, iPSC-derived neurons from patients with familial AD resulting from a duplication of the *AβPP* gene also showed significantly higher levels of the aforementioned biochemical markers [12]. This provides promising evidence that iPSC-derived models can be used to study the etiology of AD, as well as test potential treatment *in vitro.* However, several studies have created models of small sample size, especially in regards to sporadic AD, which are insufficient to fully understand the frequency of certain genomes that can generate neuronal phenotypes in this form of the disease. Thus, a larger sample size should be used to create an iPSC-derived neuron disease model, and current obstacles regarding time for cell maturity still remain to be overcome [12]. In addition, future molecular genetic studies need to be completed in order to fully understand the different variants in the genome of sporadic AD patients as well as understanding whether neurons themselves are altered or if other cell types play a role in the onset of AD [12].

In this regard, a collaborative project between our lab and a NCI, NIH lab has also recently contributed to the investigation of AD/ND pathogenesis and used IPSCs to help model disease [30]. Rather than focusing on neuronal neurodegeneration as an initiating event in ND pathogenesis [10], we hypothesized that astrocytes undergo an early senescence in AD and amyotrophic lateral sclerosis (ALS) patients setting up an unhealthy "neighborhood" in the brain. By manipulating IPSCs to develop into neurons we tested late passage ("old") astrocytes and early passage ("young") astrocytes in co-culture experiments and found that the late passage astrocytes produced neurotoxic cytokines that induce apoptosis in the human IPSC generated neurons. This *in vitro* phenomenon could be altered to promote survival or enhance death by driving or blocking expression of variable key p53 isotypes in the astrocytes. Because these isotypes are not expressed normally in rodents, using human IPSCs was the only way to study and show this change in an *in* vitro model. We are currently screening molecule libraries to find agents that can make this

molecular switch in p53 isotypes as possible ND therapeutics and screening the compounds using IPSCs.

It is clear that the use of iPSC-derived neurons and glia will help to fill in the gap of defining molecular pathogeneses for the different forms and stages of AD and other NDs.

Therapeutic applications of iPSCs

Since, the hallmark findings in AD include Aβ deposition and neurofibrillary tangles (NFTs) caused by abnormal tau proteins, many interventions are selected for as a means to eliminate either or both pathological findings [10]. Furthermore, both factors may contribute to the loss of neuronal-interconnectivity that ultimately results in neuronal cell death. It follows that any agent with the ability to ameliorate these findings could potentially be beneficial in slowing the disease progression of AD. However, the isolating presence of the blood brain barrier (BBB) makes therapy selection more difficult because it excludes many systemic therapeutic agents from physically reaching the brain. Fortunately, creative uses of iPSCs may allow for circumvention of this barrier [2, 28].

NSC and sNEP

A recent study investigated the use of NSCs as a means to directly synthesize therapeutic agents within the brain. Through modifying NSCs to over-secrete neprilysin (sNEP)-a specific enzyme that degrades Aβ-investigators hypothesized that transplantation of these NSCs could slow the progression of AD [2]. Using mouse models of AD and stereotactic transplantation approaches, sNEP-secreting NSCs were transplanted into the mouse's hippocampus or subiculum while non-modified NSCs were transplanted into the contralateral locations. Following sacrifice, researchers demonstrated that the sNEP-secreting NSCs successfully implanted into both transplantation locations, and that these structures showed a significant decrease in the Aβ plaque density when compared to the control contralateral transplant sites. Additionally, nearby regions, including the amygdala and medial septum, showed regression of Aβ deposits, which provides proof-of-concept for NSCs that secrete sNEP and decrease Aβ deposits in both proximal and distal structures [2]. As Aβ is only one factor in AD progression, the researchers also noted a 31.8% increase in synaptic density in the subiculum, but this synaptic density increase did not occur in the medial septum or amygdala [2].

iPSCs and MWM test

A 2015 study investigated the link between stem cell transplantation and cognitive change. Researchers used human iPSCs to derive neural progenitor cells and subsequently transplanted them into a mouse model of AD [5]. Then, the group measured the change in cognitive ability through the well-accepted Morris water maze test (MWM). The experimenter's adaptation of the MWM test concealed a platform submerged slightly under water in a small pool. The mouse was placed in one of the four quadrants of the pool and taught to find the submerged platform using visual clues. By measuring the amount of time the mouse used to orient itself and determine the location of the platform, the experimenters had a quantitative approximation of learning and memory [5]. Specifically, in this experiment, the mouse population that received the neural progenitor cell transplant used significantly less time to find the platform than the control group did. Ultimately, this increased ability to quickly complete the MWM test indicates an improvement in the cognitive dysfunction that is associated with both AD and other forms of dementia [5]. While a multitude of experiments on specific biochemical markers of AD pathology show therapeutic promise, the ultimate goal of any human intervention is to reverse or slow the symptomatic progression of human AD. By using iPSCs to derive neural progenitor cells and then quantifying a symptom that relates to the patient's quality of life, this experiment effectively focused on a more clinical aspect of AD stem cell research.

Use of iPSCs of personalized/precision medicine

Along with the promising use of iPSCs in creating ideal disease models to further our understanding of AD, is the possibility of their use in precision medicine. Conventionally, treatments have been derived through preclinical research studies using mouse models, but these models exclude the patient-unique genetic information of AD treatment. Great variability in the bio-

chemistry of neurons has been observed based on the patient's unique genetic make up, a finding paramount in the further understanding of sporadic AD [12]. Currently available drugs for AD, claim to boost neurotransmission or protect cells from neuronal excitotoxicity yet often only provide temporary clinical symptomatic relief in patients [35]. With the promising effectiveness of iPSC-derived neuron models, studies are currently investigating the development of treatments that can alter the disease course in patients, especially if patient-specific neurons will recapitulate variability in individual genetic backgrounds [1].

Conclusion

iPSCs are becoming an important tool in advancing both AD modeling and therapeutic intervention. By utilizing these cells as models to gain a greater understanding of the etiology of familial and sporadic AD, researchers are honing in on the intricate molecular mechanisms and therapeutic targets of these conditions. Naturally, as more is understood about the causes of both types of AD, there are more potential uses of iPSCs as interventional agents. This promising new frontier in iPSC research combines the benefits of pluripotent cells with the inherent advantages of using induced cells instead of embryonic cells. iPSCs eliminate the ethical concerns related to embryo destruction, and they can be obtained without depending upon the naturally limited harvest of ESCs. Perhaps the most powerful advantage of iPSCs-both for modeling and clinical intervention-is unique to the field of personalized, precision medicine. Namely, iPSCs eliminate most of the immunological problems that may arise from other forms of stem cell therapies since they are autologous and genetically identical to the patient.

Nonetheless, challenges do remain in iPSCs' development, use in disease modeling, and therapeutic application. Animal research showing the efficacy of ESCs as potential clinical agents is promising for the future of iPSC-based medicine, but how well ESC-based experimentation will immediately translate into iPSC human therapy remains to be established. At the moment, ESCs show proof-of-concept for iPSC therapy, but they do not completely demonstrate identical properties between the two. Further, there is debate regarding the tumorigenic properties in stem cell therapy overall with specific concern to the use of viral vector iPSCs. Fortunately, the gap in the differences between these two types of stem cell lines continues to narrow, and it is quite feasible that iPSCs will soon be as close to perfectly interchangeable with ESCs as is possible. Until we reach this point, ESCs will continue to be heralded as the gold standard for true pluripotency. As the scientific community moves closer to complete mimicry of ESCs with IPSCs, more researchers will find it prudent to pursue the ethically less controversial and therefore more clinically relevant iPSC-based experimentation.

Once these challenges are addressed, iPSCs have the potential to bring in a new era of AD treatment. From unlocking mysterious aspects of neurodegeneration to acting as a means of precisely individualized intervention, the inherent blank canvas characteristics of these cells will soon give the medical community an unprecedented level of control and creativity in disease therapy.

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