The Future of Neuroregeneration

Hanifa Mohammed

Abstract

As the average lifespan for humans is growing, neurodegenerative diseases are becoming more common. The two most common neurodegenerative diseases are Alzheimer's disease (AD) and Parkinson's disease (PD). Both of these diseases cause the progressive degeneration of the central nervous system (CNS). To combat the effects of these disorders it's crucial to be able to regenerate the lost neurons. The issue is that cells in the brain reduce their plasticity as age increases, leading to little to no regeneration of the lost cells. Induced pluripotent stem cell (iPSC) is a new avenue of research regarding neuroregeneration as the ability to specialize them into neurons provides a method to combat the progressive degenerative nature of neurodegenerative diseases. Research in AD has shown successful experiments in specializing iPSCs into glial cells and cholinergic neurons to improve memory loss in AD mice. Research in PD has shown a method to specialize iPSC to neurons and thereby obtaining patient-specific transplants. The transplants and their effects have been successful in many animal models, leading to the potential of clinical trials in the near future.

Neurodegenerative diseases are disorders in which the structure and function of the central nervous system (CNS) and/or the peripheral nervous system (PNS) are degraded. Neurodegenerative diseases arise in mid to late life, and with the increasingly aging population in the world it's predicted that more than 12 million Americans will have neurodegenerative diseases by 2030 (The Challenge of Neurodegenerative Diseases, n.d.). In mammals the neurons in the CNS do not spontaneously regenerate which leads to multiple complications related to diseases that affect brain or spinal cord repair. These complications lead to disorders such as Alzheimer's disease (AD) and Parkinson's disease (PD) (Huebner, E. A., & Strittmatter, S. M. (2009)). Currently there are multiple conceptual solutions to battle these disorders, all of which focus on replacement of lost neuronal cell bodies, or in other words neuroregeneration.

Studies have shown that the "lack of intrinsic regenerative ability of CNS axons" is due to the expression of adult neuronal genes (Nagappan, P. G., Chen, H., & Wang, D. Y. (2020)). Neuroregenration of CNS neurons requires tenascin-binding integrin to assist in the organization of the extracellular matrix glycoprotein, tenascin, which modulates cell adhesion (Tucker & Chiquet-Ehrismann, 2015). The tenascin-binding integrin is not made in a fully differentiated adult neuron cell and thus adult neurons cannot undergo mitosis and produce more neurons (Nagappan, P. G., Chen, H., & Wang, D. Y. (2020)). Embryonic axons can make tenascin-binding integrin as they remain undifferentiated, meaning they have better chances to grow new neurons in the CNS than adult axons (Nagappan, P. G., Chen, H., & Wang, D. Y. (2020)).

The use of pluripotent embryonic stem cells (ESC) could be the answer to treat many conditions that often have only palliative care as a treatment. ESCs though come with ethical limitations since they are derived from human embryos. Induced pluripotent stem cells (iPSC), are a novel system that can overcome this limitation by generating a pluripotent stem cell from a somatic cell, a cell that is not a gamete or an undifferentiated stem cell. They also have the added benefit of being created per-patient so that immune system rejection is minimized. In the laboratory, iPSCs have shown great promise in regenerative medicine as they can give rise to any cell type in the body, including neurons (Fig 1).

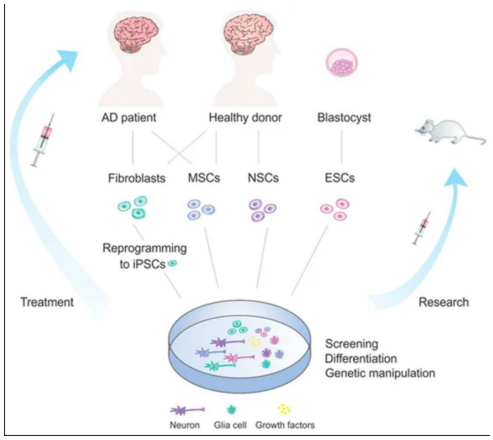

Figure 1: Cells can be gathered from the patient in concern. They can then be reprogrammed to iPSCs and used to differentiate into the desired cell for treatment. This creates iPSCs specifically tailored to the patient, thus reducing chances of immune rejection. Image from Vasic et al., 2019.

With the growing interest in the possibilities iPSCs provide to many neurodegenerative diseases, many researchers have begun to investigate how iPSCs can mitigate the two most common neurodegenerative diseases Alzhemier's Disease (AD) and Parkinson's Disease (PD).

AD is the most common neurodegenerative disease, with a predicted 6.2 million people in the US alone suffering from the disease (Alzheimer's Disease Questions and Answers, n.d.). AD is sporadic and age-related and results in the gradual deterioration of cognitive functions . The brain of an AD patient has a noticeably reduced volume (Fig 2).

This reduction in brain volume was particularly centered at the hippocampus, which is responsible for learning and memory, the loss in volume was attributed to death of neurons and degeneration of synapses. Moreover, research suggests the unique occurrence of amyloid plaques, aggregated misfolded protein that cannot be broken down by the body, in the extracellular space of the AD brain is a causative factor in the development of this disease (Vasic et al., 2019;Cha M. Y. et al. (2017)).

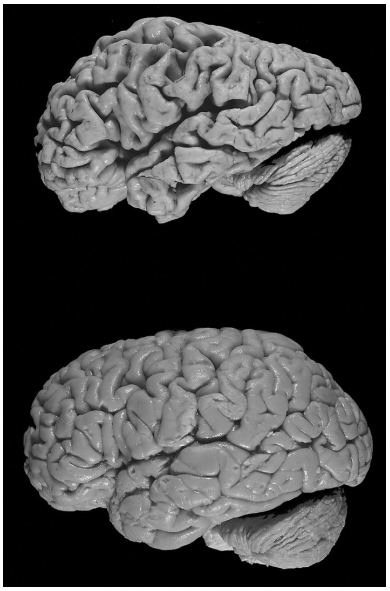

Figure 2: The brain volume of an AD patient (top) as compared to the brain volume of a normal patient (bottom). Image from: Hersenbank. (2008).

In order to study how iPSCs could reverse the effects of amyloid plaques, a study by Fujiwara et al., treated transgenic mice with amyloid plaque build up with an iPSC therapy.

Firstly, amyloid plaques have been duplicated in PDGF promoter driven amyloid precursor protein (PDAPP) transgenic mouse models. PDAPP is a mutant of the human amyloid precursor protein (APP) which results in the formation of amyloid plaques, and the buildup of amyloid plaques when mutated.

Now that the transgenic mice had a build up of amyloid plaques Fujiwara et al. implanted human iPSC into these mice and attempted to regenerate cholinergic neurons, common nerve cells that serve as acetylcholine neurotransmitters, to ascertain the role iPSC cells may have in reversing the buildup phenotype.

The iPSC cells that were injected had been specialized into neuronal precursors that would display a cholinergic neuron phenotype and were injected into the bilateral hippocampus of mice that had high levels of amyloid plaques (Fig 3A-C) (Fujiwara et al., 2013). As a control, some PDAPP mice were injected with PBS (Fujiwara et al., 2013).

In order to measure neuroregeneration, the spatial memory function, a skill that utilizes the hippocampus, of the PDAPP mice was measured prior to and after the injection at various intervals (Fujiwara et al., 2013) (Fig 3D-G).

If the spatial memory function of the PDAPP mice, with the transplanted iPSC cell, improves after the injection, it's indicative of neuroregeneration in the hippocampal region which could point to reversal of AD phenotype (Fujiwara et al., 2013).

Upon grafting the neuronal precursors into the mice, it was noticed that they dispersed throughout the hippocampus and became cholinergic and

GABAergic neurons (Fujiwara et al., 2013). GABAergic neurons are hypothesized to have similar growth conditions as cholinergic neurons and as a result have also regenerated (Fujiwara et al., 2013). Regardless, the spatial memory function of the mice with the iPSC transplantation was seen to improve after the grafting (Fujiwara et al., 2013) (Fig 3D-G).

The exact mechanisms involved in the improvement of spatial memory function in these mice are unknown. Although, it is thought that the "neuronal precursors reconstruct neural networks essential for spatial memory function" (Fujiwara et al., 2013). This implies that iPSCs can be used to alleviate and delay the symptoms of AD.

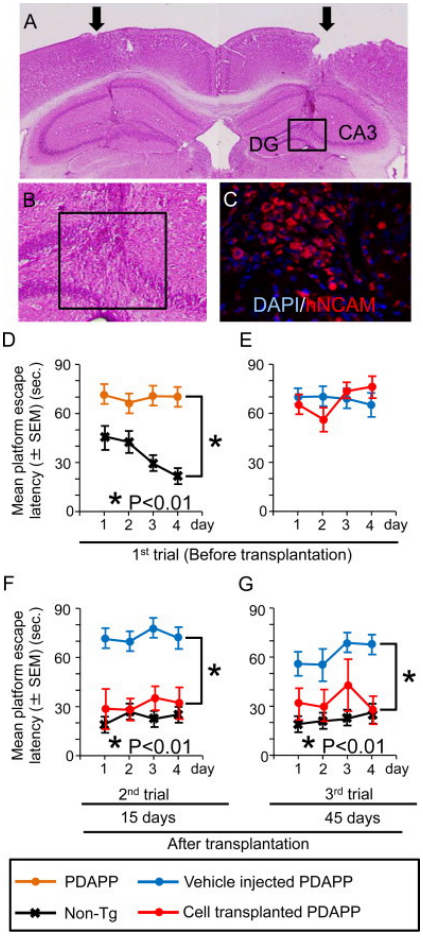

Figure 3: iPSCs were transplanted in the hippocampus as indicated by the black square on panel A and B. The neurons were fluorescently labelled by DAPI to check for cell viability as seen n panel C. Mean platform escape measures the time it takes for the mice to escape a maze, effectively measuring spatial memory. Before the transplantation both the PDAPP with the iPSC cell transplantation and the control with the PBS have similar, comparable spatial memory (D-E). Within the second and third trial the PDAPP with the cell transplantation shows an improved spatial memory (F-G). Image from Fujiwara et al., 2013.

Another study, Cha et al., has shown that iPSCs can be used to prevent neurodegeneration without regenerating neurons. Research on neuron-astrocyte interactions has suggested that glial cells take part in regulating nerve activity and intracellular signalling by releasing neuromodulatory factors. (Cha M. Y. et al. (2017)). Glial cells can be a causative factor to AD as their hyperactivation leads to formation of excess amyloid plaques (Types of glia. (2016)).

5XFAD is a transgenic mouse model that has five mutations that result in formation of amyloid plaques (Shin et al., 2021). Protein-iPSCs are adult cells that were injected with the proteins of an ESC, resulting in the adult cell gene expression being that of an ESC. Protein-iPSCs is another method of obtaining iPSCs. Cha et al., injected protein-iPSCs into 5XFAD mice for the purpose of improving AD pathogenesis, and specifically focusing on their effect on amyloid plaques. To test the effects of protein-iPSCs on the 5XAD mice, spatial memory functions were tested. Mice with the injected protein-iPSCs displayed better spatial memory then those mice with no injected protein-iPSCs (Cha M. Y. et al. (2017)). Furthermore, these protein-iPSCs were found to have become glial cells, mostly oligodendrocytes, implying that healthy oligodendrocytes, which create support for axons in the CNS by producing myelin, may “improve memory function by maintaining axonal integrity” hence reducing the neurodegenerative effects of the AD (Cha M. Y. et al. (2017)).

Parkinson’s Disease (PD) is another neurodegenerative disorder that affects more than 10 million people worldwide (Statistics, n.d.). PD involves the degradation of the dopaminergic neurons of the substantia nigra pars compacta (SNc) (Elkouzi et al., 2019). This degradation of dopaminergic neurons cuts off the connection between the SNc and the striatum, effectively reducing dopamine source. This results in the distinct tremors, rigidity, bradykinesia and postural instability that is noticeable in PD patients (Elkouzi et al., 2019). The challenge with PD is the heterogeneity of the disease due to common genetic variants (Greenland et al., 2019). This results in difficulty in finding one treatment that works for all PD patients, so to effectively treat PD a method to establish individualized treatments is necessary (Elkouzi et al., 2019). While it’s possible to take ESC and specialize them to dopaminergic neurons to replace lost dopaminergic neurons, it’s difficult to reach the desired level of specification that is required for an adept PD treatment (Elkouzi et al., 2019).

iPSCs were used to establish a method to treat PD subtypes that arose from the common genetic variants. Because iPSCs can be generated from the patient, individualization of the treatment is possible.

The blood of PD patients has been collected for research into the disease for a long period of time, resulting in a large data bank of different types of PD haplotypes (Deleidi et al., 2011). This large data bank can be used to create iPSC lines specific to variants of PD.

Deleidi et al., used Mauritian cynomolgus macaques (CM) to generate iPSCs. CM has seven holtypes, so if iPSCs can be generated by all seven haplotypes and still be able to regenerate neurons, then this process would indicate that individualized treatment for PD is plausible (Deleidi et al., 2011).

CM iPSCs went through in vitro differentiation into dopaminergic neurons (Deleidi et al., 2011). 400,000 differentiated iPSCs were then transplanted into the striatum of 6-OHDA rats (Deleidi et al., 2011). 6-OHDA rats is an animal model that has neurotoxin-induced neurodegeneration, behavioral deficits and motor dysfunction to model PD in a rat (Simola et al., 2007). These models were used to examine how the PD phenotype would change after iPSC transplant.

Upon examining the dopaminergic neural graft after 4-16 weeks post-transplantation, it was found, through amphetamine and apomorphine responses, that the dopaminergic neurons were able to rebuild the connection between the striatum and SNc that 6-OHDA rats did not have (Deleidi et al., 2011) (Fig 4). The disconnection between the striatum and SNc is a common cause of PD in humans So this finding implies the possibility that iPSCs could be used to create dopaminergic neural grafts and combat the pathogenesis of PD (Deleidi et al.). Having successfully repeated this experiment on monkeys, human clinical trials are the next step in creating an adept treatment for PD (Deleidi et al., 2011). Kyoto, Japan has begun clinical trials, which have shown both long-term survival and good integration into brain networks (Elkouzi et al., 2019).

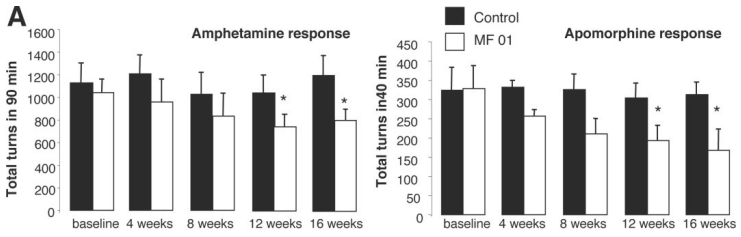

Figure 4: The amphetamine and apomorphine levels were recorded between before transplantation of the iPSCs and after the said translation at weeks 4, 8, 12, 16. It is clear that both amphetamine and apomorphine decrease after transplantation. Image from Deleidi et al., 2011.

To conclude, iPSCs show great promise in neuro-regeneration due to their autologous translatability and pluripotency. When it comes to treating neurodegenerative diseases, iPSCs can be used to regenerate or restore lost and damaged brain networks. Alzehimer’s Disease, the most common neurodegenerative disease, is marked with amyloid plaques, which aid in progression of the disease.

The effect of these amyloid plaques can be removed by regenerating cholinergic neurons at the hippocampus, helping restore lost neurons and allowing the individual to retain their memory and learning skills. Furthermore, the effect of amyloid plaques can also be mitigated by protein-iPSCs turned glial cells. Glial cells, like oligodendrocytes, play a major role in regenerating axons in the CNS. Parkinson's Disease, another common neurodegenerative disease, is heterogenous and so no one treatment exists to help all those who are suffering from it. To develop a method for individualized and personalized treatment, iPSCs were used to create custom dopaminergic neural grafts. These grafts could then be transplanted into the striatum where they rebuild the connection between the striatum and SNc that many PD patients lack. This experiment yields high success rates in animals, and so it moves onto clinical trials. Many researchers have been using iPSCs to both regenerate neurons as well as other cells that slow down the degeneration of neurons.

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