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Recent advances in stem cells therapy: A focus on cancer, Parkinson's and Alzheimer's

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ABSTRACT

Stem cells serve as potential therapeutics due to their high proliferative capacity, low immunogenic reactivity and their differentiating capabilities. Several pre-clinical and early-stage clinical studies are carried out to treat genetic diseases, cancers and neurodegenerative disorders with promising preliminary results. However, there are still many challenges that scientists are trying to overcome such as the unclear expression profile of stem cells *in vivo*, the homing of stem cells to the site of injury and their potential immune-reactivity. Prospective research lies in gene editing of autologous stem cells *in vitro* and safe injection of these modified cells back into patients. Here, we review the clinical trials executed using stem cell therapy in an attempt to cure challenging diseases like cancer, Parkinson's and Alzheimer's diseases.

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1. Introduction

Stem cell therapy is emerging profoundly, raising hopes for curing diseases that were once thought to be incurable. Founding concepts behind the use of stem cells in therapy include their ability to

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regenerate original body tissues, their ability to be modified to deliver potent drugs or nanomaterials, and their immune modulation capability [1].

According to the National Cancer Institute, it is estimated that 1,735,350 new cases of cancer will be diagnosed in 2018 in the US and 609,640 patients will die from cancer [2]. Scientists spare no effort to deeply understand the biology of tumors to develop new cancer treatments. However, major challenges include insufficient and unspecific delivery of drugs to target sites, in addition to their short half-lives [3]. Stem cell therapy has recently arisen as a promising means to tackle such challenges.

Slowly progressive, degenerative neurological diseases such as Parkinson's disease (PD) and Alzheimer's disease (AD) elicited the urge for novel therapeutics. Research in the last couple of years was shifted towards transplant therapy for these diseases and stem cells therapy is emerging to alleviate symptoms or even reverse disease progression [4–6]. Here, we review the latest stem cell therapy advances in treatment of cancer, Parkinson's disease and Alzheimer's disease.

2. Stem cells in treatment of the disease of the century

Scientists have employed different strategies to make use of stem cells in combating cancer. For example, stem cells were modified to express anti-cancer effector proteins such as pro-apoptotic and anti-proliferative proteins or express anti-angiogenesis factors to limit cancer cells' blood supply and create a non-supportive microenvironment for the tumor [7–9]. Other strategies include modification of stem cells such as Mesenchymal Stromal/Stem Cells (MSCs) that naturally show tumor-tropic properties, to stimulate the immune response against cancer [10]. For example, human MSCs were modified to secrete IL-12 and IL-18, shifting the immune response against cervical cancer, renal cell carcinoma and glioblastoma in mice through the activation of natural killer cells and tumor-specific T cells [11–14].

2.1. Stem cell-mediated suicide gene therapy

An interesting strategy to achieve cancer cell death involves genetic engineering of stem cells to express specific enzymes that convert inactive forms of anti-cancer drugs into their active forms only at the tumor site. This strategy that confers high specificity and efficacy is known as stem cell-mediated suicide gene therapy.

Three main suicide gene systems are utilized: (1) Conversion of 5-fluorocytosine (5-FC) to the toxic anti-metabolite 5-fluorouracil (5-FU) via cytosine deaminase (CD) enzyme, (2) Conversion of the inactive Irinotecan drug to the topoisomerase inhibitor SN-38 by carboxylesterase enzyme, (3) Conversion of the Ganciclovir (GCV) drug to its phosphorylated forms to block DNA synthesis through the action of the thymidine kinase enzyme.

The three systems have been used to modify MSCs, and showed efficacy in treatment of brain, breast, ovarian and prostate cancers in mouse models [10,15–17]. In another study, neural stem cells were modified to express the CD enzyme in addition to IFN-β that induces apoptosis. The cells were then injected in mice with metastatic breast cancer and subsequently, the inactive 5-FC prodrug was injected to the mice. Results showed that the combined treatment suppressed the growth and metastasis of the cancer cells [18]. Moreover, 18 patients with high-grade glioma were enrolled in a clinical trial that has started in 2014 and is expected to be complete by 2019. The tumors were surgically resected and the patients' brains were injected with neural stem cells which were modified to express the CD enzyme. After a few days of the cellular injection, the patients were treated with 5-FC, which was then converted into 5-FU (Clinicaltrials.gov, NCT02015819).

2.2. Stem cells as delivery vehicles for oncolytic viruses

Oncolytic viruses confer selectivity to tumors since they replicate within cancer cells only. However, the host immune system rapidly recognizes the virus and clears it, reducing its therapeutic efficacy. Stem cells have been studied as potential delivery vehicles for these viruses, showing promising results in pre-clinical studies.

MSCs have been used as effective carriers of attenuated oncolytic viruses to hepatocellular carcinoma and ovarian tumors in mice and humans, respectively [19,20]. MSCs selectively localized to the tumor xenografts in mice and infection of tumor cells with the viruses was subsequently confirmed. Tumor volumes were significantly reduced and the survival rate of mice increased remarkably [21]. In 2014, MSCs were loaded with oncolytic Herpes Simplex Virus (oHSV) which increased the anti-tumor efficacy of the virus in a mouse model of glioblastoma compared to the direct injection of the oHSV [10,22].

In a study performed in 2010, researchers explored the efficacy and safety of injecting autologous MSCs, which were genetically modified to carry oncolytic adenovirus (ICOVIR-5), in four young patients who suffered from metasatatic neuroblastoma. Fortunately, one case out of the four showed no evidence of metastatic disease and was in remission for three years post-treatment [23].

The first oncolytic virus accepted by the FDA was the Talimogene Laherparepvec (Imlygic®), or T-VEC which was used for the treatment of melanoma [24]. Since the microenvironment of tumors is relatively "cold"; lacking immune cells, it has been recently postulated that the oncolytic viruses can induce a systemic immune response through secreting danger signals. These signals turn the local microenvironment of tumors into hot areas of immune cells recruitment [25,26]. For example, pre-surgical treatment with oncolytic viruses was shown to sensitize triple-negative breast and brain cancer cells to immunotherapy [27,28]. Moreover, a recent clinical study showed that combining T-VEC oncolytic virotherapy with immunotherapy increased the infiltration of T cells to the tumor vicinity [29].

2.3. Stem cells role in post-cancer treatment

In hematological cancers, bone marrow transplantation is a conventional protocol used to restore the cellular components of the blood after chemotherapy rounds. However, a major drawback associated with the use of bone marrow transplantation is the development of immune reactions such as graft-versus-host disease (GvHD) [30]. This immune condition is treated with immunosuppressive drugs, which unfortunately have adverse effects and can be toxic to patients [31,32]. It was shown that MSCs suppress lymphocyte proliferation, manifesting immune suppressive properties and thus, they are being tested as co-therapy with Hematopoeitic Stem Cells (HSCs) to prolong the graft survival of the HSC transplant. The MSCs immunomodulatory mechanisms include inhibition of T cell proliferation and induction of T regulatory cells [33]. In addition, it has been shown that MSCs provide a cellular support for the HSCs niche [34]. In a phase I/II clinical trial assessing the effect of MSCs on GvHD acute and chronic cases, complete response was achieved in one acute and one chronic case, partial response was achieved in six acute and three chronic cases, and no major adverse effect was observed after MSCs therapy [35].

2.4. Potentiating the effect of stem cell-based therapy in fighting cancer

Despite the promise that stem cell-based cancer therapy holds in cancer treatment, some challenges still exist. For instance, the use of allogenic cells can mount severe host immune response, limiting their therapeutic potential. In 2013, a group of researchers

managed to edit HLA genes in human Embryonic Stem Cells (hESCs) *in vitro* using zinc-finger nucleases, enabling the ESCs to evade the HLA-restricted cytotoxic T-lymphocytes. This paved the road towards generating universal cells from allogenic donors [36]. Also, in a study in 2014, CTLA4-Ig fusion protein and PD-L1 were knocked in hESCs to allow for their constitutive expression before and after differentiation. The knock-in of CTLA4-Ig disrupts the co-stimulatory pathways and that of PD-L1 activates the inhibitory pathways of T cells. Therefore, the modified hESCs were immune-protected when injected into humanized mice. These humanized mice were reconstituted with a human immune system that normally elicits an immune reaction against hESCs. This finding can lead to developing ways to protect hESCs from allogenic immune rejection without the need for systemic immune suppression [37].

The tumor environment is a heterogeneous pool of cells baring different mutations. In addition, cancer cells tend to gain resistance to therapies, which adds up to the complication of fighting cancer with a single type of therapy [10,38]. Therefore, the need for combinatorial therapy arises. As previously discussed, the combinatorial therapy strategies could include combination of immunotherapy with oncoloytic virotherapy [18]. Other strategies include combining radiotherapy, chemotherapy and oncolytic virotherapy [10,39,40].

3. Stem cells against neurodegenerative disorders

3.1. Stem cell therapy for Parkinson's disease (PD)

PD is the second most common neurodegenerative disease, that affects 2–3% of the elder population PD is characterized by the loss of dopaminergic nigral neurons, formation of α-synuclein-containing Lewy bodies and extensive extra-nigral pathology [41–43]. Its symptoms include motor and non-motor features [44] that respond well to dopaminergic agents in the early stages. However, these medications fail overtime and produce adverse effects, such as dyskinesia and neuropsychiatric complications [45].

In 1987, a team led by professor Madrazo recognized neural grafting as a novel approach for replacing lost dopaminergic cells. Adrenal medulla tissues were autografted into the brain of two young PD patients, which led to the amelioration of PD signs including tremors, rigidity and akinesia. Neural transplantation and cell-based therapy have, since then, been considered as possible therapies for PD since it is a good candidate as a focal degeneration disorder [46]. This study was supported by a pilot study held 2 years later on 18 patients confirming Madrazo's results. However, this approach was stopped due to limited pre-clinical data and patients developing post-operative psychiatric disturbances [47].

Studies on neural transplantation continued during the 90s but were conducted using different source of cells: fetal ventral mesencephalic (fVM) instead of adrenal medulla. Earlier studies showed promising results; however, the technique wasn't yet optimized [47]. In 1993, the NIH funded two trials, where the enrolled patients with moderately advanced PD were grafted with human fVM. The results were published in 2001 followed by another one in 2003 conducted as a double blind placebo control trial [48,49]. Both trials reached the same conclusion that human fVM transplants didn't ameliorate the symptoms of PD compared to the dopaminergic medications, in addition, the patients exhibited Graft-Induced Dyskinesia (GIDs) [50–52]. Although in these trials some of the subjects showed encouraging signs of improvement, the consensus at that time was to discard this approach.

New approaches were pursued to find better source of cells for transplantation. The emergence of human embryonic stem cells (hESCs) in 1998, unlocked the scope for several research teams to generate dopaminergic neurons [53–55] that functioned *in vitro* or in animal models of PD [48,56–58]. Although hESCs provided unlimited source of cells, they failed to produce proper midbrain dopamine (DA) neurons resulting in little improvement in addition to tumor formation in incompletely differentiated cells [59,60].

The failure of hESCs was owed to the fact that the DA neurons have been erroneously generated. This was discovered in 2007 and 2008, when two studies reported that the DA neurons are not derived from neuroepithelial cells like all neurons but from a different source of cells [61,62]. This insight inspired researchers to develop new hESCs differentiation protocols to generate the correct DA neurons [63–66]. Hence, the floor-plate-derived cells expressed specific markers of midbrain DA neurons, released dopamine and restored the motor functions after transplantation into rodent models of PD similar to that attained by human fetal DA neurons [64,66,67].

Clinical trials with stem cell (PSC)-derived DA neurons have witnessed a new and an exciting era of stem cell-based therapy for Parkinson's disease. Therefore, guidelines and strategies were set for clinical translation to patients [68–70]. For instance, in 2018, a group of Chinese researchers could transplant neural-precursor cells derived from embryonic stem cells in PD patients. In this trial, the cells are intended to develop into mature dopamine-producing neurons [71]. Also, Australia have witnessed the world-first neural stem cell transplant by lead researcher Dr Andrew Evans, Royal Melbourne Hospital (RMH) Neurologist, and his team. Tens of millions of parthenogenetic neural stem cells were transplanted directly into the brains of 12 Australian patients suffering from moderate to severe PD as part of a phase I safety clinical study (Clinicaltrials.gov, NCT02452723).

The generation of the induced Pluripotent Stem Cells (iPSCs) by Takahashi and Yamanaka, provided a source of patient-specific and disease-specific neurons and avoided many of the ethical issues associated with the hESC lines [72,73]. Although there is a vast development in iPSC research, a recent commentary discussed the major shift in the main goals of iPSC research from personalized cell therapy to a tool for mechanistic studies of human diseases [74]. That is the reasons why clinical trials using iPSCs are scarce.

In 2017, Kikuchi et al., a Japanese team established a successful protocol for transforming iPSCs derived from both healthy individuals and PD patients, into dopamine-producing neurons. They grafted human iPSC-derived midbrain dopaminergic progenitors into monkeys that had been treated with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a toxin which ablates nigral dopaminergic neurons. They continued to monitor their behavior for two years in an attempt to alleviate PD symptoms [75]. A major boost to researchers' hope was attained as the monkeys survived with improved motor function and crucially no signs of tumor development for both sets of cells: derived from healthy individuals and PD patients [75]. These results fortified the confidence of this team to test this approach on humans.

3.2. Stem cell therapy for Alzheimer's disease

Alzheimer's disease (AD) is a progressive neurodegenerative disorder, affecting more than 46.8 million elders above the age of 65 worldwide. AD is considered the main cause of dementia. It is associated with impairments of memory, thinking, language, and reasoning [76–79]. AD is considered challenging as it involves extensive attacks on neurons in the brain [80]. The accumulation of Amyloid beta (Aβ peptides), lipid-carrier protein apolipoprotein E (apoE), microtubule associated protein Tau, and the presynaptic

protein α-synuclein are enumerated as the prominent neuropathological symptoms of the AD [79,81–83]

As a result of the significant neuronal loss in the brain at the time of diagnosis, the treatment interventions for AD patients with the available drugs are considered too late. Therefore, new pharmaceuticals that target earlier stages before widespread neurodegeneration and overt dementia occur, are crucially needed [84]. Despite the long-term focus on the pathology of AD, still no efficient treatment that can stop or reverse the progression of the disease is present. Moreover, symptomatic treatments are modestly effective and offer only temporary benefit. In this view, the stem cell therapy such as ESC or iPSC-derived neural cells emerged from the early 2000s as a potential idea to replace destroyed neurons beyond the aid of pharmacological therapies [85].

Stem cell therapy as an approach to treat AD was first tested on animal models [84]. Neural stem cells (NSCs) is an example of cell therapy derived from ESCs, which aims to restore the function of the damaged nervous system. For example, NSCs derived from neonatal rats' hippocampus were implanted into the brain of AD rats and were able to differentiate into the new cholinergic neurons and improved the ability of spatial learning and memory for AD rodent models [86]. Also, neuron-like cell (NLC)-derived mouse embryonic stem cells (mESCs) that were injected into AD rats resulted in the repair of brain lesions [87].

Human NSCs (hNSCs) were also transplanted intracerebroventricular in transgenic AD mice models to ensure the wide spread of the engrafted cells to repair the multiple affected regions of the brain [88,89]. The study revealed migration, engraftment and differentiation of hNSCs into three CNS neural cell types. The developed synaptic plasticity and anti-apoptotic function, also decreased tau-phosphorylation, Aβ production, and neuroinflammation. The transgenic mice showed long term survival, no adverse effects were shown and the mice had an improved spatial memory. However, the cognitive recovery following hNSC grafting was not maintained in the long term [89]. These results were further proved in a similar study where the cognitive function of the mice was improved through synaptogenesis [88,89].

In addition, scientists transplanted human umbilical cord mesenchymal stem cell-derived neuron-like cells (HUMSC-NCs); another stem cell source into AD mice. This activated microglia (M2-like microglia) and its associated anti-inflammatory and immune-modulatory responses that beneficially enhanced the cerebral function, augmented synapsin I level, and reduced Aβ deposition in the mice [90]. In another study, systemic transplantation of bone marrow-derived mesenchymal stem cells in AD mice showed reduction in pE3-Aβ plaque size and an immune-modulation effect [91]. Also, MSCs could be used for modulating adult neurogenesis endogenously, since co-culturing of Aβ-treated neuronal progenitor cells (NPCs): an AD disease model with MSCs stimulated hippocampal neurogenesis and enhanced NPCs differentiation into mature neurons [92]. Furthermore, placenta-derived mesenchymal stem cells improve memory dysfunction in an Aβ1-42-infused mouse model of AD [93]. AD mice models were also transplanted with precursors of cholinergic neurons which were derived from human iPS (hiPS) cells. The mice survived and showed improved memory function [94].

Owing to the promising results manifested in the pre-clinical trials executed on AD animal models using MSCs, they were sufficient to approve the initiation of clinical trials in patients with AD. A phase 1 clinical trial using human umbilical cord blood–derived mesenchymal stem cells (hUCB-MSCs) was first conducted in 2015 on nine patients with mild-to-moderate AD. In an attempt to treat AD, the patients were stereo-tactically injected with hUCBMSCs into the hippocampus and precuneus: the brain parts mostly affected during the earlier phases of AD. It was proven that the administration method of the stem cells was safe, practicable and showed no adverse effects; however, it should be further tested for its clinical effectiveness on AD pathogenesis. The study paved a new road for future AD cell therapies studies using larger sample size and placebo controls to test the efficacy of this treatment in the long term [95].

Several clinical trials are being conducted on AD patients and their results are yet to be published (Clinicaltrials.gov, NTC01547689, NTC02672306, NTC02054208, and NTC02600130). However, all these trials are limited by the diversity of the damage in the neurons of AD patients.

The most recent phase I/II clinical trial that started in 2017, is held by the biotechnology enterprise Nature Cell. It is conducted on 60 AD patients using 'ASTROSTEM': a stem cell drug for AD treatment which consists of stem cells derived from autologous adipose tissue and intravenously injected ten times into the patients (200 million cells/injection) (Clinicaltrials.gov, NCT03117738).

4. Challenges facing stem cell therapy

Although stem cells hold great promise in therapeutics, it is necessary to provide scientific evidence on the safety and effectiveness of their usage [96]. Moreover, several challenges are yet to be overcome in order to translate experimental lab studies into clinical applications [10]. One challenge that scientists face is the ability to correctly isolate and identify stem cells from the patients' tissues [97]. Stem cells need to be expanded on a large-scale to have a significant efficacy when injected into patients. The expansion and passaging of the cells might affect their phenotype and render the pool of the cells heterogeneous in their properties, which adds more challenge to systemize any genetic manipulation [98]. The stem cells also need to differentiate properly into the required cell type *in vitro* and maintain their cellular identity when transferred into patients; express the same genomic and metabolic profile [96].

Other issues include the use of viral vectors to integrate pluripotency genes into differentiated cells to reprogram them, which may increase the risk of tumor formation [99]. Thus, the safety of the oncolytic viruses carried on stem cells need to be well studied to avoid any complications in its clinical studies [10]. In addition, the epigenetic memory of the differentiated cell may confer repressive epigenetic mark that may not allow for the expression of the reprogramming factors [100]. Another issue is the difference between the niche of the host cells and that of the *in vitro* cultured cells, which decreases the proliferative and differentiating capacity [96]. Another major challenge is the immune rejection, where transplanted stem cells can mount severe host responses [101]. Finally, treatment of complicated diseases such as cancer or neurodegenerative disorders requires targeting multiple defective pathways simultaneously which hints at the necessity to search for combinatorial therapies. Selection of the right therapies to combine is still a big challenge that remains to be addressed [10].

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