Neurologic Stem Cells:

A Potential Therapy for Pathologies of the Brain

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Abstract:

The burden of neural degeneration is one that is becoming more steadfast in humans as we are beginning to live longer lives. Combating the entropy of being can, potentially, be accomplished using embryonic stem cells as they differentiate into neural stem cells via the exposure to certain mutagens like retinoic acid (RA), BMP2 antagonist noggin, and a variety of different tropic factors. Using these molecules in a precise manner can allow us to guide stem cells to the identity of our choosing, thus allowing us the opportunity to replace damaged brain tissues. The addition of RA, BDNF, and GDNF show to effectively increase dopamine production from progenitor cells, which has promising implications for patients suffering from Parkinson Disease. Huntington Disease also proves to be amendable to cell therapy as GABAergic neurons are efficiently regenerated in a rat striatum lesioned with quinolinic acid. Furthermore, multiple sclerosis benefits from the addition of stem cells as they secrete neuroprotective agents like CNTF that attempt to shield existing myelin from the attack of autoimmune antibodies. Stem cell research can be a fickle process that yields positive results less frequently than desired. However, we are beginning to know more about stem cells than ever before, and if we keep pushing the limits of our knowledge, the return will far exceed the investment.

Introduction:

Stem cells are best characterized by their capacity to differentiate into multiple types of cell lineages. Different degrees of this ability exist: totipotentcy, pluripotency, and multipotentcy. The first of which has the highest potential to produce progeny from all cell lineages. Cells of this classification only exist a few days after the formation of a zygote. As the zygote replicates and forms a blastocyst, this ability is lost; the cells of the inner cell mass are now pluripotent and can give rise to all three germ layers[1]. These cells are now deemed embryonic stem cells (ESCs), and they are the focal point of cell therapy due to their differential plasticity.

Stem cell research has exploded in the past couple of decades as the excitement regarding their potential continues to build. The optimism surrounding ESCs is predicated on their ability to serve as potential therapies for congenital and degenerative pathologies. The reputation of stem cells appears to be something of science fiction, however there appears to be a likely explanation for the presence of stem cells in the human body. It is hypothesized that the restorative ability of stem cells rests on their evolutionary upbringing. It seems probable that natural selection would favor individuals who possessed the capability to repair/replace damaged tissues from acquired lesions; thus, over time, this ability would become fixed in the human species[26]. All humans, young and old, have a certain number of adult stem cells (ASCs) spread throughout their body working constitutively with everyday functioning[2].

The supposition regarding the presence of stem cells dates more than a half a century prior to the verification of their existence. In the early 1920s, Alexander Maximow, a Russian embryologist, was puzzled by the idea of a single cell creating all the different types of cells found in the mammalian body. He envisioned that these cells must have some capacity to change or morph into other cell types, he referenced them as "polyblasts"[3]. Indeed, Maximow's idea was based on sound logic, but he had no idea that he had just described the ESCs that would later become a medical sensation. Even with Maximow's intriguing speculation regarding ESCs, it was not until 1981 that the first ESC lines were characterized from mouse embryos[4]. Even then, it was not until 1998 that the first derivation of human ESC lines were developed and subsequently documented[1,5].

Although ESCs are the highlight of cell therapy, there are two other broad classifications of stem cells. The first are the aforementioned ASCs which exists in all humans throughout their lifespan. The second type is the novel induced pluripotent stem cells (iPSCs). These are somatic cells that have been genetically altered to behave like ESCs with regards to pluripotency. The mechanism to accomplish this tells scientists a great deal about stem cells differentiation and will be discussed

later. iPSCs are an attempt to circumvent the ethical concerns surrounding ESCs. Other stem cells will be referenced; however, ESCs still show the most promise, thus this paper will emphasize these cells as they are the precursors to neural stem cells (NSCs).

Potential Treatments using ESCs and their Stem Cell Progeny:

ESCs represent a section of cells from the inner cell mass that has unlimited differential potential. They are essentially immortal as they can divide an infinite amount of times if they are not shunted into certain cell lineages. But, even when differentiation occurs, there are many subsets of multipotent stem cells that can be produced. These subsets, like hematopoietic and mesenchymal to name a couple, have the potential to treat a large list of diseases. This list may include diabetes, osteoporosis, and kidney, lung, liver, heart, and some autoimmune diseases[6].

Perhaps the largest interest regarding ESCs is their ability to differentiate into NSCs. By traveling the neural route on the cell pedigree, the stem cells are now able to serve as a likely cure for many congenital and acquired neurological diseases. These would include: Parkinson, Huntington, and Alzheimer's Disease, along with amyotrophic lateral sclerosis (ALS), multiple sclerosis (MS), spinal cord injury (SCI), and stroke[7]. An interesting finding from the research of NSCs is that the mechanisms of remedy seems to differ among certain pathologies. For example, treatment for Parkinson and Huntington Disease functioned best when the stem cells regenerated lost or degenerate neurons. Furthermore, patients suffering from MS showed the most improvement when the NSCs enriched the remaining neurons by the secretion of tropic factors[7-8]. As of now, scientists do not know why different conditions benefit better from different mechanisms of therapy. The best guess is that it has something to do with the stem cell differentiation in their respective niche, which highlights the paramount aspect regarding the attempt to harness their remedial power.

Stem Cell Differentiation:

An intuitive notion in utilizing stem cells for therapeutic purposes lies behind understanding the mechanisms of differentiation. Scientists are exploring two aspects of differentiation that supersede all others: the maintenance of pluripotency in ESCs and NSCs responses to different paracrine signals and transcription factors to specify neural subtype. The former is warranted if scientists are to continually grow ESCs that will then differentiate into NSCs, so this will be explored first.

Maintaining ESCs for Pluripotency:

The ability to grow ESCs in culture is predicated on the prevention of spontaneous differentiation. There is an interplay between many intrinsic and extrinsic factors that control cell morphology. There are several transcriptional homeoproteins that control differentiation, but the most imperative is Leukemia Inhibiting Factor (LIF)[9]. This cytokine is released by adjacent cells and functions via juxtacrine interactions. Notably, the undifferentiated cells seem to be incapable of secreting this, thus requiring the presence of fibroblasts or differentiated cells with this capacity[9].

The warrant of feeder layers, composed of cells that supply LIF, has been accomplished using mouse fibroblasts. This layer of nutrient-rich mouse cells has been effective at supplying LIF to keep the cells in an undifferentiated state, however it comes with threatening consequences[10]. If ESCs are to be commercialized, all potential malignancies must be eradicated. In the case of mouse feeder layers, the worry surrounding retroviruses should not be overlooked. To approach this concern, scientists have proposed using human feeder layers from infant foreskins, as they have shown to be effective at giving rise to fibroblast-like progeny that are capable of supplying LIF[10].

Although LIF is necessary to prevent differentiation in most ESCs, it is not exclusionary to other transcriptional regulators. Scientists have shown that bone morphogenic protein-2 (BMP2) increases the efficacy of LIF via synergistic mechanisms. BMP2 works to activate inhibitor-of-

differentiation (Id) genes via the Smad signal propagation pathway[9]. Due to the fact that many signals and pathways contribute to differentiation, BMP2 blocks one of these pathways and indirectly increase the expression of signal transducer and activator of transcription-3 protein (STAT3), which is activated via LIF and its propagatory pathway[10-11].

Previously, there was no known transcription factors that led to the maintenance of pluripotency without the help of LIF/STAT3 pathway. However, scientists have now discovered a protein that seems to control differentiation independent of LIF/STAT3[12-13]. After mutating the gp130 receptor that is the initiator of the STAT3 pathway, the addition of homeoprotein Nanog was observed to maintain pluripotency 60% better than LIF even in the presence of known differentiating ligands like retinoic acid (RA)[12]. Furthermore, this eliminates the need for feeder layers as this pathway is independent of LIF. Even though Nanog has shown to increase self-renewal, the mechanism is not yet well understood.

There are still a large number of other ways to control transcription of genes and maintain pluripotency, but the aforementioned two (Nanog and LIF) seem to be the most inclusive. Oct-3/4 are POU transcription factors that assist in maintaining an undifferentiated state, but the overexpression of this protein provokes differentiation to embryonic meso/ectoderm[14]. This is the epitome of the intricacies surrounding ESC differentiation and the balance of contributing factors. Furthermore, scientists have also reported the addition of telomerase can help the ESCs to maintain optimal potential. This enzyme protects cells from senescence since ESCs undergo massive replication that could compromise the integrity of their telomeres[15].

The knowledge with regard to the maintenance of pluripotency is novel and needs to be better understood. Progress is being made, however, as scientists are starting to maintain more lines of cultured ESCs than ever before[1]. But, ESCs provide no benefit to science if the power of

directed differentiation cannot be exploited, thus the realm of neural subtyping is next to be discussed.

Differentiation into Neural Subtypes:

The maintenance of pluripotency serves only as a prerequisite to guided differentiation into other cells types. To be effective, scientists must control cell morphology into neuroectoderm and subsequently into specific subtypes of neurons and glial cells. It has been shown that the largest contributors to the development of neuroepithelia is the administration of RA and BMP2 antagonist: noggin[11,16-17]. Schuldiner et. al effectively demonstrated that the exposure of ESCs to RA increased the expression of mature neuron marker neurofilament heavy chain protein (NF-H). This protein was found at a 25% increase in embryoid bodies treated with RA as opposed to the untreated control group. Furthermore, the same group found that ESCs treated with RA expressed dopamine receptor DRD1 and two serotonin receptors (5HT2A and 5HT5A) and tested positive for dopa-decarboxylase enzyme, a protein that is critical in the synthesis of both dopamine and 5HT[16].

Analogous to ESCs, NSCs display vulnerability to intrinsic and extrinsic factors, but their fate seems to be particularly dependent upon the time of exposure to different morphogens[18]. It was found that the exposure of neuroprogenitor cells (NPs) to fibroblast growth factor-8 (FGF8) at the beginning of development favors the production of midbrain-type dopaminergic neurons, whereas the deferred addition of the protein does not seem to influence the NPs in the same manner[19]. This highlights how timing has just as great an effect as the type of morphogen used.

Intuitively, the identity of the morphogen will prove to be significant also. In the developing fetus, rostral circuitries and connections seem to be more primitive than their more caudal counterparts[18]. If chemical guidance is stopped after the formation of NPs, almost all of the cells will turn into neurons and glial cells with more anterior properties[20]. The addition of RA was shown to effectively caudalize the cells by inducing the expression of certain Hox genes; additionally, RA

was shown to inhibit the formation of forebrain identities through the suppression of Otx and Bf-1 operons[27]. However, in the unhindered brain, cells begin to secrete factors such as Wnt glycoproteins which effectively modify some existing entities into a more caudal identity[21]. Furthermore, it is suggested that forebrain tissues retain their primitive identity by resisting Wnt signaling through soluble antagonists released by adjacent entities[22]. The discrimination between rostral and caudal tissues is important as they carry different properties[18]. It may seem curious to suggest that neurons of the same classification (i.e. dopaminergic) can be strikingly different solely on the basis of their spatial positioning in the brain; however, it is not disputed that dopaminergic neurons have a unique role in the substantia nigra (projection area) when compared to the somatosensory cortex (association area). Against the best attempts to recapitulate the native niches of NSCs, scientists have only been able to successfully subtype small amounts of neurons and glial cells. This is a testament to the multifaceted nature of the human brain. The delicate balance of neuron morphology is two-fold: it depends on the morphogen identity as well as the timing of ligand binding. As such, this is tough to reproduce *in vitro*.

Neurons in the brain are subject to their respective signaling pathways and transcription factors. Generally, they all emulate the same basic outline as any other somatic cell: ligand binding, tyrosine kinase activation, scaffolding, and then propagation which ultimately leads to the expression of some proteins that give the neuron its subtype distinctiveness. For the purposes of this paper as an overview, it suffices to appreciate that each subtype expresses a unique protein pattern at very specific times that ultimately guides it to synthesize its respective neurotransmitter. The non-motor CNS neurons are astonishingly complex thus most results are rather cumbersome. However, motor neurons in the CNS and PNS seem to be better understood.

Although it has been tough to recreate the conditions of the brain for specific subtypes. Motor neurons have perhaps made the greatest progress regarding growth and guided development,

as the attempts to recreate *in vivo* conditions have been more successful. It has been found that Sonic the Hedgehog (Shh) signaling protein plays a large role in the development of motor neurons from caudal prerequisites[22-23]. Specifically, motor neuron progenitors (MNPs) are regulated by Shh via homeodomain (hD) and basic helix-loop-helix (bHLH) transcription factors[23]. It has been shown that the continued addition of exogenous Shh produced high levels of two hD proteins, Pax6 and Nkx6.1, and Olig2, a bHLH peptide[17, 24]. The high concentrations of these regulators drives the cell to become a motor neuron and removes the possibility of it differentiating into other types of neural progeny[25]. The aforesaid pathway is not demonstrative of the entire process, but it illuminates the key characters at play. Nevertheless, the progress that has been made with regards to motor neuron development sheds light on potential to eradicate pathologies that involve the loss of these neurons.

The complexities surrounding neural circuitries renders any meaningful progress infrequent. Currently, scientists have ideas of transcription factors and signaling pathways that are important to gross arrays of neurons, but neural subtyping is very specific and must be better understood to be a real asset to potential therapies. Regardless of the lack of knowledge in some areas, there are a variety of cell therapies that have been successful on many different pathologies, and these will now be investigated.

Neurologic Pathologies Potentially Amendable to Cell-Based Therapies:

In the previous section, the differentiation of ESCs was explored along with the guidance into fore/hindbrain and motor classifications. Here, the highlights of cell-based therapy will be exploited to show there is a great deal of potential in this field of science. Specifically, Parkinson Disease, Huntington Disease, and multiple sclerosis will be presented in depth.

<u>Therapy for Parkinson Disease:</u>

Parkinson Disease (PD) is categorized as the loss of dopaminergic (DA) neurons from the substantia nigra and other projection areas in the brain. Patients with this disease present with motor

difficulties and also exhibit problems with speech and fatigue. To combat this neurodegenerative disease, scientists have questioned whether the transfection of DA progenitors into key areas could show any remedial results.

The success of any cell-based therapy relies on the ability to grow a large quantity of cells *in vitro* so they may be transfected into human (or mouse) patients. This is where the aforementioned knowledge regarding differentiation and survivorship becomes imperative to the future of stem cell endeavors. Morizane et al. showed that large scale production of DA neurons is possible when ESCs are grown with PA6 feeder layers. It was discovered that these stromal cells secreted soluble factors such as glial cell line-derived neurotrophic factor (GDNF), brain-derived neurotrophic factor (BDNF), dibutyryl cyclic AMP (dbcAMP) and RA-analog ascorbic acid (AA), all of which have been found to assist cells in the production of dopamine[28]. A large portion of the cells grown tested positive for tyrosine hydroxylase immunoreactivity, which is a key enzyme in the pathway that catalyzes the formation of dopamine; this would indicative of DA progenitors. Furthermore, another study has success growing vast amounts of DA neurons *in vitro* for transfection. In this study, it was found that BDNF and transforming growth factor (TFG) – α were the major players that induced higher levels of dopamine production[29]. The exact influence of these factors on dopamine production has yet to be fully classified and reported, but what is known is that the above components have an effect in pushing NSCs to form DA neurons.

Once large amounts of healthy progenitors have been identified, they are ready for transplantation. Zhang et al. performed an experiment were the injectable neurons were fluorescently labeled so they could be detected after transfection. The researchers showed that after *in situ* injection into the brain, the progenitor cells actually migrated very effectively and incorporated into many areas of the brain including the hippocampus and the striatum[30]. The state of stem cell development during transfection is very key: the neurons need to be near the median of a nascent

NSC and fully differentiated DA state. This allows to adherence to certain lineages depending on their respective niche[30]. Terminally differentiated cells do not perform well under *in vivo* conditions not suitable to their exact subtype. It seems successful transfection with completely mature neurons is more difficult to obtain as their reduced malleability has large repercussions on their viability.

One of the most successful trails on PD was performed on rats that have acquired lesions that allowed them to serve as a model of patients with PD. In this experiment, Björklund et al. displayed that the transplantation of progenitor cells fully differentiated into DA neurons in the rat model. After time, the transfected neurons tested positive for TH-immunoreactivity and proliferated into healthy midbrain-like DA cells. The cells also showed to migrate heavily into many areas of the brain and respond to cues from endogenous NSCs which makes this a promising study for future research. The authors also reported that the rats showed vast improvements in motor competence as well as a large reduction in extrapyramidal effects and asymmetries, thus reducing the effect of PD in rats[31].

Therapy for Huntington's Disease:

Huntington's Disease (HD) is a neurodegenerative condition in with the brain exhibits loss of crucial medium-spiny, GABAergic neurons in the striatum, a deep tissue layer in the midbrain[32]. It has been found that the disease is brought on by an autosomal dominant mutation in the huntingtin (htt) or IT15 gene which is located on chromosome 4[33]. Although scientists have been able to locate the exact location of the mutation, the method of action of the alteration is widely unknown thus hampering effective treatments. However, cell therapy does provide some promising upside for patients with this terminal disease.

In order to study HD, or any pathology, scientists must be able to recreate the diseased condition in a lab setting. This was particularly challenging for researchers looking to induce Huntington-like lesions in the brains of rats. After careful consideration and many biochemical test,

it was found that injection with N-methyl-D-aspartate (NMDA) receptor agonist quinolinic acid (QA) most closely provides a neurochemical benchmark that seems to emulate conditions of HD[34]. The NMDA receptor works to bind very excitatory neurotransmitters like glutamate, and thus act to oppose inhibitory GABA molecules in the brain. The continued antagonism of these GABAergic neurons eventually leads to atrophy with closely mimics the loss of these neurons in HD. Furthermore, Beal et al. solidified the use of QA showing that it also led to increases in aspiny neurons like somatostatin and neuropeptide Y which are also commonly seen in HD patients[34].

As displayed with the DA neurons in PD, scientists now know they can grow large amounts of ESCs on feeder layers. The next step with regard to treatment of HD is that of administration. As aforementioned, the tissues affected by this disease are deep within the brain, and this renders patients vulnerable to collateral trauma if probing instruments damaged viable tissues on their way to the striatum. Promisingly, it has been found that NSCs can actually migrate relatively vast distances to areas of lesion and integrate into the affected tissues[39]. Specifically, this seems to eliminate the need for invasive techniques that could be more malignant than beneficial.

Due to the curiosity regarding the mechanism of HD, many researchers have attempted to find a therapy for the condition even if they were not precisely certain on the course of action. Promisingly, it was found that NSCs that have not been terminally differentiated actually exhibited GABAergic phenotypes when xenografted into the striatum[35-37]. Specifically, Aubry et al. found that the progression of NSCs into striatal progenitors was marked by the expression of GSH2 and DLX2 genes, along with the detection of DARPP32 protein which is indicative of terminally differentiated neurons[35]. Furthermore, Song et al. found that ESCs grown on PA6 feeder layers prompted positive results when transfected into rats with unilateral QA lesions; the rats showed vast behavioral and histological improvements in as little as one week[36]. Similarly, McBride et al. found the same positive result with regard to NSCs integration and differentiation into GABAergic neurons.

However, here they report that cytokine ciliary neurotropic factor (CNTF) seemed to speed the recovery processes in rats with QA lesions; however, it was also shown that neurons in the CNTF treatment group actually responded less to cytokine and chemotaxic cues[37]. This hinders them from migrating to areas of lesion, thus CNTF is hypothesized to have a nonselective desensitization effect on neurons.

A notable difference between PD and HD is that the latter has a genetic origin. This brings different treatment options as scientist can attempt to preemptively combat the disease depending upon whether someone is at risk due to family history. Interestingly, in a study using rats with induced QA lesions, Ryu et al. found that cell therapy was extremely effective in reversing the effects of neuronal loss and degradation of GABAergic neurons if the NSCs were administered proactively as opposed to subsequent to lesioning. They, then, hypothesized that the cause of this was the neuroprotective release of BDNF from the exogenous NSCs. BDNF was shown to have a protective effect on cells against mutagens *in vitro* and secretion of this agent was very high following administration of NSCs, leading the researchers to this conclusion[38]. With an onset in the mid 20s, HD could be effectively reduced or eradicated with proactive treatment.

Therapy for Multiple Sclerosis

Multiple sclerosis (MS) is an autoimmune disease in which antibodies are made against certain components of the myelin sheath that wraps neurons. As a result, episodes of vicious demyelination characterize the life of patients with this disease. Treatments are limited to plasmapheresis and symptom alleviation rather than halting the progression of the disease. Cell therapy gives a promising insight as to the future of disease prevention and amelioration.

To recreate the neuroenvironment, scientists induce experimental autoimmune encephalomyelitis (EAE), which is an inflammatory and demyelinating disease of the CNS that closely imitates what is seen in the CNS plagued with MS[40]. Subsequent to lesioning, the scientists

transfected NSCs intravenously and monitored their migrations. Most (~90%) showed expressions of key proteins like CD44 and very late antigen (VLA) -4 while also lacking a class of common glycoproteins and selectins; the combination of present and absent proteins appears to be consistent with molecules that can pass the blood brain barrier (BBB)[40]. Later, they found injected cells sufficiently migrated to areas of destruction and began secreting immunomodulatory actors such as CNTF. Furthermore, it was discovered that CNTF's immunosuppressive effect functions by inhibiting the transcription of the proinflammatory cytokine TNF- α, and it was also shown to increase the efficacy of oligodendrocyte progenitors (O-2A)[40]. As a result, the scientists of this study suggest intravenous administration of NSCs may allow passage of the BBB and effective remyelination without the need for invasive procedures.

In another study of MS, EAE rats were injected with NSCs and the differentiation of the stem cells to O-2A was monitored. This was done by observing expression of genes through their protein product. An $A2B5^{+}PSA^{-}NCAM^{-}$ phenotype was needed to confirm that the cells were indeed O-2A cells and not cell adhesion progenitors that show similar protein patterns[41]. Here, survivorship was not recorded due to the fact that all mice were euthanized and brain tissues were harvested. Upon examination, it was found that O-2A cells effectively migrated to areas and began to rebuild axonal myelination[41]. A point of contention with the aforementioned study could regard whether or not perceived O-2A cells were of exogenous origin. To combat this doubt, another team modified the O-2A genome to express bacterial β-galactosidase; this created unique oligodendrocytes that could be distinguished from the indigenous cell population. Promisingly, the researchers found a large population of β-galactosidase positive O-2A cells integrating into the neural circuitries in areas of vast demyelination[42].

Previously, it was mentioned that stem cell therapies can differ in their means of remedy. It has been suggested that stem cell therapy is not a one-size-fits-all, but rather an intricate balance of

unique rehabilitating factors. Whereas most of cell therapy is grounded in the replacement of degenerate cells, Lee et al. found that, in some conditions, the nourishment and biotic assistance from exogenous NSCs actually proves to be a better therapy than cell replacement[43]. In the case of MS, the tropic enrichment of existing myelinated axons and oligodendrocytes has proved to be a superior treatment than the regeneration of the cells themselves[7-8]. Furthermore, ALS seems to benefit from similar treatments showing widespread remyelination of axon from injected stem cells[44-45].

In a rare clinical trial of NSCs on MS and ALS, the researchers found that 3 out of the 4 patients exhibited progression in MS symptom relief following injections; although, only one patient noted permanent progression and remyelination[46]. This elucidates the ambiguous nature of NSCs treatment. The very fact that improvement was shown is encouraging; however, continued improvement is warranted if cell therapy is to become commonplace and a pragmatic treatment for congenital and degenerative diseases.

Challenges Facing Cell-Based Therapy:

Aside from the fact that scientists do not know enough about stem cells, there are still a number of concerns regarding the effectiveness and safety of cell therapy. The implications of these concerns have caused hesitation from organizations like the FDA and other agencies that are imperative to the funding of stem cell endeavors. In fact, in 2009, the FDA put a complete halt on clinical trials of ESCs as the threat posed by certain problems seemed too great to continue[47]. They have since reinitiated clinical trials, however lots of individuals in the scientific community remain apprehensive.

Perhaps the most imposing hazard with regards to ESCs is their tendency to over-proliferate in their new niche, consequently resulting in teratomas[48]. The drive to replicate is an innate feature of ESCs, as just a few cells are to eventually become an entire organism. Thus, scientists must find a

way to suppress this natural inclination by further characterizing mechanisms involved and finding a way to antagonize this primordial characteristic. Perhaps, ASCs would be a more suitable alternative as they no longer have the intrinsic drive to multiply to rapidly.

As with any transplant, the risk regarding immunological rejection is always high. This has significantly impeded the process of cell therapy as the quest to find a compatible donor is no small feat. This has pushed scientists to overcome this concern with the invention of iPSCs[47]. This characterization of iPSCs is extremely novice and predicated on the researcher's ability to revert a somatic cell back to its embryonic origin through the addition of certain transcription factors; however, work done in this field is novel and results are very unconvincing as of yet. It may seem that the introduction of iPSCs is invulnerable to complications, yet these autologous stem cells still carry the same genetic predispositions as the degenerate cells of which they are trying to replace[7]. Furthermore, the process of reversion in iPSCs is not well understood and thus carries the potential to reactivate oncogenes that could lead to the formation of teratomas[49].

It is important to appreciate that scientific research cannot exist independently of those organizations that are able and willing to fund these undertakings. Moreover, the government has a large impact of the progress of science through the allocation of funds. Seeing as the President and congress are not permanent positions, elected officials move in and out of those seats carrying their personal view on stem cell research with them as they come and go. As such, this has a profound impact on research ability depending on the current views of those in office. The ethical and moral obligations of politicians is a large factor in maintaining public image, thus they are normally contempt to deviate from their promises. In 2001, President Bush signed an executive order banning the use of federal funding for stem cells research, an order than President Obama overturned in 2009[50]. This one example is illustrious of the haphazard nature of stem cell funding, and since

research depends on these politicians, it highlights exactly why research efforts can be rather precarious.

Conclusion:

ESCs show the greatest promise for the treatment of neurologic diseases as their ability to differentiate into NSCs is key to the regeneration of functional cerebral capacities. As discussed, these cells exhibit the ability to amend severe degenerative and congenital pathologies of which we only have treatments for the symptoms. Great progress is being made on the understandings of pluripotency and the mechanisms of differentiation; while harnessing this knowledge, it is possible to divert stem cells from their evolutionary fate and redirect them to the identity of our preference. In the coming decades, it is possible for the likes of PD, HD, MS, ALS and others to be obsolete or as rudimentary as the common cold.

Moving forward, we must better understand the local niche as to properly recapitulate conditions *in vitro*. We must create better methods of transfection as to minimize the potential of collateral trauma and transplant rejection. We must identify potential oncogenes, silence them, and prevent their reactivation in iPSCs and transplanted ESCs. With technologies improving every year, it is not outrageous to suggest that these challenges can be overcome sooner rather than later. If scientists can accomplish these seemingly insurmountable feats, the human race will have taken a giant leap in the direction of improving the quality of life for hundreds of thousands of people all over the world. The implications of this paints a vivid picture of what could be; the miraculous restorative ability of stem cells remains virtually untapped. This fact keeps us optimistic, it keeps us going, and it keeps us hungry to know more.

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