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As the mean age of the world population rises and people's life expectancies increase, the prevalence of Alzheimer's disease will increase and will pose a threat to society in terms of the people affected and the money used. Current therapies are not adequate in that they are unable to extend life beyond a few months. This review will both overview the predominant hypotheses about how to approach the treatment of Alzheimer's as well as discuss some of the treatments available and those in preclinical phases. Finally, we will discuss the ideas and recent research behind gene therapy and stem cell therapy as well as the new ways in which these two methods have been combined and could be combined in the future to create a more effective approach to treating Alzheimer's.

Alzheimer's disease is the most common neurodegenerative disorder worldwide (Jakob-Roetne & Jacobsen, 2009). As it is most frequent in old age, the changing population demographics lead to concerns regarding the occurrence rate and lack of a cure.

Alzheimer's is a type of dementia and normally presents symptoms of memory loss and ability to learn and reason. This can cause patients to lose understanding of who they are, who others are and where they are, causing panic and distress (Chen & Blurton-Jones, 2012). This creates many problems for Alzheimer's patients and their loved ones and these issues have prompted much research in the field of Alzheimer's disease over the past few decades.

Amyloid Hypothesis

Much of the research regarding Alzheimer's has focused on the workings and implications of the amyloid hypothesis. The hypothesis posits that the accumulation of the amyloid-beta peptide (Aβ) in the central nervous system (CNS) triggers a cascade of events that leads to cell death. The series of events that leads to the excess Aβ begins with the β-amyloid precursor protein, which occurs in different variants, all of which can be spliced into different versions of Aβ (Jakob-Roetne et al., 2009). Although Aβ is not toxic at the standard physiological concentration, with the increased concentration present in Alzheimer's, it causes neuronal degeneration. However, all processes that cause increased APP levels and therefore Aβ levels are not currently known, though mutations in the APP gene play a factor in genetic cases of AD (Borlongan, 2012).

Aβ42 is a longer variant of the Aβ peptide, which is most susceptible to aggregation, which is the process that eventually leads to cell death. Instead of forming an α-helix as the peptides usually do, they form β-sheets, which come together to form toxic amyloid plaques (Jakob-Roetne et al., 2009). The accumulation of these plaques then set off a chain of events that lead to neuronal dysfunction, inability to perform long- term potentiation (a function that is critical for memory) and finally neurodegeneration (Jakob-Roetne et al., 2009, p. 3041).

Although the buildup of toxic amyloid plaques clearly plays an important role in the pathology of Alzheimer's, there are also clearly other important co-pathologies. The best researched among them is the buildup of neurofibrillary tangles caused by the over-phosphorylation of the tau protein (Jakob-Roetne et al., 2009). However, this is only one of the many other observed pathological

differences in AD and it is not yet known which change is the root cause. Because of lack of definitive information regarding the initial trigger and cause of Alzheimer's, many current treatments function as palliative treatments and attempt to work to stop the process of neurodegeneration not by addressing the underlying pathology but instead looking to aid and replenish the areas of the brain most devastated by neuronal death. Although palliative treatments will never constitute a permanent cure for Alzheimer's, because of the current lack of a permanent cure, they provide an important means by which to delay the progression of the disease. Even upon the eventual discovery of the treatment for the underlying pathology of Alzheimer's, it will be critical to have palliative treatments available to slow the progress of the disease in order to give time for the cure to function.

Cholinergic Hypothesis

The most popular palliative treatments for Alzheimer's are directed towards the cholinergic hypothesis, which posits that a significant loss of cholinergic neurons in the CNS is a primary contributor to the cognitive symptoms of Alzheimer's (Bartus, 2000). The treatments directed at this hypothesis, which include cholinesterase inhibitors, are one of the few current treatments for Alzheimer's that have been approved by the FDA.

There exists much evidence that points to the idea that the loss of cholinergic neurons in the CNS and particularly in the basal forebrain contributes to a loss of cognitive function. Since the mid-1990s, studies have shown that the severity of degradation of cholinergic neurons correlates to the clinical severity of Alzheimer's before death, the deposition of neurofibrillary tangles and well as amyloid plaques (Frölich, 2002). This suggests that the amyloid hypothesis and the cholinergic hypothesis are connected and that both are somehow related to the pathophysiology of the disease.

Furthermore, recent longitudinal studies have indicated an initial plasticity and subsequent loss of plasticity in the cholinergic system. The study looked at the levels of choline acetyltransferase (ChAT), which is an enzyme that facilitates the creation of acetylcholine from acetyl-CoA and choline. The results showed an increase in ChAT levels in cases of mild cognitive impairment and mild AD, but normal levels in cases of moderate to severe AD. Scientists concluded from this data that the brain initially attempts to compensate for lower acetylcholine (ACh) levels by upregulating ChAT, but that as the disease progresses the brain is unable to sustain the process of compensation (Frölich, 2002). Therefore, palliative treatments that continue to compensate for ACh reduction would be effective in suppressing the cognitive symptoms of AD.

Treatments

Drug treatments

After the identification of Alzheimer's as a disease, the search for a cause and subsequently a treatment began. However, since research into causes has not led to one distinct answer but instead multiple observations about the neural systems effected during AD, drug treatments of three distinct types were developed – drugs that acted as indirect ACh agonists and inhibited the action of ACh esterases, drugs that block the formation of amyloid plaques, and drugs that inhibit NMDA receptors (Taupin, 2009). But while all the three types of drugs are effective to some extent in alleviating the symptoms of Alzheimer's, they are unable to stop the progress of the disease.

Gene therapy

Alzheimer's has been observed to be heritable to an extent, and that has spurred research on possible genetic markers. While different genes have been associated with Alzheimer's, there has not been enough research to identify definite genetic causes.

Therefore, current gene therapy research instead focuses on the causes proposed by the different hypotheses. One type of gene therapy that has been developed recently involves genetically modifying cells to produce more acetylcholine. Fu et al. (2004) transplanted a ChAT overexpression gene in bacteria and injected the vector bacteria into mice treated with Aβ to induce Alzheimer-like cognitive deficiencies. The researchers observed that the vector bacteria successfully crossed the blood-brain barrier and post mortem neural analysis shows successful expression of the gene in some neurons in the brain. The mice treated with the bacteria vector also showed improved cognitive abilities. In addition to testing gene therapy, this study also showed that increasing ChAT levels in specific areas of the brain could help alleviate cognitive deficiencies induced by Alzheimer's.

Other proteins currently being targeted in gene therapy are nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF). Both of these molecules have been associated with synaptic plasticity (Chen et al., 2012), and NGF has been shown to decrease neuronal cell death (Kim, Lee & Kim, 2013).

Stem-cell treatment. Stem cells are one of the most recent treatment options for neurodegenerative diseases. The cell death in AD is not limited to a specific type of neurons. Since stem cells can divide and differentiate into a variety of cell types, they seem a reasonable solution this widespread neuronal cell death. Different groups of researchers have independently found that transplantation of multipotent stem cells (MSCs) transplanted into the brains of rat models of AD led to both reduction of Aβ deposition and tau hyperphosphorylization. Furthermore, this treatment also led to improvements in memory and spatial learning among the rats (Glat & Offen, 2013).

Additionally, stem cells can be genetically modified in order to also counter some of the chemical effects of Alzheimer's, like the decrease in cholinergic sensitivity.

Genetic modifications can also increase the levels of specific neurotrophic factors by increasing production of those molecules. By combining stem cell treatment and gene therapy through genetic modification of stem cells, it is possible to take advantage of both the benefits of using stem cells to replace areas of prevalent neuronal death as well as the benefits of increasing the production of specific proteins known to help treat the cognitive symptoms of AD.

Recent research has found that transplanting stem cells producing more ChAT helped improve working memory in mice with lesions imitating neuronal cell death in Alzheimer's (Wang et al., 2006; as cited in Kim et al., 2013), transplanting cells with increased production of NGF improved memory in rats (Wu et al., 2006; as cited in Kim et al., 2013), and transplanting cells producing more BDNF improved working memory in mice (Blurton-Jones et al., 2006; as cited in Kim et al., 2013). In 2012, Park et al. conducted an experiment with rats that were injected with kainic acid to induce neuronal cell death akin to that in Alzheimer's. Four weeks later, neural stem cells (NSCs) genetically modified to produce excess of ChAT were transplanted in the lesioned areas. The rats were tested for learning and memory. Over a few weeks, the rats showed full restoration of ACh levels in the brain and their learning and memory capacities improved to a level comparable to normal rats (Glat et al., 2013, p. 1492). A similar study conducted that

same year found that transplanting NSCs producing more NGF in mice had similar effects as those seen when ChAT overexpressing NSCs were implanted (Lee et al., 2012; as cited in Kim et al., 2013).

However, stem cell treatment faces its own obstacles. Stem cells have been the center of ethical debates for a while now. While the human brain does have some amounts of naturally occurring NSCs, suggesting that inducing neurogenesis in AD patients could be a possible cure (Glat et al., 2013), the possibility of AD having genetic markers diminishes the capacity for these NSC to successfully fill in for areas of neuronal death. It is possible to take the naturally occurring NSCs and genetically modify them, but the amount of such NSCs available is still low compared to the levels of cell death.

Umbilical cords have some amounts of MSCs, but not a lot of research has used umbilical cord MSCs. Stem cells can be harvested from embryos but there has been much pushback on this subject because of issues of ethics.

A further problem regarding stem cell transplant is that it is unpredictable. Since stem cells can differentiate into a variety of different types of cells, there is no way to fully regulate the differentiation process to only form the specific subtype or subtypes needed. Recent research has focused a lot on generating specific subtypes of neurons from stem cells. Bissonnette et al. (2011) generated functional basal forebrain cholinergic neurons from embryonic stem cells with a success rate of 85%. They also stated that with additional controls, the success rate could be increased to 94%. However, this differentiation was carried out in a culture medium and not after transplantation. In 2013, Crompton et al. carried out successful controlled differentiation of transplanted NSCs using sonic hedgehog (SHH) signaling, the basic premise being that in an embryo the stem cells differentiate into specific neuronal subtypes due to SHH signaling. This research suggests that it may be possible to take advantage of the chemicals involved in SHH signaling to control the differentiation of stem cells.

Once transplanted, it is difficult to ensure that the cells travel to the appropriate places and form the appropriate neural connections. Without the appropriate neural connections, the overexpression of chemical factors will not be as effective. Though Park et al. (2012) found a more than 96% success rate in the appropriate migration and differentiation of transplanted NSCs, this research was done on an animal model and such high success rates need to be replicated by multiple studies before the treatment can be tried on humans. Some of the pathologies associated with AD might be affecting the success of proliferation and differentiation of transplanted cells. Few studies have investigated the effect of Aβ on transplanted cells and while some showed that it hindered proliferation and transplantation (Haughey et al, 2002; as cited in Glat et al., 2013), another study showed that it helps in successful proliferation and degradation (Jin et al., 2004; as cited in Glat et al., 2013), suggesting that Aβ oligomers help in proliferation and differentiation, but as the disease progresses, the fibrils formed from Aβ lead to cell death.

It is additionally possible that if embryonic stem cells from a stem cell bank are used, the transplanted cells will also be unrecognizable to the immune system and therefore may lead to an immunological response. Because of this complication, researchers have been focused on learning to use stem cells found naturally in the muscles, or on taking skin cells and inducing them to pluripotency, creating induced pluripotent stem cells (iPSC). Since these stem cells have the same genetic makeup as the rest of the body's cells, the body recognizes them as its own and therefore no immunological

response is elicited. However, the other problems of specific differentiation and formation of appropriate connections remain.

Discussion

The lack of clarity in terms of the causes of Alzheimer's has hindered the search for a cure till now. Palliative drug treatments have helped in alleviating the symptoms of the disease, but there is still no cure for AD. While gene therapy has had some success on animal models, more research is needed on the specific functionality of these different proteins and how they affect the pathophysiology of AD. Furthermore, studies should be done to address the potential benefits of combining different gene therapies and using them together to create a multi-pronged approach to treating AD. More research also needs to be done on the combination of gene therapy and stem cell treatment in Alzheimer's. Of particular interest is how different genetic changes can be incorporated together to affect the same group of stem cells in order to create a multi-pronged approach to the treatment of Alzheimer's. The idea would be that hopefully by combining these smaller scale successes, a more long-lasting and overarching treatment could be found which could potentially arrest the progress of the disease. Despite the problems associated with the use of stem cells in neurodegenerative diseases, these approaches to treating Alzheimer's are incredibly promising. The use of stem cells is currently the only known method of reversing the effects of neuronal death, and when those effects are combined with the effects of gene therapy using genetically modified cells, there is a great potential for a the creation of a treatment more effective than anything that is currently available. The goal of this new treatment would be to create a long-term palliative treatment that could arrest the rapid advance of Alzheimer's, and possibly add years onto the lives of patients. There is still much investigation that needs to be done, including moving the research from animal models to human trials and further experimenting with the combination of the different genetic alterations. However, the research already completed provides a solid foundation that shows great potential for the development of a new, cutting-edge treatment for Alzheimer's.

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