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Mini review: Prospective therapeutic targets of Alzheimer's disease

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Abstract:

Alzheimer's disease is a neurological condition that causes the disruption of neuronal connections in the human brain. It is progressive and targets about 10% of the United States population over the age of 65.3 to date, there is no cure to the disease. Physicians can treat symptoms but lack the ability to stop the progression of the disease. However, promising research has come to the surface in recent years. A collection of these therapeutic targets, which have yielded positive results in mice models, are presented in this article. They include targets such as meningeal lymphatics, mitochondrial homeostasis, genomic instability, calcium homeostasis, and cold-shock proteins such as RNA-binding motif protein 3 and reticulon-3, high-density lipoprotein, and antibodies.

Keywords:

Calcium homeostasis, cold shock proteins, donanemab, genomic instability, HDL, mitochondrial homeostasis, meningeal lymphatics

Introduction

Alzheimer's disease (AD) is a progressive neurological disease with no known cure. It affects almost 6 million people in just the United States and continuously deteriorates their quality of life.[1] The current treatments for AD are centered on slowing disease progression rather than curing the illness. AD is caused by the buildup of beta-amyloid (Aβ) plaques that disrupt neurochemical signaling. Amyloid precursor protein (APP) is cleaved to form these (Aβ) plaques. This disruption causes atrophy and ventriculomegaly leading to a myriad of issues including cognitive decline and onset of behavior and psychological ailments. Acetylcholine is overall decreased while there is N-Methyl-D-aspartate (NMDA) overactivity, so those are the main current therapeutic targets. The most common symptoms are memory loss and confusion, which are usually treated by NDMA antagonists such as memantine or central acetylcholinesterase inhibitors such as rivastigmine.[2] These work to increase the amount of neurotransmitter signaling in the declining regions in the brain. This treatment may cause the working neurons to fire more often but will not stop the number of neurons from continuously diminishing. There have been many new theoretical solutions to treat AD instead of targeting only its symptoms or progression. This article looks at a collection of prospective therapeutic targets of AD and provides a comprehensive look on the findings of each.

Meningeal Lymphatics

Meningeal lymphatics are promising targets to combat aging and AD. The brain has always been considered to be an immune-privileged organ because it has very limited interactions with the immune system.[3] Da Mesquita *et al*. looked at the role of meningeal lymphatics in clearing central nervous system (CNS) waste. The parenchyma of the CNS was thought to not have lymphatic vasculature, so we previously believed the brain solely removes its debris by transport mechanisms in across

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the blood–brain barrier and into the cerebrospinal fluid or phagocytosis by microglia. The (re) discovery of meningeal lymphatics gave a new area to study the clearance of Aβ peptides.

Groups of mice were assigned to two groups consisting of meningeal lymphatic ablation and control. The procedure was done twice with a 3-week gap and images were taken 6 weeks after the initial intervention. Younger mice seem to rely of meningeal lymphatics for learning and memory cognition, while older mice have natural disruption of this system. This article ultimately shows evidence that age contributes to dysfunction of meningeal lymphatics is one of the major underlying factors for worsening Aβ peptides accumulation. Therapeutically targeting the decline of these lymphatics may help with age-related cognitive decline.

As promising as this new therapeutic target sounds, there are still some limitations to the study. In mouse models of AD, the deposits of Aβ peptides in the dura are not mimicked the way they are in patient models. In addition, the less aggressive phenotypes of AD were not included in this study and can affect the level of decline of the meningeal lymphatics. Future studies should be conducted studying the decline of the blood–brain barrier with age and how that contributes to the progression of the disease. Ultimately, however, augmentation of the drainage of meningeal lymphatics may improve cognitive function in those mice models of AD.[3]

Cellular Interventions

Recent evidence has indicated that the amyloid precursor protein, a known precursor to Aβ peptides, can be cleaved to form C-terminal fragments, which ultimately disrupt mitochondrial homeostasis. Mitochondrial dyshomeostasis is also considered to be an early event in AD development. The specific contributions of APP-C-terminal fragment (CTF) remain unknown, however, Vaillant-Beuchot *et al*. conducted a study to determine the role of APP-CTF in the pathogenesis of AD.[4]

The mitochondrial structure and function in neuroblastoma cells in mice were studied and found to have a dramatic increase in the production of APP-CTFs. The participants were classified into four categories based on mitochondria morphology. Class 1 was the healthiest and 86% of control cells fall in this class. APPs were mouse (AD mice model) displayed a drastic reduction of class 1 mitochondria and an enhancement of the other 3 classes in over 20 different fields of mitochondria. This reduction and damage of mitochondria lead to mitochondrial structure alternation and mitophagy failure due to the increased release of reactive oxygen species into the neuronal cells. APP-CTFs accumulation was shown to happen both independently of AD and in conjunction to the progression of the disease. Overall, there may be potential here to protect cell degradation and the progression of AD.[4]

By enhancing mitochondrial proteostasis, the pathogenesis of AD can be delayed. This would reduce overall amyloid aggregation and decrease APP-CTF accumulation. This would be a valuable therapeutic target for AD treatment. This study's limitations revolve around them choosing to use AD mice models that specifically produce high volumes of APP-CTFs, and other versions of the disease may not respond the same to the therapy. In addition, it is unknown exactly how the accumulation of APP-CTFs could be targeted rather than just promoting mitochondrial numbers.

Genomic instability also has the potential to play a role in brain aging and neurodegeneration. In AD, patient brains display elevated DNA double-strand breaks and downregulated double-strand break repair pathways. Histone deacetylases (HDACs) are enzymes that remove acetyl groups from the histones that DNA wraps around, regulating DNA processes.[5] HDAC1 stimulates OGG 1 (DNA glycosylase), to remove 8-oxoG lesions, a guanine-rich region which is particularly susceptible to oxidative DNA damage. Pao *et al*. conducted a study to evaluate HDAC as a therapeutic target in DNA repair.

HDAC1 knockout mice (KO) displayed astrogliosis, increased glial fibrillary acidic protein, and DNA damage at only 3 months' old with no other gross abnormalities in brain organization. The mice were all primed with fear conditioning and placed in the water maze task to assess hippocampal spatial learning. HDAC1 deficiency did not alter cognitive performance in the younger mice but did in the older mice, showing an age-dependent deficit in memory after HDAC1 KO. There was also a significant correlation with 8-oxoG accumulation and gene repression by HDAC1 ablation leading to elevated 8-oxoG at all four downregulated gene promoters. By deacetylating OGG1, HDAC1 stimulates its cleavage activity and directly affects the 8-oxoG levels and gene expressions.

This study was unique in naming a way to affect the proposed target. Small molecule activator of HDAC1, exifone, has been shown to have procognitive effects in patients with Alzheimer's type dementia in the past by causing a 50% reduction in acetylation done by HDAC1 KO. HDAC1 was overall proven to affect cognition and memory in aged mice. By finding a therapeutic target and proving its effectiveness, this study does a great job at pointing to future directions that HDAC1 can

be studied and targeted. Exifone should be studied to discover potential side effects of using a small molecule activator.[6]

Calcium Homeostasis

AD alters neuronal calcium homeostasis by increasing the levels of calcium that are found in the neurons closer to the amyloid plaques.[2] The decreased expression of the calcium-binding protein may be a susceptibility factor for AD as well as demonstrating the importance of neuronal calcium homeostasis. Angulo *et al*. proposed that the expression of the calcium-binding protein, calbindin-D_{28k} (CB), is associated with AD pathophysiology.

Calcium dyshomeostasis is associated with network hyperexcitability. Although it is known that calcium homeostasis is altered in AD, the characterization of calcium dynamics of subicular neurons in AD mice is unknown. AD mice express presenilin mutations that can cause an increase in calcium through IP3 receptors, but the activation of ryanodine receptors is altered in AD. CB is found in the dentate gyrus and subiculum and some studies have found it decrease in AD mice while others say there is increased CB expression. This study put two groups of mice, control and CB KO, into fluorescent calcium imaging to look at its differing dynamics.

J20 (AD mice model) mice were tested for age and CB expression and found that there was a significant difference in the DG between young/old J20 mice and old J20/WT mice in the bursting cells. In addition, peak Ca^{2+} transients were found to be significantly smaller in old CB KO mice compared to wildtype (WT). Finally, there was a significantly lowered calcium plateau level in subicular BC dendrite in the WT mice compared to the J20 CB KO mice. Since there were so many differences in calcium levels found in the hippocampus and surrounding regions, this is an important area of study in future of AD research. Increased calcium in these regions may be able to mimic non-AD brains. This study used very specific parameters and could be done again with different mice models of AD and different stages of AD disease. The age range was also extreme, from 1 to 2 months declared young and 14–18 months mice declared old and middle-aged mice could be studied for early onset. Overall, calcium homeostasis is found to be age-dependent and regulating calcium levels in the hippocampus, DG, and subiculum can be seen as a potential treatment for AD.[2]

Cold-Shock Proteins

Structural plasticity is one of the main targets of AD as it progresses. Bastide *et al*. conducted a study on how reforming lost synapses is made possible by cold-shock proteins. When hibernating mammals go into hibernation, the cooling induces the loss of their synaptic contacts, which are reformed as the mammal rewarms. This is a form of structural plasticity, and it was found to be mediated by cold-shock proteins. This study found that the same happens in cooled laboratory rodents.[7]

RNA-binding motif protein 3 (RMB3) is an RNA-binding protein that was evaluated for its role in structural plasticity and the role it plays on neurodegeneration during cooling. 5XFAD mice were the AD-type mice used in this experiment. Their capacity to regenerate synapses after cooling was found to be lost with RBM3 was lost. RBM3 KO increased synaptic loss, accelerated the disease, and prevented the neuroprotective effects of cooling. When RBM3 was overexpressed through boosting endogenous levels through hypothermia of lentiviral delivery, however, the results showed sustained synaptic protection in 5XFAD mice. This prevented both behavioral deficits and neuronal loss, and it significantly increased the life span of the mice. This leads to impaired synapse regeneration in mice after cooling and protecting the mice against synaptic toxicity during the progression of disease. Therefore, RBM3 is both important to the protection of neurons during cooling and to the protection of neuronal synapses with age and AD-related neurodegeneration.

This study was found to be one of the more promising ones. It had many significant findings in an area of study rarely explored. There were, however, a few limitations. There was a smaller number of participants with only 2–3 mice per condition and the data collected from solely these participants. The study also focused on prion-infected mice and their synaptic degeneration. If this study was repeated to focus on large groups of AD mice, there may be more certainty of the effects of RBM3. This is a hopeful therapeutic target for future AD treatment.[7]

A newer cold-shock protein, reticulon 3 (RTN3), was evaluated in another study by Bastide *et al*. Since RBM3 has been already by proven by these authors to play a critical role in mediated synaptic repair processes, but the mechanisms by which micro ribonucleic acid (mRNA) encoding cold-shock proteins escape repression is unknown, they wanted to focus on another cold-shock protein. This study shows that the cooling-induced reprogramming of the translatome and ultimately leads to the upregulation in RTN3, a reticulon protein that acts on synapse formation. RMB3 binds to RTN3's mRNA and drives its increased expression.

This study investigated the posttranscriptional response to hypothermia *in-vitro* in a mouse model of neurodegeneration. HEK293 cells were cooled because these cells have a well-documented response to cooling. Specific mRNAs during cooling were hypothesized to be able to evade a global reduction in translation elongation, but identifying these specific mRNAs proved difficult. After Western analysis, it was found that the expression of Noggin and RTN3 increased at freezing temperatures. RBM3 is then bound to RTN3 mRNA and increased its translation through trans-acting effects on initiation, while the cis-acting elements help to evade the initiation block.

The results conclude that if RTN3 is overexpressed, then there is a less synaptic loss in mice with neurodegenerative diseases, but knockout of RTN3 eliminates cooling-induced neuroprotection, even in the absence of changes in RMB3 levels.[8] After cooling, there is translational reprogramming during which cold-shock proteins are overexpressed. By enhancing RTN3 expression, RBM3 would be more effective in neuroprotection. This study adds to the potential effectiveness of targeting cold-shock proteins. It gave another option if RBM3 could not be safely modified, and it also looked at the cis-and trans-acting factors of RTN3. Therefore, RTN3 is another route of the study that could be a target for AD therapies.[8]

The Role of High-Density Lipoprotein

High-density lipoprotein (HDL) is usually only considered in patients who are struggling with high cholesterol and dysfunctions of the liver. HDL's main role is transporting cholesterol from the tissues back toward the liver, along with protease inhibition, complement regulation, hemostasis, and reducing inflammation.[1] Button *et al*., however, show that HDL's established vasoprotective properties may help provide resilience to cerebrovascular dysfunction that is present in AD.

This study created a three-dimensional (3D) model of a bioengineered human vessel and tested the beneficial functions of HDL on these vertebral vessels. The functions include preventing Aβ-induced endothelium activation, reducing Aβ's vascular accumulation, maintaining Aβ in a soluble state, and inducing endothelial NO secretion.[1] The assays of HDL function suggest that they are biomarkers for cerebrovascular disorder. This study elaborated on the known properties of HDL and ultimately led to the conclusion that targeting HDL could result in specific therapeutic interventions in diseases such as AD. HDL can protect against memory deficits, neuroinflammation, and cerebral amyloid angiopathy.[1]

Targeting Antibodies

One of the defining features of AD is the accumulation of the Aβ peptides. These accumulate and disrupt synaptic transmission. Donanemab is an antibody that targets a form of deposited Aβ. Mintun *et al*. studied this antibody in an attempt to evaluate its ability to degrade these plaques and increase cognition in AD patients.

A phase 2 trial was conducted with 257 patients, each assigned to two groups of either receiving donanemab or the placebo. They were all given a positron emission tomography scan to confirm tau and amyloid deposition before the study, and those in the donanemab group were given treatment every 4 weeks for up to 72 weeks. Their cognition and Mini-Mental State Examination (MMSE) were tested with AD progression-specific tests. Although there was a significant improvement in the primary outcomes tested, the secondary outcomes did not show significant difference. The primary outcomes were integrated AD rating scale and mixed model for repeated measures scores, while the secondary outcomes were the MMSE and AD Cooperative Study-Instrumental Activities of Daily Living Inventory. This study concluded that there was an overall reduction of amyloid plaque level in the donanemab group compared to the placebo group but there was no significance at an individual level.

The limitations of this study include the requirement for participants required the highest levels of tau protein, which may have made them partially resistant to anti-amyloid treatments. In addition, with the donanemab group, the treatment was so aggressive that the majority of the plaques had been reduced in the first half of the study. The extra time hurt the statistical numbers and should be shortened when repeated. Donanemab still has significant promise to be a therapeutic target for the progression of AD if this study could be repeated with slightly different parameters. It provides hope for the elimination of amyloid plaques.[5]

Conclusion

There are many promising prospective AD treatments in the process of being studied. These treatments aim to stop the progression of the disease and try to conserve the synapses between neurons and emphasizing neuroprotective functions. The main treatments discussed in this article are meningeal lymphatics, mitochondrial homeostasis, histone deacetylases, calcium homeostasis, RBM3 and RTN3, HDL, and donanemab [Table 1]. These targets have all been shown to play a role in the development of AD through the use of mice models. They should be explored further as

Table 1: Summary of the main points covered by each study and the test participants used

Intervention	Test subject studied	Findings
Meningeal lymphatics	AD mouse model	Impairing meningeal lymphatics affects brain CSF influx and ISF diffusion and worsens cognitive function. Improving meningeal lymphatic function in aged mice increases brain perfusion and alleviates cognitive deficits
Mitochondrial homeostasis	AD human brains postmortem	Altering mitochondrial structure leads to the exacerbated production of APP-CTFs and Aβ peptides
Genomic instability	5XFAD mouse model (5 AD-linked mutations)	HDAC1 knockout mice exhibit age-dependent cognitive decline, accumulation of 8-oxoG, and impaired memory. HDAC1 protects against 8-oxoG lesions in 5XFAD mice and improves cognition
Calcium homeostasis	J20 AD mouse model	Calcium-binding protein knockout mice show changes in their calcium buffering capacity in the subicular dendrites. Age-related decline in CB shown in non-AD mice suggests that AD causes preemptive aging
RBM3 (cold-shock protein)	5XFAD mouse model	Failure to induce RMB3 parallels lost the capacity for synaptic recovery in neurodegenerative disease models. Overexpression of RMB3 restores structural synaptic plasticity and is neuroprotective in neurodegenerative disease
RTN3 (cold-shock protein)	5XFAD mouse model	RBM3 binds to RTN3 mRNA and increases its translation through trans-acting effects on initiation. RTN3 overexpression increases synapse number in the wild mice and increases the survival of affected mice
HDL levels	AD mouse model and 3D bioengineered human arteries	Increases in HDL levels in AD mice protect against cerebrovascular dysfunction, as intravenous administration reduced Aβ levels and memory deficits. A 3D bioengineered human artery modeled vascular inflammation and was able to show that HDL functions in these vessels reduce Aβ accumulation
Donanemab	AD human patients	Donanemab targets Aβ peptides and can increase cognition in AD patients and decrease the tau and amyloid deposition shown on PET scans

HDL: High-density lipoprotein, AD: Alzheimer's disease, CSF: Cerebrospinal fluid, ISF: Interstitial fluid, APP: Amyloid precursor protein, Aβ: Beta-amyloid, HDAC: Histone deacetylases, CB: Calbindin-D_{28k}, mRNA: Micro ribonucleic acid, PET: Positron Emission Tomography, CTFs: C-terminal fragments, 5XFAD: Mice with 5 Alzheimer's Disease linked mutations, RTN3: Reticulon 3, RBM3: RNA-binding motif protein 3, 3D: Three-dimensional

they have the potential to provide new avenues for new neuroprotective therapies.

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Conflicts of interest

Dr. Yuchuan Ding is an Associate Editor of *Brain Circulation*. The article was subject to the journal's standard procedures, with peer review handled independently of this Editor and their research groups.

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