Application of microRNA (miRNA) in Alzheimer's Disease (AD)

By

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A thesis submitted to the School of Pharmacy in partial fulfillment of the requirements for the degree of Bachelor of Pharmacy

School of Pharmacy
Brac University
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Declaration

It is hereby declared that

1. The thesis submitted is my/our own original work while completing degree at Brac University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. I have acknowledged all main sources of help.

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Approval

The thesis/project titled “Application of microRNA (miRNA) in Alzheimer’s Disease (AD)” submitted by Rabea Halim (18146006) of Summer, 2021 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy (Hons.) on March 10th, 2022.

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Ethics Statement

No human or animal tests are involved in the study.

Abstract

Alzheimer's disease is a fatal illness that primarily affects older generations; its pathology pathways include beta-amyloid, tau protein, and activated microglia, destruction of synapse and no disease-modifying medication has been found. MicroRNA has the potential to be a novel therapeutic method by targeting certain mRNAs that cause the disease to worsen, so their overexpression causes a reduction in disease, and another class of microRNA targets those mRNAs that are required for a healthy brain, but their overexpression causes the disease to worsen. There are several microRNAs, and each one has a different target and impact in the brain, with some having a therapeutic effect and others having a pathogenic effect. MicroRNA is a promising therapeutic agent and drug target which needs to be implemented in human trials, not just animal models.

Keywords: MicroRNA, Alzheimer’s disease, beta-amyloid, tau, synapse

Dedication

I want to dedicate my thesis to my parents, guardian and respected supervisor for their support, without all of their blessings it would have been quite impossible to complete my work.

Acknowledgement

I would like to give my gratitude to Almighty Allah for giving me the ability, the strength, wisdom and patience for me to complete this body of work. I am ever so grateful to be able to finish this work, I am grateful for all experience I have gain through this work and with the blessing of my loved ones, I will implement these valuable lessons in both personal and professional scenarios.

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List of Acronyms

Aβ	Beta-amyloid
APP	Amyloid precursor protein
AD	Alzheimer's Disease
MCI	Mild cognitive impairment
EOAD	Early-onset AD
LOAD	Late-onset AD
NFT	Neurofibrillary tangle
NPS	Neuropsychiatric symptoms
MiRNA	MicroRNA
CSF	Cerebrospinal fluid
MSC	Mesenchymal Stem Cells
EV	Extracellular vesicles
WT	Wild-type
KO	Knockout
TG	Transgenic
BBB	Blood–brain barrier
ADAD	Autosomal dominant Alzheimer's disease
TBI	Traumatic brain injury
CI	Cognitive impairment
CVD	Cardio vascular disease
CAA	Cerebral amyloid angiopathy
T2D	Type 2 diabetes
CNS	Central Nervous System
ISF	Interstitial Fluid
ChEI	Cholinesterase inhibitor
BACE	Beta-secretase
FAD	Familiar Alzheimer's Disease
BAD	Beta-amyloid dysfunction
SVC	Synaptic vesicle cycle

TLR Toll-like receptors

Chapter-1 Introduction

1.1 Alzheimer's Disease (AD)

Alzheimer's disease (AD) has complex pathobiology, and it is a heterogeneous disease in nature. For the diagnosis of AD the main neuropathologic indicator is the presence of beta-amyloid (Aβ) in appearance of neuritic plaques and hyper-phosphorylated tau accumulation as neurofibrillary tangles (NFTs) (Long & Holtzman, 2019), along with these there is brain atrophy which causes neuronal and synapse loses. The patient's brain also undergoes innate immune response together with huge changes within microglia's and astrocyte's phenotype. The amyloid cascade hypothesis is widely accepted for pathogenesis of AD, this hypothesis states that any variation in Aβ triggers a series of events that leads to toxic tau accumulation that later causes disease initiation and leads to downstream neuron death. The genetical implication of AD supports this hypothesis in that where Aβ production and aggregation are main disease causing agent and their accumulation occurs way before the symptoms of AD emerges in a patient and along with Aβ accumulation the brain loses synapse. In AD patient non-neuronal changes such as glial inflammatory response around the plaques and vascular function abnormalities, including diminished cerebral blood flow and blood–brain barrier (BBB) disruption occurs (Henstridge et al., 2019). The pyramidal neurons and synapses death occurs in the cerebral cortex and within specific area of the subcortical region, and then there is senile plaque (SP) deposition, which is composed of Aβ and microtubule-associated protein tau being hyper-phosphorylated, is associated with AD. The loss of neurons and synapse mainly occur in the hippocampus and the disease when progressed further causes damage in cognition and behavioral function. (Moore et al., 2019). Dementia is an intra-individual pattern where a person's memory is diminishing and impaired thinking of at least two domains of cognition, thus for this reason, the most common cause of dementia is AD, where a person's over the age of 65 have personality changes, amnesia, and impaired memory (Long &

Holtzman, 2019). The dementia caused by AD causes the patient to suffer significant disability that progresses further throughout disease course and patient might die in 5 to 12 years after the first symptoms are identified (Vermunt et al., 2019). AD is currently incurable and only options of treatment is to prevent the disease progression further. The two categories of AD and those are the Early-onset AD (EOAD) and late-onset AD (LOAD), most of the AD cases are LOAD which occurs in patient age being more than 65, EOAD cases are rare, where below 65 age patients are found be less than 5%, another subtype of EOAD is autosomal dominant Alzheimer's disease (ADAD), where patient age is below than the range of EOAD and this kind of cases are found at only 1% to 2% cases, these early-onset cases have a swift disease progression and few symptoms are found to be in connection with other different neurologic symptoms that is not found in sporadic AD commonly (Long & Holtzman, 2019). Preclinical prodromal, mild dementia, and moderate-to-severe dementia are the four clinical stages of Alzheimer's disease. Amyloid accumulation and normal cognition characterize preclinical Alzheimer's disease. Amyloid build-up and moderate cognitive impairment, both amnestic and non-amnestic, characterize prodromal AD (Vermunt et al., 2019). Even though the symptoms of AD are similar to normal ageing cognitive decline, they are not interchangeable with AD cognitive impairment. Patients with very mild to mild AD dementia have substantial changes in multiple cognitive, functional, and behavioural domains, ranging from mild to moderate impairments. People whom are aging normally will still have their personality and life-long interest intact and their certain characteristics such as levels of initiative, motivation, sociability, empathy and behaviour, and those patients whom are being diagnosed with dementia will have shown changes in their mood, anxiety, and sleep and also increased anxiety, depressive symptoms, apathy, and withdrawal symptoms, these are common in patients who are in early stages of AD also known as preclinical AD. As the disease progress further, the patient later has late-stage AD symptoms such as judgment impairment, disorientation, and

confusion and long with it their behavioural will have a substantial change such as aggression and irritation; and neuropsychiatric symptoms (Atri, 2019). Prevention of AD is very important as it has been identified about 60 environmental factors along with co-morbidities that increases the risk of AD. As currently most AD treatment do not improve much of patient's life and it is increasingly expensive to treat the patient and taking care of older patients who have lost all cognitive function becomes dependant on healthcare personnel completely and this causes an immense pressure in healthcare facilities, for this reason managing the risk factors is the first step to reduce the occurrence of AD. The risk factors include quality of air, heavy and other sort of metals, trace elements, exposure due to occupation, lifestyle causes like diet, sleep disturbance, smoking etc., and co-morbidities like vascular diseases, type-2 diabetes mellitus and adult-onset diabetes, traumatic brain injury (TBI), epilepsy, depression etc., (Armstrong, 2019; Edwards et al., 2019). Bangladesh has less concern of Alzheimer's disease as it is an overpopulated country and most dementia patients are treated in home under family care. According to WHO the number of deaths caused by AD is 1.26% of total death. In Bangladesh the availability of treatment of AD patients is very scarce and not to mention only 0.09 neurologist are available. In a country with rising issue of patients with dementia, there is lack of initiative by healthcare facilities to deal with the issue, there only few NGOs are available, to help in dementia related cases. It is widely known that dementia is a disease related to AD, so an increasing importance is needed to provide disease modifying therapeutics and various options that are available currently is only to treat the cognitive impairment symptoms, don't target the disease pathologies. AD is a fatal disease and it has increased burden in healthcare facilities along with the family of the patient, this disease should be given more resources for research regarding treatment, diagnosis and prevention of the disease (Roy et al., 2020). From a global perspective, nations such as the United States have around 5.8 million Alzheimer's disease patients, with 5.6 million of them over the age of 65. The medical expense of treating

Alzheimer's disease is predicted to reach one trillion dollars, putting tremendous strain on the medical industry. Loss of synapse and its function, disruption in mitochondria's structure and function, inflammatory actions, and neurofibrillary tangles (NFTs) that is intracellular and neuritic plaques that is extracellular are all linked to AD. Despite significant advances in our knowledge of synaptic defects and AD aetiology, no observable diagnostics, medicines, or treatments exist to prevent or delay the course of AD (Kumar & Reddy, 2020).

1.2 Symptoms of Alzheimer's Disease

Table 1: Signs and Symptoms of Alzheimer's Disease (AD) (Atri, 2019)

Functions	Alzheimer's Disease Symptoms
Memory	Patients have memory loss, and forgetting new information along with important dates and appointments, asking same question repeatedly within short period of time and depends on memory aids for daily tasks, which they didn't need before.
Planning or problem-solving	The patient has difficultly in doing the following tasks: developing plan, following a plan or known recipe, working with numbers and paying bills etc., starting, focusing or completion of a project.
Familiar task completion	The patient has difficulty doing common task they used to do early, like using appliances, devices, not being able to drive like before or forgetting familiar locations, and having problems with playing common games and managing budget.
Recognition of time or place	The patient forgets address, even if it is the same route they take daily and also gets confused about time, date, day and seasons, this occurs in later stages of AD. The patient mixes up timeline of events and people along with inability to process future and present events.

Comprehension of visual images and spatial relationships	The patient has inability to judge differences between size, shape, colour and identify common known objects and are not able to remember which location they are in and how they got there from the start.
Communication, use and recall of words and names during talking and writing	The patients have difficulty to converse with someone normally and forget meaning of certain words and have speech slurring.
Ability to retrace steps	Forget where they keep things, and misplace close personal items that they just kept aside few moments ago.
Judgment and interpersonal interactions	The patient become vulnerable to scam and other activities where an ill person might take their negative advantage, and they lost proper judgment in decision making whether personal, finances etc.
Work or social activities	Patients lose all interest in hobbies they used to do earlier in life and may even forget how to do them, also loose social life as they have behavioural changes and might become reclusive day by day.
Mood and personality	Patient becomes anxious, depressed, less motivated, fearful, suspicious, or having labile affect, they become very uncomfortable in new places and becoming more agitated for not remembering things, which results in misplaced anger and overreaction, and becomes impulsive in their actions and words.

1.3 Risk factors of Alzheimer's Disease

There are co-morbidities like vascular diseases, traumatic brain injury, type 2 diabetes mellitus that pose as a risk factor for AD. In case of vascular diseases, the control mechanism of cerebrovascular network and the neurovascular ensures constant blood flow which is an important function, in the brain the neurovascular unit consists of a specialized groups of neurons, astrocytes and vascular endothelial cells, all have important functions and any changes to vascular system causes a reduced cerebral perfusion leading to brain, giving rise to vascular pathologies gives rise to cognitive impairment and brain dysfunction related to AD. CVDs increase AD risk due to amyloid plaques and NFTs in the brain and hippocampus. Not to mention hypertension leads to atherosclerosis that causes blockade of cerebral blood supply which leads to cognitive impairment. It is hypothesised that Aβ causes hypertension before dementia onset, which leads to high blood pressure and cerebrovascular impairment. Hypertension increases risk of brain strokes which leads to dementia and thus increases risk of AD. It is suggested that strokes could initiate Aβ production, Aβ clearance is disturbed, and synaptic and neuronal loss is made worse, which has been already initiated by Aβ and tau pathology. Hypertension is found to cause intracranial atherosclerosis which lowers the blood perfusion of the brain, and this is connected with neuritic plaque increment. Cholesterol level increased in blood causes an increase in Aβ, which creates an imbalance. In Cerebral amyloid angiopathy (CAA) vessel the Aβ is accumulated causing constriction of vascular lumen inducing likely-hood of micro-aneurysms, this damages the cerebral functions leading to cognitive impairment and thus it is a huge risk factor in AD patients, where in 90% cases of AD patients have CAA. In CVD patients there are risks of protein misfolding and deposition, and increased amyloid accumulation. Vascular inefficiency generates hypoxia and hypo-perfusion, which activates the amyloid precursor protein (APP) cleavage enzyme α-secretase and promotes fibrillar amyloid deposition. Ischemia upregulates Aβ cleavage and

accumulation, whereas Aβ begins cerebrovascular dysregulation, increasing the brain's vulnerability to ischemia. When there is vascular dysfunction, the important Aβ clearance mechanisms are altered and impaired, resulting in Aβ build-up in the parenchyma and arteries. Tau hyper-phosphorylation and NFTs have also been linked to vascular risk, as well as the synergistic effect of increased Aβ burden, which means that increased Aβ plasma levels are linked to vascular diseases in both the brain and the periphery. To sum up, improving vascular health early on will reduce AD developing risks, as vascular diseases are becoming increasing common in every household of Bangladesh, this might cause an elevation of AD patients in future. Type 2 diabetes (T2D) is common cause of AD, being that AD is now referred as type 3 diabetes. Insulin receptors are found throughout the central nervous system (CNS), and hyperinsulinemia is linked to an increased risk of AD. The receptors modulate synapse density by regulating circuit function and plasticity and play a role in the cholinergic system. Insulin receptor impairment and hyperinsulinemia have been connected to aging and AD, and increased insulin and its receptor sensitivity is linked to the impairment of Aβ and tau expression and metabolism. Insulin assists in activating choline acetyltransferase, therefore unpredictable insulin levels can disturb the cholinergic system, in AD. Insulin degrading enzyme (IDE) is required for both insulin and Aβ degradation. As a result of the struggle between insulin and Aβ for IDE, hyperinsulinemia can result in an increase in amyloid levels in the brain. In rats with IDE there is loss-of-function, mutations, glucose intolerance and the accumulation of Aβ aggregates are seen. T2D causes protein misfolding and aggregation, which is common to AD. All these confirms that T2D is heavily correlated with the development of AD, and diabetes being very common in Bangladesh that it needs to be minimized to lessen the risk of AD development. Traumatic brain injury (TBI) causes damage to neurons and synapses, hippocampus, cortex and other medial temporal lobe structures, total brain volume loss, and ventricular volume increases. Due of mechanical trauma, oedema,

elevated intracranial pressure, and ischemia, thus TBI can cause fast necrosis. In patients with TBI, Aβ plaque level increases, especially the Aβ42 type and also tau levels and NFTs increases. Patients with Mild cognitive impairment (MCI) have their age of onset of AD accelerated if they have history of depression. Patients with depression have higher levels of Aβ in their brains, as a result increases the formation of amyloid plaques through increasing the corticotropin-releasing factor release, which leads to an increase in neuronal activity, stimulating the production of Aβ, and this displaying an association between depression and AD development. Stress causes neuronal loss in the hippocampus, which is affected by AD neuropathology, through increasing glucocorticoid release and decreasing neurotrophic factor levels. Finally, lifestyle factors like physical activity, sleep disturbance, diet and smoking also are risk factors for developing AD. Elderly people who does regular exercise particularly aerobic exercise has increased hippocampus density and brain volume. Exercise is shown to increase neuroplasticity, which improve cognitive functions and thus slower the development of dementia, eventually AD. Sleep disturbance has shown to increase risk of AD. Sleep disturbances are linked to an increased risk of cognitive impairment, such as MCI and dementia, whereas Aβ deposition appears to affect sleep efficiency (the amount of time spent sleeping in bed), particularly in the preclinical stages of AD. Because continuous light exposure reduces the synthesis of the hormone melatonin, which governs the sleep-wake cycle, a rise in insoluble tau and memory impairment occurs when the quantity of light is increased during the sleep-wake cycle. Sleep has been discovered to improve the rate of Aβ clearance in the brain via the glymphatic system. Indeed, when awake individuals were compared to sleeping humans, the interstitial concentration of Aβ was greater, indicating that alertness is related with increased Aβ production. It has been demonstrated that a single night of sleep disruption induces an increase in Aβ levels in the brain as well as an increase in ISF tau levels, demonstrating that sleep has a direct role in AD pathogenesis. Smoking increases the risk of

hypertension which results in vascular disease and causes cognitive impairment and declines in verbal memory and slower visual search speeds and increasing amyloid deposition, promoting tau hyper-phosphorylation, and worsening the inflammatory response, but smoking is not associated with dementia. Controlling all the lifestyle factors can lead to reduced risk of developing AD or lowers the devastating effects of the disease (Edwards et al., 2019).

1.4 Current treatment Option

FDA has approved drugs to manage cognitive impairment and symptomatic AD malfunction, those drugs are cholinesterase inhibitors (ChEIs) like donepezil, rivastigmine, and galantamine, and also have approved uncompetitive NMDA receptor antagonist such as memantine. Currently there is no disease modifying drugs. In AD pathogenesis there is presence of cholinergic deficit, where cholinergic neurons in Meynert's nucleus basalis and other septal nuclei distributed mainly in the cortex are lost. The cholinergic input loss is theorized to cause AD's early attention and memory deficits and ChEIs recovers deficiency by increasing acetylcholine at synapse, and most are used mild to moderate AD. These medications do not modify the disease pathology rather treat the cognitive symptoms associated with the disease. Each ChEIs have different pharmacokinetic profiles, for example donepezil has longer half-life in comparison to others and are given as single dose and formulation are also different, rivastigmine is given as transdermal patch which releases continuously but their efficacy does not vary significantly. Meta-analysis of cognitive functioning and global functioning, the drug efficacy is measured and confirmed. They have small beneficial effect and in long run do not help slowing the disease progression. ChEIs are not given for preclinical AD stage as it might cause disease to worsen instead. Another drug is anti-NMDA which is memantine. Memantine is NMDA receptor antagonist that inhibits neurotoxicity mediated by glutamate, and shows down neuron death in AD and by measuring the cognition, activities of daily living , and NPS, the efficiency of the drug is analysed, but its effect is short term and potency is less, and are

employed in AD at the mild to moderate stages and are not given to patient with mild AD. Aβ-therapeutics, such as active and passive immunization strategies, secretase inhibitors that target Aβ, NFTs, and inflammation are needed for creating disease modifying therapeutics. Among these passive immunizations such as aducanumab has been used in patients recently as disease modifying treatment, and to clear Aβ there is passive immunization. Monoclinal antibodies have less adverse effects and variability in their efficacy and titre levels is managed strictly. Aducanumab is human Immunoglobin1 antibody that binds to Aβ epitope specifically and clears amyloid plaques and this is consistent in APP animal model and human subjects with prodromal or mild AD (Long & Holtzman, 2019). Aducanumab binds mainly to the parenchymal Aβ not vascular Aβ, and they mainly target pathogenic Aβ oligomers and clear them as they are responsible for cognitive decline and dementia in AD patients. Aβ plaque burden is reduced after aducanumab was given in dose dependent manner, this stabilizes the cognitive decline after a year of treatment. Aducanumab is selective for insoluble fibrillar aggregates and soluble Aβ oligomeric depositions found in the amyloid plaques, aducanumab do not bind to non-pathogenic Aβ monomers and is only binding with pathological Aβ aggregations. Despite the positive effects the clinical improvement of AD patients is not consistent throughout all the clinical trials and the different dose-groups, this limitation caused it not be fully approved as a potential AD drug, as there is still more research and data is needed to ensure that this drug is able to completely combat the Aβ plaque aggregations, without any drawbacks. Not to mention aducanumab is only approved for patients with MCI, and the drug cost is extremely high at the moment and patients taking aducanumab has developed ARIA associated with oedema, microhaemorrhage, or superficial siderosis, and this has to be monitored regularly, increasing the cost of the treatment even further (Mukhopadhyay et al., 2021).

1.5 MicroRNA and its Application in Alzheimer's Disease

MicroRNAs are small RNA molecules of 22 to 25 nucleotide long and are expressed in humans, and these microRNAs are involved in development and pathological processes via regulation of the gene expression through binding at 3 prime untranslated region (UTR) of mRNA in a sequential manner. The microRNAs are found throughout the human body but some are localized in specific tissues. By the nuclear genome microRNA are made in the nucleus and they modulate host cell's mRNA and protein levels. The microRNAs that are circulating are released from cells and enter extracellular spaces and here they manage intra-cellular communication. MicroRNA expressions is imbalanced in the brain, extracellular fluid in patients with AD. In the brain microRNAs are overexpressed and they are major CNS regulators. Any impairment in microRNA biogenesis disrupt nervous system development in terms of its ability for differentiation, learning, memory, and neuronal survival. They are found in cell compartments such as rough endoplasmic reticulum, multivesicular bodies, lysosomes, mitochondria, etc, and the microRNA also micromanages the cells. The microRNA bind with hundreds of different mRNA or genes targeted, not all binding is of physiological importance, not to mention not all microRNAs acts as switch on and off factors they also fine-tune gene expression profiles in the absence of mRNA degradation, with the regulation of local dendritic mRNAs or proteins during storage and or activation at the synapse (Kumar & Reddy, 2020). MicroRNA has a potential to be revolutionary therapeutic method, this is due to its ability to target many mRNA, and switch on and off certain gene which can be modified to target genes that further worsens the AD pathology. They can be given to increase the level of microRNA that is beneficial to AD or as a knockdown of microRNA, and they are currently in clinical trials for cancer therapy but not yet for AD. But, multi-targeting microRNAs such as microRNA mimic oligonucleotides which is microRNA supplementation, microRNA antisense oligonucleotides which acts as microRNA knockdown are in preclinical trail, tested

for its ability to simultaneously target multiple pathways of AD pathology related (Walgrave et al., 2021).

1.6 Objectives of the Study

In this study the roles of microRNAs in the Alzheimer's disease are explored and identified and this way the treatment of patients with AD are made easier by identifying novel pathway of AD treatment. In this study first objective is to identify microRNAs that can help treat the neurodegeneration through mRNA regulation and deregulation and explore its full potentials in slowing down the disease progression and also ways microRNA can further hamper the disease, this way the microRNAs can be manipulated to treat the patients. Finally, this study will compile how the different pathologic aspects of AD can be treated through microRNA, and how it can be formulated to be administered in patients and provide a hope for patient suffering from the disease, which currently has less treatment options, so that further study can be conducted and implement the research in human trials not limit to animal models.

1.7 Aim of the Study

Aim of the study is to provide explanation and compilation of how microRNAs provides a therapeutic effect for the treatment of Alzheimer's disease patients, and provide insight to a novel way to treat Alzheimer's disease.

Chapter 2 Methodology

This review paper has been conducted based on recent and relevant research papers and articles from high-impact factor journals. A comprehensive search has been performed through peer-reviewed journals, official reports, and articles. To enrich the review paper, basic and additional information have been collected from different books. Following search engines have been used to collect data for this paper- ResearchGate, Google Scholar, Science Direct, PubMed,

Cell Press, Elsevier, etc. in which the major publications include- Nature, ACS (American Chemistry Society), AACR (American Association for Cancer Research), Molecular Cell, Cancer Cell, Journal of Molecular Biology, Journal of Medicine, Science, etc. In-depth screening of the journals followed by narrowing down to the most recent (within the last 5 years) and relevant ones was done to create an ideal quality review on Application of microRNA (miRNA) in Alzheimer's Disease (AD).

Chapter-3 Pathology of Alzheimer's Disease

3.1 Beta-amyloid (Aβ) Pathogenesis

Beta-amyloid also known as A4 amyloid protein is a peptide which is the target of protein of insoluble fibril-type within the Alzheimer's disease's cerebrovascular amyloid angiopathy. Aβ is the target molecule for the treatment of the with the disease. The amyloid precursor protein (APP) and its processing by the Beta-secretase (BACE) and Gamma-secretase complexes PSEN1 and PSEN2 have been studied genetically, resulting in a fundamental molecular knowledge of its bioprocessing. In Familiar Alzheimer's Disease (FAD), there will be rare mutations in the PSEN genes and APP genes which causes abnormal amyloid beta formation or an unfavourable shift in the ratio of Aβ40/Aβ42, and in certain occasions, there is an increased Aβ production. The Amyloid cascade is where the Aβ accumulation causes a triggering of a series of events that lead to plaque formation and results in the pathology of AD, this hypothesis does not take into account of Aβ's different forms, such as beta-amyloid monomer, misfolded soluble beta-amyloid oligomers, and fibrillar beta-amyloid. The Beta-amyloid dysfunction (BAD) hypothesis focuses more on these different aspects and its effect on Alzheimer's disease progression. Aβ is produced in synaptic process at elevated production and turnover rates. It's produced as an Aβ monomer by excited neurons and is proven that both in time and concentration dependent manner, it is able to affect synaptic activity. In acute brain

damage, Aβ dynamics are related to neurological state. It's thought that Aβ monomers Aβ1–40 and Aβ1–42, when released in a well-controlled manner by BACE- and Gamma-secretase, are important facilitating factors for synaptic vesicle cycling in neurons. One of the early and best biomarkers for people Alzheimer's disease is a decrease in CSF Aβ-42 monomer levels. Aβ species that have structurally change into at least dimers or multimers, thus forming 3D structure that becomes detergent soluble amyloid Aβ species, is characterized as a misfolded Aβ oligomer. The bulk of deposited Aβ oligomers in Alzheimer's disease brains may be precisely described alongside monomers and fibrils by immunoprecipitation with unmovable Aβ oligomer isotope specific antibodies from Alzheimer's disease brains and then quantified separately as Aβx-y oligomers in a separate step. Aβ fibrils are made up of Aβ monomers and grown in number, oligomers are not responsible for their folding pathways. Aβ fibril are the major plaques found in Alzheimer's disease brain. The correlation of the different forms of beta-amyloid with Alzheimer's disease pathophysiology is explained in the figure no.1.

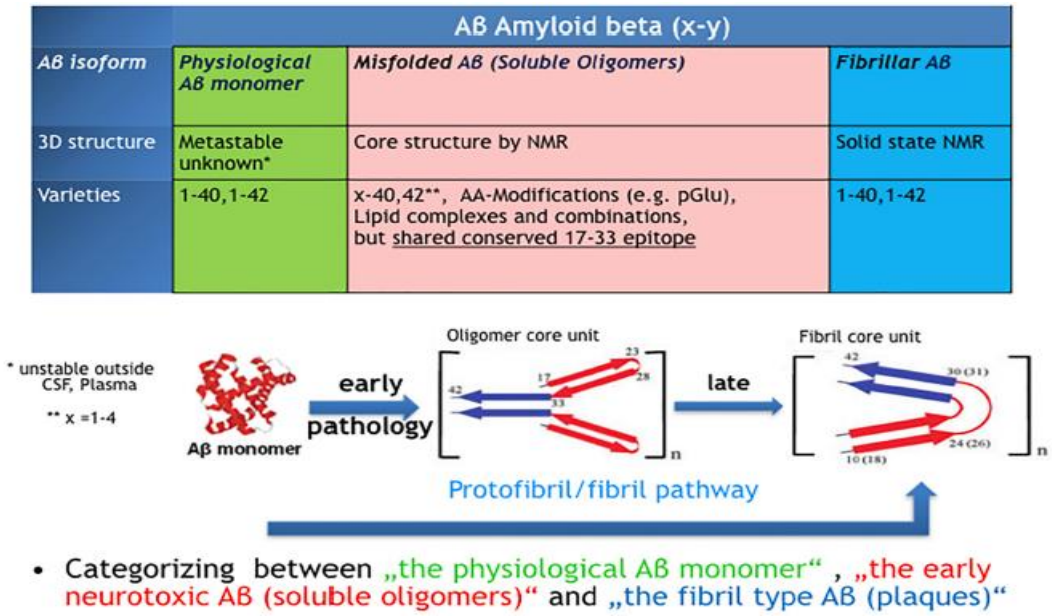

Figure 1: The relation between different types of beta-amyloid (Hillen, 2019)

The process of biogenesis and turnover of Aβ is physiologically essential and occurs on neurons of vertebrates. The difference in Aβ Cascade hypothesis and BAD hypothesis is that

in BAD, the Aβ is assumed to have physiological importance in healthy people, and it has been shown to have important duties in regulation of synaptic activity. The Aβ monomer homeostasis in the synapse is complex and essential and the complexity is because of the metastable hydrophobic peptide with membranes, lipids, and vesicles interplay. When formation and metabolism of Aβ is disrupted it causes Aβ pathophysiology. When the Aβ turnover mechanism is altered, misfolded neuropathogenic species are produced early, which hampers the homeostasis of Aβ. According to BAD hypothesis, the binding of neuropathogenic types of Aβ oligomers with the receptors induces toxic effects on cells and the imbalance of Aβ homeostasis causes the pathology of AD. It is assumed that if the optimum concentration of Aβ is not met through the positive feedback mechanism, of APP processing and BACE up-regulation, the Aβ is replenished and this can be detrimental as increased production and turnover rate of Aβ will cause an increases mild-folding of Aβ. In the CSF of AD patients and pre-synaptic terminals of TG models, this phenomenon can be detected through the increment of BACE levels. For the synaptic vesicle cycle (SVC) process, the peptide Aβ monomer is an important source of cofactor physiologically, and is considered to be harmless peptide. The SVC is mainly found in the pre-synaptic terminals and is the main site for Aβ production, and this can be said the Aβ monomer to be physiologically essential, but its other forms such as misfolded oligomers and fibril are harmful. It had been found that AD patients have 2.5 fold less Aβ turnover, and this means that the optimum Aβ concentration is hampered in the synapse, along with the gamma-secretase and beta-secretase activities, that ensures the regulation of Aβ levels according to different individual neuron needs. It is already known that, Aβ misfolding and lowering of Aβ monomer1–42 CSF level due to Aβ evading the in-vivo folding by FAD chaperone control due to the mutation of APP or over-production of Aβ monomer that triggers the formation of oligomers, causes the Aβ pathology in AD patients. The misfolding of Aβ monomer causes the depletion of the monomer in the synapse, and as its

important for controlling the synaptic activity, this loss is compensated through the positive feedback of BACE up-regulation. But this further caused increased production of Aβ and more misfolding, more formation of oligomers, and deficiency of monomers. All these suggest that the misfolding of Aβ monomer, formation of misfolded oligomers in the neuronal sites is the cause of Aβ pathology that leads to AD. The BAD hypothesis proved that lowering the production of Aβ by the use of secretase inhibitors is useless as BACE activity is elevated in first place due to reduced Aβ monomer, gamma secretase modulators that will maintain the ratio of Aβ42/Aβ40, and reducing the chances of Aβ aggregation (Hillen, 2019).

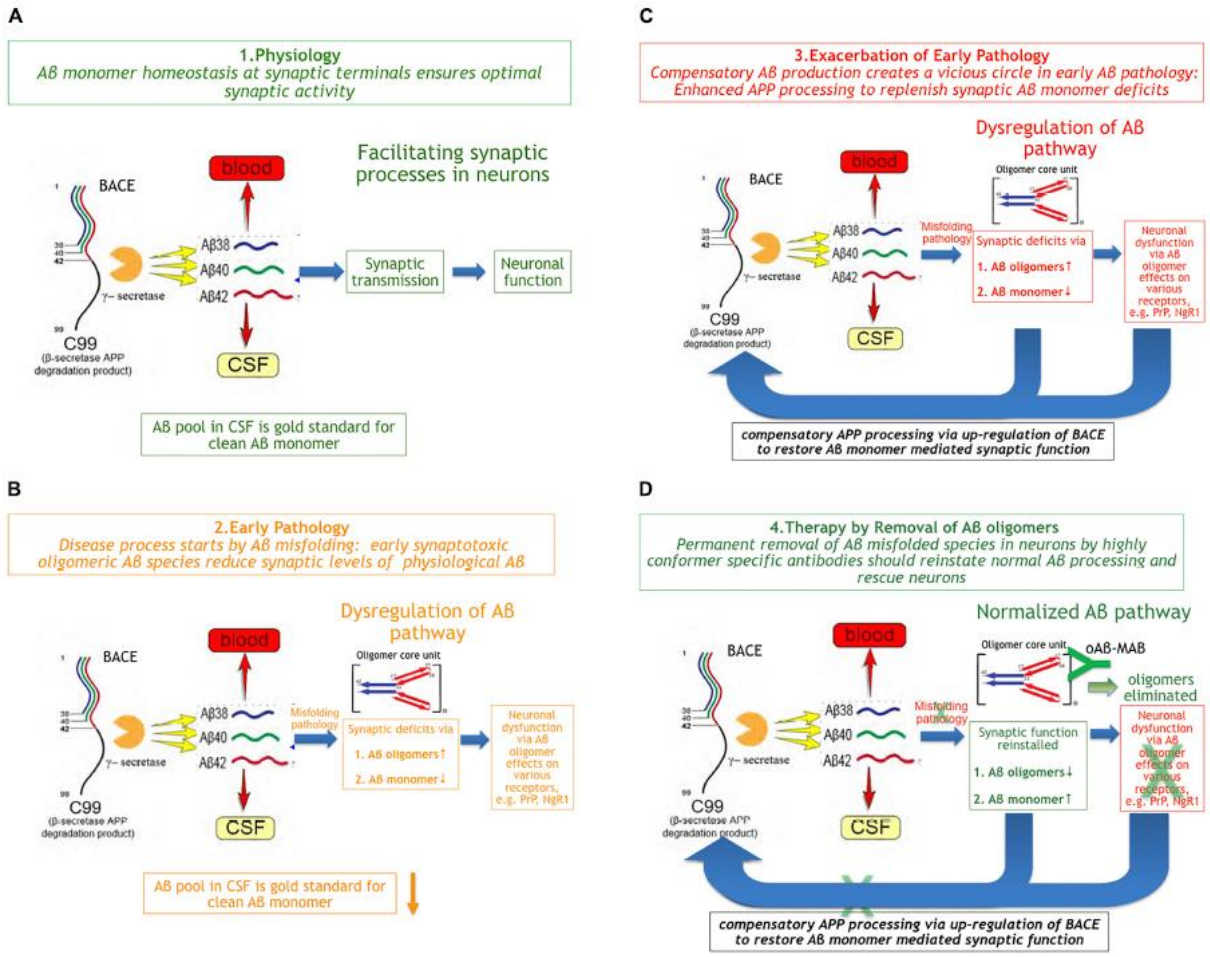

Figure 2: The process of early Aβ monomer misfolding causing Aβ pathology in AD (Hillen, 2019)

3.2 Tau Pathology

Neurofibrillary tangles (NFTs) is present within the brain of AD patients and are responsible for neurodegeneration and it made up of tau proteins in hyper-phosphorylated and aggregated form. The pathology of tau correlated to the AD brain functional deficits. Propagation of tau follows neuroanatomical pathways and it shows that the abnormal tau transmission is prion-like manner from cell to cell. This is assumed to trigger accumulation and normal soluble tau proteins are transformed into pathologically aggregated tau and this is done though the endocytic mechanisms (Ando et al., 2021). The major constituent if NFTs in AD brain is tau, and this tau is axonal microtubule–binding protein possessing many physiological functions and consist of subdivision like structurally disordered N-terminal region, the proline-rich mid-domain, and the highly conserved C-terminal domain. The microtubule binding repeats, which are partially integrated into the center of tau filaments, are found in the C-terminal portion. The Tau protein have post-translation modifications like phosphorylation, acetylation, and truncation and hyper-phosphorylated tau proteins are integrated into the fibrils. It is assumed that truncation of tau at N- and C- terminal have necessary role in the tangle formation and this truncated tau induces neurofibrillary degeneration within the transgenic animals. The tau protein which originally was not folded, the tau has physiological structure where the C-terminal is folded over the microtubule binding repeats and each end of the molecule later conjoins, this microtubule repeats is made up of two hexapeptides that is made up of intermolecular β-sheet rich structures. Tau might develop an irregular shape under pathological situations, revealing these residues and increasing its susceptibility for self-aggregation. Through the process of templated misfolding or seeded nucleation, physiological tau monomers is able to be integrated into the aggregates and this causes formation of aggregates elongation rapidly. The seeding process begins with the misfolding and aggregation of tau monomers, which subsequently serve as the basic building block for the development of

oligomers and, eventually, the formation of detergent insoluble, highly structured fibrils. In AD, these particular tau filaments form a paired helical filaments or straight filaments, and they form a mixture within the cell creating the disease causing NFTs. Neurons with NFTs can live for decades in AD brain. In addition, neuronal loss in Alzheimer's disease correlates with NFTS but their amount outnumbers NFTs. In an animal model with aggressive tauopathy, suppressing tau overexpression prevented neurodegeneration and cognitive impairments. NFTs keeps on growing, as a result soluble tau which are not sequestered by insoluble tau fibrils creates toxicity. When an aggregation-prone type of tau overexpression causes the formation of Gallyas-positive NFTs quickly, while soluble tau neurotoxicity was decreased. It's still possible that bigger tau aggregates cause toxicity by occupying space in the cell's congested environment. The soluble oligomers, on the other hand, may easily diffuse throughout the cell, interact non-physiologically with a wide range of cellular proteins, and induce synaptotoxicity. Smaller tau species can also spread from one cell to another, spreading physiological tau in healthy neurons. Synaptic activity increases the secretion of both physiological and accumulated tau into the interstitial fluid. After entering the neuron, tau seeds can leak into the cytosol via damaged vesicles and seed physiological monomers, propagating the pathological process. Microglia and astrocytes can also phagocytose extracellular tau and play a role in its growth. The spread of tau is now thought to be at the root of tau disease extending throughout the brain, as demonstrated in figure no.3.

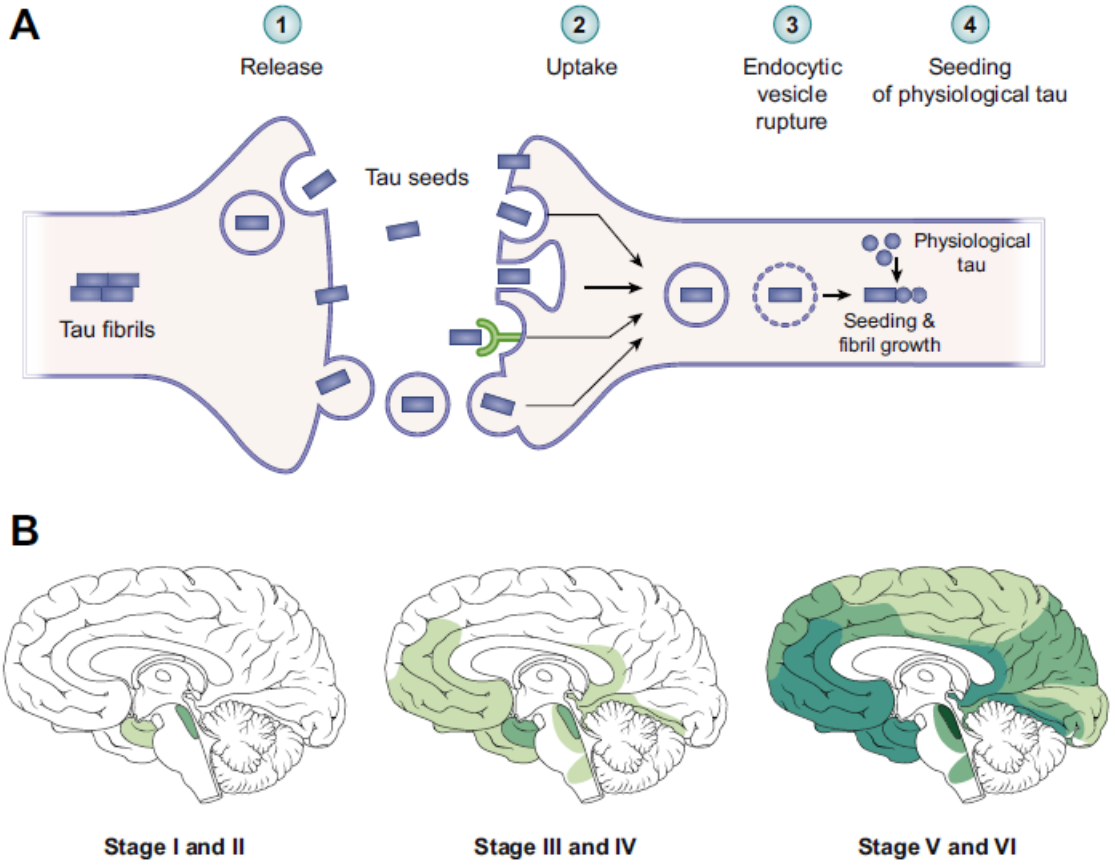

Figure 3: Demonstration of Tau release in part (A) and Part (B) exhibits its pathology in AD Brain (Vogels et al., 2020)

Tau disease is assumed to start in the rostral medial temporal lobe (particularly the entorhinal cortex, Braak stage I/ II) and spread to limbic areas (Braak stage III/IV), including the hippocampus, tau disease eventually reaches the neocortex (Braak stage V/VI), which is always accompanied by cognitive symptoms, as shown in figure no.3. The entorhinal cortex neurodegeneration occurs tau causes the functional disconnection of the hippocampal formation from the cortical association areas, which is assumed to be the cause of cognitive symptoms in AD. In AD, entorhinal cortex is vulnerable to tau pathology and neurodegeneration. Tau pathology in Alzheimer's disease appears to invariably impact the same cell type, like the big excitatory pyramidal cells in layer II of the entorhinal cortex that project to the hippocampus through the performant pathway. In tau pathology the tauopathies

start in different part of the brain and affect the glial cells. For age related tau astrogliopathy the staging progression of astrocytic tau pathology has been made, and it is said that there is selective regional vulnerability to tau pathology and it differs between different tauopathies. It was previously mentioned that tau pathology vulnerability depends on the regional vulnerability of the brain but now evidence shows that tau aggregates spreads through neuronal connections and causes misfolding in healthy cells, which is confirmed by the finding of seed-competent tau from white matter tracts, and also from synaptosomes from patients with AD, in the region of brain where tau pathology was not detected. Tau obtained from AD patients with Aβ plaque pathology was much more seed-competent than tau isolated from patients without plaques; this is explained experimentally by an increased fraction of high molecular-weight tau among the presence of plaque pathology. Using the used Förster resonance energy transfer–based cellular biosensor assay, it was demonstrated that Primary microglia collected from AD patients, secret seed-competent tau into extracellular region, this concludes that despite microglia phagocytose extracellular tau, it is not fully destroyed and being secreted back into extracellular region thus microglia contributing to the propagation of tau pathology. The region-dependent differences in phagocytic capacity and the process being age sensitive, this connects to the relation between regional vulnerability and tau spreading. Transgenic animal model shows that human tau has the ability to spread to neighboring and synaptically connected neurons, this models also shows that Aβ deposition accelerates the propagation of tau, and increasing the neuron activity through optogenetically the spreading of tau in entorhinal cortex speeds up. AD-derived tau filaments are more aggressive than recombinant tau fibrils, according to these research. When AD-derived tau filaments were injected into transgenic mice with Aβ plaque pathology, all three primary kinds of AD tau pathology (NFTs, neuropil threads, and tau aggregation in dystrophic neurites) were formed in an animal model. Individuals with dementia has highest tau longitudinal PET signal, and these studies on human

conflict with the idea that tau spread from affected area to another unaffected area in brain synaptically. Lesion propagates over limbic and adjacent cortices, forming a pattern that is regarded a hallmark for neuropathological diagnosis of the condition. Neuronal loss induced by disconnection and differentiation of important brain circuits causes defects in memory and higher-order cognitive skills in Alzheimer's disease. The structural patterns of tangle build-up in AD are caused by local tau aggregation inside neuronal cells that is passed from cell to cell, leading to the concept that circuit-based patterns of neurodegeneration are a significant role in tau pathology. Tau pathology is caused by two sources. It appears that tau spreads to downstream linked neurons despite regional and cellular restrictions, as seen in transgenic mice with Alzheimer's disease. This results in extremely gradual synaptic, axonal, and somatic degeneration, all of which are related with the build-up of misfolded tau. Tau fibrilization begins when extracellular tau aggregates penetrate cells. In astrocytes and oligodendrocytes, this tau is found. Tau is released into the synapse as hyperphosphorylated tau, misfolded tau, or a fragment of tau, and tau is also transmitted over the synaptic connection. The build-up of tau in the synapse causes synaptic damage (Vogels et al., 2020).

3.3 Microglia and Inflammation

Inflammation in Alzheimer’s disease is detrimental and chronic in nature, and post-mortem of AD patients shows increased levels of microgliosis and astrogliosis surrounding Aβ plaques, with increased levels of inflammatory cytokines and chemokines, which connects with the AD disease progression and cognitive impairment. Microglia is main contributor for inflammation within the AD. Microglia are innate immune cells located within the CNS, unlike other glial cell, microglia with peripheral macrophages shares amyeloid lineage. In mice model, due to neurodegenerative conditions such as AD, proliferation of microglia increases. Within the CNs microglia has functions such as the immune response, extracellular signalling, phagocytosis, antigen presentation and synaptic pruning, these numerous functions of microglia indicate that

even under normal physiological conditions there is limited self-renewal. It is known that in different region of the brain and in different environment, microglia present will have different function, phenotype and markers, thus being heterogeneous in nature. Microglia is identified as M1 and M2 based on their differences in phenotype and its marker. Microglial cells that are aged shows an increase pro-inflammatory response in case of injury. Peripheral lipopolysaccharide (LPS) injection to young mice, in comparison to adult mice caused an increased level of interleukin (IL)-6 and tumour necrosis factor (TNF)-α, which is pro-inflammatory cytokines. These alterations are predominantly IFN-dependent, according to gene ontology analysis of transcriptome data from young and old mice. These modifications are accompanied by morphological changes, the most prominent of which is hypertrophy. Along with morphological changes all these corresponds to hypertrophy, and phagocytic functions are also altered, its function is decreasing by age, it has not been confirmed whether its due to AD or not. Genes that were changed in total brain tissue were shown to be substantially concentrated in microglia and astrocytes in an AD model including APPSWE and the N141I mutation in PS2. However, only a minority of these genes were really up-regulated when investigated in these cell types as a distinct population and microglia makes the majority of the cells. It is known that microglia have ability to morph and this morphological structure is based on physiological environment, such as microglia that are 'quiescent' have a ramified morphology, whereas those that are 'active' have an ameboid shape, and this changes are dependent on regions. In mice model, those mice that are treated with LPS have different cell perimeter, roundness and soma size, in AD model of dual Indiana and Swedish APP mutation their microglia morphology are different from those that are wild-type mice, as there is changes in branch length, area and this has negative effects. The human subjects with AD microglia are observed to have five distinct morphological type in various sections of hippocampus. Microglia with similar morphologies might, however, have different underlying phenotypes.

Aβ peptide and its aggregates triggers neuroinflammation through the microglia and this process varies. This sensing occurs through receptors like, Toll-like receptors (TLRs), nucleotide-binding oligomerization domain (NOD)-like receptors, receptors for advanced glycation end-products, formyl peptide receptors, scavenger receptors, pentraxins and the complement cascade. All these initiates neuroinflammation via signalling pathways. These sensing can also occur through the NOD, leucine-rich-containing family, pyrin domain containing-3 inflammasome, multiprotein complexes involved in the maturation and secretion of pro-inflammatory cytokines IL-1β and IL-18. TLRs recognizes both DAMPs and pathogen associated patterns and TLR2 and TLR4 are important for Aβ recognition, and the TLR2 is responsible for triggering the neuroinflammatory response. The downstream TLR signalling through the NFκB, activator protein 1 and IFN regulatory factor (IRF) pathways causes proinflammatory gene transcription, by endocytosis microglia takes part in Aβ clearance which depends on whether Aβ is in fibrillar or soluble oligomeric which causes in phagocytic or macropinocytic processes respectively. Fibrillar A is identified by a cell surface receptor complex made up of class A scavenger receptor, class B scavenger receptors, α6β1 integrin, CD14, CD36, CD47, TLR2, TLR4, TLR6, and TLR9 receptors. By increasing TLR activation the Aβ fibrillary clearance occurs through phagocytosis. Removal of TLR2 and TLR 4 causes ineffective cell receptor complex to identify Aβ for it to initiate clearance via phagocytosis. Aβ that is soluble are cleared through fluid phase macropinocytosis. If there is Aβ deposits and neurofibrillary tangles in excess these impair the Aβ-related clearance abilities by the recruited microglia and astrocytes. Microglia releases extracellular microvesicles (MVs) through exocytosis which is potent modulators of inflammation and immunity, and can stimulate synaptic activity, this MVs is elevated in AD patient. MVs is neurotoxic and increase Aβ production. MVs, also drives tau related pathology, and inflammation caused by microglia causes both aggregation and tau phosphorylation. The senescent morphological microglial is

associated with tau and this is observed from the human brain sample analysis. In wild-type mice, LPS-induced inflammation has been shown to induce tau aggregation with this further enhanced in the microglial fractalkine receptor CX3CR1 lacking transgenic mice. Abnormal engulfing of synapsing during synaptic pruning by microglia leads to dysfunction. Microglia causes abnormal phagocytosis of healthy neurons due to the inactivation of signals present on neurons that prevents its phagocytosis under normal conditions, and this is observed in AD cases (Moore et al., 2019).

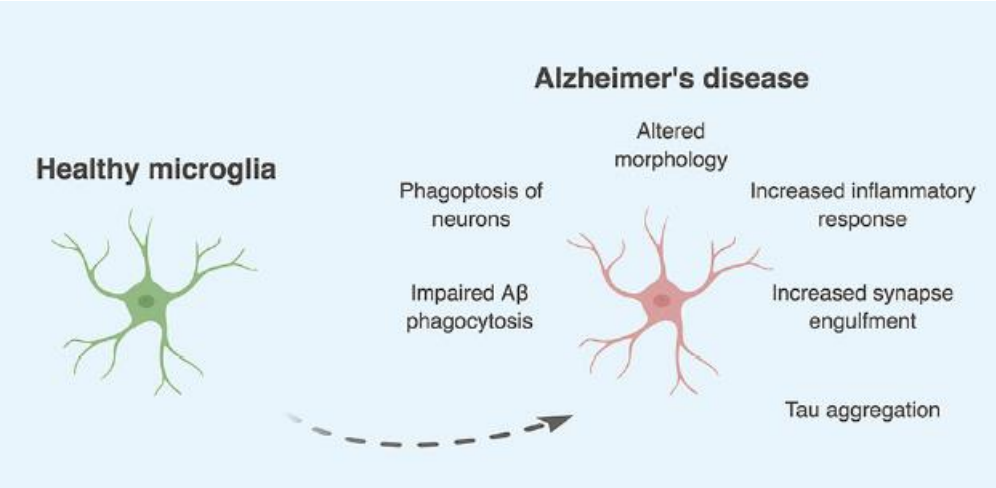

Figure 4: Alterations in microglia in AD (Moore et al., 2019)

Chapter-4 MicroRNA application in Alzheimer's Disease

4.1 Beta-Amyloid

In the degradation of APP and Aβ metabolism, microRNA have roles in regulating genes that are related and associated pathways. The degradation of APP occurs through secretase pathway. The α-secretase hydrolyzes APP to produce fragments (sAPP) which has external functions, P3 and AICD, that is soluble and has neuroprotective action, in comparison to APP hydrolyzed by β-secretase gives forms of Aβ40 and Aβ42. Aβ42 is accumulated more than Aβ40, and this causes formation of plaques and thus giving neurotoxic effects, and the ration

of Aβ40/Aβ42 is dependent on the enzyme γ-secretase. In APP lysis the key enzymes' activity is regulated by microRNA, and various microRNA takes part in Aβ metabolism through adjusting β-secretase activity such as the BACE1, and those microRNAs are microRNA-339-5p, microRNA-29c, microRNA-15b, microRNA-195, and microRNA-124. The BACE1 expression increases if the microRNA-339-5p is downregulated, and this induces Aβ deposition. BACE1 expression are negatively regulated by microRNA-29c and microRNA-135b, and gives neuroprotective effects. In APP transgenic mice, the BACE1, Aβ and neuro-inflammation levels due to the overexpression of microRNA-188-3p in the hippocampal. ADAM metallopeptidase domain 10 (ADAM10) hydrolyzes the APP which gives non-pathogenic Aβ, and the AD-related ADAM10 is a part of ADAM family of α-secretase. In AD, the microRNA-221 is downregulated and this results in ADAM10 content to increase. Aβ activates microRNA-140-5p, and it is both the ADAM10 negative regulator and the transcription factor of SOX2. BACE1 and ADAM10 and APP metabolism regulation are targeted by microRNA-107, thus confirming the facts that a single microRNA has the ability to target various genes or pathways and causing combined effects. MicroRNAs like microRNA-29c, microRNA-107, and microRNA-339-5p and 10 other microRNA regulates BACE1, and in AD these microRNAs are downregulated giving negative correlation with BACE1. ADAM10 is negatively regulated by microRNA-221 and microRNA-140-5p, but in AD microRNA-221 is downregulated, meanwhile microRNA-140-5p is upregulated, and thus playing different roles. In, γ-secretory proteolytic system the PS-1 is important component and neuron is protected by the regulation of microRNA-212 and PEA15 by the PS1/γ-secretase system. The APH1 subtype and γ-secretase complex subunit is the Aph-1 homolog A (APH1A). The γ-secretase complex activity and Aβ levels is increased due to the APH1A overexpression. In the hippocampus, the long-term episodic memory formation, is achieved by targeting APH1A protein by microRNA-151 and causes decreases in the protein level. AICD

is elevated due to the increased level and activity of β-secretase, which later stimulates APP and BACE1 expression and thus for amyloidogenic pathway more enzymes and substrate are available. Through the amyloidogenic pathway the AICD is formed and this has the ability to translocate nucleus and acts as transcription regulator. The expression of FBXL18 and CDK6 is directly downregulated by the AICD/miR-663 and this affects neuronal cell growth and differentiation. In the Aβ metabolism the microRNAs are also involved. In hippocampus of AD the family member of microRNA-15 or microRNA-107 consist of including microRNA-103 and microRNA-107 are downregulated, this intensifies the Aβ generation and APP phosphorylation. This results in activates cyclin-dependent kinase 5 (CDK5) due to increased levels of CDK5R1/p35 and proceeds to stagnation. The microRNA-107 is inhibited by the APP overexpression and microRNA-188-5p expression in the neurons of hippocampal is reduced by Aβ42 oligomerization. In APP/PSI their cortical neurons are exposed to Aβ, after 48 hours, this causes microRNA-34a to first increase and then decrease in level (Wei et al., 2020). In animal models with AD the microRNAs such as microRNA-9, microRNA-29, microRNA-29a/b-1, microRNA-124, microRNA-101, microRNA-107, microRNA-298, and microRNA-328 expression is reduced by regulating BACE1 and or APP expression as they participate to increase Aβ production. From clinical studies of AD patient's peripheral whole blood shows a downregulation of microRNA-29a/101. For the regulation of APP levels come microRNAs takes part it in. As an example the microRNA-106a and microRNA-520c overexpression causes marked reduction of APP level in HEK-293 cells. In both humans and transgenic mice, the microRNA-29 overexpression causes increased Aβ production but decreased endogenous BACE1. On the other hand, the microRNA-17, microRNA-101 and microRNA-16 reduced expression is followed by increased APP level, thus concluding that those microRNAs are involved in APP suppression. In N2a/APP695 cells the microRNA-195 overexpression gives reduced Aβ level meanwhile inhibition of the microRNA increases Aβ. The reduction of these

microRNAs causes increased BACE1 expression and function which leads to uncontrolled Aβ production and this is the common in AD brains of humans and mice. Along with it, microRNA-186 expression in neuronal cells causes Aβ level to decrease through the suppression of BACE1 expression, but endogenous microRNA-186 downregulation creates an opposite effect. These researches focus on the molecular processes behind BACE1, APP, and Aβ dysregulation in Alzheimer's disease, as well as fresh insights into the illness's etiology. MicroRNA-128 increases Aβ levels and causes the development and progression of AD. In of 3xTg-AD mice in comparison to Wild-type mice there is an increase of Aβ and microRNA-128, where as in knockout mice there is improved cognitive function. The microRNA-126 inhibition creates a neuroprotective function against Aβ42 toxicity, linking them with AD progression (Kou et al., 2020).

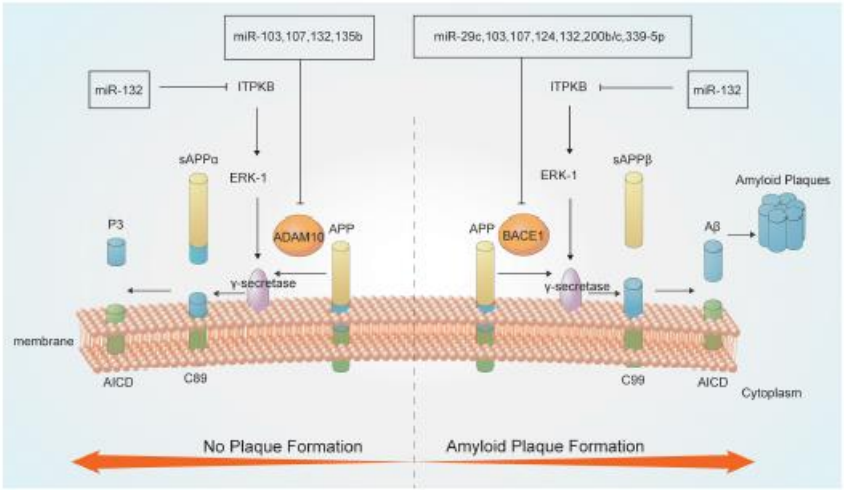

Figure 5: MicroRNAs (miRNAs) are involved in Aβ metabolism (Wei et al., 2020)

4.2 Tau pathology

The microRNAs such as microRNA-129 targets MAPT and affect tau protein synthesis and by regulating important enzymes' activity it also affects tau phosphorylation. The protein kinases like GSK3, PKA, and CDK5 have the ability to manipulate the phosphorylation sites exposure and thus modulating tau protein phosphorylation, meanwhile at multiple sites tau protein is

dephosphorylated at different amount by phosphatases. Tau phosphorylation is also regulated by proline-directed serine/threonine kinase known as CDK5. The CDK5 is activated by P35/P25 and p35 cleavage, induced by calpain (CAPN) giving the p25, which causes an increment of CDK5 activation uncontrollable and thus inducing tau hyper-phosphorylation. In AD, the microRNA-124-3p is reduced and they function in preventing the transformation of p35 to p25 by inhibiting the CAPN1 mRNA translation, as a result abnormal tau phosphorylation is reduced. For tau phosphorylation the GSK3 kinase is needed and it is downregulated by microRNA-219-5p and thus in AD tau phosphorylation is reduced. For memory formation and maintenance, the microRNA-132/212 has major role, and downregulating them causes the S-nitrosylation balance to be affected and promotes in vivo tau phosphorylation along with aggregation. In neurons the tau hyper-phosphorylates due to the upregulation of microRNA-146a through the ROCK1/PTEN signaling pathway modulation. The microRNA-138 overexpression targets the RARA/GSK3b pathway and promotes increment of tau hyper-phosphorylation in Thr231, Ser396, and Ser404. The microRNA-106b by targeting Fyn inhibits tau phosphorylation induced by Aβ42 at Tyr18, meanwhile by suppressing CACNA1C expression the microRNA-137 inhibits tau phosphorylation. The microRNA expression is affected by tau protein level changes. In the mouse model, the microRNA-92a expression increases in AD due to tau accumulation, thus promoting anxiety via the microRNA-92a/vGAT/GABA signal (Wei et al., 2020). In Knockout mouse model of microRNA-132 and microRNA-212, there marked cognitive decline in the recognition, new object recognition and spatial memory. In AD subjects with mild cognitive decline the downregulation of microRNA-132/-212 in the frontal cortex, confirming their important regulatory action in cognitive capacity. Meanwhile in AD mice model, tau pathology dendritic abnormality, and memory deficits is recused by microRNA-101b mimic. In transgenic mice the microRNA-137 level decreases APP/PS1 but in SH-SY5Y cells, their

mimics inhibits phosphorylated tau proteins, promoted by Aβ1-42. Of microRNA-15 family, the microRNA-15a is downregulated in AD, they target ERK1 for their participation of Tau hyper-phosphorylation, decreasing microRNA-15 levels causes neuronal tau hyper-phosphorylation. The data from clinical trials of AD patients shows that microRNA-106b is down-regulated, and at the site of Tyr18 can inhibits tau phosphorylation induced by Aβ42. Patients with advanced AD brain is tau protein rich and the microRNA-512 is reduced expression thus indicating that its natively regulates Tau protein. In the frontal cortex of AD patents the microRNA-153 is reduced when compared with aged-matched control groups. While abnormally increased microRNAs are also involved in the hyper-phosphorylation of Tau protein, and in animal models with AD microRNA-125b is significantly increased. Injection of microRNA-125b into C57BL/6 wild-type mice results in increased phosphorylation of Tau protein and decreased learning and memory function. Correspondingly, overexpression of microRNA-125b in primary hippocampal neurons can cause Tau hyper-phosphorylation, alter synaptic morphology, and quicken apoptosis. In primary neurons, blocking microRNA-125b can lower Tau phosphorylation and kinase expression or activity. The preventive role of microRNA-125b in AD is uncertain. (Kou et al., 2020).

4.3 Synaptic plasticity

In AD pathogenesis the microRNA-34c overexpression in neurons of hippocampal negatively regulates dendritic length and spine density. Increased soluble Aβ levels promote glutamate release and excitatory toxicity. The Aβ mediated synapse toxicity and plasticity is regulated by microRNAs, and this causes synapse to be weak to the neurotoxicity caused by Aβ. For synaptic toxicity caused by Aβ42 oligomer is mediated by CAMKK2/AMPK/Tau pathway. From the toxic effects of Aβ, the synapse and neurites are protected by microRNA-431 through the Wnt/b-catenin signaling pathway. The synaptic damage and dysfunction caused by Aβ42 is relieved by microRNA-188-5p. In the post-synaptic membrane important “molecular

switch" for learning and memory is localized and known as N-Methyl-D-aspartate receptor (NMDAR), which is an ion channel protein, exciting then increases calcium concentration and impairment of long-term potentiation (LTP), toxic damage, and loss of synapses and they drive neuro-excitotoxicity caused by glutamate, and various other factors such as p39 kinase, contributing to Aβ induced neurotoxicity. Synaptic function is regulated by BDNF, and expression of BDNF-dependent postsynaptic protein is increased due to microRNA-132. In early AD, the MicroRNA-132/212 family members are downregulated and they have major role in neural function and synaptic plasticity. The microRNA-132 potential target is the regulator of cholinergic transmission and synaptic plasticity in the cholinergic neurons and this induces Aβ42 production and lead to cholinergic neurodegeneration. In AD brain's frontal and temporal lobes, the microRNA-200c is downregulates thus giving a positive protective effect against endoplasmic reticulum stress (ERS)-induced loss of cholinergic neurons (Wei et al., 2020). Restoring the reduced levels of microRNA acting at synaptic level, recovers the cognitive function. In AD patients, in comparison to age-matched control group there is abnormal level of microRNA-188-5p downregulation on the cerebral cortices and hippocampus, and in rat model of the primary hippocampal neurons cultures, with exposure of Aβ, the microRNA-188-5p overexpression will remove the decreasing dendritic spine density, and in 5XFAD mice AD mouse model recovery of the same microRNA improves behavioral outcomes and synaptic activity, cognitive function. But health. despite all beneficial effect, elevation of certain microRNA can deteriorate the neuron, and through exogenous means they can be downregulated. In the study of AD patients, in hippocampal tissues the microRNA-34a/p73 expression is increased, which is involved in modulating synaptic activity by reducing synaptotagmin-1 expression. On Wild-type animal model, in hippocampal tissues the overexpression of microRNA-30b can damage the synaptic structure and neuronal functions, causing cognitive decline. But in contrast, in transgenic mice knocking down the microRNA-

30b prevents synaptic and cognitive decline. Thus alluding the fact that imbalance and changes in microRNA causes memory decline in AD. At 3xTg-AD mice dorsal and ventral hippocampal tissues the level of c-Fos protein is reduced due to upregulation of microRNA-181 and SIRT1, and the SIRTI and c-Fos transcription factors are involved in memory consolidation and are potential targets of microRNA-181. In brain of Tg2576 mice the microRNA-124 upregulation is promoted Aβ. In the hippocampal synapse formation and learning capacity the PTPN1 is implicated. The 3 prime-UTR of PTPN1 is targeted by microRNA-124 and their translation is suppressed thus disrupting synaptic transmission, plasticity and memory. The neurotrophic factor BDNF is important for synaptic plasticity and cognition. In animal's models with AD with reduced BDNF in the prefrontal cortex and hippocampus is associated with cognitive decline. In AD rats, microRNA-10a is negative regulator in synapse remodeling due to BDNF-TrκB signals reduction. Similarly, in APP/PSEN1 transgenic mice the microRNA-206 upregulation and changes in their level, at the hippocampal tissue, cerebrospinal fluid, and plasma is involved in the AD pathology through downregulation of BDNF (Kou et al., 2020).

4.4 Immuno-Inflammatory Responses

Toll-like receptors (TLR) plays a major role in neuro-inflammation which is under certain conditions is activated by microRNAs. In microglia TREM2 an immunoglobulin superfamily receptor is found and any mutations in this increases risk of LOAD, in 299 nucleotides of 3 prime UTR of TREM2 mRNA is targeted by microRNA-34a which leads to its downregulation and microglia phagocytosis. In AD mice, knocking out TREM2 reduces neuro-inflammation. Through influencing the responses of inflammation-associated cytokines, microRNAs give both protective and pathogenic roles. MicroRNA-139 inhibits AD development via regulating cannabinoid receptor type 2 (CB2)-mediated neuro-inflammation and has a negative regulatory influence on responses to pro-inflammatory stimuli. The inflammatory response is mediated

by prostaglandin E2 (PGE2) (Wei et al., 2020). In PC12, microRNA-132 has a role in inflammation control and is a negative regulator of the inflammatory response. Using resveratrol treatment, the upregulation of microRNA-132 causes the inflammatory response to be ameliorated in the PC-12 cells, and microRNA-132 also supposedly targets IL-1β, IL-6, and TNF-α, and it is downregulated in neuron HT-22 cells with LPS-induced inflammatory injury, and microRNA-132 overexpression attenuates inflammatory response. MicroRNA-132 target is TNF receptor associated factor 6 (TRAF6) which is associated with inflammation induction. MicroRNA-206 enhances LPS induced inflammation and causes the release of Aβ in microglia via binding to the 3 prime-UTR of insulin-like growth factor 1 (IGF-1), and as the result IGF-1 exposure mitigates microRNA-206-induced inflammation in microglia, thus indicating that in AD the micrpRNA-206/IGF-1 signaling pathway may be associated with microglial inflammation. In 3xTg AD animal model, microRNA-155 is significantly overexpressed, and upregulating will increase activation of microglia and astrocytes, thus triggering inflammatory mediator productions and take part in regulation of AD through activating different T cell functions during inflammation. Clinical data from human AD brains shows that higher levels of microRNA-125b and microRNA-146 worsen neuro-inflammation and diminish complement factor H, which is linked to neuronal release of microRNA-146a and microRNA-155 and inflammatory propagation in the AD brain. In LPS-treated microglia, microRNA-32-5p knockdown can reduce the production of inflammatory cytokines, and microRNA-32 targets directly the dual specificity phosphatase 5 (Dusp5). By controlling Sirt1 levels, microRNA-204 inhibition might suppress inflammation in LPS-induced mouse microglial cell lines (N9 and BV2). The loss of microRNA-29a impairs the function of neuronal navigator 3, which serves as guidance, and it is abundant in degenerating pyramidal neurons in AD (Kou et al., 2020).

Chapter-5 MicroRNA as Therapeutics and Pathologic

5.1 Extracellular Vesicles (EVs) and Mesenchymal Stem Cell (MSC) role as delivery system

Mesenchymal stem cells (MSCs) are produced and localized within the stroma of all organs and have the ability to secrete extracellular vesicles (EVs), most MSCs main functions are performed by these EVs. The function of MSCs is mainly for self-replication and multidirectional differentiation, and its function in physiology and in pathology of diseases, along with altering the mechanism of immunological and inflammatory. The MSCs had the ability to play a role in recombinant proteins in bone and cartilage healing. MSCs has the ability for self-renewal and repair so they are employed in organ diseases, and this ability was assumed for the MSCs paracrine fusions with target cells but MSCs alone could not perform such fusions. It was assumed that bioactive and soluble factors like cytokines and growth factors are responsible for the fusion but it was later established that it was the EVs released by the MSCs, that helped in the fusion. MSCs is known to cause immunological response in patients and this reason many of MSCs are still under research to make them safer but employing EVs along with MSCs are opened a door for them to be much safer and with faster tissue penetration than the single MSCs and this MSCs-EV lacks the self-renewal so there is less risk of tumour formation due to uncontrolled cell division. These EVs contains molecules such as non-coding RNAs, microRNAs that can help in inflammation and tissue regeneration as mention earlier on regards of microRNA function for treating Alzheimer's Disease (Racchetti & Meldolesi, 2021). MSC-released EVs bind to neurons in the brain and spinal cord, through microRNAs in their cargo. The presence of EVs in brain tissues can affect a variety of functions, including synaptic and impacts of inflammation. When MSC-EVs are given in low doses there is activation of receptors and phosphorylation of kinases by microRNAs that helps in recovery of patients with

AD, not to mention they mitigate immune response produced by innate cells with distinct repair (Meldolesi, 2022).

5.2 Animal Model Tests of microRNA as therapeutic in Alzheimer's Disease models

5.2.1 MicroRNA-146a

In the hippocampus of AD model mice, Exosomes coming from Bone marrow (BM)-MSC transfers the microRNA-146a into the astrocytes and suppresses inflammatory NF-kB expression, thus reduce the astrocytic inflammation in AD model through the attachment of BM-with the choroid plexus (CP) of the brain. Upregulation of expression in the APP/PS1+ MSC group and the APP/PS1+vehicle group is contrasted. No variation in IRAK1 expression in Wild type plus MSC or vehicle and APP/PS1 plus MSC or vehicle groups of mice and TRAF6 expression in the subiculum area is decreased in the APP/PS1 + MSC group compared to the WT+ vehicle and APP/PS1 + vehicle groups, and NF-κB The expression increased in the APP/PS1 +vehicle group compared to the WT+vehicle and WT + MSC groups. But no increase in expression of NF-κB in the APP/PS1+MSC group compared to the WT+vehicle and WT+MSC groups. In the astrocytes, the conditioned medium (CM) with microRNA-146a transfected BM-MSCs with exosomes has higher free-floating than those non-transfected BM-MSCs, and transfection causes an increase in microRNA levels within astrocytes, and in return reduces the TRAF6 and NF-κB expression, adding exosomes in BM-MSCs increases microRNA-146a expression and reduced TRAF6 and NF-κB expression. This improved cognitive function and reduced astrocyte inflammation and induced synaptogenesis. The microRNA-146a is a negative feedback regulator of NF-κB activation and upregulated in the AD patient's temporal cortex and it is found in the astrocytes and microglia, but in APP/PS1+vehicle has both increased expressions of microRNA-146a and NF-κB, so the

negative feedback regulation do not work well enough. Microglia of M1 types has microRNA-146a expression and this is reduced by treatment of BM-MSC and astrocytes are a cause for microRNA-146a increase, and APP/PS1 mice treated with BM-MSC has increased microRNA-146a as well as a decrease in TRAF6, and no increases NF-κB compared to WT mice, thus for expression of microRNA-146a BM-MSC is needed. CM cultured BM-MSCs has free-floating microRNAs and to rule its effect, microRNA-146a mimic is added to astrocytes' culture and expression of microRNA-146a is observed and exosomes from microRNA-146a transfected BM-MSCs are added to cultured astrocytes and these showed mimic has no effect while exosomes has positive effect, thus indicating that microRNA-146a packed in exosomes but not free-floating microRNA-146a collected from BM-MSCs helps in ameliorating astrocytic inflammation, helping in treating AD (Nakano et al., 2020).

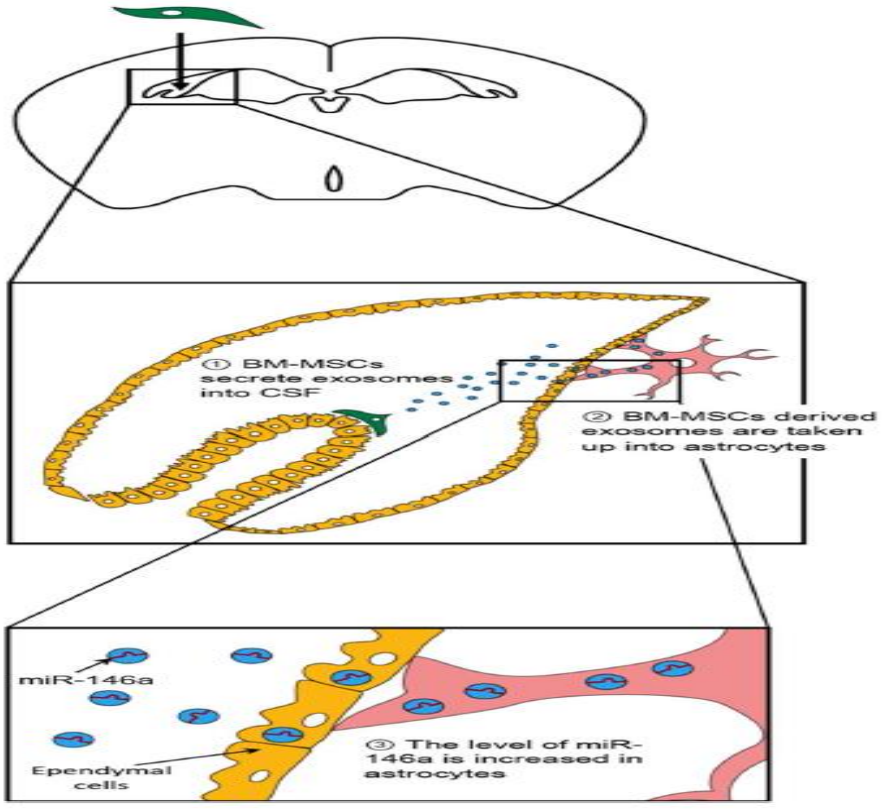

Figure 6: How BM-MSC therapy transfers the microRNA (Nakano et al., 2020)

5.2.2 MicroRNA-22

Through the application of adipose-derived mesenchymal stem cells (AD-MSC), microRNA-22 is added to the AD mice model, this when observed under water maze test, the behavioral and memory abilities of mice were improved significantly, and when observing the mice escaping time, time it has stayed in the platform and time is has crossed platform, and the time of the escape was shortened, and on the other hand the results were increased in mice of the group with AD-MSC loaded with microRNA-22, referred as exo-microRNA-22 group and thus the mice can be concluded to have improved behavioral and memory abilities, and Interleukin-1β, Interleukin-6 and Tumor Necrosis Factor-α inflammatory factors expression level is decreased, in the peripheral blood and cerebrospinal fluid, in comparison to control group. When Nissl staining was conducted of both control and exo-microRNA-22 group, the nerves cell was damaged and sparsely arranged in cortex and hippocampus in the control group whereas in exo-microRNA-22 group they were densely arranged and positively stained and are improved, so microRNA-22 can inhibit nerve damage and inflammation, to further justify the effect of microRNA-22, exo group with only ADMSC have lower reduced inflammation and nerve damage inhibition than exo-microRNA-22 group. The activation of microglia, which increases the inflammation, is dependent on the expression of CD206 and IBA-1, and their expression is high in control group compared to the exo-microRNA-22. The expression of protein, GSDMD and p30-GSDMD was high, along with it is the NLRP3 inflammasome is activated in control group, in exo-microRNA-22 both p30-GSDMD and GSDMD and NLRP3 are downregulated, higher than exo group. Inflammatory factors such as IL-1β, IL-6 and TNF-α and pyroptosis was reduced in exo-microRNA-22 group (Zhai et al., 2021).

5.2.3 MicroRNA-455-3p

The microRNA-455-3p targets the APP gene, and its overexpression in mouse neuroblastoma cells reduces mutant APP circular DNA expression and Aβ toxic effect on synaptic activities,

mitochondrial biogenesis, cell viability, mitochondrial dynamics, and apoptosis, were also reduced. In comparison to wild-type (WT) mouse, the Transgenic mouse (TG) has upregulation of microRNA-455-3p and down regulation of APP mRNA, while in Knockout mice (KO) where the expressed of microRNA-455-3p is suppressed, reducing the microRNA-455-3p expression caused an increase in APP expression. Maze water test of both TG and KO mouse showed that spatial learning and memory were higher in TG mouse, as their latency time for platform finding, distance travelled, time spent in target quadrant were lower than KO mouse. As shown in figure no.7, the WT, TG and KO mouse brain were immune-stained using cell type-specific markers like the NeuN, GFAP and Iba1 and the staining assay shows that there is increased immune-reactive intensity of NeuN in TG in comparison to WT and KO mouse and reduced astrocyte population, thus confirming that overexpression of microRNA-455-3p increases neuron levels and knockdown of microRNA-455-3p enhances astrocyte and microglial activity, which causes further inflammation and nerve cell damage.

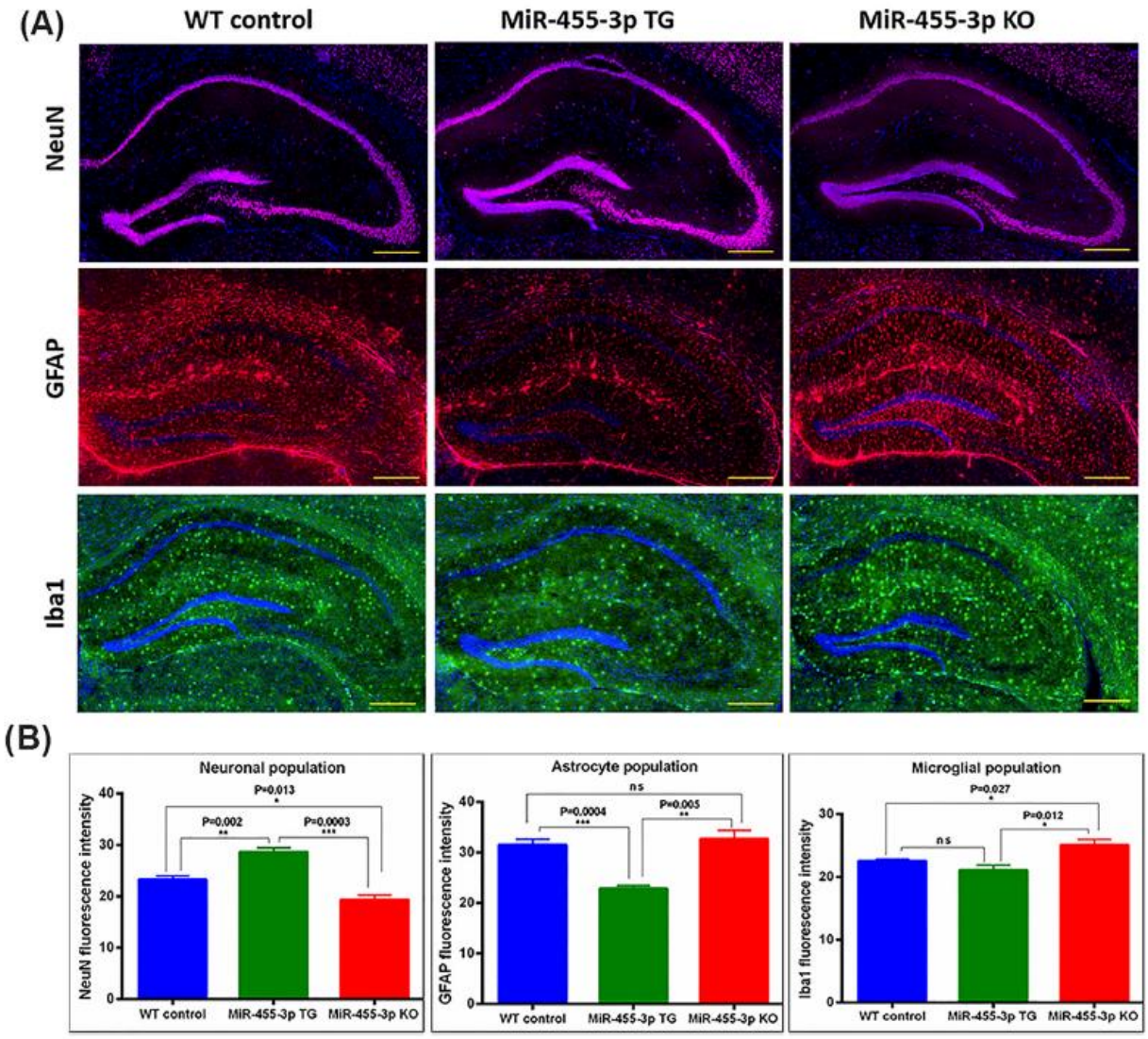

Figure 7: The immunoassay of WT, TG and KO mouse (Kumar et al., 2021)

Using Golgi-Cox staining in the hippocampi of 12-month-old WT, TG and KO mice the dendritic length and number of spine are calculated, and as shown in figure no.8, the TG mouse had dense and elongated dendrites and increased length and increased amount of dendritic spines compared to both WT and KO mice, whereas in WT the result is higher than KO as it has further reduced microRNA-455-3p expression.

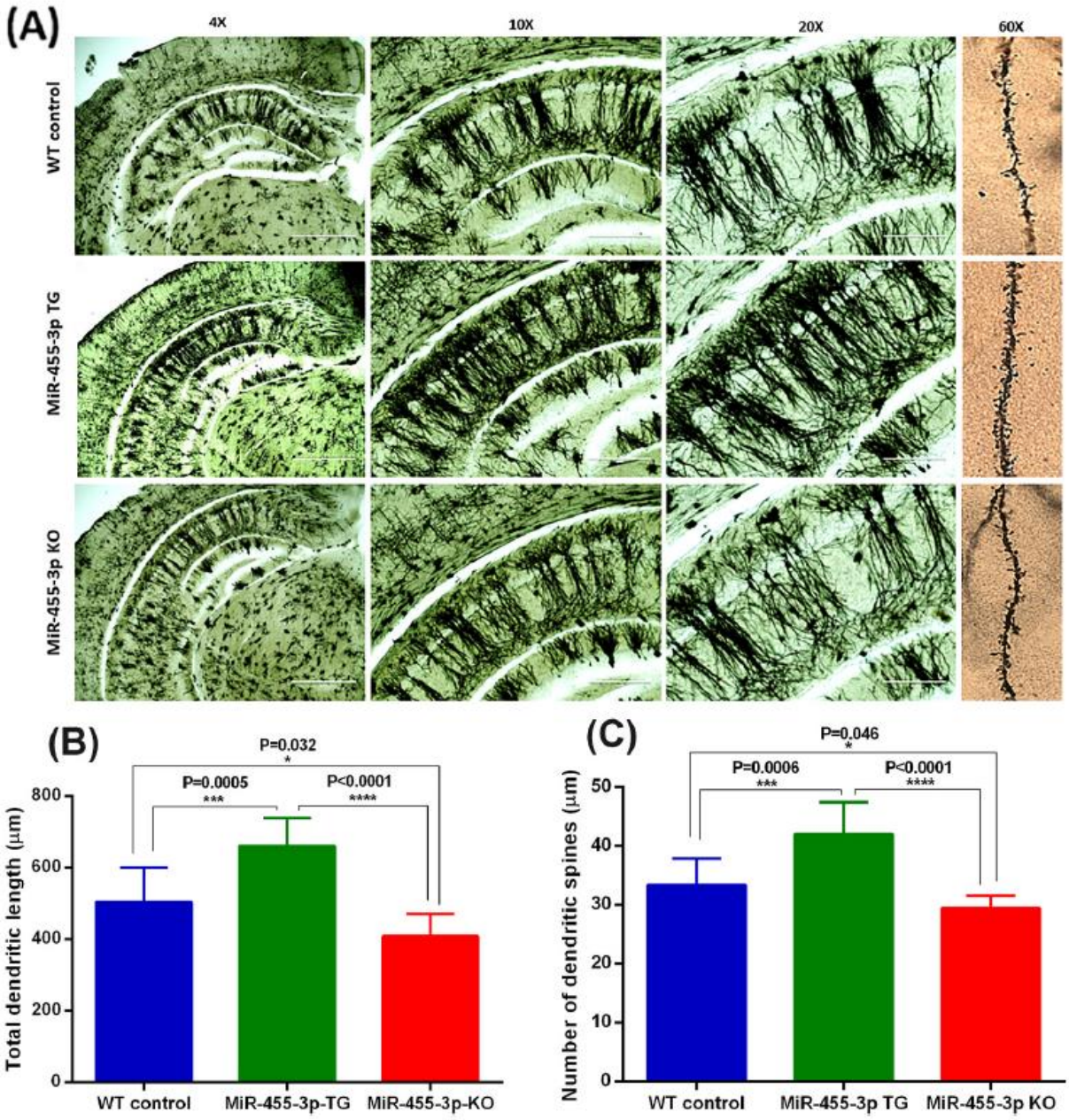

Figure 8: Hippocampal neurons dendritic spine density of Wild-Type, Transgenic and Knock-Out mice (Kumar et al., 2021)

As shown in figure no.9, the TG mouse has improved mitochondrial number and length in comparison to the WT and KO having worse result, thus proving importance of microRNA-455-3p importance in mitochondrial morphology. As shown in figure no.9, synapse organization and numbers in both the cortex and hippocampus of WT, TG and KO mice, are

examined and the results shows TG has higher than both KO and WT. All these data shows the importance of microRNA-455-3p in the AD brain (Kumar et al., 2021).

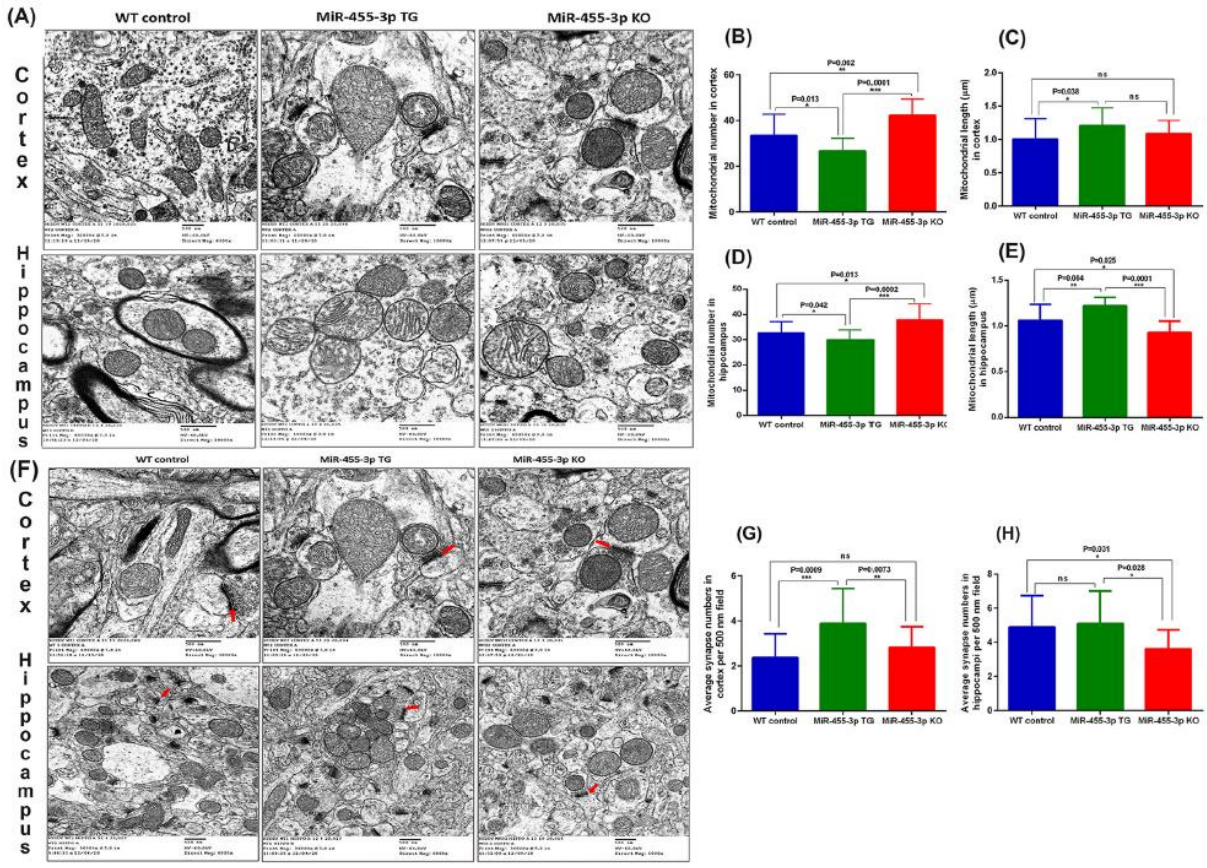

Figure 9: Morphology of mitochondria and number of synapse in Wild-Type, Transgenic and Knockout mice (Kumar et al., 2021)

5.3 Animal Model Tests of microRNA as pathologic in Alzheimer's Disease models

Not all microRNA is beneficial for AD patients many of the microRNA has pathologic effect rather than therapeutic which can worsen the patient condition, rather many medications has to be developed which can target these microRNAs and block them from harming the patient. One of them being, microRNA-30b which targets receptors such as, sirtuin1 (sirt1), ephrin type-B receptor 2 (ephB2) and glutamate receptor subunit 2 (GluA2), and these are responsible for maintaining the synapse stability and they are downregulated when in the hippocampus of

AD brain there is upregulation of microRNA-30b. In the wild-type mouse the over expression of miR-30b causes impairment of basal synaptic transmission and long-term potentiation (LTP), decreases the learning and memory and density of dendritic spines and synaptic proteins expression, which is consisted of PSD-95 and subunits of glutamate receptor, all are reduced, and these results are similar to that found in TG mouse with upregulated microRNA-30b, knocking down their expression prevents cognitive and synaptic decline, and decrease synaptic proteins expression and dendritic spines densities, thus improving the condition of AD. The fluorescent in-situ hybridization (FISH) assay of AD port-mortem patient showed that in the neurons there in upregulation of microRNA-30b, and or regulation of synaptic maturation, synaptogenesis, dendritic spines formation, guidance of the axon, and, expression and function of receptors such as AMPA and NMDA glutamate, synaptic plasticity for long term, and formation of memory, there is a need for EphB2 and sirt1 expression, and it has been predicted that microRNA-30b is founded bound in the 3 primer UTR of EphB2 and sirt1, so blocking them from functioning normally. To test the hypothesis that there is AD patient and TG mice reduction in the hippocampal tissues expressions of ephB2, sirt1 or GluA2, and in WT and TG mouse are injected in the hippocampus of 4-month-old with, lentiviral vector (LV) expressing microRNA-30b, LV-microRNA-30b, LV-microRNA-30bs (which knockdowns the endogenous microRNA) or LV-scramble control was stereotaxically injected into the WT or TG mice and the analysis was performed 2 months after LV injections are given. In the hippocampal, the WT mice injected with LV-microRNA-30b the level microRNA-30b increases and in TG mice endogenous microRNA-30bs is upregulated along with it in WT injected with LV-microRNA-30b the ephB2, GluA2 or sirt1 expression is reduced in the hippocampus whereas in TG mice injected the LV-microRNA-30bs expression returned to normal, thus supporting out hypothesis, but blocking the expression of microRNA-30b does not affect the Aβ42, Aβ plaques, or BACE1 levels. The WT mice is injected with LV-

microRNA-30b or TG mice is injected with LV-microRNA-30bs, and their hippocampal basal synaptic transmission are assayed in terms of input-output function and synaptic plasticity are assayed in terms of long-term potentiation (LTP), the result shows that in WT mice the microRNA-30b overexpression causes impaired input-output function similar to TG mice. In TG mice knocking down microRNA-30bs improves the impaired basal synaptic transmission and long-term synaptic plasticity, thus microRNA-30b do cause synapse dysfunction. Using Morris maze water test in WT mice-treated with LV-microRNA-30b or in TG mice-treated with LV-microRNA30bs are analyzed, results showed that during the probe trials, the WT mice had hard time in find the hidden platform during the learning acquisition sessions and number of time platform is crossed are reduced and target quadrant time spent is also reduced, whereas the TG mice had improved spatial learning and memory. The WT mice injected with LV-microRNA-30b, in the neurons of hippocampal and neuron granules in dentate gyrus area and CA1 region and neurons of pyramidal of the mice have reduced dendritic spines, whereas in TG mice LV-microRNA-30bs has returned to normal. The inflammatory factors such as NF-κB signaling, pro-inflammatory cytokines and Aβ42, causes the upregulation of microRNA-30b thus targeting them can also reduce its expression. All these data showcases that the pathologic side of microRNA needs to be explored and there is are far more microRNA whose deregulation will benefit the AD patients (Song et al., 2019).

Chapter-6 Future Prospects

6.1 Future Prospects

Future prospects of microRNA as therapeutic is a hopeful approach, microRNA mimics are being experimented as combination therapy for AD treatment and on animal models the results are positive. Despite the research, the full potential and biology of microRNA are still unclear and it has to be further studied to provide concrete evidence of its therapeutic effect.

MicroRNAs regulation and deregulation are very sensitive, any high and low can cause more harm to AD patients than benefit and certain microRNAs has several different targets that provide different response, this creates a gap in the development of microRNA as a therapeutics. Alzheimer's disease research for therapeutics are still long way to go and even current time there is not enough disease modifying therapeutics and to combat this issue and thus, vast and vigorous research is needed and which is being conducted currently. It was established that to deal with Alzheimer's disease related pathology, combination therapy is the best method and for this reason microRNAs are promising enough to be employed as one of its therapy or as a target for drug products to act on. MicroRNA, has the ability to switch on and off certain gene or mRNA by binding to them and their effect depends on physiology affected. Manipulating them is highly desirable but flaws remains as most study are done in animal model and are currently in pre-clinical stage of clinical trials and even then not much progress are being done in research but microRNA has immense potential and through the employment of stem cells of various types as their delivery system to study their potential, and it is under the radar of research community. To sum up, further research is highly needed for microRNA for treatment of Alzheimer's disease to be able to use in clinical trials on humans.

6.2 Conclusion

Alzheimer's disease is a debilitating illness that is affecting many older generations, mostly in urban city and developed countries, their pathology pathways are numerous such as beta-amyloid, tau protein, activated microglia and for their treatment no disease modifying method have been established, so far aducanumab have been approved for treating the disease. MicroRNA has potential to be a novel therapeutic method, by targeting certain mRNAs that causes the disease progress further to worse position, so their overexpression causes a reduction of disease, and another class of microRNA targets those mRNAs whose protein expression is needed for a healthy brain, rather the overexpression of those microRNA causes the disease to

worsen. There are many microRNAs and single microRNAs have different targets and gives different effect in the brain, while some have therapeutic and some have pathologic effect on Alzheimer's disease brain. MicroRNAs are analyzed in preclinical stage and microRNAs such as microRNA-146a, microRNA-22, microRNA-455-3p have positive effect in the AD mice model, meanwhile the microRNA-30b has negative effect in Alzheimer's disease brain and it has to be blocked from further deteriorating the Alzheimer's disease situation. But all these result and data are in pre-clinical stage, and further study needs to be conducted, for them to be formulated, adjusted and designed so that they do task they are intended for with minimum side effects on the body, and then they can move to higher phases in clinical trials, without further study microRNA are still not ready to be used as therapeutic, and for those microRNAs which needs to be deregulated, their target drugs needed to be further studied to be formulated and without conclusive result in animal trails, the study will not progress further.

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