

Role of Protein's and Future Treatment Options for Alzheimer's Disease

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 (Hons.)

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Declaration

It is hereby declared that

1. The thesis submitted is my 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.
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Approval

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Ethics Statement

This is to certify that this project titled “Role of proteins and future treatment options for Alzheimer’s disease” is submitted for the partial fulfillment of the requirements for the degree of Bachelor of Pharmacy (Hons.) from the School of Pharmacy, Brac University constitutes my own work under supervision of Dr. Md. Aminul Haque, Assistant Professor, School of Pharmacy, Brac University and I have given appropriate credit where I have used language, ideas or writings of another.

Abstract

Alzheimer's disease is a progressive brain disorder that slowly destroys the memorizing skill, decline in cognitive function and thinking ability which is one of the main causes of dementia. The exact cause of AD is still unknown but several studies suggest that extracellular accumulation of amyloid-beta oligomers and intracellular hyperphosphorylated tau peptides mainly responsible for this disease. Amyloid beta is 40-42 amino acid long polypeptide chain that is produced amyloid precursor protein through β -secretase and γ -secretase enzymatic cleavage. Impaired amyloid accumulation causes synaptic dysfunction, mitochondrial dysfunction that cause neurodegeneration. Tau is intrinsically disordered protein which is required for stabilizing microtubules but hyperphosphorylation of tau protein cause them to dissociate from microtubules causes axonal loss. Acetylcholine esterase inhibitor and NMDA receptors antagonist are currently available drugs for AD that reduce AD symptoms. Currently scientists are working several AD drugs targeting the pathological hall marks of AD ($A\beta$ and tau).

Keywords: Alzheimer's disease; APP; Amyloid-beta, Tau hyperphosphorylation, Synaptic dysfunction, Treatment.

Dedication

Dedicated to my parents and respected teachers.

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Table of Contents

Declaration.....	ii
Approval	iii
Ethics Statement.....	iv
Abstract.....	v
Dedication	vi
Acknowledgement	vii
Table of Contents	viii
Table of Figure	x
List of Acronyms	xi
Chapter 1 Introduction.....	1
Chapter 2 Amyloid precursor protein and Amyloid-β biogenesis	3
Chapter 3 Components of Aβ plaques	6
3.1 Mutations causing impaired A β production in AD.....	7
3.2 Central and peripheral A β pools	9
Chapter 4 Toxicity of Aβ.....	12
4.1 Synaptic dysfunction.....	12
4.2 Mitochondrial dysfunction.....	16
4.3 Oxidative stress.....	17
4.4 Neuro-inflammation.....	18
Chapter 5 Neurofibrillary tangle.....	20

5.1	Tau in synaptic function.....	22
5.2	Hyperphosphorylation.....	23
5.3	Acetylation.....	25
Chapter 6 Tau aggregation		27
6.1	Tau propagation	28
6.2	Interaction between A β and tau	29
Chapter 7 Current treatment options for AD		31
7.1	Cholinesterase inhibitors.....	31
7.2	NMDA receptor antagonists	31
Chapter 8 Future treatment options for AD		33
8.1	Anti – A β	33
8.2	Anti – tau.....	35
8.3	CRISPR/Cas9 gene therapy	36
Chapter 9 Challenges in AD treatments		38
Chapter 10 Conclusion		39
References.....		40

Table of Figure

Figure 1: Proteolytic processing of Amyloid precursor protein and A β biosynthesis.....	4
Figure 2: Enzymatic cleavage of Amyloid Precursor Protein	8
Figure 3: Multiple mechanisms of amyloid-beta toxicity.....	15
Figure 4: Healthy and pathological tau inside the neuron.	20
Figure 5: Six isoforms of tau.	21
Figure 6: Formation of PHF or tau aggregation.....	27
Figure 7: Targeting β -secretase and γ -secretase to reduce A β burden.....	35

List of Acronyms

Ach	Acetylcholine	CFTs	C-terminus fragment
AD	Alzheimer's disease	CNS	Central nervous system
AICD	APP intracellular domain	CRISPR	Clustered Regularly Interspaced Short Palindromic Repeats
ALP	Amyloid precursor-like proteins	CSF	Cerebrospinal fluid
AMPA	α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid	DIAD	Dominantly inherited Alzheimer's disease
ApoE	Apolipoprotein E	DISC	Death inducing signaling complex
APP	Amyloid precursor protein	ECD	Extracellular domain
ASP	Aspartyl protease	EGFP	Enhanced green fluorescent protein
ATP	Adenosine tri-phosphate	EMA	European medicine agency
A β	Amyloid beta	EPR	Electronic paramagnetic analysis
BACE	β -site amyloid precursor protein cleaving enzyme	FADD	Fas-associated death domain
BBB	Blood brain barrier	FasR	First apoptosis signaling receptors
CBF	Cerebral blood flow	FDA	Food and drug administration
CDK-5	Cyclin dependent kinase 5	GSK-3 β	Glycogen synthase kinase-3 β
CECs	Cerebral endothelial cell	HAT	Histone acetyl transferase
IL	Interleukin	PHF	Paired helical filament
iPSC	induced pluripotent stem cells	PNS	Peripheral nervous system

LDL	Low density lipoprotein	PP	Protein phosphate
LRP	LDL-related protein	PSD	Post-synaptic protein
LTD	Long-term depression	PSEN	Presenilin
LTP	Long term potentiation process	RAGE	Receptor for advanced glycation end product
MAP	Microtubules associate protein	ROS	Reactive oxygen species
MAPK	Mitogen-activated protein kinase	sAPP α	Soluble α APP fragments
MAPT	Microtubule-associated protein tau	sAPP β	Soluble β APP fragments
MBD	Microtubule binding domain	sGAGs	Sulfoglycosaminoglycans
MnSOD	Manganese superoxide dismutase	sgRNA	single guide RNA
MOT	Mitochondrial outer-membrane translocase	SIRT1	Sirtuin1
mtDNA	Mitochondrial DNA	SPs	Senile plaques
NFTs	Neurofibrillary tangles	TMs	Transmembrane segments
NMDA	N-methyl-d-aspartate	TNFs	Tissue necrosis factors
OCR	Oxygen consumption rate	UCPs	Uncoupling proteins
PER	Para-magnetic electron resonance	UDP-GlcNAc	Uridine diphosphate N-18 acetylglucosamine

Chapter 1

Introduction

Alzheimer's disease is a progressive brain disorder that slowly destroys the memorizing skill, decline in cognitive function and thinking ability which is one of the main causes of dementia. Currently an estimated 46 million people are being suffering with Alzheimer's disease and among which approximately 6 million patients are from America and the number is expected to rise 13 million by 2050 ("2021 Alzheimer's disease facts and figures," 2021). Deaths from cardiovascular disease have decreased 7.5 % while deaths from Alzheimer's disease have increased 145 % between 2000 to 2009 which shows the severity of this problem. Moreover, during Covid-19 pandemic there is a 16 % increase in the mortality rate in people with Alzheimer's disease. At first it was thought that Alzheimer's disease is a rare disease but later found that age is the major prevalence factor in Alzheimer's disease as the severity or progressiveness of the disease is proportional to aging (Dos Santos Picanco et al., 2018). Although people of all ages can develop Alzheimer's but almost 90 % of the patients who shows the symptoms of Alzheimer's are 65 years or older (Dennis J Selkoe & Hardy, 2016). The exact cause of AD is still under investigation but several research and studies suggested that extracellular senile plaques (SPs) also known as accumulation of amyloid- β peptides, intracellular neurofibrillary tangles (NFTs) or accumulation of hyper-phosphorylated tau proteins and dystrophic neuritis results in the loss of neuronal function and shrinkage of brain (Sun, Chen, & Wang, 2015). Extracellular accumulation of A β plaques interfere with neuron to neuron communication at the synapses also inside the neurons the accumulation of tau proteins block the nutrients essential for normal functioning which lead to the damage and death of neurons. Microglia is the immune cell of the central nervous system (CNS) that acts like macrophages; microglia protects the brain from infection and inflammation (Kinney et al., 2018). But the toxic metabolite of A β and tau activates the

microglia which tries to remove the accumulated plaques from the neurons that set the chronic inflammation that kills the cells of the brain resulting in the atrophy (decreased brain volume) of brain (Ayubcha, Rigney, Borja, Werner, & Alavi, 2021; Kinney et al., 2018). Mitochondrial dysfunction is also observed due to A β plaques that causes phosphorylation of tau and increased the free radical formation that lead to mtDNA damage. Abnormalities in mitochondrial dynamics also known as co-ordinated cycles of fission and fusion that maintain the shape and size of mitochondria is also impaired in AD due to A β accumulations (Carrillo-Mora, Luna, & Colín-Barenque, 2014). So, the presence of A β causes the excessive mitochondrial fragmentation and reduced mitochondrial fusion that lead to the reduction in size of mitochondria. Impaired mitochondrial function contributes to the synaptic dysfunction because ATP level is reduced due to declined mitochondrial biogenesis which is essential for the delivery of neurotransmitters into the synapses (Donner et al., 2021) (Kolarova, Garcia-Sierra, Bartos, Ricny, & Ripova, 2012). So, the role that A β plays in the pathogenesis of AD makes it one of the potentially efficient targets for the treatment for AD.

Chapter 2

Amyloid precursor protein and Amyloid- β biogenesis

The exact factors that have been involved in the pathogenesis of Alzheimer's diseases is still not fully understood and debated but there are strong evidence that A β is the initiator of subsequent events that ultimately lead to the AD. A β peptides aggregate to form soluble oligomers, SPs which then alter microglial and astrocytes activity as well phosphatase activity that causes neuronal death (Sun et al., 2015).

Amyloid precursor protein (APP) is a trans-membrane type-1 glycoprotein that is expressed in many tissues especially in the synapses of neurons. It also plays several biological activities such as intracellular transport, neuronal development, signaling pathway and maintains neuronal homeostasis (Zhou et al., 2011). APP is one of the three genes that present in mammals and the other two genes are amyloid precursor-like proteins (APLP1 and APLP2) (G.-f. Chen et al., 2017). Both the ALP1 and ALP2 lack of genes that is required for encoding A β sequence. APP contains membrane tethered A β domain, it also has long extracellular N-terminus and short cytosolic C-terminus, the C-terminus is process through non-amyloidogenic pathway whereas the extracellular N-terminus is process through amyloidogenic pathway which is the main pathway of A β biosynthesis (Figure-1) (G.-f. Chen et al., 2017; Sun et al., 2015). Proteolytic cleavage of APP by β -secretase or BACE1 (β -site amyloid precursor protein cleaving enzyme 1) produces 40 to 42 amino acid residue known as A β which is the main component of amyloid plaque in the brain of AD patients (Nalivaeva & Turner, 2013; Sun et al., 2015). Cytosolic C-terminus of APP is first cleaved in the A β domain by α -secretase to produce soluble APP fragments (sAPP α) and 83 amino acids C-terminus fragments (C83). It was reported that α -secretase of APP is increased with the increase of electrical, muscarinic and neuronal activity. C-terminus fragment (CFTs) is further processed by γ -secretase to produce non-toxic P3 (3 kDa) and APP intracellular

domain (AICD) respectively (Zhou et al., 2011). This non-amyloidogenic pathway is the innate way to prevent intracellular accumulation of A β (O'Brien & Wong, 2011; Swerdlow, 2007; Westmark, 2013). On the other hand extracellular N-terminus is cleaved by β -secretase to produce membrane tethered 99 amino acid C-terminus fragments β (CFT β or C99) and soluble β APP fragments (sAPP β). CFT β or C99 is further processed by γ -secretase to produce AICD that can regulate gene expression such as induction of apoptotic genes in the nucleus. Also cleavage of C99 produces neurotoxic extracellular A β that aggregates to form senile plaques (Figure-1) (G.-f. Chen et al., 2017; Westmark, 2013). Several experiments shows that the enzyme that cleave extracellular A β domain present in abundant amounts in neurons which accelerate the amyloidogenic pathway of APP processing and impair neuronal function in AD patients (Sun et al., 2015).

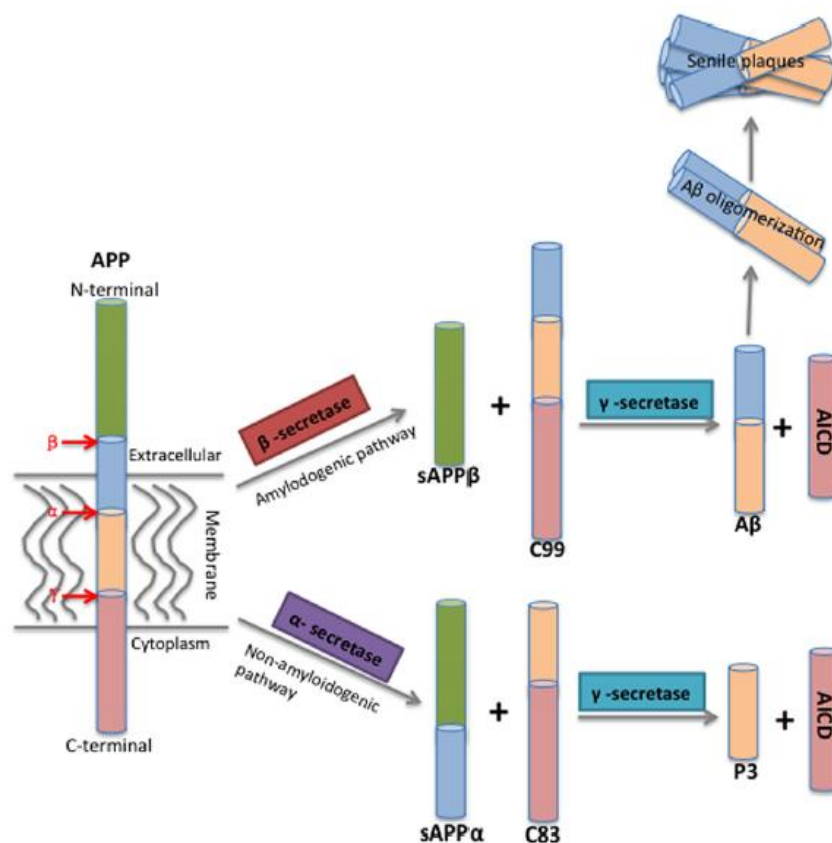


Figure 1: Proteolytic processing of Amyloid precursor protein and A β biosynthesis (Sun et al., 2015).

Recent studies conducted by single particle cryo-electron microscopy in 2014 reveal the three dimension complex of γ -secretase which comprises a horseshoe-shaped transmembrane domain containing 19 transmembrane segments (TMs) and a large extracellular domain (ECD). Presenilin 1(PSEN1), nicastrin, presenilin enhancer 2 (PSEN2) and anterior pharynxdefective1 proteins form the γ -secretase complex (G.-f. Chen et al., 2017). Aspartyl protease that forms from the auto-processing of N and C terminal cleavage of APP activates the presenilin which is required for the activity of mature γ -secretase. The other three proteins nicastrin, presenilin enhancer 2 and anterior pharynxdefective1 modulate activity of γ -secretase enzyme in response to physiological stimuli. Due to this unique processing of APP it is one of the main targets for AD drugs (Swerdlow, 2007).

Chapter 3

Components of A β plaques

Improper cleavage at the C-terminus of A β region of APP by γ -secretase produces two major isoforms of A β , one is with 40 amino acid long residue (A β_{1-40}) and 42 amino acid long residue (A β_{1-42}) which is less abundant than A β_{40} but more toxic (Hampel et al., 2021). Mutation in the APP and PSEN1 and PSEN2 alters the proteolytic cleavage of APP that increase the level of longer A β peptides mostly A β_{42} , A β_{43} . These peptides are more aggregating and hydrophobic in nature whereas A β_{40} is less aggregating and consider anti-amyloidogenic in nature (Dos Santos Picanco et al., 2018). The A β_{42} peptide form have cytotoxic properties that involves in neurodegeneration and facilitate the formation of oxiradicals which is toxic to the neuronal cells. This isoform form the insoluble structure and plays role in the deregulation of calcium homeostasis due to lipidic dysregulation of cell membrane that ends up in neuronal death (Bharadwaj, Dubey, Masters, Martins, & Macreadie, 2009; Dos Santos Picanco et al., 2018). The oligomerization of A β peptides is determined by the relative proportion of A β species in the brain and periphery. In-vivo studies showed that A β_{40} level in the brain can inhibits the fast aggregation of A β_{42} kinetics and have a protective effect (Gu & Guo, 2013; Mawuenyega et al., 2010). A β level in both brain and periphery is known to lower in normal elderly people than patients with AD and aggregation of A β in patients with or without AD is important factor that can influence the A β_{40} :A β_{42} ratio in both brain and periphery. A β_{40} is the most abundantly generated isoforms by neurons but A β_{42} isoforms is more prone to self-aggregation due to toe C-terminus hydrophobic residue as a result A β_{42} is more immunoreactive than A β_{40} (Leong, Ng, Chye, Ling, & Koh, 2020). Electronic paramagnetic analysis or EPR studies revealed the interaction between A β_{40} and A β_{42} . According to EPR analysis it was found that A β_{40} and A β_{42} may coexist in the extracellular space and generates three different types of A β population, they

maybe present as $A\beta_{40}$ alone, $A\beta_{42}$ alone or in form of $A\beta_{40}/A\beta_{42}$ mixture (Gu & Guo, 2013). The aggregation of $A\beta_{40}$ in the brain is too slow compared to $A\beta_{42}$ to develop AD as overexpression of $A\beta_{40}$ did not develop amyloid pathology in the transgenic mice and AD brain contains mostly or sometimes only $A\beta_{42}$ (Jankovska, Olejar, & Matej, 2021). Mutations in the APP and PSEN genes altered the proteolysis of APP that increases the production of $A\beta_{42}$ that progressively accumulate and rise the level of $A\beta_{42}$ in the brain interstitial fluid. Aggregated $A\beta_{42}$ plaque act as a reservoir that continuously release diffusible oligomers that activate cellular dysfunction and injured the surrounding cells (Hunter & Brayne, 2018; D. J. Selkoe, 2001).

3.1 Mutations causing impaired $A\beta$ production in AD

Alteration in the main four genes (APP, Apoe4, PSEN1, PSEN2) products have been linked to the increased production and cerebral deposition of $A\beta$ plaques (Farzan, Schnitzler, Vasilieva, Leung, & Choe, 2000; Swerdlow, 2007). Total nine mutations in the APP have been found to increase $A\beta$ production. Among those nine mutations, double missense mutation in the two amino acids at β -secretase cleavage site of the APP induces increased β -secretase cleavage activity, as a result it generates more $A\beta_{40}$ and $A\beta_{42}$ (Kametani & Hasegawa, 2018; D. J. Selkoe, 2001). Five mutations at the $-\text{COOH}$ terminal of the γ -secretase site selectively increase the production of $A\beta_{42}$. The remaining two mutations occur at the $A\beta$ coding region of the APP which enhance the aggregation properties of the $A\beta$ species (Kametani & Hasegawa, 2018; D. J. Selkoe, 2001). This is also known as E693Q mutation of APP that causes severe amyloid deposition in the cerebral vessels especially in the meningeal arteries that lead to the brain hemorrhage with amyloidosis (Hunter & Brayne, 2018; Kametani & Hasegawa, 2018; D. J. Selkoe, 2001). Another mutation in the 21st amino acid of the $A\beta$ coding region also known as A692G mutation is found to be involved in the $A\beta$ production and tangle formation which may cause dementia, this mutation also cause

occasion cerebral hemorrhages with severe microvascular β -amyloidosis. A692G mutation is also involved in the changes of the heterogeneous NH_2 terminal of β -secretase cleavage site that accelerate the production of full length peptides (Hunter & Brayne, 2018). More recently a studies in the transfected cells showed that a novel membrane anchored aspartyl protease (also called BACE2 or AspI) cleave the $\text{A}\beta$ region of APP more efficiently so A692G mutation shift the cleavage site towards ASP1 NH_2 terminus and increase the proportion of APP cleaved product generated by BACE2. All of these APP mutations heightened production of various $\text{A}\beta$ species (Hunter & Brayne, 2018; Tcw & Goate, 2017)

Mutations of the PSEN genes also increase the production of $\text{A}\beta$ species, especially $\text{A}\beta_{42}$. Assay of $\text{A}\beta_{40}$ and $\text{A}\beta_{42}$ from the plasma and skin fibroblast showed that PSEN1 mutations selectively increase the production of $\text{A}\beta_{42}$ by two fold levels (Hunter & Brayne, 2018). An important observation found that expressing human APP with PSEN1 mutations in the transgenic mice lead to the accelerated AD like phenotype in the offspring and $\text{A}\beta_{42}$ plaque accumulates as early as 3-4 month of age (Hunter & Brayne, 2018; Westmark, 2013). PSEN mutation increase the γ -secretase cleavage of the C99 that produce more peptides ending at $\text{A}\beta_{42}$ (Figure 2) (Lazarov & Demars, 2012).



Figure 2: Enzymatic cleavage of Amyloid Precursor Protein (Lazarov & Demars, 2012)

Apolipoprotein (ApoE4) has been recognized as a genetic risk factor as a high percentage of ApoE4 is found in the $\text{A}\beta$ deposit of AD brain. Immunohistochemical studies showed that inheritance of ApoE4 protein is linked with higher levels of $\text{A}\beta$ plaque than in people who are

lacking with ApoE4 protein (Kim, Basak, & Holtzman, 2009). People with ApoE4 inheritance who died (mostly nonagenarian) without showing any symptoms of AD have demonstrate higher amount of A β plaque in the brain (D. J. Selkoe, 2001). It suggest that effects of elevate level of A β could be observed in people or host with ApoE4 allele inheritance would not necessarily develop AD. Currently there is no concrete evidence why cells that co-express APP with ApoE4 protein lead to increased production of A β but cells with ApoE2 or ApoE3 does not (Sarkar, Choudhury, & Avinash, 2002). According to some theory ApoE4 protein decrease the clearance of A β peptides from the brain and enhance the steady state level of A β . Effects of AD promoting ApoE4 inheritance have been observed by Expressing ApoE3 and ApoE4 protein in the transgenic mice (Wildsmith, Holley, Savage, Skerrett, & Landreth, 2013). Mice expressing ApoE4 protein showed decreased neuronal growth as well as reduced maintenance of established neurons. Moreover mice that are crossed with mutant human APP with deleted ApoE4 genes showed decrease A β plaque burden, it suggest that absence of ApoE4 genes decrease the deposition of A β plaque in the brain (Leong et al., 2020; Wildsmith et al., 2013).

3.2 Central and peripheral A β pools

People with AD have higher level of A β in both central and peripheral tissue than normal elderly people. Peripheral tissue is the major source of A β as skeletal muscle counts about one-quarter of total body weight but it is found that A β level is higher in brain than in peripheral organs (G.-f. Chen et al., 2017; Wang, Gu, Masters, & Wang, 2017). There are three types APP isoforms that involve A β production (APP695, APP750, APP751) among which APP695 is highly expressed in the neuron that involve in the production of A β (mostly toxic A β_{42}) (Nalivaeva & Turner, 2013). Other two isoforms, APP750/51 mostly expressed in the peripheral organs specially leukocytes and platelets (G.-f. Chen et al., 2017). AICD is intracellular APP metabolites which acts as a gene regulator and involve in various function

such as cell signaling, cell cycling, protein metabolism etc. Neprilysin is a protein that reduces neuronal A β level by cleaving A β . So dysregulation of AICD could hamper neprilysin expression that can lead to increased neuronal A β accumulation. Transthyretin is another extracellular protein regulated by AICD that helps to inhibit A β aggregation, so alteration of AICD function can also increase the extracellular A β level. AICD is primarily produced by cleaving APP C-terminal with β -secretase, so effect of neprilysin and transthyretin expression is somewhat controlled by β -secretase (Murphy & LeVine, 2010; Silva et al., 2017). In the neuron β -secretase cleavage of APP695 is much higher than the APP750/51 and peripheral APP is primarily processed via α -secretase which result increased A β production in the neuron and decreased A β production in periphery. Lipoprotein, albumin these are A β binding protein that present in abundant amount in the peripheral organ, these protein binds with A β plaque and contribute it's transportation and clearance from the peripheral organs. Also dilution effect of blood reduces A β concentration form the circulatory system. These reasons explain why A β plaques rarely deposit in the peripheral organ but mostly in the brain and cerebral wall vessel (Wang et al., 2017). A β generated in the brain can be transported in the peripheral pool by using blood brain barrier (BBB) or by using glymphatic-lymphatic pathway. Several transporter proteins such as LDL-related protein-1 (LRP1), p-glycoprotein mediates the flow of A β from brain to peripheral pool through BBB. Arachnoid villi also plays role in the transportation by absorbing A β from the CSF and release it into the circulatory system (Wang et al., 2017). Peripherally generated A β can also enter into the brain and plays role in the pathogenesis of AD. Some studies suggested that receptor for advanced glycation end product (RAGE) might be responsible for transporting peripherally derived A β from blood to brain across the BBB as RAGE acts as binding site for A β and in AD brain the expression is increased by several fold (Askarova, Tsoy, Shalakhmetova, & Lee, 2012). RAGE promotes aggregation and accumulation by

internalizing A β into neuron which causes rapid activation of p38 mitogen-activated protein kinase (MAPK) signaling pathway leading to mitochondrial dysfunction and cell death. Also impaired A β clearance from the peripheral tissue might be another reason A β efflux from periphery to brain (Jarosz-Griffiths, Noble, Rushworth, & Hooper, 2016).

Chapter 4

Toxicity of A β

The deleterious effect of AD depends on the toxicological properties of AD as it explains other pathological effects of AD. There are several mechanisms through which A β exerts its toxic effects such as synaptic dysfunction, inflammation, oxidative stress, mitochondrial dysfunction, altering membrane permeability etc.

4.1 Synaptic dysfunction

Decline of cognitive ability in AD patients is closely related with synaptic loss due to deposition of A β plaque and NFTs (H. Zhang et al., 2021). Several studies reveal that there is 20-30% decrease in the number of cortical synapses and 10-15% decrease in the number of neuron synapses in AD patients (Carrillo-Mora et al., 2014). Also AD patients shows reduced amount of synaptophysin (pre-synaptic protein), synaptopodin and PSD-95 (post-synaptic protein), these protein are important for normal function of neurons (Carrillo-Mora et al., 2014; Dore et al., 2021; H. Zhang et al., 2021). Synthetic and naturally secreted A β oligomers reduces the long term potentiation process (LTP) that lead to the development of neuropathological lesions and disturbance in synaptic transmission in several transgenic models (Li et al., 2018; Westmark, 2013). Scaffolding protein such as PSD-95 plays an important role in the synapses by localizing many signaling proteins, receptors and channel in the synapses thus regulate synaptic strength and plasticity. It also protects synapses from soluble A β oligomers, so low level of PSD-95 may make the synapses vulnerable to A β . Due to intensive accumulation of A β oligomers PSD-95 level decrease in the brain that also negatively regulate the α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and N-methyl-d-aspartate (NMDA) type glutamate receptors that are required for synaptic strength and controlling synaptic transmission across the synapses (Carrillo-Mora et al.,

2014; Sarkar et al., 2002; Shankar & Walsh, 2009). LTP protein are also depend on the NMDA receptors for signal transmission from neuronal circuits that play role in learning and memory and soluble A β oligomers are thought to capable of inhibiting NMDA dependent LTP proteins (Carrillo-Mora et al., 2014; Rudy, Hunsberger, Weitzner, & Reed, 2015). Another studies showed that A β oligomers facilitate the movement of AMPA receptors away from the synaptic junction by promoting dephosphorylation of receptors that initiate long-term depression (LTD) and decrease post-synaptic strength of neurons in hippocampus region. A β interaction with several neuronal receptors is one of the leading pathological event of AD as it lead to direct neuronal injury causing cognitive impairment (Salvadores, Gerónimo-Olvera, & Court, 2020). It was reported that extra synaptic NMDA receptors increase the A β production by shifting α -secretase activity toward β -secretase activity. Intracellular NMDA receptors also plays role in the internalization and accumulation of A β peptides in the neurons. It was found that low concentration of A β could increase the LTP but long exposure of high concentration A β can decrease NDMR dependent LTP that overexcite the neurons (Klyubin et al., 2014). A β is also increase the extracellular glutamate concentration by reducing glutamate reuptake which inhibit the LTP and improve the LTD (figure 3) (Wei et al., 2010). Experiment on AD brains showed that intra neuronal and extra neuronal A β peptides were in dynamic equilibrium which lead to the memory deficit. Spine density is also reduced at nearby dendrites as overexpression of APP causes more A β secretion that lead to the loss of spines on overproducing APP neurons. Imaging assay also revealed that neighboring neurons (that do not produce A β) close to overproducing APP neurons could also face robust effects of A β oligomers therefor their neurons can be damaged also. To confirm this scientist infected the neurons of hippocampal CA1 region with two viruses- enhanced green fluorescent protein (EGFP) virus which did not express APP gene and another one is double promoter APP/tomato virus. CA1 dendrites that were labeled with

EGFP were divided into two segments- one segments is located 50-100 μm away from the dendrites infected with double promoter APP virus that overexpress APP and another segment is located 10 μm away from APP overexpressing dendrites. Two photon-laser scanning microscopy images showed that spine density of EGFP labeled dendrites that were 50-100 μm away from the infected APP overexpressing dendrites was normal. But EGFP labeled dendrites that were close to (10 μm away) infected APP overexpressing dendrites showed significant reduction in spine density. Overexpression of APP and $\text{A}\beta$ can also initiate several cell signaling pathway that lead to neuronal cell death such as apoptosis, necrosis, autophagy etc. (Wei et al., 2010).

Neuronal cell membrane contains several types of death receptors such as first apoptosis signaling receptors (FasR), tumor necrosis factor receptors (TNFs) for programmed cell death. These receptors have cysteine rich extracellular domain and intracellular death domain in the cytoplasm. $\text{A}\beta$ peptides bind with these death receptors which lead to the recruitment of fas-associated death domain (FADD) that aggregate with pro-caspase 8 and form a death inducing signaling complex (DISC). This complex further activates the caspase-3 and initiate neuronal cell apoptosis (Leong et al., 2020). $\text{A}\beta_{42}$ also has greater affinity for the $\alpha 7$ - nicotinic and cholinergic receptors and promote $\text{A}\beta$ peptides accumulation inside the cholinergic neurons (Kar, Slowikowski, Westaway, & Mount, 2004; Romoli, Sen, Parnetti, Calabresi, & Costa, 2021). By studying rat's hippocampal slices it was found that $\text{A}\beta$ peptide reduces the release of acetylcholine (Ach) from cholinergic neurons. Signal transduction of muscarinic receptors is also affected by soluble $\text{A}\beta$ oligomers (Wei et al., 2010).

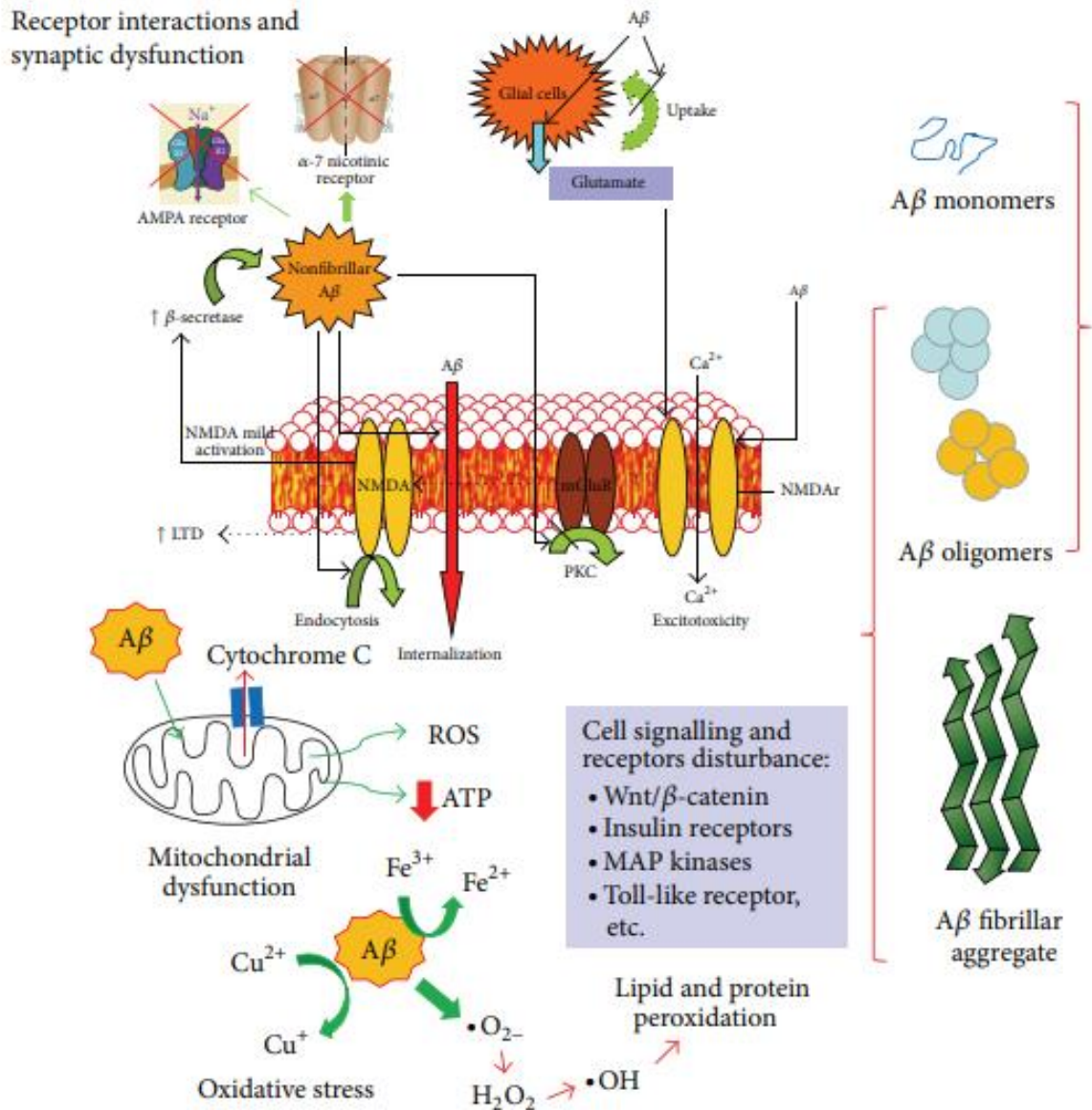


Figure 3: Multiple mechanisms of amyloid-beta toxicity (Carrillo-Mora et al., 2014).

A β also inhibits astrocyte reuptake and thus increases the extracellular concentration of glutamate, which increases the duration of action of glutamate. Entire neuronal activity is also altered due to spreading of glutamate from one synapse to another synapse. Prolonged duration of action of glutamate in the synaptic cleft causes receptor desensitization that leads to synaptic depression (Carrillo-Mora et al., 2014; Rudy et al., 2015).

4.2 Mitochondrial dysfunction

A β is also involved in mitochondrial dysfunction as high level of APP and A β found in external mitochondrial membrane (Donner et al., 2021; Swerdlow, 2007). Although generation of A β inside the mitochondria is not well understood yet as there is not β -activity is not found in the mitochondria though it has γ -secretase activity. So sAPP β from other sources might transported into the mitochondria where it processed by γ -secretase to produce A β or maybe A β oligomers from outside sources might transported into the mitochondria by using some transport protein such as mitochondrial outer membrane translocase (MOT) (Carrillo-Mora et al., 2014; Picone, Nuzzo, Caruana, Scafidi, & Di Carlo, 2014). A β oligomers inside the mitochondria decreases its respiratory states as oxygen consumption rate (OCR) is decreased along with activity of kreb's cycle enzyme activity and cytochrome-c oxidase activity is also decreased (Donner et al., 2021). A β aggregates also destroy the mitochondria by creating pores in the mitochondrial membrane that initiates apoptosis. Mitochondria is also known as cell power house as they generate most of the ATP which is required for glucose metabolism. A β peptide is thought to interfere with mitochondrial enzymatic machinery such as ATP synthase that hamper the mitochondrial ATP synthesis (Agrawal & Jha, 2020; Carrillo-Mora et al., 2014; Donner et al., 2021). Some animal studies showed that introduction of toxic A β aggregates into the mitochondria induce the generation of reactive oxygen species (ROS) that led to the impaired membrane potential (figure 3). Manganese superoxide dismutase (MnSOD) which is required to protect mitochondria from ROS also becomes the target of A β as a result protection against ROS reduced significantly therefore compromising mitochondrial function (Bell et al., 2021; Y. Zhao & Zhao, 2013). Another way of A β mediated mitochondrial dysfunction is by blocking activity of mitochondrial uncoupling proteins (UCPs). UCP2 and UCP3 protein protect mitochondria from ROS by reducing membrane potential toward ROS and diminishing proton motive

force. AD brain showed that accumulation of A β peptides causes the down regulation of UCPs and mitochondrial protective mechanism against ROS becomes dysfunctional (Carrillo-Mora et al., 2014; Y. Zhao & Zhao, 2013). A β also activates cytosolic and calcium dependent phospholipase A, NADH oxidase that produce more ROH causing mitochondrial dysfunction. A β can damage mitochondrial DNA and causes mitochondrial dysfunction. Reduced mitochondrial fusion due to A β peptide lead causes extensive mitochondrial fragmentation (mitophagy). Due to excessive A β , level of PTEN-induced kinase 1 is also reduced causing decrease clearing of damaged mitochondria from neuronal cell. Mitochondrial dysfunction also cause synaptic dysfunction because due to A β plaques mitochondrial ATP production is reduced and ATP is essential for the release of neurotransmitter into the synaptic cleft by synaptic vesicle (Chung, Lee, & Lee, 2018; Donner et al., 2021; Leong et al., 2020).

4.3 Oxidative stress

Para-magnetic electron resonance (PER) examination showed that aggregated or fibrillar form of A β is responsible for showing pro-oxidant effects by generating free radicals (Carrillo-Mora et al., 2014). There are several metal binding sites in the first 15 amino acid position (mostly histidine 6, 13, 14 and tyrosine 10) of A β peptide chain especially for Cu²⁺ ion. Several studies revealed that imidazole ring's nitrogen of histidine amino acid strongly bind with the Cu²⁺ ion and tyrosine 10 provides the necessary oxygen for this binding interaction. With the help of molecular oxygen A β can also generate superoxide anion by reducing Cu²⁺, Fe³⁺ into Cu⁺ and Fe²⁺ which later form hydrogen peroxide by reacting with molecular hydrogen (Carrillo-Mora et al., 2014; Gella & Durany, 2009; Y. Zhao & Zhao, 2013). A β also involve in the lipid and protein peroxidation by extracting proton from neighboring lipid and proteins (Carrillo-Mora et al., 2014). Metal reducing capability of A β peptides significantly reduced when these amino acids is substituted with other amino acids.

Metal ions specially Cu^{2+} and Zn^{2+} ions plays catalytic roles and are essential for regulating synaptic function. Cu^{2+} limits calcium entry into the cells by blocking NMDA receptors and regulates synaptic activation. Upon neuronal activation Zn^{2+} ions is released into the synaptic cleft from the presynaptic neurons and inhibit the excitatory NMDA receptors (Carrillo-Mora et al., 2014; Huang, Zhang, & Chen, 2016). On the other hand Fe^{3+} is important for generation of new synapses and maintaining synaptic plasticity. So deficiencies of these ions can alter the neurochemical environment of neurons that can hamper memory function. Zn^{2+} ions also involves in the cross-bridge dytyrosine linkage between two $\text{A}\beta$ molecules thus helping in the formation of $\text{A}\beta$ oligomers (Butterfield, 2002; Carrillo-Mora et al., 2014; Huang et al., 2016).

4.4 Neuro-inflammation

$\text{A}\beta$ oligomers also involve in the neuroinflammation causing neuropathological changes that are observed in the progression of AD (Leng & Edison, 2021). BBB plays crucial role in normal physiologic function as it maintain cerebral homeostasis by separating the brain from the circulatory system and prevents entering any harmful materials inside the brain (Askarova et al., 2012). Cerebral endothelial cell (CECs) that are connected by tight junction form the layer of BBB and this CECs layer is rich in various components such as mitochondria, astrocytes, pericytes, perivascular macrophages etc (Askarova et al., 2012; Kinney et al., 2018). These cells modulates the cerebral blood flow (CBF) as they surrounds the brain capillaries and influence cerebrovascular tone (Kinney et al., 2018; Michalicova, Majerova, & Kovac, 2020). Although brain inflammation is important in neuroprotective role when it is in acute phase but chronic inflammation can cause serious brain damage which is observed in the pathogenesis of AD (Carrillo-Mora et al., 2014; Kinney et al., 2018). Brain immune cells release cytokines such as IL-1, 6 including reactive oxide and nitric oxide during any injuries in the brain. Cerebral $\text{A}\beta$ deposit was increased in AD patient's brain who were suffering

head injuries, this is due to IL-1 increased the production of APP and A β burden in the brain (Askarova et al., 2012; Carrillo-Mora et al., 2014; Leng & Edison, 2021). Microglia remains inactive in normal healthy brain but becomes active when encounter any threat or injury (Carrillo-Mora et al., 2014; Leng & Edison, 2021; Sun et al., 2015; Walker, 2020). According to some investigation it was demonstrated that A β might be responsible for the activation microglial cells resulting in the migration of other immune cells to phagocyte A β plaque. But sustained activation of microglial cells lead to the microgliosis resulting in an increased number of microglial cells and release of inflammatory cytokines at the A β accumulated sites. As a result efficiency of microglial cell is decreased and they no longer clear the A β (Carrillo-Mora et al., 2014; Heneka et al., 2015; Kinney et al., 2018; Leng & Edison, 2021). Moreover, due to microglial over activation inflammatory cytokines starts to damage the neurons as well as increasing A β accumulation. Neuroinflammation exacerbate due to continuous release of pro-inflammatory cytokines that accelerate neurodegeneration. As microglia becomes less efficient in clearing A β and continuously release pro-inflammatory cytokines which recruits additional immune cells into the sites. According to some recent data it was found that peripheral macrophages starts to migrate into the A β deposited plaque and tries to clear A β but recruitment of peripheral macrophages tends to cause more damage to the brain by exacerbating the already sustained inflammation (Kinney et al., 2018; Kurz, Walker, Rauchmann, & Pernecky; Leng & Edison, 2021; Shankar & Walsh, 2009; Wildsmith et al., 2013).

Chapter 5

Neurofibrillary tangle

Other than A β another protein that plays major role in the pathogenesis of AD is tau protein. Abnormal accumulation of tau inside the neurons lead to the formation of neurofibrillary tangles (NFTs) which impaired normal neuronal function (Brunello, Merezhko, Uronen, & Huttunen, 2020).

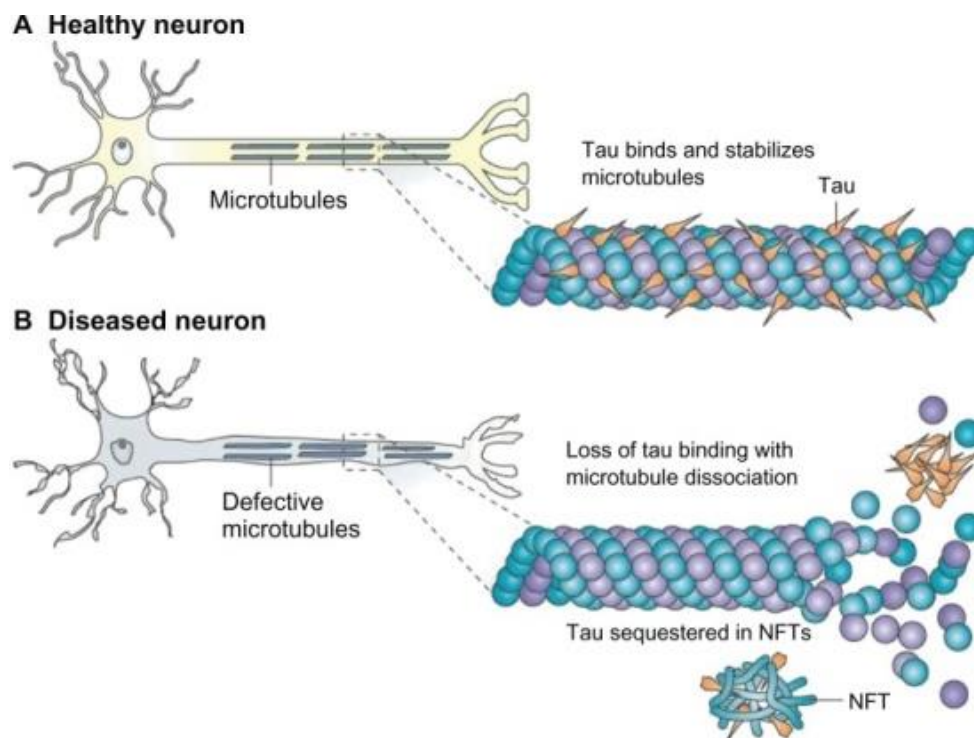


Figure 4: Healthy and pathological tau inside the neuron (S. Chen et al., 2013).

Microtubule helps to support neurons internally and plays role in signal transduction, synaptic plasticity, synapses formation and transport of nutrients from cell body to axon and dendrites (Kolarova et al., 2012). In healthy neuron tau proteins binds with microtubules to stabilize it (figure 3) (Ghosal, Fan, Dawson, & Pimplikar, 2016). Tau proteins are intrinsically disordered proteins that are abundant in central nervous system more specifically in the frontal, temporal, entorhinal and hippocampal region of the brain (Michalicova et al., 2020). Axons microtubules predominantly contains tau so, when these tau proteins detach

from the microtubules and join together to form tangles inside the neuron which causes disturbance in the synaptic communication between neurons by blocking neuron's transport systems (Naseri, Wang, Guo, Sharma, & Luo, 2019). This is one of the pathological features of AD. The structure of tau is regulated by several post-translational modifications such as glycation, glycosylation, polyamination, ubiquitination etc (Michalicova et al., 2020). But abnormal modifications such as hyper-phosphorylation of tau plays detrimental roles in neural function that causes neurodegeneration (Gong & Iqbal, 2008). As of now total six isoforms of tau have been identified that are expressed by human brain and the gene responsible for encoding tau is microtubule-associated protein tau (MAPT) gene containing 16 exons. Among these 16 exons alternative splicing of 8 exons generate 6 main isoforms in the CNS and another 6 additional isoforms also produced in the peripheral nervous system (PNS) (Goedert & Spillantini, 2017; Saito et al., 2021). Tau protein is generally 350-441 amino acid long and contain four primary domains (Figure 4). These are N-terminus domain (projection domain), proline rich domain, microtubule binding domain (repeated domain, R1 to R4) and C-terminus region (flanking region) (Kent, Spires-Jones, & Durrant, 2020; Yu et al., 2019).

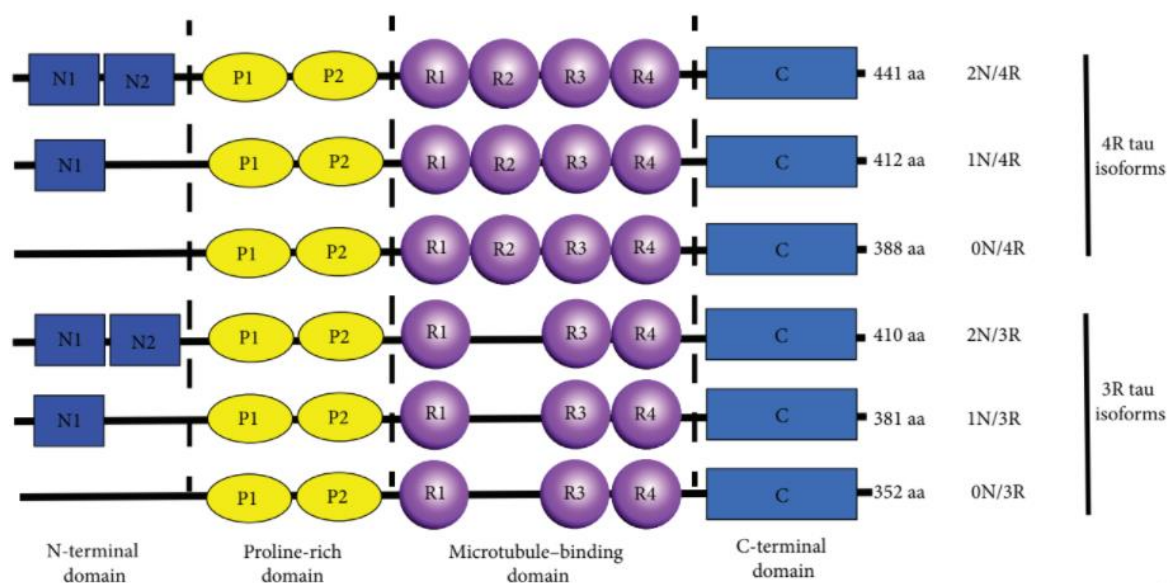


Figure 5: Six isoforms of tau (Yu et al., 2019).

Alternative splicing of microtubule binding domain (MBD) produces 3R and 4R tau isoforms (Kolarova et al., 2012). 3R tau isoform is produced during development whereas 4R isoform is produced during adulthood and these two isoforms are maintained in balanced ratio (1:1) in normal human brain. Though these two are present in equal amount in the brain but 4R tau isoform is strongly involved in the assembly of microtubules in axon. Imbalance of 3R and 4R ratio can cause axonal dysfunction leading to dementia and AD (Kolarova et al., 2012; Naseri et al., 2019; Yu et al., 2019; H. Zhang et al., 2021). Recent studies found 50 mutations that interfere with splicing of exon 10 in the MAPT gene that might be responsible for abnormal 4R elevation causing aggregation of tau. On the other hand 50% reduction of tau level in brain is observed when chromosome region containing of MAPT gene is deleted. In cases of AD and other neurodegenerative disorder tau protein loses it's capacity to bind with microtubules and become ineffective to keep cytoskeleton well organized (Naseri et al., 2019).

5.1 Tau in synaptic function

It is already confirmed that defects of synaptic function in the hippocampal region of brain causes memory deficit. Tau support neuron by stabilizing microtubules thus maintaining normal neuronal function. Therefore, failure of tau to bind with microtubules affect the synaptic function. Impaired motor coordination and morphological synaptic defects have been observed due to tau knockdown in the hippocampal region (Kolarova et al., 2012; Naseri et al., 2019). Transgenic mice expressing human wild type tau shows that tau hyperphosphorylation and aggregation reduce LTP level in mouse hippocampus (Busche & Hyman, 2020; Naseri et al., 2019). Though exact mechanism of tau mediated synaptic function or dysfunction is unclear but there are some possible explanation. At presynaptic neuron normal synaptic vesicle release might be impaired due to interference with pathological tau. Synaptogyrin-3 which is a synaptic vesicle anchored transmembrane protein

might be responsible for the interaction between pathological tau and synaptic vesicle release resulting in decreased neurotransmission (Kent et al., 2020; Naseri et al., 2019). Under normal or pathological condition axonal tau protein can move to dendrites at the post synapses where it interacts with post-synaptic density proteins (PDS). So during pathological condition tau can dissociate from the dendritic microtubules causing dendritic loss and aberrant postsynaptic function (Brunello et al., 2020). Neurons are highly polarized cell, so efficient transport system is very important to deliver proteins, lipid and other organelles from cell body to axon and dendrites. Therefore, alteration of axonal transport system can trigger axonal degeneration which can affect entire nervous system (Naseri et al., 2019; Salvadores et al., 2020).

5.2 Hyperphosphorylation

Post-translational modification of tau (mostly phosphorylation) is extensively studied as AD brain contains NFTs three to four times higher concentration than normal brain and these NFTs are highly enriched with hyperphosphorylated tau proteins (Gong & Iqbal, 2008; Naseri et al., 2019; H. Zhang et al., 2021). Biochemical studies at the disease related sites showed that phosphorylation of tau reduces its ability to bind with microtubules and propagates self-aggregation of tau to form toxic tangles (Kolarova et al., 2012). Scientists have found approximately 85 phosphorylation sites in tau protein by using mass spectroscopy analysis, among those 85 sites 45 are pathological phospho-sites (Naseri et al., 2019). Approximately 81 serine or threonine phosphorylation sites have been found in the longest tau protein (441 amino acid long) (Gong & Iqbal, 2008; Kolarova et al., 2012). These phosphorylation sites are located in the proline rich region near the vicinity of MBD and in the motif KXGS near the C-terminus. KXGS motifs are conserved amino acid residue in the MBD (R1 to R4) region, hyperphosphorylation and hypoacetylation of serine amino acid in KXGS motif disrupts binding of tau with microtubules (Kolarova et al., 2012). Upregulation

of tau kinases or downregulation tau phosphatase are thought to be responsible for abnormal tau phosphorylation. Glycogen synthase kinase-3 β (GSK-3 β) plays key in regulation of tau phosphorylation. In-vivo analysis showed that GSK-3 β can phosphorylate ser199, thr231, ser396, ser404, ser406 and ser413 residue of the longest tau isoform. Phosphorylation of thr231 further increase the GSK-3 β level and causes conformational changes of tau isoforms (Gong & Iqbal, 2008; Kolarova et al., 2012; Korte, Nortley, & Attwell, 2020; H. Zhang et al., 2021). On the other hand protein phosphatase such as PP1, PP2A, PP2B regulates synaptic plasticity and learning by dephosphorylate tau protein (Kolarova et al., 2012). In vitro analysis showed that the level of PP has been significantly reduced in AD brain which indicate that phosphorylation rate is increased several fold compared to dephosphorylation during neurodegenerative disease (Ayubcha et al., 2021). Tubulin (proteins that polymerizes into long chain to form microtubules) is present over ten fold excess of normal tau that ensure all the tau bind with microtubule to stabilize it but during AD these tau proteins do not bind with tubulin instead they inhibits the assembly of tubulin to form microtubules (Kolarova et al., 2012). It was reported that AD patient contain 40% more cytosolic tau that do not form polarized into paired helical filament (PHF) moreover, these hyperphosphorylated tau removes microtubules associate protein (MAPs, MAP2, MAP3) from microtubule lattice (Barbier et al., 2019; Kolarova et al., 2012). Another study revealed that phosphorylation of tau at the proline rich region near the MBD promotes self-aggregation of tau to form PHFs, NFTs. In vitro kinetic studies showed that mutation of thr231, ser396, ser422 into Glu increases the aggregation tendency of tau (Gong & Iqbal, 2008; Miao et al., 2019). Despite extensive studies the exact cause of tau hyperphosphorylation is still in debate. Substrate of kinase and phosphatase such as oligonucleotide, P-nitro phenyl phosphatase also alter the regulation of tau protein by promoting hyperphosphorylation and aggregation. Beside these enzymes, a monosaccharide called β -N-acetylglucosamine (GlcNAc) modifies the

serine/threonine residue of tau chain via glycosidic bond known as O-GlcNAcylation. Both in-vitro and in-vivo studies showed that this O-GlcNAcylation process help to regulate tau phosphorylation by acting as nutrient/stress sensor. O-GlcNAcylation also involve in regulating protein structure, enzyme activity, protein-protein interaction also modulating protein stability. In AD brain a decrease in O-GlcNAcylation rate was observed due to tau hyperphosphorylation. Glucose metabolism helps to regulate O-GlcNAcylation process by supplying uridine diphosphate N-acetylglucosamine (UDP-GlcNAc) which is donor protein for O-GlcNAcylation. So, decrease O-GlcNAcylation rate due to impaired glucose metabolism in AD brain might facilitate tau hyperphosphorylation causing neurofibrillary degeneration (Cantrelle et al., 2021; Gong & Iqbal, 2008; Liu, Iqbal, Grundke-Iqbal, Hart, & Gong, 2004).

5.3 Acetylation

Acetylation of tau is important for normal regulating neuronal function. Tau contains many serine residue particularly in MBD region. Abnormal acetylation of at serine residue (mostly serine281) contribute to tau mediate neurodegeneration (Brunello et al., 2020; Naseri et al., 2019). Mass spectroscopy analysis of tau in both wild type and APP transgenic mice showed that multiple lysine residue have been acetylated (Kolarova et al., 2012; Naseri et al., 2019). The enzyme that facilitate tau acetylation is histone acetyl transferase P300 (HAT-p300) and CREB binding protein, whereas enzymes that deacetylate tau in brain is sirtuin1 (SIRT1) and HDAC6. Acetylation of tau within MBD region increases phospho-tau level and alter the structure of tau which might increase aggregation property and also makes tau more vulnerable to misfolding. Studies on PS19 transgenic mouse model showed that acetylation of serine280/281 residue in MBD region increases insoluble tau aggregation (Lucke-Wold et al., 2017; Naseri et al., 2019). Memory impairment and locomotor dysfunction have been observed in drosophila model when acetylated tau level increased. Mislocalization of tau to

somatodendritic compartment and deficit in LTP level have been observed when serine274 and 281 residue is acetylated. SIRT1 protein helps to deacetylate tau, it also activate transcription of α -secretase thus reducing A β generation. Neurodegenerative disease reduces the activity of SIRT1 protein thus exacerbate A β generation and further increase tau phosphorylation and acetylation which have deleterious effect on neuronal health (Cohen et al., 2011; Kolarova et al., 2012; Naseri et al., 2019).

Chapter 6

Tau aggregation

MBD region spanning from ser214 to glu372 residue is thought to mediate tau aggregation. Tubulin polymers tightly bind in the MBD region and tether tubulin dimer together. Hexapeptide motif VQIVYK present in the third repeat (R3) of MBD region is crucial for fibril assembly. A second motif VQIINK located in second repeat (R2) binds with R3 motif to form β -sheet structure that facilitate tau aggregation (Brunello et al., 2020; Walker, 2020). Mutation in VQIVYK motif can destabilize tau structure that promote rapid aggregation. Cryogenic electron microscopic study revealed that amino acid residue within the MBD region forms β -helix structure that makes up the core of tau filaments (Goedert & Spillantini, 2017; Kolarova et al., 2012; Oakley et al., 2020; Yan et al., 2020).

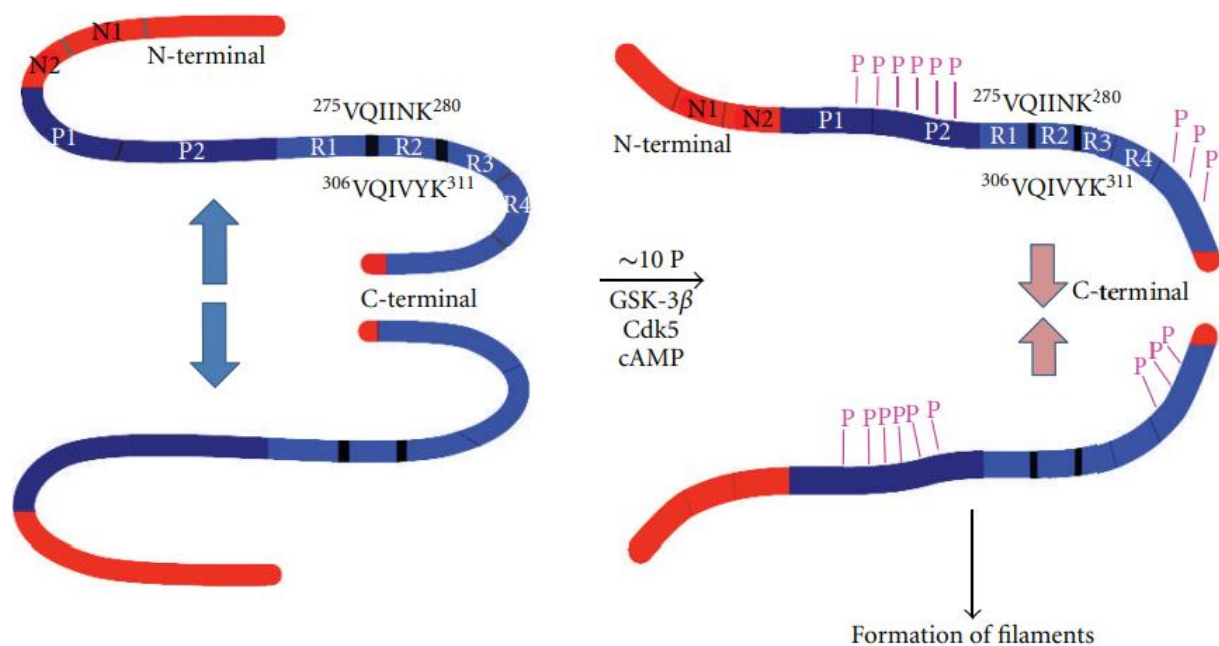


Figure 6: Formation of PHF or tau aggregation (Kolarova et al., 2012).

The first step of tau aggregation is formation of tau dimer via exposure of VQIINK/VQIVYK motif that forms a nucleation center. Tau monomer is then recruited into this nucleation center to form oligomers (Gong & Iqbal, 2008; Kolarova et al., 2012; Zhang, Cao, Ma, Wei,

& Li, 2021). Disulfide bridge is formed between two cysteine residue located in R2 and R3 during dimerization process which is a critical step in tau fibril formation. In vitro studies showed that N-terminus and C-terminus region of tau monomer is inhibitory in nature that helps to prevent interaction between these two sticky regions (Kolarova et al., 2012; Saito et al., 2021). But abnormal phosphorylation in tau protein neutralizes the inhibitory feature of N and C-terminus region that induce a relaxed structural conformation. Due hyperphosphorylation the sticky domain interact with each other to form PHF. NFT obtained from AD brain showed that deamidation process further increase tau polymerization by converting amide group from amino acid side chain into acidic group. Polyanionic molecules such as sulfoglycosaminoglycans (sGAGs), heparin also increases tau polymerization, oxidation of cysteine residue in 3R region increases disulfide cross linking thus facilitating self assembly of tau monomer (Gong & Iqbal, 2008; Kolarova et al., 2012).

6.1 Tau propagation

Tau is found in the CSF at higher concentration that facilitate cognitive decline. Memory impairment along with decreased synaptic plasticity was observed when NFTs derived from AD brain was injected into the mouse hippocampus. It was also observed that those injected NFTs spread into the corpus column, cortex and hypothalamus of brain which were independent from tau origin (Pooler et al., 2013; Sengupta, Nilson, & Kaye, 2016; Takeda, 2019). By using trans-synaptic mechanism of release tau can spread from one neuron to another neuron. It was reported that tau can be secreted into the synaptic cleft by interacting with NMDA receptor of neuron (Brunello et al., 2020; Mamun, Uddin, Mathew, & Ashraf, 2020). Membrane bound vesicle such as exosomes are used in intracellular communication and these exosomes are used to deliver different cellular content from one cell to another cell. Scientist found the presence of tau in exosome derived from AD patients which indicate that tau can use exosome to move from one neuron to another neuron. Tau inside the exosomes

phosphorylated at faster rate and capable of propagate those phosphorylated tau into neighbouring cells (d'Errico & Meyer-Luehmann, 2020; Naseri et al., 2019). Cellular uptake of tau oligomers might another possible explanation of tau propagation. Healthy neurons can take extracellular tau secreted during synaptic transmission by using endocytosis process. Adjacent cells are connected with each other via tunneling nanotubes. Tunneling nanotubes are filamentous actin containing channels that are used in intracellular protein transport. Intracellular tau might use these tunneling nanotubes to transfer into the healthy cells. Tau can penetrate cell membrane directly by interacting with lipid bilayer causing membrane disruption. Heparan sulfate proteoglycan (HSPGs) helps tau to penetrate cell membrane, so tau can enter into the healthy cells by macropinocytosis mediated by HSPGs (Mamun et al., 2020). Post-mortem studies on AD patient brains showed that microglia contains highlyphosphorylated tau but microglia do not express tau inside them. The possible explanation is that microglia might engulf tau from the extracellular space in an attempt to degrade it. Toxic tau oligomers appears in the cytoplasm of microglia when the amount of hyperphosphorylated tau surpasses it's degradation capabilities that lead to microglial dysfunction. When microglia is no longer able to degrade tau it acts reservoir of toxic tau aggregates. Microglia actively secret tau into the neuron and promote neuron to neuron transfer of toxic tau aggregates (Naseri et al., 2019).

6.2 Interaction between A β and tau

Post-mortem of AD patient brain and AD transgenic mouse brain A β co-localize with phosphorylated tau synaptic nerve terminal . In-vitro and in-vivo studies demonstrated that A β oligomers can increase tau phosphorylation and oligomerization. Tau can increases A β toxicity because knockout of endogenous tau in transgenic mouse model reduces cognitive dysfunction and synaptic toxicity mediated by A β (Li et al., 2018). As per discussion it is confirmed that GSK-3 β and CDK-5 enzymes is required for tau phosphorylation at ser/thr

amino acid residue, aggregated A β increases the activity of GSK-3 β and CDK-5 enzymes that ultimately causes tau hyperphosphorylation. Other than increasing tau hyperphosphorylation, A β aggregates increase tau oligomerization and aggregation. A β oligomer also induces the activity caspase 3 and calpain-1 that produce N-terminus tau fragments by cleaving tau at Asp421 of C-terminus region. This tau fragment lacking of C-terminus aggregates more rapidly than full length tau that cause degradation of both neuronal and non-neuronal cell death (Kolarova et al., 2012; H. Zhang et al., 2021). It was reported that impaired metabolism of APP or A β oligomers can trigger tau mis-localization by inducing axonal defects. APP might promote propagation of pathological tau by acting as a receptor of abnormal tau fibril. Interaction of tau with A β oligomers lead to AMPA receptors mediated endocytosis and also facilitate LTD expression (Busche & Hyman, 2020; Chung et al., 2018). These interactions between tau and A β oligomers causes cognitive decline and memory impairment that exacerbate AD pathogenesis. Synergistic effects of A β and tau fibril increases the number and activity of microglia and astrocytes which trigger inflammatory cascade. Inflammatory cytokines induces neuronal injury by releasing tau into the extracellular space which further interacts with already existing NFTs and A β oligomers ultimately lead to cell death (Busche & Hyman, 2020; Chung et al., 2018; Kent et al., 2020).

Chapter 7

Current treatment options for AD

The severe consequences of AD lead researchers to focus on the discovery of effective drugs to prevent the disease. Despite all efforts unfortunately there are no pharmacotherapeutic options to prevent or cure the disease completely. Current treatment options focused on the reduction of AD symptoms.

7.1 Cholinesterase inhibitors

Neuropathological and imaging studies showed that AD patients have significant reduction in cholinergic activity in the hippocampus region of brain that involve in memory impairment and cognitive decline. So, improving cholinergic function in brain may help to treat memory impairment and cognitive deficit. Several cholinesterase enzyme (more specifically acetylcholinesterase) is responsible for degradation of Ach neurotransmitter once it is released into the synaptic cleft (Dos Santos Picanco et al., 2018; Szeto & Lewis, 2016). Cholinesterase inhibitor drugs block the activity of Ach-esterase thus increasing concentration of Ach into the synaptic cleft thus helps to improve cognitive behavior. Rivastigmine, Galantamine, Donepezil and Tacrine are currently approved cholinesterase inhibitor drugs to treat behavioral symptoms of AD. Other than blocking the activity of Ach-esterase in the central nervous system these drugs specially Rivastigmine also inhibit the activity of butyrylcholinesterase (BuChE) in the amygdala, thalamic nuclei (Grossberg, Tong, Burke, & Tariot, 2019; Schachter & Davis, 2000; Yiannopoulou & Papageorgiou, 2013).

7.2 NMDA receptor antagonists

A β oligomers are able to decrease NMDA dependent LTP level in the brain thus affecting learning and memory. NMDA receptor can also increase A β production by shifting α -secretase activity towards β -secretase activity. So, inhibition of NMDA receptor activity help

to improve learning and memory function. Memantine is the FDA and EMA approved NMDA antagonist that reduces excessive glutamate action thus decreasing excitotoxicity and neurodegeneration (Dos Santos Picanco et al., 2018; Grossberg et al., 2019; Szeto & Lewis, 2016). This drug also reduces A β toxicity and decrease tau phosphorylation. Bis(propyl)cognitin is another non-competitive NMDA receptor antagonist used to treat mild to moderate AD (Dos Santos Picanco et al., 2018).

Chapter 8

Future treatment options for AD

From the current discussion it is already confirmed that the main cause of AD is abnormal functioning of A β and tau peptides. So currently researchers are trying to develop new treatment strategies targeting A β and tau proteins. Moreover, modifying genes that are responsible for abnormal accumulation of A β peptides and tau fibril might be another way to prevent AD. Several clinical trials are ongoing to imply these strategies but so far no drugs have been approved. The success ratio of AD drug is very low compared to other disease. About 40% of drug candidates pass phase 1 and phase 2 clinical trials and success ratio at phase 3 clinical trials is near zero.

8.1 Anti – A β

The above discussion tells us that one of the pathological hallmarks of AD is accumulation of A β oligomers. Monomeric A β is not toxic to the brain but when these A β monomer form oligomers they start to disrupt normal brain function by slowly destroying synapses. So, inhibition of A β oligomers formation is one of the key strategy to stop the progression of AD (Weller & Budson, 2018). One therapeutic approach of AD is to prevent A β oligomer formation or facilitate A β clearance from the brain by using monoclonal antibodies without eliminating β -secretase or γ -secretase activity in patients with minimal cognitive dysfunction (Lozupone et al., 2020). Solanezumab was one of the most promising drugs for the treatment of AD designed by Eli Lilly and company. This drug neutralizes A β_{42} mediated toxicity by binding with A β monomers and allow A β peptides clearance from the CNS before it clumps together to form oligomeric A β . Despite of it's promising therapeutic activity the drug Solanezumab was declared as failure in 2017 after it's 4 years long phase 3 clinical trial due to it's ineffectiveness in dominantly inherited Alzheimer's disease (DIAD) (Sengupta et al.,

2016; Weller & Budson, 2018). Roche pharmaceutical has been working on another monoclonal antibody called Gantenerumab (human IgG1 antibody) that works similarly like Solanezumab by enhancing its clearance from the brain. Roche pharmaceutical claims that Gantenerumab is able to neutralize $A\beta_{42}$ mediated LTP inhibitory effects on the brain. Currently this drug is in the phase 3 clinical trial and FDA has awarded this drug candidate as a breakthrough therapy designation for AD treatment which brings the drug closer to get full FDA approval (Weller & Budson, 2018). Besides monoclonal antibodies another strategy for decreasing $A\beta$ plaque is to inhibiting enzymes (β -secretase and γ -secretase) that produces $A\beta$ peptides from APP (figure 7). These enzymes inhibitor drugs do not help to reduce already existing $A\beta$ plaque from the brain but decrease production of new $A\beta$ peptides. Over activation of BACE1 enzyme increase production of toxic $A\beta$ peptides both centrally and peripherally, so targeting these enzymes is most effective way to control $A\beta$ production. So, researchers are testing several compounds that interfere with activity with these enzymes in an attempt to prevent to progression of AD. Most of these inhibitor compounds bind with the secretase enzymes through covalent interactions. Maximum inhibition is confirmed by increasing the number of covalent interaction between the enzymes (BACE1 and γ -secretase) and the inhibitor drugs so that secretase enzymes have greater affinity for the inhibitor compounds than APP (Menting & Claassen, 2014). P10-P4' StatVal is the first BACE1 inhibitor developed by Elan pharmaceuticals to treat AD. Although the drug seemed promising in it's in-vitro studies but failed to form a stable BACE1/ P10-P4' StatVal in it's in-vivo studies (Menting & Claassen, 2014). NCT01496170 is an BACE 1 inhibitors that entered in clinical trials with a single dose of 450 mg per day had a success ratio of 90% in phase 1 clinical trials but failed during phase 2 clinical trials. Another drug NCT01739348 was 80% successful to reduce $A\beta_{40/42}$ burden during phase 1 and phase 2 clinical trials but phase 3 clinical trials of this drug was terminated in 2018 as it failed to improve cognitive

function in AD patients (Das & Yan, 2019). LY2811376 is another non-peptide BACE1 and γ -secretase inhibitor that entered in phase 1 clinical trials but failed due to its excessive toxicity and detrimental effects on cognitive ability (Dos Santos Picanco et al., 2018; Westmark, 2013). Verubecestat was another BACE1 inhibitor with potential therapeutic effects, this drug candidate reduced CSF $A\beta$ level significantly but in phase 3 clinical trials the cognitive function slightly worsen and the study failed (Lozupone et al., 2020; Weller & Budson, 2018). JNJ-54861911 developed by Janssen pharmaceuticals is currently undergoing phase 2 clinical trials is successful to reduce CSF $A\beta$ by 90% at a dose of 50 mg per day during Phase 1 clinical trial and the study is expected to complete in October 2022 (Weller & Budson, 2018). Combination therapy with anti- $A\beta$ and monoclonal antibodies could be a potential therapeutic strategy to treat AD that will accelerate $A\beta$ clearance and prevent new $A\beta$ peptide formation. Extensive studies need to be conducted to prove the effectiveness of combination therapy. Till now drugs that aim for $A\beta$ to treat AD is not successful yet.

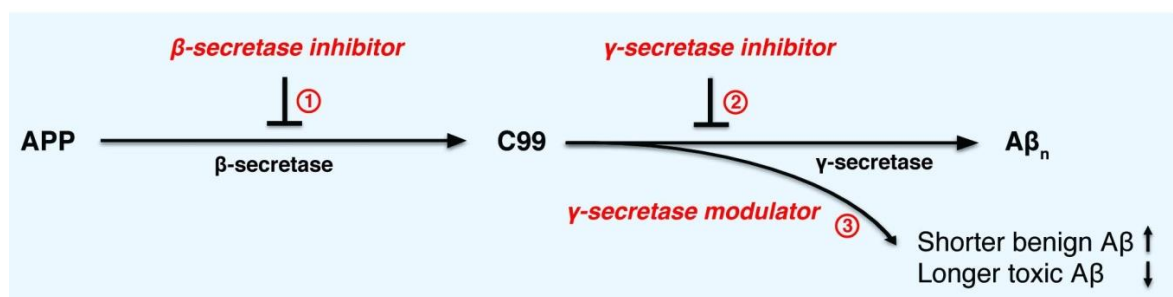


Figure 7: Targeting β -secretase and γ -secretase to reduce $A\beta$ burden (J. Zhao, Liu, Xia, Zhang, & Wang, 2020).

8.2 Anti – tau

As discussed above it is confirmed that accumulation of hyperphosphorylated tau in the CNS impairs the cognitive function and causes memory deficit and directly related to AD pathology. So, preventing tau hyperphosphorylation or spreading of pathological tau in the CNS is the good way to stop AD progression. GSK-3 enzyme is responsible for regulating glycogen metabolism, cell signaling and normal tau phosphorylation. It has two isoforms-

GSK-3 α and GSK-3 β . Negative regulation of GSK-3 β is responsible for tau hyperphosphorylation at ser/thr residue. So, targeting this GSK-3 β enzyme could be effective to reduce toxic tau phosphorylation. Lithium and valproate inhibit GSK-3 β and reduce tau phosphorylation in transgenic mouse model so these compounds could be effective in treating tau mediated toxicity (Nalivaeva & Turner, 2013). mts-L803 is GSK-3 β inhibitor currently under clinical trials, this compound showed promising effect by reducing tau hyperphosphorylation as well as inhibition of A β accumulation (Dos Santos Picanco et al., 2018). Another target for the treatment of AD is cdk5 enzyme. Cdk-5 also involves in tau hyperphosphorylation that increases toxic tau aggregates in CNS. A purine derivative named Roscovitine showed promising effect in AD treatment, it has an IC₅₀ of 0.16 μ M for cdk-5 enzyme and can cross BBB. Administration of cdk-5 inhibitors reduces tau burden along with other neuro-toxic proteins from brain. But this roscovitine has not been approved by FDA as its safety and toxicological data has not been completed yet (Gong & Iqbal, 2008; H. Zhang et al., 2021). RO7105705 is a monoclonal antibody developed by Genentech (a member of Roche pharmaceuticals) that targets tau oligomers and prevents cell-to-cell tau spreading. This drug candidate is currently under phase 2 clinical trial and is expected to complete the study in September 2022 (Weller & Budson, 2018).

8.3 CRISPR/Cas9 gene therapy

Currently one of the promising approaches in AD treatment and research is gene editing technology. Repairing pathogenic genes or screening mutated genes that involve in the pathogenesis of AD could be effective in AD treatment. Clustered Regularly Interspaced Short Palindromic Repeats or CRISPR gene editing technology is showing great potential in the research field to treat AD. CRISPR/Cas9 cuts DNA in a precise manner and takes the advantage of the body's natural DNA repair mechanism to correct the mutated genes. It has two parts- a single guide RNA (sgRNA) that guides the system towards the target and a Cas9

protein that cleaves that DNA strands (Bhardwaj et al., 2021; Rohn, Kim, Isho, & Mack, 2018). CRISPR/Cas9 technology can be used to develop AD models which will give scientist an opportunity to study the disease more precisely. By using this technology scientist can develop induced pluripotent stem cells (iPSC) model. Scientist can introduced mutated genes responsible for AD into the iPSC lines from healthy individual to generate A β peptides so that they can run experiment on these disease models (Duan et al., 2021; Penney, Ralvenius, & Tsai, 2020). So, mutations in genes such as APP or PSEN mutation can increase A β production that trigger AD. PSEN1 and PSEN2 gene mutation can increase A β_{42} production by altering APP cleavage site. CRISPR/Cas9 can correct PSEN autosomal dominant mutation and decrease the A β production and normalize A $\beta_{40/42}$ ratio (Bhardwaj et al., 2021; Lu, Yu, Cai, Sun, & Yang, 2021; Rohn et al., 2018).

Chapter 9

Challenges in AD treatments

Despite of intensive research and investigation, little progress have been made to prevent or find a cure for AD. Some potential drug candidates showed promising result in several animal models during pre-clinical trials but ultimately failed in large scale clinical trials. Early onset of AD which appears before the age of 65 years could lead physicians to misdiagnose the AD because in these younger patients cognitive complaints is often unrelated to memory dysfunction. So diagnosis of AD in young patients could be a challenge for physicians. Pathological changes may begin many years before first clinical symptoms of AD. For example, CSF A β ₄₂ accumulation may begin as long as 20 years and CSF tau level have been developed 15 years before the onset of AD. This AD spectrum of AD from clinically asymptomatic to severely impaired cognitive function is not well defined in current understanding of AD (Frozza, Lourenco, & De Felice, 2018). Current animal models for studying AD pathophysiology need to be improved so that their physiologic condition can be extrapolated to the human condition, this will help the researchers to predict the effect of drug candidates in human more efficiently. Limitations in early diagnosis is another challenge in AD treatment because eligible candidates do not enroll in clinical trials until symptoms of AD appears, this causes difficulties to run clinical trials (Pharma, 2018).

Chapter 10

Conclusion

The prevalence of AD is increasing every year worldwide and cost of AD is staggering. In the future, special risk-assessment profile will be offered to people reaching their 50s to determine their possibilities of developing AD. What is clear from the above discussion is that A β and tau plays major roles in the development of AD. Synergistic effects of both A β and hyperphosphorylated tau increases the neuronal degradation. But many mysteries and questions regarding AD is still unresolved. A β and tau proteins normally synthesize within the brain and plays normal physiological roles but why they build up abnormally in large amount in elderly people not in young people is still not clear. Why neuronal cells are mostly affected by these toxic proteins? Extensive studies are being carried out to know exact mechanism of AD pathology. Although there is no concrete treatment to eradicate this disease completely but recent studies are showing hope to an integrated and therapeutic approach to cure this disease. Current treatment options for AD is supportive without attenuation of the disease progression. Clinical trials of some potential compounds are being conducted targeting pathological hallmarks (A β and tau) of AD. Several disease modifying treatment (such as monoclonal antibodies, immunotherapies etc.) that target A β can alter the progression and complication. If this can be accomplished then preventive treatment could be offered to people with higher risk of developing AD. Future studies should focus on early detection of AD so that preventive measure could be taken. Moreover, changes in lifestyle such as proper diet, regular exercise and moral practice could be useful to relieve mental stress during AD.

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