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Dysfunctional dendritic and axonal activity in Alzheimer's Disease - identifying possible therapeutic targets

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Contents

List of Abbreviations

ABCA7	ATP-binding cassette transporter A7
ACE	Angiotensin converting enzyme
AChEI	Acetylcholinesterase inhibitors
AD	Alzheimer's disease
ADAM10	Metalloproteinase domain-containing protein 10
AEP	Asparagine endopeptidase
AICD	APP intracellular domain
Akt	Protein kinase B
ALS	Autophagy lysosome system
AMPARs	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors
APFs	Annular protofibrils
APH1A or APH1B	Anterior pharynx-defective 1 A or B
APOE	Apolipoprotein E
APP	Amyloid precursor protein
ATP	Adenosine triphosphate
AVs	Autophagic vacuoles
Aβ	Beta-amyloid
BACE1	Membrane-bound enzyme β-secretase 1
BBB	Blood brain barrier
BCSFB	Blood-cerebrospinal fluid barrier
BDNF	Brain-derived neurotrophic factor
BFCN	Basal forebrain cholinergic neurons
CaMKII	Calcium-dependent protein kinase II
CaMKIV	Calcium-dependent protein kinase IV
CDK5	Cyclin dependent kinase 5
CK2	Casein kinase 2

CREB	cAMP response element-binding protein
CSF	Cerebrospinal fluid
CypD	Cyclophilin D
DLP-1 or Drp1	Dynamin-like protein 1
DMTs	Disease modifying treatments
EC	Entorhinal cortex
ECE	Endothelin converting enzyme
EE	Early endosome
EOAD	Early onset Alzheimer’s disease
ER	Endoplasmic reticulum
ERK1/2	Extracellular-signal-regulated kinase 1 and 2
ETC	Electron transport chain
FAD	familial AD
FAT	Fast axonal transport
GLT-1	Glutamate transporter 1
GLUT-1	Glucose transporter 1
GSK3β	Glycogen synthase kinase-3β
HDAC6	Histone deacetylase 6
IDE	Insulin degradation enzyme
IMM	Inner mitochondrial membrane
IP3	Inositol trisphosphate
ISF	Interstitial fluid
JIP1	JNK interacting protein 1
KIF5	Kinesin-1 protein
KIFs	Kinesins
LE	Late endosome
LOAD	Late onset Alzheimer’s disease
LRP1	Lipoprotein receptor-related protein 1

LTD	Long-term depression
LTP	Long-term potentiation
mAb	Monoclonal antibody
mAChR	Muscarinic acetylcholine receptor
MAP	Microtubule-associated protein
MAP1	Microtubule-associated protein 1
MAP2	Microtubule-associated protein 2
MAPT	Microtubule-associated protein tau
MBOs	Membrane-bounded organelles
Mdivi-1	Mitochondrial division inhibitor 1
Mfn 1	Mitofusion 1
Mfn 2	Mitofusion 2
mGluR5	Metabotropic glutamate receptor 5
MMPs	Metalloproteinases
MT	Microtubule
mtDNA	Mitochondrial DNA
NCSTN	Nicastrin
NEP	Neprilysin
NFTs	Neurofibrillary tangles
NGF	Nerve growth factor
NMDARs	N-methyl-D-aspartate receptors
NT	Neurotrophin
NT-3	Neurotrophin-3
NT-4	Neurotrophin-4
OMM	Outer mitochondrial membrane
OPA-1	Optic atrophy protein 1
OXPHOS	Oxidative phosphorylation system
P3	3 kDa fragment

p38MAPK	p38 mitogen-activated protein kinase
PEN-2 or PSENEN	Presenilin enhancer 2
PHFs	Paired Helical Filaments
PI3K	Phosphoinositide 3-kinases
PICALM	Phosphatidylinositol-binding clathrin assembly protein
PIP3	Phosphoinositide-3 phosphate
PLCγ	Phospholipase C-γ
PP1	Protein phosphatase 1
PP2A	Protein phosphatase 2A
PP2B	Protein phosphatase 2B or calcineurin
PrPc	Cellular prion protein
PSD	Postsynaptic density
PSD-95	Postsynaptic density protein-95
PSEN1	Presenilin 1
PSEN2	Presenilin 2
PTVs	Piccolo-bassoon transport vesicles
RAGE	Receptor for advanced glycation end products
RE	Recycling endosome
Rip	Regulated intra-membrane proteolysis
ROS	Reactive oxygen species
SFs	Straight filaments
SNPH	Syntaphilin
SORL1	Sortilin-related receptor 1
SPs	Senile plaques
SS	Szeto-schiller
SVPs	Synaptic vesicle precursors
TGN	Trans-golgi network
TLR	Toll-like receptor

TREM2	Triggering receptor expressed on myeloid cells 2
Trk	Tropomyosin receptor kinase
UPS	Ubiquitin-proteasome system
α-CTF or C83	α-C-terminal fragment with 83 amino acids
βCTF or C99	β-C-terminal fragment with 99 amino acids

Abstract

Alzheimer's disease is the most common neurodegenerative disorder in the world, characterized by cognitive deficits and dementia. Amyloid-beta peptide and tau protein aggregate are the two disease hallmarks, forming plaques and neurofibrillary tangles, respectively. These insoluble structures have been recognized to cause synaptic and neuronal dysfunction, progressively leading to neurodegeneration. This review aims to describe the processing of these proteins and their modifications leading to aggregation, as well as synaptic-based mechanisms, that result in behavioral changes and memory loss in AD. Part of these mechanisms include modifications in axonal transport and dendritic activity, due to hyperphosphorylation of tau protein, as well as activation of glutamate receptors preceding excitotoxic events, which involve calcium dyshomeostasis and mitochondrial dysfunction, closely linked to synaptic dysfunction. Understanding how these processes intricately work is expected to bring about new therapeutic strategies worth to be explored in the near future.

Keywords: Alzheimer disease; neurofibrillary tangles; tau protein; amyloid plaque; cognitive dysfunction; axonal transport; glutamate; mitochondria

1. Introduction

Alzheimer's disease (AD) is a chronic neurodegenerative disorder of the central nervous system. It is the most common dementia among elderly people (1). In developed countries, AD is one of the major healthcare problems, becoming a social and economic challenge. Currently, there are 30-35 million people affected worldwide (2). By the year of 2030 it is estimated that 65.7 million people will suffer from this progressive deterioration of cognitive function, if there is no effective treatment in the meantime. Current therapies based on acetylcholinesterase inhibitors (AChEI) or glutamate receptors antagonist (e.g. memantine) are only able to attenuate AD clinical symptoms (3), namely deficits in language, attention, orientation and memory, and behavioral manifestations, like irritability, agitation, sleeping changes and delusions (4).

AD pathogenesis implicates the loss of cholinergic and glutamatergic neurons and synapses, mostly in the temporal and parietal lobes (5), starting in the transentorhinal and entorhinal areas and spreading to the hippocampal region and neocortex (6,7). Changes in these areas are responsible for memory failure, behavioral and linguistic changes and progressive cognitive impairment (1). AD can be classified into two distinct types, depending on the age of installation. In patients with less than 65 years, early onset AD (EOAD) is mainly autosomal dominant familial AD (FAD) (8), corresponding to 2-3% of all cases (2). Several mutations have been found in amyloid precursor protein (APP), presenilin 1 (PSEN1) and presenilin 2 (PSEN2), which will be discussed later (9,10). FAD is characterized by less hippocampal volume loss, increased parietal atrophy and enhanced white matter damage when compared to the most common type, late-onset AD (LOAD) or sporadic AD (8). Several risk factors, either genetic or environmental may influence the development of LOAD (11). Apolipoprotein E gene (APOE) with ε4 allele has a major influence in LOAD, since it is found in approximately 25% of sporadic AD cases, although the main risk factor is still aging (12). Both EOAD and LOAD have been described to cause similar cytopathological features, including synaptic dysfunction and loss, decreased ion homeostasis or microglial activation (13), as summarized in **Figure 1**.

AD progression has been linked to multiple cellular changes, including mitochondrial impairment and oxidative stress, mechanisms involving metal ions overload and cell membrane disruption caused by amyloid-beta peptide (Aβ) overload and neuroinflammation (14).

This review aims to describe the mechanisms underlying Aβ generation and tau aggregation in AD, and further define the pathways leading to pre- and post-synaptic dysfunction in AD, both accounting for the application of several therapies, some of them undergoing human testing in clinical trials.

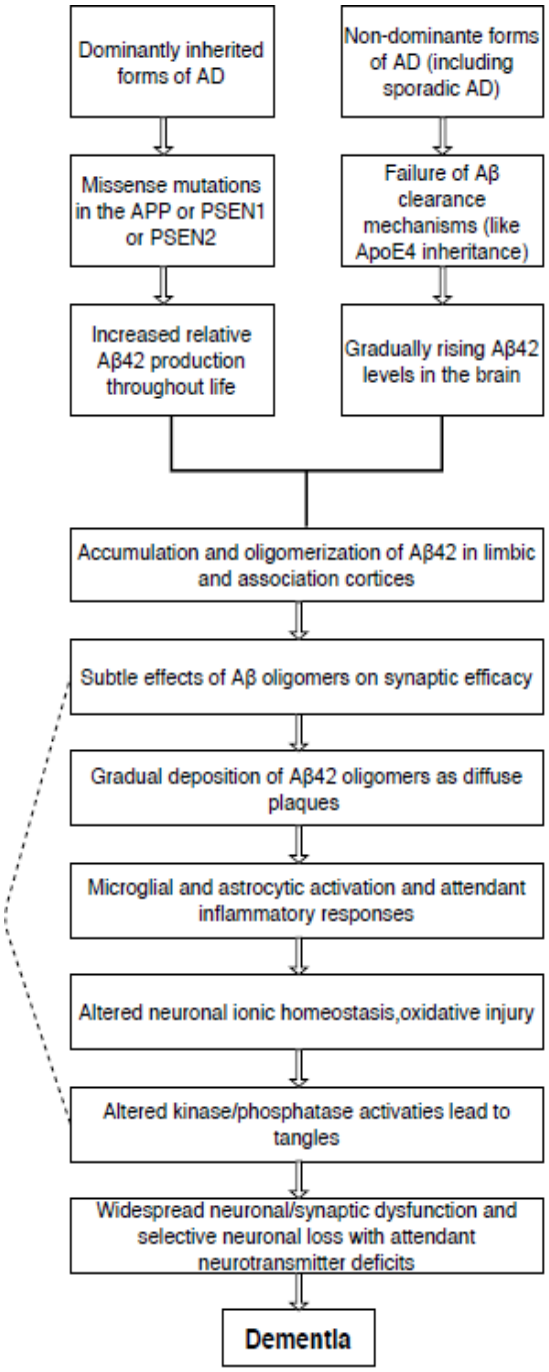

Figure 1. Both LOAD and EOAD increase Aβ42 production, which results in its aggregation and plaque formation, promoting an inflammatory response, ion dyshomeostasis and oxidative damage. Consequently, tau protein is hyperphosphorylated, affecting synapses, leading to spine and neuronal loss. All these changes result in cognitive deficits and dementia. Adapted from: (13)

2. Methods

Research was made on PubMed using MeSH terms: Alzheimer Disease/physiopathology*, Glutamic Acid/metabolism*, Neuronal Plasticity/physiology*, Synapses/metabolism*, Mitochondria/metabolism*, Dendritic Spines/physiology* and Axonal Transport/physiology*. There were 207 results until the 18/07/2019. The filter used was English language.

After reading the *Abstracts* of each article, I chose the ones that were more relevant to the theme and according to publication date, having excluded articles prior to 2014.

Pertinent articles cited on those previously chosen were also included.

3. Aβ generation and tau aggregation

In 1906, Alois Alzheimer recognized the two pathological hallmarks of the disease (15), which are extracellular senile plaques (SPs, formed by aggregation of Aβ, generated from abnormal cleavage of APP), and intracellular neurofibrillary tangles (NFTs) constituted by misfolded/hyperphosphorylated microtubule-associated protein tau (MAPT), which form toxic deposits (16), as schematized in **Figure 2**. The amyloid cascade hypothesis was formulated in 1992, becoming one of the most accepted hypothesis on AD pathogenesis (17).

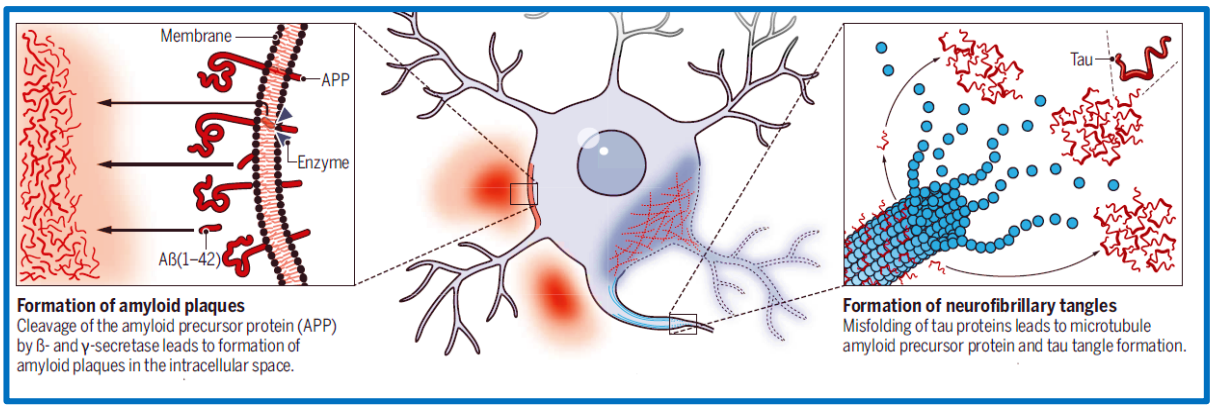

Figure 2. Scheme representing the formation of amyloid plaques and neurofibrillary tangles. Adapted from: (15)

3.1. Aβ generation– involvement of selective enzymes

APP is a type I transmembrane protein that undergoes a sequential proteolytic processing, resulting on the formation of Aβ peptide (18). APP is expressed in different cell types, namely neurons and glial cells (12). APP is anterogradely transported in neurons along axons and dendrites to synapses, where the production and release of Aβ is predominant (9), as discussed in section 5.2. Apart from being able to generate Aβ, APP was previously described to have a role in axonal transport, neuronal development, metal ion homeostasis, neuroprotection or repair and promotion of neurite outgrowth (6,16). APP transport to the plasma membrane is driven by the early secretory pathway from the endoplasmic reticulum (ER), mostly via trans-golgi network (TGN) (18). Only approximately 10% of APP arrives to the plasma membrane, while the rest remains in the TGN (9,19). Once in the plasma membrane, APP that was not proteolytically processed by α-secretase (non-amyloidogenic pathway) or β-secretase (amyloidogenic pathway) is internalized into the endosome and can follow different pathways (**Figure 3**), namely the late endosome-lysosomal pathway, in which APP is degraded (19), the retrograde pathway back to the TGN, in which APP binds to sortilin-related receptor 1 (SORL1), and the recycling pathway, back to the plasma membrane (9,19).

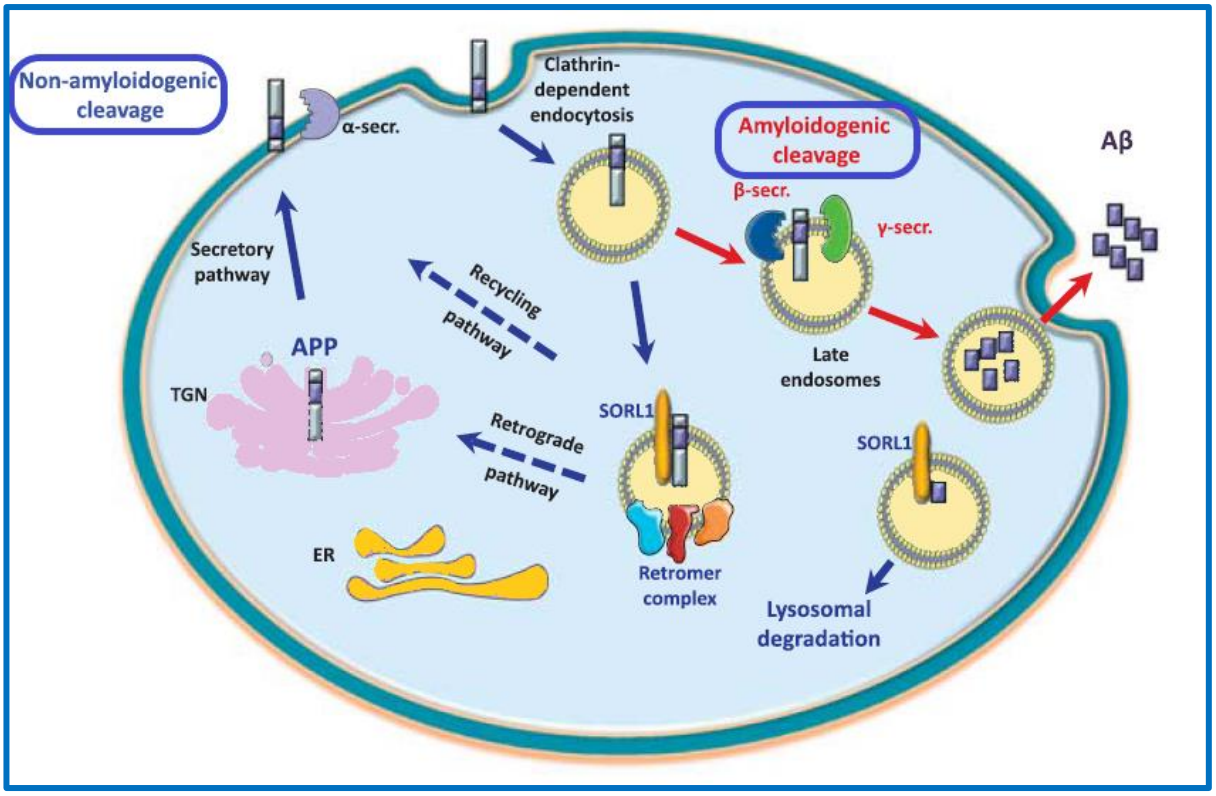

Figure 3. APP can be moved from TGN into the plasma membrane, where it can be proteolytically processed via non-amyloidogenic by α-secretase or via amyloidogenic by β-secretase, releasing Aβ peptide. APP that does not follow these pathways is internalized and may follow the recycling, retrograde or lysosomal pathways. Adapted from: (9)

APP can follow a non-amyloidogenic or an amyloidogenic pathway, in order to be proteolytically processed (20) (**Figure 4**).The non-amyloidogenic pathway occurs mainly in the plasma membrane and is responsible for more than 90% of APP cleavage. It starts with the cleavage of APP within the Aβ domain (6,11) by α-secretase metalloproteases, being metalloproteinase domain-containing protein 10 (ADAM10) the most important (19). Cleavage by α-secretase results in the release of its large N-terminal ectodomain named soluble α-APP and α-C-terminal fragment with 83 amino acids (α-CTF or C83) (18,21). α-APP is secreted into the extracellular compartment, instead of staying in the plasma membrane, as α-CTF (12). Soluble α-APP is neuroprotective and participates in neuronal excitability by increasing several physiological functions, such as synaptic activity, learning, memory and resistance to oxidative and metabolic stress (6). C83 is then processed by γ-secretase (a mechanism named regulated intra-membrane proteolysis (Rip) (18)), leading to the release of two soluble products, 3 kDa fragment (p3) and APP intracellular domain (AICD). P3 is released into the extracellular compartment, where it participates in synaptic signaling, whereas AICD is translocated into the nuclei and has a role in regulating gene expression (6). γ-secretase is a transmembrane multiprotein complex constituted by four transmembrane proteins: PSEN1 or PSEN2, nicastrin (NCSTN), subunits anterior pharynx-defective 1 A or B (APH1A or APH1B)

and presenilin enhancer 2 (PEN-2 or PSENEN). This complex is an aspartyl protease inserted in the membrane that cleaves transmembrane substrate proteins. Presenilins are the catalytic component of γ-secretase and its autocatalytic cleavage is facilitated by PEN-2, whereas NCSTN works as a substrate recruiter (18,22).

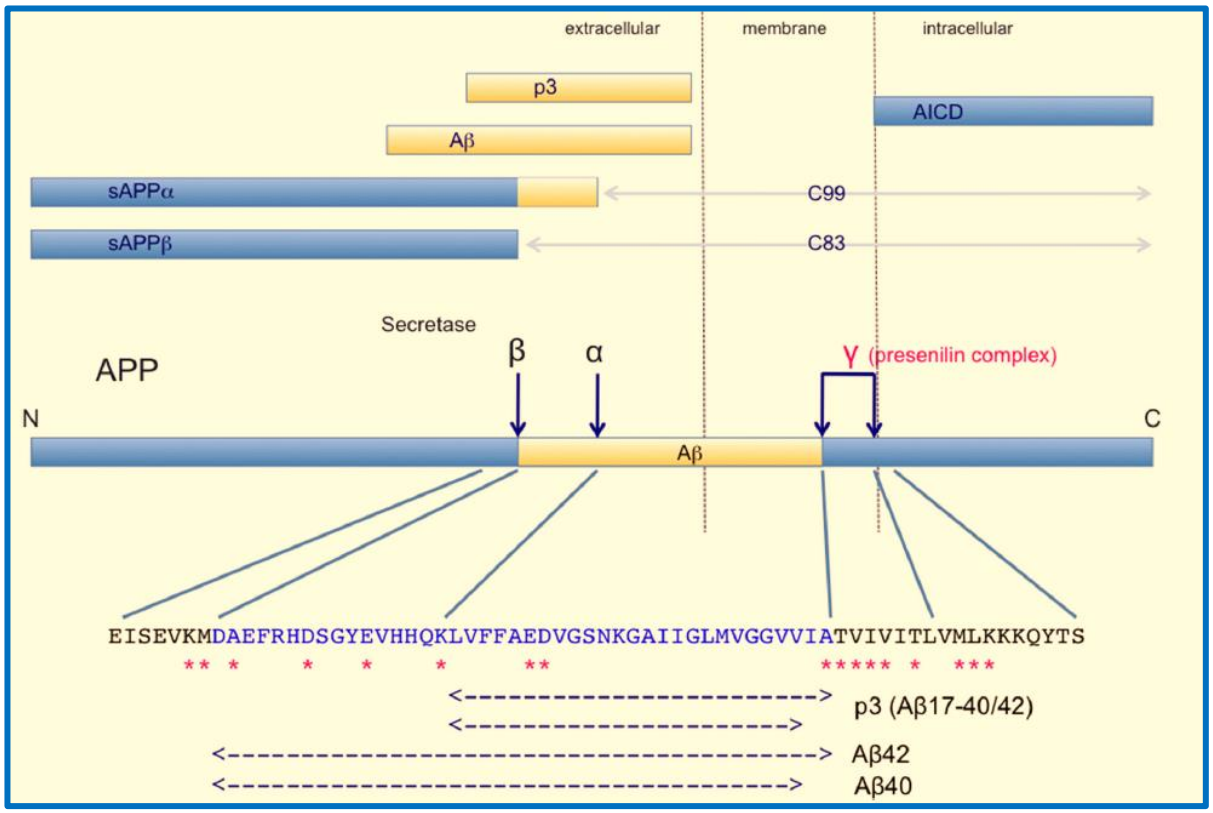

Figure 4. Amyloidogenic pathway in which full length APP is cleaved by β-secretase, resulting in soluble β-APP and C99 that is then cleaved by γ-secretase, releasing AICD and Aβ. Non-amyloidogenic pathway in which α-secretase cleaves full length APP leading to soluble α-APP and C83 that is then cleaved by γ-secretase, releasing AICD and p3. Adapted from: (20)

The amyloidogenic pathway mostly occurs at lipid rafts (9) and under pathological conditions (12). The proteolytic processing of APP occurs either in axons or dendrites, but mostly in axons. This means that Aβ is generated in higher amounts presynaptically. APP is first endocytosed from the axonal and dendritic plasma membrane into the endosomal compartments, more specifically into the early and late endosomes (LE). Membrane-bound enzyme β-secretase 1 (BACE1) is a membrane protein also transported through the endolysosomal pathway. The interaction between BACE1 and APP inside endosomes defines the rate of Aβ production (23). BACE1 becomes mature in ER and TGN and can go through the retrograde pathway back to the TGN, be recycled to the plasma membrane or suffer lysosomal degradation (9,19). Aβ sequence is cleaved at a specific site, 99 residues away from the C-terminus, by BACE1 (11). Cleavage of APP results in the release of its soluble ectodomain β-APP and β-C-terminal fragment with 99 amino acids (βCTF or C99) (18). Soluble β-APP is released into the extracellular space and has a neuroprotective function, regulating

synaptic plasticity and acting like a microtubule associated protein (MAP) (16). C99 stays in the membrane (11). Then, C99 is cleaved by γ-secretase, resulting in AICD and Aβ peptide (24). Aβ peptide can be cleaved by γ-secretase in different sites, resulting in Aβ40 (around 90%) and Aβ42 (18,25). Aβ42 is insoluble, has a higher tendency to aggregate due to its hydrophobic properties, being more pathogenic (6). An increase in Aβ42/Aβ40 ratio, due to, for instance, mutations responsible for FAD, enhances Aβ aggregation and oligomerization, culminating in insoluble plaques in synaptic clefts, and disturbance of synaptic signaling (11).

Under pathological conditions, besides the increase in Aβ production, its clearance mechanisms (**Figure 5**) are also impaired (26). When Aβ peptide is in the intracellular compartments, it can be degraded by the ubiquitin-proteasome system (UPS) or autophagy lysosomal system (ALS) (9).

After being released into the extracellular compartment, Aβ peptide can be degraded by different mechanisms, such as extracellular proteolysis or phagocytosis by microglia (26). Proteolytic clearance uses degrading enzymes secreted by neurons or astrocytes, such as neprilysin (NEP), metalloproteinases (MMPs), insulin degradation enzyme (IDE), endothelin converting enzyme (ECE), plasmin, angiotensin converting enzyme (ACE) and cathepsin-B (cat-B) (12,25). Microglia are innate immunity myeloid cells that internalize Aβ by phagocytosis into early endosomes (24), after recognition by cell-surface receptors, such as toll-like receptors (TLR1, 2, 4, 6). Aβ internalization, causes a concomitant inflammatory response (9). Aβ phagocytosis is inhibited by CD33 and enhanced by ATP-binding cassette transporter A7 (ABCA7) and triggering receptor expressed on myeloid cells 2 (TREM2) (11,27).

There is also the efflux receptor-mediated transcytosis pathway across the blood brain barrier (BBB). This mechanism happens through generation of complexes between Aβ peptide and chaperons, such as apoE2, 3 or 4 (25,28). The complex binds with lipoprotein receptor-related protein 1 (LRP1), which is an efflux transporter protein (12,25) and is internalized by phosphatidylinositol-binding clathrin assembly protein (PICALM), which is involved in clathrin-dependent endocytosis. Despite influencing Aβ clearance, PICALM also promotes APP and γ-secretase internalization, reducing Aβ production. However, its levels are reduced in AD (29). The endocytosed ApoE is mainly recycled, whereas Aβ is mostly degraded in lysosomes (28). Glucose transporter 1 (GLUT-1) increases LRP1 expression and is responsible for keeping the integrity of BBB, meaning that it influences the transport of Aβ peptide into the blood (26). Receptor for advanced glycation end products (RAGE) is an Aβ influx transporter receptor that promotes its flux from the blood into the brain. In pathological conditions that promote Aβ accumulation, the balance between influx and efflux systems is impaired (12,25). In the periphery, Aβ peptide will be degraded by blood cells, such as monocytes and red cells, or even in the liver or kidney (12,26).

Furthermore, Aβ peptide present in the interstitial fluid (ISF) can be translocated into the blood by BBB or into the cervical lymph nodes by both perivascular drainage and glymphatic system, Aβ released to the cerebrospinal fluid (CSF) can be transported into the blood, across the blood-cerebrospinal fluid barrier (BCSFB) and the arachnoid vili or into the cervical lymph nodes, through lymphatic clearance (26).

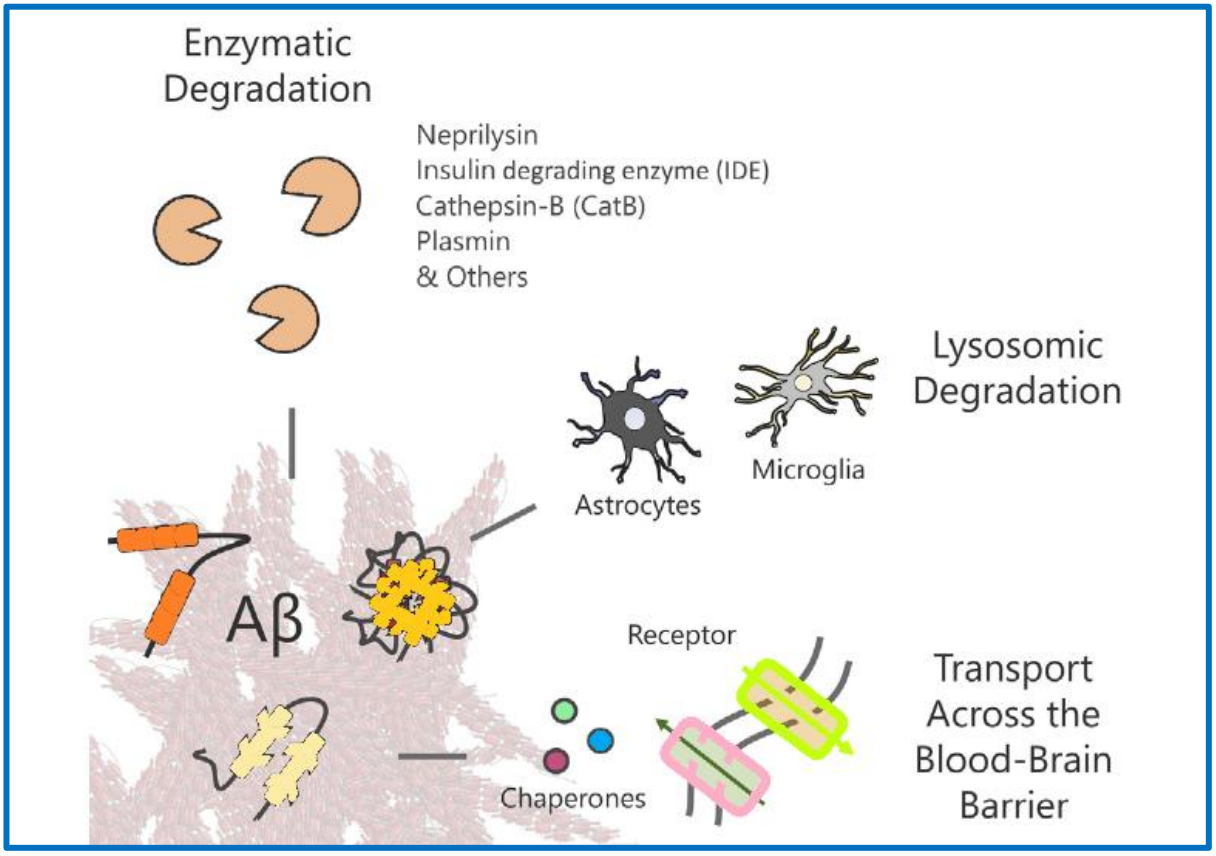

Figure 5. Beta-amyloid clearance mechanisms. Adapted from: (25)

Under pathological conditions, when clearance mechanisms fail, Aβ accumulates and monomeric Aβ may assemble into dimers, trimers and lead to the formation of oligomers and, consequently, insoluble fibrils and senile plaques (**Figure 6**) (21). It can also originate annular protofibrils (APFs), which may form membrane pores, allowing excessive influx of calcium, which can have severe consequences in neurons (24,30).

Aβ extracellular pathway is toxic namely because it can interact with N-methyl-D-aspartate receptors (NMDARs), an ionotropic glutamate receptor that, when overactivated, impairs intracellular calcium homeostasis, resulting in mitochondrial dysfunction and oxidative stress (16,31), as described in section 5.1.

Aβ cytotoxicity does not happen *per se*. Indeed, Aβ requires tau to be toxic, whereas tau can cause neurodegeneration by itself (16). Of relevance, the brain areas in which amyloid deposition is increased do not correspond to those in which there are synapses and neuronal

loss, suggesting tau aggregates can be the responsibles for these consequences (3). Based on tau-mediated neurotoxicity, tau spreading affecting different brain areas along disease progression, as well as the fact that all therapeutic strategies based on Aβ production (e.g. BACE inhibitors) have failed in AD, more recently the Aβ cascade hypothesis has been putted aside.

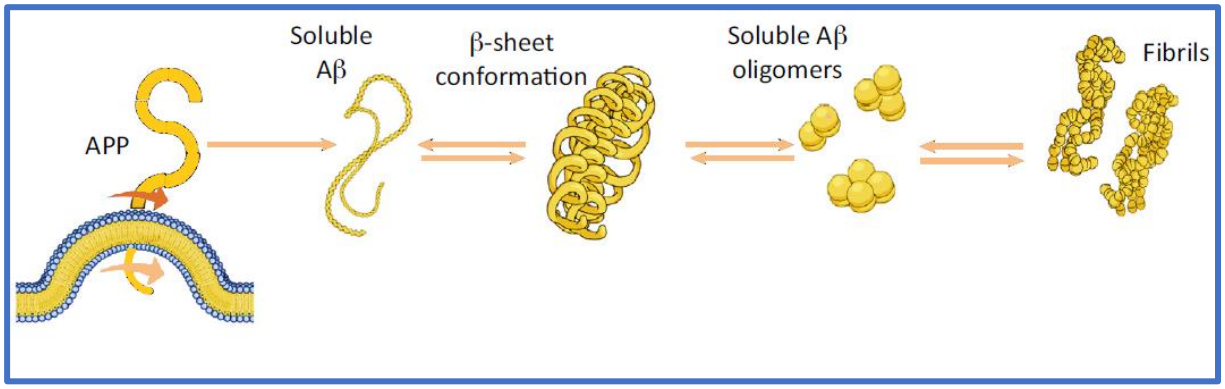

Figure 6. Different stages lead to the formation of Aβ insoluble fibrils. Adapted from: (21)

3.2. Tau hyperphosphorylation and aggregation - involvement of selective enzymes

Tau is a MAPT, mainly located in axons (80%) under normal/non-pathological conditions, having a role on maintaining the microtubule (MT) structure and stabilization, keeping the integrity of the cytoskeleton and allowing axonal transport (3,32). Its physiological functions also include stimulation of neurite growth, interaction between membranes, as well as anchoring enzymes and axonal transport of organelles to nerve terminals (7). Tau allows polymerization of tubulin in microtubules, keeping the cellular microarchitecture. Tau is also located in synapses and dendrites (smaller amounts, than in axons), the latter mostly at post-synaptic terminals (33), influencing neuronal maturation and synaptic function (3). Tau can be also found in the plasma membrane (mostly non-phosphorylated) and in the nucleus, where tau is responsible for keeping DNA integrity (21,34).

Under a hyperphosphorylation state, tau detaches from the microtubules, leading to the interruption of MT stabilization (14); consequently, in the nucleus excessive phosphorylated tau also loses its attachment to DNA (35). In AD, 95% of tau filaments are aggregated into paired helical filaments (PHFs), being the other 5% straight tau filaments (SFs), which are its primary structure (32).

Tau gene has 16 exons and full-length tau protein (**Figure 7**), the largest brain isoform (2N4R), has 441 amino acids. Tau has two major domains: projection domain (two thirds of the molecule) and microtubule binding domain (named repeat domain). The projection domain is

constituted by the N-terminal region containing acidic amino acid residues, that allow the interaction with other molecules (14), cell membrane and organelles, like mitochondria and may associate microtubule to actin (32). Between the projection domain and the repeat domain, there is a proline-rich region that has several sites available for phosphorylation and interacts with the microtubule-surface, mainly contributing for microtubule stabilization and promotes tau's interaction with the plasma membrane. Within the microtubule binding domain there are the basic tubulin binding region and the acidic C-terminal, which is responsible for the link with tubulin. This domain has a 'tau 'site', which is enough to promote tau aggregation and is the place where several pathogenic mutations occur (14,32). The repeat domain catalyzes aggregation of native tau into oligomers (14), because truncated tau acts like an assembly model into which native tau aggregates, forming new tau seeds, inducing its aggregation (35,36).

The protein is bipolar, with the N-terminal being negatively charged, and positively charged residues, like proline-rich domain and microtubule-binding repeats (32). Only some residues are hydrophobic, meaning that tau is highly hydrophilic (3).

By alternative splicing of mRNA of the MAPT gene on exons 2, 3 and 10, six splicing isoforms of tau are generated, each of them with distinctive physiological functions and all present in the adult brain. The number of repeats of microtubule-binding domains can either be 3R or 4R, which distinguishes the six isoforms at the C-terminal (17,33), as only some have the exon 10 (14). In AD, the ratio of the six isoforms is 1:1 of 3R and 4R, distinguishing it from other tauopathies (32). Each one of them can either have none, one or two projection domains at the N-terminal domain (0N, 1N or 2N) (17). Both the N- and C-terminal projections are external to the microtubules and both are needed to stabilize the microtubules (32).

Under physiological conditions, MAPT allows signaling molecules, trophic factors and organelles, like mitochondria, to travel along axons (17). Since tau is also located in the post-synaptic terminals (33), it can interact with cell membrane complex Src kinase Fyn, as described in section 6.3. Under disease conditions, there is an abnormal mitochondrial accumulation of tau, that interferes with oxidative phosphorylation system (OXPHOS) and increases reactive oxygen species (ROS), leading to greater oxidative stress and also interfering with mitochondrial dynamics, as detailed in section 6.2. (32,37). This ends up affecting the entire neuronal health, structural and regulatory cellular functions (17) and even APP processing is impaired due to changes in axonal transport. Pathogenic/hyperphosphorylated tau is redistributed from axons to somatodendritic areas (3).

Tau is a phosphoprotein and its physiologic function and distribution depends on its state of phosphorylation (3). Kinases and phosphatases are responsible for maintenance of phosphorylation balance (7), allowing tau to be attached to microtubules (33). The

physiological status is unfolded tau. When it is not in contact with other proteins, it might self-assemble to avoid other interactions (32). Tau is phosphorylated in several sites (hyperphosphorylated) which is caused by, for instance, an excessive activation of kinases, changes in gene expression or cellular stress (3) and hyperphosphorylated tau can aggregate into oligomers or SFs and then lead to PHFs, which will turn into insoluble neurofibrillary tangles (NFTs) (**Figure 8**) (7,33).

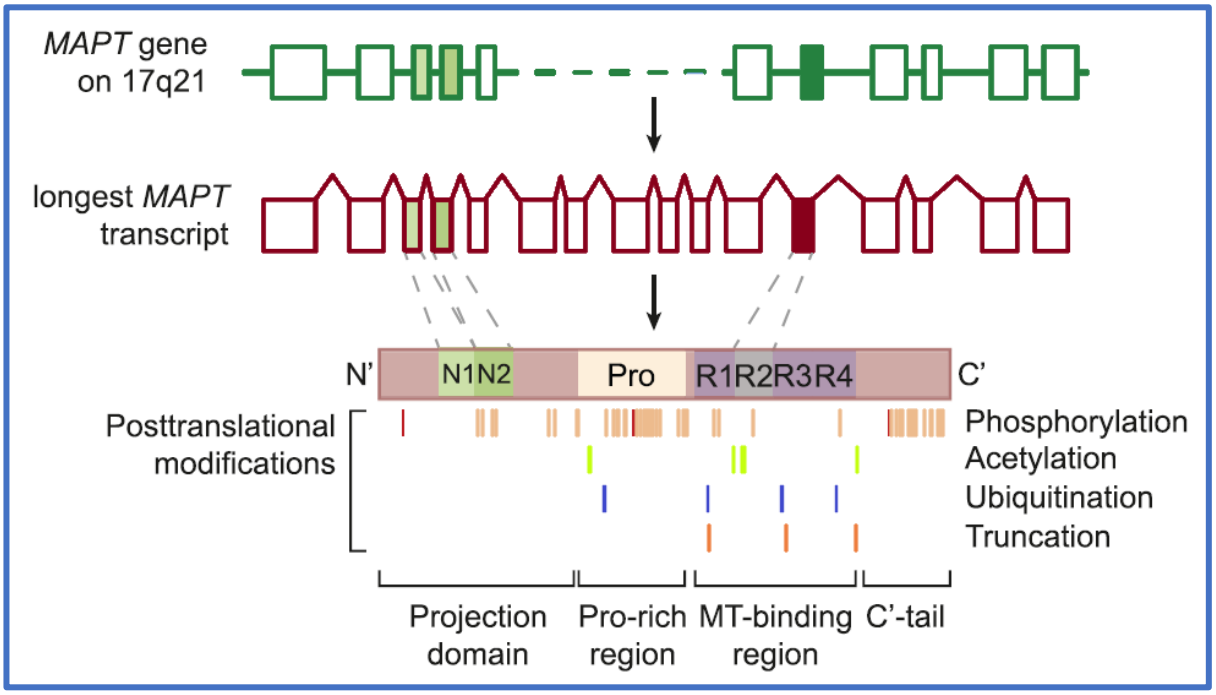

Figure 7. Schematized the alternative splicing mRNA of MAPT gene and post-translational modifications of tau protein, which is constituted by a projection and MT-binding domains and pro-rich region. Adapted from: (33)

NFTs are responsible for synaptic loss, dysfunctional axonal transport and cognitive deficits (3). The idea that NFTs are the second most important pathological hallmark in AD is changing; perhaps they are the main/initial molecular target (1). In NFTs formation, there are kinases overexpressed, such as cyclin dependent kinase 5 (CDK5) and glycogen synthase kinase-3β (GSK3β) (12). GSK3β is the main responsible for tau's phosphorylation, either in normal or disease state (38). In AD, excessively activated GSK3β (by Aβ oligomers, for instance) leads to tau hyperphosphorylation, mostly via phosphoinositide 3-kinases (PI3K)/ protein kinase B (AKT)/ GSK3β (3). A family of calcium-dependent cysteine proteases, calpains, also have a role in tauopathy, through calpain-active CDK5 and/or extracellular-signal-regulated kinase 1 and 2 (ERK1/2), leading to accumulation of hyperphosphorylated tau (21). CDK5 is a neuronal kinase that can be abnormally activated by p35 and p39, for example (3). Furthermore, the calcium-dependent calcineurin/ protein phosphatase 2B (PP2B) may be also activated causing dephosphorylation and/or inactivation of calcium-calmodulin dependent protein kinase IV

(CaMKIV)/ cAMP response element-binding protein (CREB) signaling, leading to synaptic and memory impairment (21). Moreover, calpain-1 has its activity enhanced in AD, which is associated to activation of kinases, like CDK5 and GSK3β, resulting in increased tau phosphorylation (39). Both CDK5 and GSK3β will be discussed in section 5.1.

Hyperphosphorylation of tau directly influences its distribution in dendrites and post-synaptically, since it interferes with microtubule attachment, leading to a redistribution from the axons into somatodendritic areas, where it will have harmful consequences (36), as described below. Besides, it changes tau's interaction with postsynaptic density protein-95 (PSD-95) and Fyn post-synaptically. Phosphorylation can be NMDAR dependent or not (33,36). These interactions will be further elucidated in section 6.3.

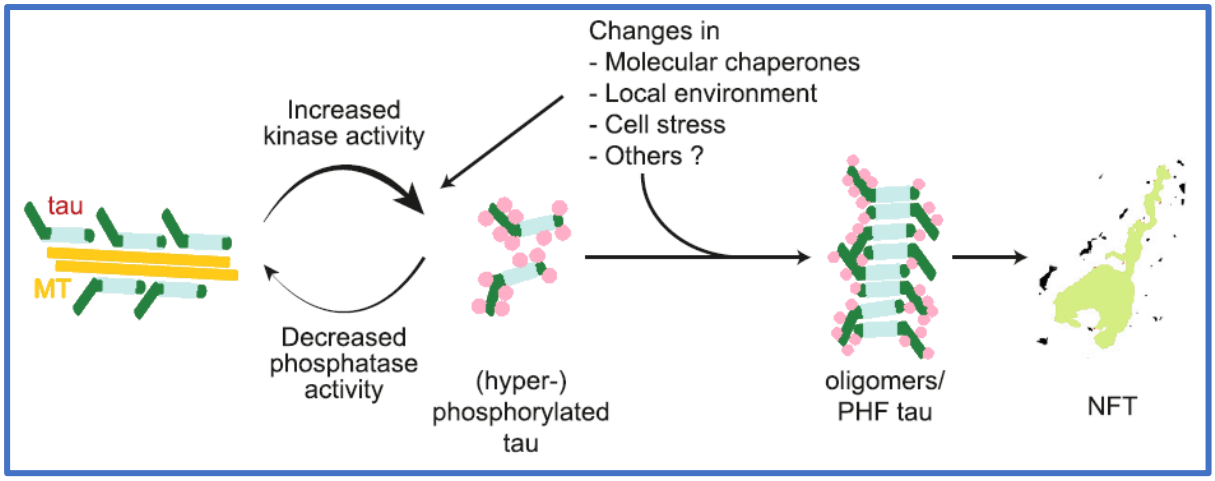

Figure 8. When there is an imbalance between kinases and phosphatases activity, tau protein becomes hyperphosphorylated and detaches from MTs, becoming susceptible to aggregate into oligomers, PHFs and insoluble NFTs. Adapted from: (33)

Phosphorylation mechanism can be reverted by doing the opposite, dephosphorylating, via phosphatases, like protein phosphatase 2A (PP2A) in dendrites (3). Aβ oligomers increases tau's hyperphosphorylation and, consequently, NFTs formation. For instance, when Aβ increases NMDAR activation, it leads to PP2A inhibition, causing excessive tau phosphorylation in dendrites (33). Besides, PP2A levels were found to be decreased in AD (38).

Post-translational modifications, such as acetylation/deacetylation, O-glycosylation, truncation/proteolytic cleavage (3) and methylation (33), allow tau protein to lose its unfolded structure, stimulating tau's aggregation (32), by losing their affinity to microtubules. This allows its redistribution from axons to somatodendritic compartments and into spines, where it has harmful effects in synaptic activity (21).

Acetylation or deacetylation depend on acetyltransferases, such as CREB. Most acetylated tau is more prone to aggregate, but there are specific tau sites, that when acetylated, inhibit phosphorylation and aggregation (34,36). Tau itself has intrinsic acetyltransferase activity. The activity of histone deacetylase 6 (HDAC6), a cytosolic histone deacetylase, is impaired by Aβ oligomers, promoting tau acetylation (33). Thus, preventing tau's acetylation, may preclude tauopathy spreading (32). When abnormally acetylated, tau can decrease synaptic plasticity and induce memory loss (21,33).

Tau self-aggregation is known to be facilitated by hyperphosphorylation and truncation (14,35). Truncation is a post-translational modification that is mediated by caspase (e.g caspase 3 and 6), calpain (e.g. calpain 1) or asparagine endopeptidase (AEP) and leads to synaptic and cognitive dysfunction (33,36). N- and C-terminally truncated fragments, trigger seeding, accumulation and spreading (32,38), because these fragments, only with repeat domain, are shorter and more prone to aggregate. Truncated tau cause neurodegeneration by itself (34). The proteolytic cleavage of tau speeds up its aggregation rate, due to oligomerization of microtubule-binding repeats (3). Truncation also allows the interaction between truncated and full-length tau, facilitating its redistribution into dendrites, where it causes synaptic dysfunction. When tau is resistant to caspase action or caspases are inhibited, the levels of dendritic tau are reduced (33). In AD brains, there is an AEP, that is moved from the lysosomes into the cytosol and becomes excessively activated and which products easily aggregate and become hyperphosphorylated. This influences microtubule stabilizing activity, increasing synaptic toxicity (3,33).

N-glycosylation also facilitates tau hyperphosphorylation and aggregation, since it changes tau's structure and decreases affinity for MTs. On the contrary, O-GlcNAcylation (O-glycosylation type) decreases tau phosphorylation, reducing aggregation (3). This post translational modification needs UDP-G1NAc, in which glucose can be converted. Since in AD affected areas (e.g. hippocampus), glucose uptake is impaired (because of damaged energy metabolism, which leads to dysfunctional mitochondrial enzymatic activity (40). There is no intracellular glucose available for this conversion, resulting in a reduction in O-GlcNAcylation and hyperphosphorylated tau (3,34,37). Besides, MAPT gene expression is different depending on the brain area, leading to susceptibility for tauopathy in specific areas, like the hippocampus (34).

4. Synaptic dysfunction in AD:

Impairment and loss of synapses have been largely described in AD (10,21). Indeed, modifications in hippocampal and neocortical networks and dysregulation in synaptic density and plasticity may constitute the main drivers of memory impairment that occurs in AD early stages (1).

Figure 9 shows a schematization of the synaptic homeostatic mechanism, and how modified synaptic plasticity underlies mild cognitive impairment, in early stages of AD (41).

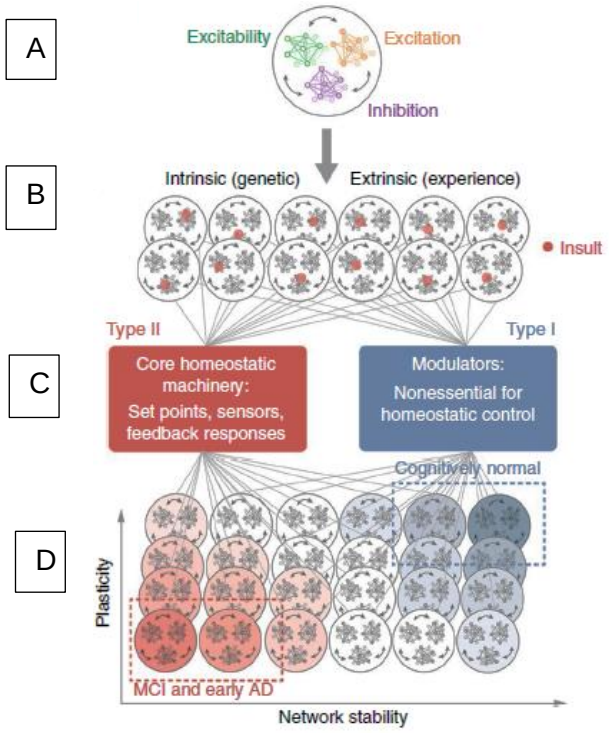

Figure 9. On part A it is represented the capacity of balance between excitation and inhibition in synapses. On B we can see several factors that will negatively affect homeostasis. Among those perturbations (part C), some will cause significant damage in synaptic plasticity and lead to early AD, while other are not able to cause cognitive deficits. Adapted from: (41)

Cognitive deficits are highly correlated to synaptic loss, than to other features of the disease, being prior to neuronal loss, The remaining synapses develop compensatory mechanisms, based on synaptic growth with increased synaptic number or size (30,42). Losing synapses in AD is an early event and both Aβ oligomers and tau have been described to be responsible for this process (30,33), possibly having a synergistic effect at the synapse (21). The influence of these proteins is evident namely through dysfunctional axonal transport of synaptic vesicles and mitochondria that cause altered presynaptic function, and dysregulation of glutamate receptors, resulting in post-synaptic/dendritic dysfunction and neuronal loss. Oxidative stress, due to excessive ROS production and neuroinflammation are also examples of mechanisms behind synaptic loss in AD (30), as summarized in **Figure 10**. There is a dynamic relationship

between Aβ peptide and tau protein, since the first promotes NFTs' formation, while tau stimulates Aβ-mediated synaptic toxicity (43). Aβ oligomers can interact with receptors on the cell surface, that activate kinases to cause tau protein hyperphosphorylation, promoting protein aggregation and redistribution in spines (21,30). These modifications result in loss of axonal transport of neurotrophic factors and mitochondrial dysfunction, leading to synaptic dysfunction and culminating in cognitive deficits (30). There is also another way of Aβ influencing tau, which is through a prion-like mechanism. Oligomers of Aβ present in synapses act like direct model for tau protein, stimulating its misfolding, which leads to its aggregation into β-sheet-rich tau oligomers (21).

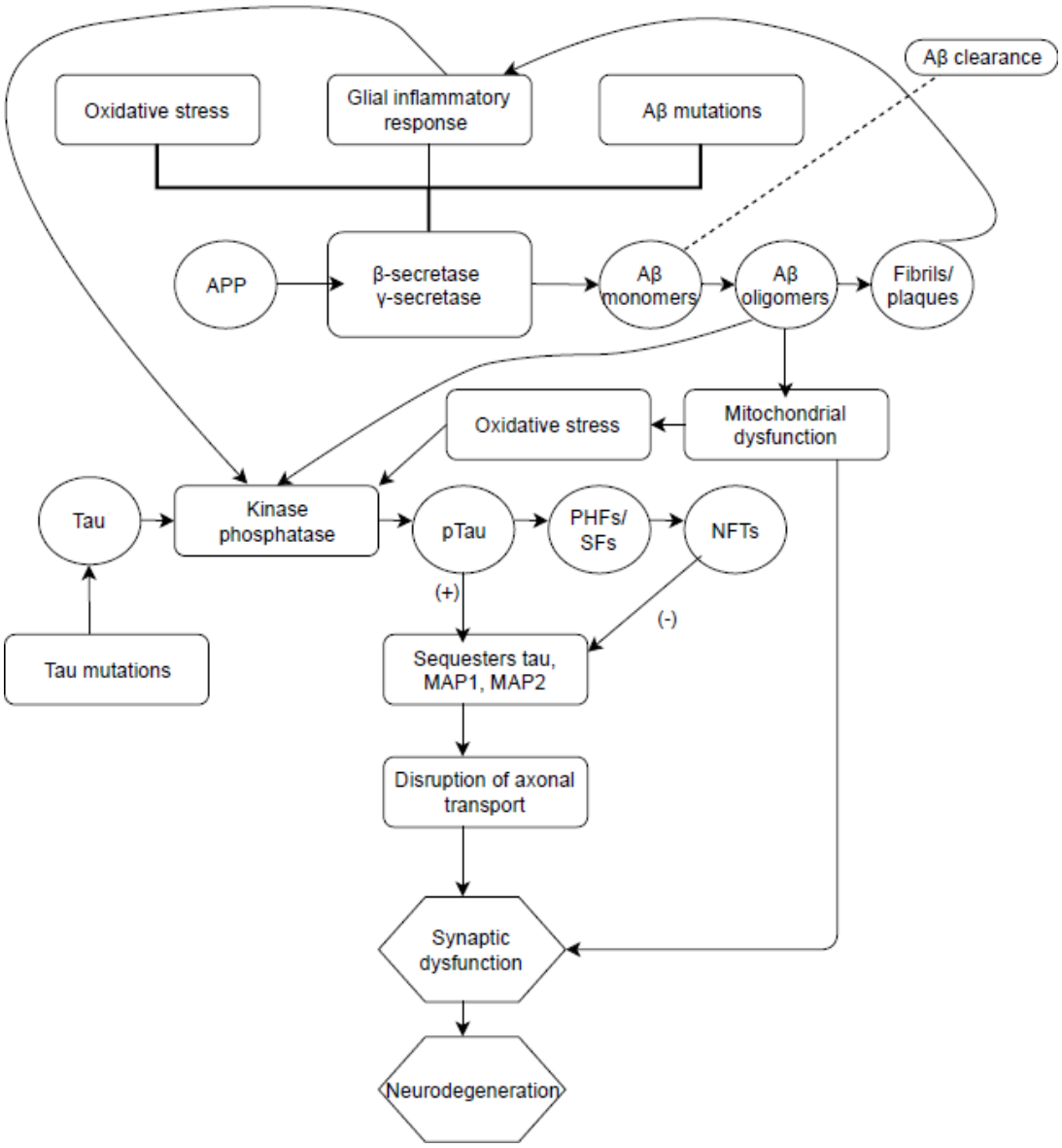

Figure 10. Oxidative stress and inflammatory response can either be a cause or a consequence of Aβ oligomers, which influence tau hyperphosphorylation, causing dysfunction of axonal transport. All these changes will lead to synaptic dysfunction, resulting in neurodegeneration. Adapted from: (9)

Aβ peptide is released in both pre- and post-synaptic terminals, being involved in the pathogenesis of dysfunctional synaptic transmission in AD (30). Aβ has a major role on the control of neuronal excitability by maintaining synaptic homeostasis, preventing excessive synaptic activity (7,9). By being released into the extracellular space in small amounts, it stimulates presynaptic function in a healthy way. When levels are excessively high, postsynaptic transmission is reduced, culminating in loss of dendritic spines, as discussed in section 5.1 (7).

There are three possible ways by which Aβ may influence synaptic and neuronal communication, by: (i) contacting directly with post-synaptic signaling complexes; (ii) interacting with glutamatergic receptors, which are mainly located in dendritic spines; and/or (iii) influencing synaptic mitochondria (3).

Aβ oligomers rather than plaques have been shown to impair synaptic activity, being closely related with cognitive changes (21). Large fibrillar plaques do not present more Aβ surface area than small oligomers, which diffuse into synaptic clefts (24). This means that the focus of researchers has changed from insoluble fibrillar Aβ to soluble oligomers, since they seem to be more neurotoxic (9). Soluble oligomers are found surrounding plaques in much higher levels, than in distant areas; since SPs maintain their stability throughout time, it is possible that there is a shift from insoluble plaques into soluble Aβ in the extracellular compartment, keeping a dynamic balance between them (24). It is still unknown the size and forms of Aβ that are synaptotoxic (21).

Neurotransmitters arrive to the synapse after being carried by synaptic vesicle transport along MTs. Excessive levels of glutamate at the synaptic cleft (mainly resulting from decreased uptake from astrocytic transporters) may cause overactivation of NMDARs, that are highly calcium permeable, causing excitotoxicity in postsynaptic terminals. Therefore, several proteins that answer to calcium stimuli are activated, such as calpains, PP2B and/or CAMKII (44).

Long-term potentiation (LTP) is a plasticity mechanism responsible for learning and memory throughout neuronal circuits (1,10). Instead of insoluble plaques, Aβ soluble oligomers, in the synaptic cleft, inhibit LTP and facilitate long-term depression (LTD) of excitatory synaptic transmission (10), by interfering with neurotransmitter glutamate, the main fast excitatory neurotransmitter in cortico-hippocampal areas, and thus key to learning and memory (9,24). Reduced synaptic plasticity linked to increased LTD has been related with the effect of Aβ soluble oligomers, which can partially inhibit synaptic NMDARs by the accumulation of extracellular glutamate, potentially resulting from decreased astrocytic glutamate uptake at relevant tripartite synapses (24). In post-synaptic areas, NMDAR signaling influences synaptic plasticity, namely by changing the location of α-amino-3-hydroxy-5-methyl-4-

isoxazolepropionic acid receptors (AMPARs) at postsynaptic membranes, which affects LTP and LTD (33). Aβ oligomers can also increase calcium levels and reduce spine density (30).

Aβ oligomers can damage plasma membrane integrity in three different ways. Firstly, extracellular oligomers can change the membrane structure itself, increasing its permeability. Secondly, plasma membrane receptors, like NMDAR can also influence this mechanism, by changing neuronal excitability by increasing calcium influx. Thirdly, intracellular Aβ has a large influence on ER and mitochondria, by increasing calcium influx, promoting a stress response (24).

Under normal circumstances, tau controls synaptic function, since it can be relocated from axons into somatodendritic compartment, where it affects synapses (21). Microtubules have a major role in spine morphology and function. When tau is in dendrites, it activates mechanisms that rely on cytoskeleton integrity to keep synaptic function. This is necessary for mature synapses to work correctly, without depending on the axonal integrity (33).

Phosphorylated tau only exists in synapses in the context of AD (3). Because tau is a MAP, it coordinates axonal transport, influencing mitochondrial trafficking and synaptic vesicle release (3,21), which results in harmful consequences for the cell, synapses and neuronal circuits (33), potentially causing impaired mitochondrial transport, changes in adenosine triphosphate (ATP) production and calcium dyshomeostasis (3,21). Without ATP, synaptic transmission is also impaired (45).

Extracellular tau protein regulates synaptic receptors signaling, like the muscarinic acetylcholine receptor (mAChR) and can modulate the targeting of glutamatergic receptors to postsynaptic sites in dendrites spines. Tau is a substrate for GSK-3β and p38 mitogen-activated protein kinase (p38MAPK) enzymes found in the post-synaptic compartment, that end up influencing LTP (21).

5. Modification of axonal function in AD

5.1. Tau hyperphosphorylation and disruption of microtubules

Under certain circumstances, such as oxidative stress and changes in phosphorylation and post-translational modifications, tau protein aggregate into oligomeric state. Oligomers can either become insoluble PHFs and NFTs or APFs, that will allow tauopathy to spread and lead to cell death (**Figure 11**) (3).

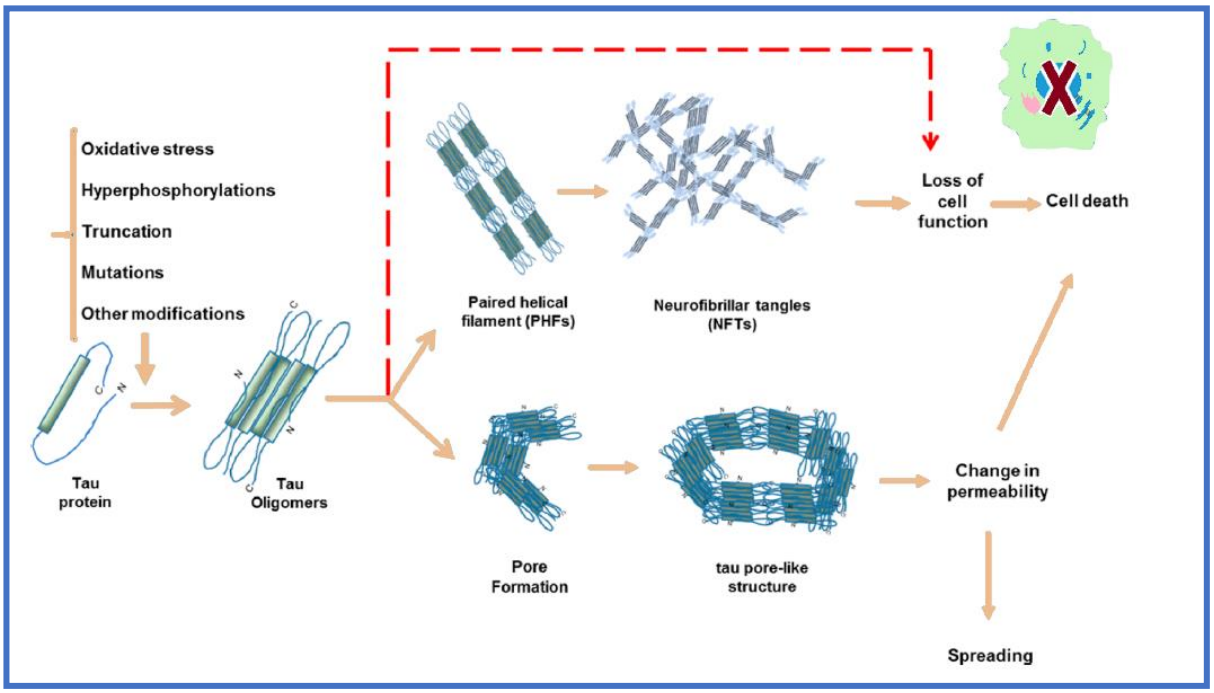

Figure 11. How tau leads to cell death. Adapted from: (14)

Microtubules serve as pathways for trafficking of cargoes in dendrites and axons. Their plus end suffers polymerization and depolymerization cycles that are regulated by microtubule-associated proteins, whereas the minus end is more stable (46). Under pathological conditions, tau protein is hyperphosphorylated, loses its affinity to microtubules, detaching from them (3,17). This happens in disease states, chronic stress or changes in gene expression, by disturbing kinases/phosphatases regulation (32).

It is believed that hyperphosphorylation of tau causes toxicity without even being aggregated, by the interaction with JNK interacting protein 1 (JIP1), which promotes the accumulation of F-actin filaments (3). Tau oligomers are the major responsible molecules for microtubule depolymerization, resulting in neurodegeneration and cognitive impairment (14). Soluble tau oligomers are probably the toxic species, which PHFs and NFTs are meant to work as protective mechanisms in affected neurons (21).

Tau phosphorylation depends on kinases, such as GSK-3β and CDK5. GSK-3β mostly uses the PI3K/AKT/GSK-3β pathway. Firstly, phosphoinositide-3 phosphate (PIP3) activate PI3K, which stimulates AKT, resulting in GSK-3β phosphorylation, which consequently results in tau hyperphosphorylation (3). CDK5 promotes neurite outgrowth and regulates axonal development and its catalytic function depends on direct association with its regulators, p35 and p39, which levels depend on calcium influx (12). When calcium levels are high, calpain cleaves p35 and p39 into p25 and p29. These will bind with CDK5 and result in CDK5-p25 and CDK5-p29 complexes, respectively, which will promote tau' hyperphosphorylation (3). Calpains are highly related to AD, since they are not only related to tau, but they can also cleave other substrates, such as APP, PICALM and GluN2B subunit (39).

Phosphatases are the responsible for reversing phosphorylation. Activation of protein PP2A avoids tau oligomerization and, consequently, NFTs formation (32). Besides, reduced phosphatase activity also results in tau hyperphosphorylation (1). The interaction between Fyn and tau can shift tau's trafficking, causing redistribution of tau to synapses and somatodendritic compartments, leading to synaptic impairment (32).

Tau oligomers are largely composed by hyperphosphorylated tau, which alters its conformation and is truncated. This makes it easier to self-assembly, since native tau is added to it and other proteins related to microtubules, such as microtubule-associated protein 1 or 2 (MAP1 or MAP2), acting as models to misfolded tau, inducing identical pathological conformation and its separation from microtubules. This progressive self-aggregation of tauopathy in a prion-like mechanism, in which there is a spreading of misfolded tau assembles from cell to cell, sequestering native tau for new seeds that will induce aggregation, until it is no longer possible to recruit normal tau (7,14,36). It is possible to spread tauopathy to unaffected areas, by forming annular pore-like structures. These are responsible for the disruption in membrane permeability, resulting in changes in ion homeostasis and cellular damage (**Figure 11**) (14). Thus, tau can enter cells by forming pore-like structures or by endocytosis (micropinocytosis or receptor-mediated endocytosis). Conversely, tau propagation in the brain appears to occur by exosomes (43).

Clearance of tau can also be dysfunctional, resulting in tau accumulation. As described previously there are two main mechanisms for tau protein degradation, the UPS for the full-length and monomeric tau and macroautophagy/lysosomal pathways for the truncated, oligomeric and aggregate forms of tau. Deficits in clearance mechanisms in AD lead to ubiquitinated protein aggregates, accumulation of autophagic vacuoles (AVs) in dystrophic neurites and neurodegeneration (14).

5.2. Changes in anterograde and retrograde transport along axons

Neurons are highly polarized cells with long projections (47). Axonal transport has a major role on neuronal homeostasis maintenance and synaptic activity (48,49). Most proteins and cellular components essential for synaptic function are generated in the cytoplasm and move along axons by cytoskeletal tracks with molecular motor proteins, such as kinesins (KIFs), dyneins and myosins, to reach synaptic terminals and backwards. The main component of cytoskeleton are microtubules, having a tubular and dynamic structure with α and β-tubulin heterodimers (49). In axons, microtubules are oriented with the plus-end pointing to the synaptic terminal, allowing it to have a specific direction, whereas, in dendrites, microtubules do not have a uniform direction (23).

Axonal transport can be classified according to movement rates and specific cargoes, into fast or slow. Slow axonal transport moves cytoskeletal elements, cytoplasmatic and neurofilament proteins, such as tubulin and actin, whereas fast axonal transport (FAT) transports membrane-bound organelles (MBOs), like mitochondria (49,50).

KIFs are the main transporters in anterograde transport (18); they move components like mitochondria, vesicles with APP, synaptic vesicle precursors (SVPs) and piccolo-bassoon transport vesicles (PTVs) from the cell body into the axon terminal (plus-end direction) (**Figure 12**) (49).

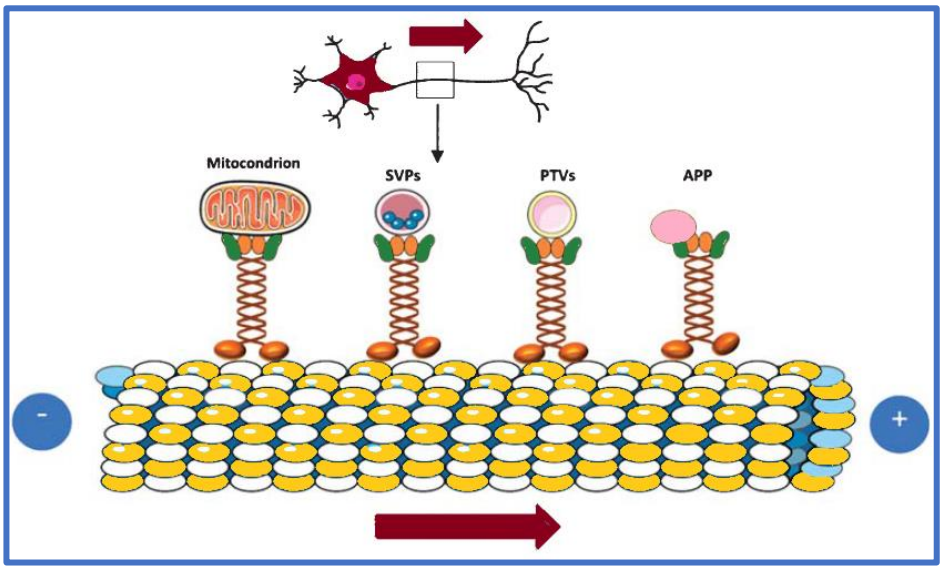

Figure 12. Anterograde transport. Adapted from: (49)

Dyneins are the main responsible for retrograde transport (**Figure 13**), from the axon terminal into the cell body (minus-end direction). Dynactin is the adapter complex that allows the link between cargoes and dyneins. Mitochondria, synaptic vesicles and endosomal recycling vesicles with neurotrophic factors, , some viruses and toxins are transported retrogradely (49).

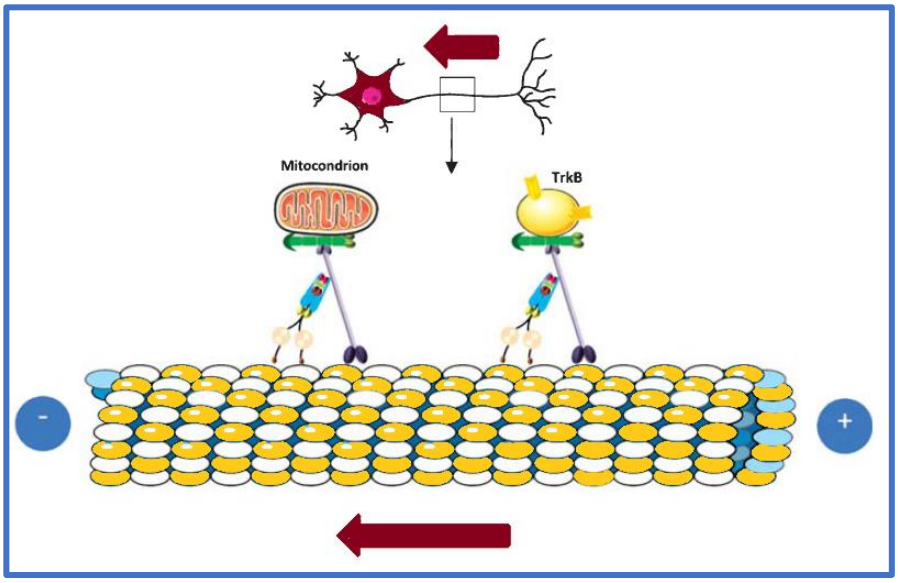

Figure 13. Retrograde transport. Adapted from: (49)

The axonal transport requires stability, which is given by the permanent connection between microtubules and MAPs, like tau (16). Underlying AD pathogenesis, either in early or late stages (48), any modification in molecular motors proteins or its connection with cargoes depends on adapter molecules (49).

Neurons, due to their morphological complexity, high activity rates and longevity have a system of membranous organelles, which can transmit signals between axons, dendrites and cell bodies, named endolysosomal trafficking mechanism, constituted by early endosome (EE), recycling endosome (RE), LE and lysosome (23,51). Rab small GTPases regulate this system and some subclasses are related to specific endosomes, like Rab5 for EE or Rab7 for LE (**Figure 14**) (23).

In early AD, accumulation of swollen EE and lysosomes in neurons (52) might be related to Rab5/EE. When in the plasma membrane, Rab5 can control endocytosis, while in EE it is able to regulate endosomal intracellular trafficking, being responsible for EE formation. Rab5 affects synapses, influences LTP and LTD of excitatory synaptic transmission and enhances amyloidogenic pathway. When excessively activated (for instance, by high levels β-CTF), promotes EE enlargement and impairs maturation from EE to LE and endolysosomal system biogenesis, besides enhancing internalization of surface receptors, like tropomyosin receptor kinase (Trk), influencing signaling endosomes. The increase in Rab5 activity can be a compensatory response to the dysfunction in dynein-dependent transport of neurotrophin (NT) signals, as well as, the decrease in Trk gene expression (51). EE become LE, which have more intraluminal vesicles, due to the conversion of Rab5 into Rab7 and LE are mostly situated in dendrites, including distal areas (23).

Dynein motor protein with Snapin adaptor protein mediate the retrograde transport of LEs (**Figure 14**). Then, LE can either fuse with each other, lysosomes or even plasma membrane (23).

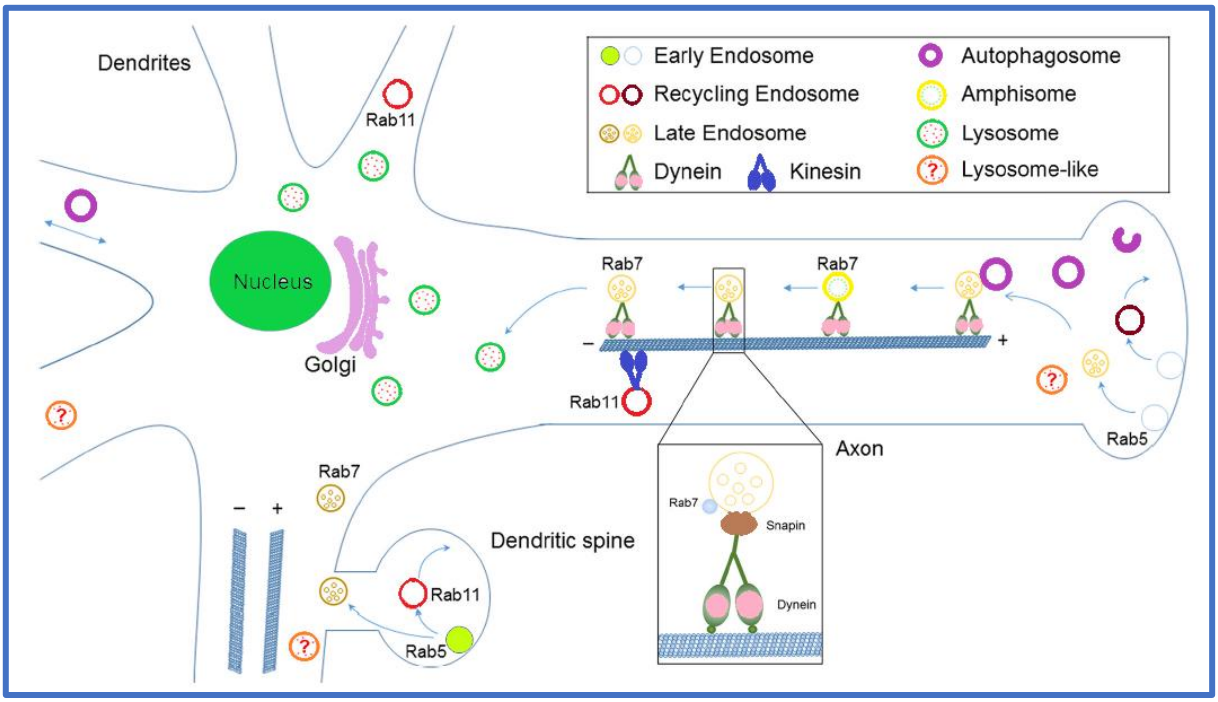

Figure 14. Endolysosomal system and autophagy in neurons. Adapted from: (23)

It is believed that tau and Aβ have a dynamic relationship, since the reduction of tau avoids the axonal transport dysfunction caused by Aβ (49). Soluble oligomers of Aβ can have an immediate effect on dissociating tau from microtubules, by interacting with NMDAR, increasing calcium influx, stimulating GSK3β to phosphorylate tau, leading to disturbances in axonal transport and more extremely into neuronal death (24). Soluble Aβ oligomers have a negative effect on LTP, since it promotes the activity of casein kinase-2 (CK2), that acts like GSK3β in the reduction of connection between cargo and motor complex (49). Only when tau is hyperphosphorylated can retain kinesin adapter-molecule JIP1 in the soma compartment, causing impairments in kinesin motor-mediated axonal transport mechanism, because the kinesin complex is not created (3,34).

In AD there is an impairment in retrograde transport of LEs, resulting in an accumulation of these in axons and presynaptic areas. The recruitment of dynein motors to LEs is impaired because axonal soluble Aβ oligomers are capable of interfering with its binding with snapin, resulting in disruption of LEs dynein-driven retrograde transport (23) and inhibition of FAT (49). Processing of APP can happen in endosomes (52), while they move fast along axons (18), since they contact with BACE1. Any change in this transport, causes dysfunctions, because they do not achieve the axon terminal. When retrograde transport of BACE1 is dysfunctional, there is an accumulation in axons, enhancing the amyloidogenic pathway (23,49).

In order to regulate processes like survival and differentiation, several neurotrophic factors released from postsynaptic neurons influence the function and structure of presynaptic neurons through a long-distance retrograde transport. They bind to cell membrane surface receptors, creating complexes between NT and Trk receptors; these NT include nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophins-3 and 4 (NT-3, NT-4) and its Trk receptors, namely TrkA selectively activated by NGF, TrkB by BDNF and NT4, and TrkA and C by NT3; all NT bind to p75 (a member of the tumor necrosis factor receptor superfamily that can mediate cell death) with low affinity or their non-matured/precursor forms (e.g. pro-NGF, pro-BDNF). The activated complex is endocytosed, resulting in a signaling endosome. Under physiological conditions, there is a constant flow of these endosomes *via* retrograde transport. Modifications of this process have been described in early stages of AD pathogenesis (51). BDNF is neuroprotector against Aβ neurotoxicity, that is decreased in AD brain, despite being an important protein in neuronal development, repair mechanisms, synaptic plasticity, and learning and memory. In axon terminals, BDNF/TrkB is a complex that activates glutamatergic transmission and promotes NMDAR subunits phosphorylation. This high affinity complex activates intracellular signaling cascades by three pathways: PI3K is activated and promotes Akt activation, which is important for neuronal survival; phospholipase C-γ (PLCγ) pathway that consists on the activation of inositol trisphosphate (IP3) receptor, leading to calcium release from ER and consequently activating calcium-dependent proteins, enhancing synaptic plasticity and MAPK/ERK pathway (42,49).

NGF is a NT that is retrogradely transported towards the nucleus, where it influences gene expression of basal forebrain cholinergic neurons (BFCN), the main cholinergic innervation in hippocampus and neocortex. BFCN synapses need a constant flow of NGF, that responds to a higher demand in brain activity. By selectively interacting with TrkA receptors, NGF promotes APP trafficking to the golgi compartment, keeping amyloid in its physiological levels in normal BFCN, by regulating APP proteolytic processing by BACE1. In early AD, TrkA and NGF levels are low, possibly due to changes in cognition and synaptic damages on cortico-hippocampal regions (10). Overexpression of APP leads to changes in structure of endosomes and differences in the axonal transport and it can be connected to dysfunctional retrograde transport of signaling endosomes with NGF (51).

6. Perturbed dendritic activity in AD

6.1. Glutamate excitotoxicity, intracellular calcium domains and dendritic spine remodeling

Understanding mechanisms behind synaptic and dendritic spine density loss is crucial, since they are the main determinants of memory impairment in AD. In the case of glutamatergic synapses, excessive glutamate levels in the synaptic cleft suppress LTP, lead to synaptic impairment (1,30), microtubule detachment and a decrease in neurite length (31).

Glutamate signal transduction at the postsynaptic terminal depends on glutamate receptors (21), namely NMDAR. Soluble Aβ oligomers were previously shown to directly interact with NMDAR, namely composed by the GluN2B subunit. Aβ oligomers also promote an excessive release of glutamate from hippocampal neurons, promoting postsynaptic activity (24). Furthermore, soluble Aβ oligomers can inhibit glutamate uptake through selective plasma membrane transporters, like GLT-1, that are largely located in astrocytes (31).

Extracellular Aβ oligomers also bind to other receptors, like cellular prion protein (PrPc) and metabotropic glutamate receptor 5 (mGluR5), forming complexes that activate intracellular Fyn kinase (24). This interaction promotes tau phosphorylation (21), while tau promotes Fyn distribution to dendritic spines and influences postsynaptic Fyn, leading to abnormal glutamatergic synaptic transmission, due to excessive activation of NMDARs (9,21).

Soluble Aβ oligomers cause neurotoxicity, which has been linked to the impairment in calcium homeostasis (24). Dysregulating in calcium homeostasis has been correlated with cognitive dysfunction. As described previously, Aβ oligomers can increase intracellular calcium by generating permeable pores to calcium in the plasma membrane, prolong the activation of ion channels, like NMDARs or AMPARs, or due to Aβ-mediated release of calcium from internal stores, like the ER, resulting in mitochondrial calcium accumulation. This will further cause mitochondrial dysfunction and oxidative stress, ultimately leading to cell death (24,31).

Glutamate can either increase dendritic spines length or decreasing it in an NMDAR-dependent pathway; morphological changes in dendritic spines largely depend on the duration of NMDAR activation (53) and, consequently, on calcium levels in dendritic spines. While synaptic NMDARs activation and high calcium levels enhance dendritic spine growth and are linked to LTP, NMDARs internalization and low calcium levels activate LTD by promoting dendritic spine shrinkage and loss of synapses (24,54). High calcium levels stimulate the activation of kinases, like CDK5, which phosphorylate tau and leads to its misplacement in dendrites, causing destabilization of axonal transport, loss of mature spines and decreased synaptic activity (21).

Besides NMDARs, AMPAR at synapses are also responsible for synaptic plasticity and they are stabilized by the PSD-95. AMPAR is highly present at spines and regulates postsynaptic strength. If all the factors that influence AMPARs are kept stable, synapses communication is preserve; on the contrary, if AMPARs become saturated or inactivated, LTP mechanisms decrease and synapses become silenced, altering synaptic connections and causing memory deficits. Consequently, spine number can differ due to (mal)adaptative synaptic mechanisms (41). Aβ oligomers inhibit AMPAR-mediated synaptic responses and ionic flux, by removing AMPAR from the synaptic cleft. Endocytosis of AMPAR leads to a depression of excitatory synaptic transmission and diminishes spine density (9). Soluble Aβ oligomers-induced increase in cytosolic calcium in neurons activate calcineurin, which further activates protein phosphatase 1 (PP1). When in excess, PP1 dephosphorylates calcium-dependent protein kinase II (CaMKII) and, consequently, dephosphorylates AMPAR, deregulating AMPAR-based LTP in the hippocampus. In addition, CaMKII levels are lower in synaptic clefts of cortical AD neurons and in the presence of Aβ oligomers. Activated calcineurin leads to dystrophic neuritis (24). This impairment in LTP mediated by Aβ also involves p38MAPK activation and CREB downregulation. The first stage of spine formation is named filopodia, which appears from dendrites and creates synapses with adjacent axons. After synaptic contact, a small percentage become protospines, that will either become mushroom or thin spines. Spine maturation depends on synaptic strength and activity rate. Dendrites spines can be classified as mushroom spines, which have a large head and narrow neck, giving them stability to create strong synaptic networks, or as stubby spines, with no distinction between its parts, both named as memory spines. There are also thin spines, which have a smaller head and narrow neck, and branched spines that have two heads and a narrow neck, both called learning spines, due to their ability to easily change their conformation (46,53).

Opening of NMDAR-associated ion channels after synaptic stimuli promote long-last modifications in the number of postsynaptic AMPAR, leading to spine growth. During synaptic activity, spines extend and contract and generate/stabilize new spines. During LTP, spine heads enlarge, their length is reduced, and neck's diameter is enhanced, whereas in LTD there is a decrease in spine number and size (spine 'shrinking') (53–55) This dynamics (**Figure 15**) during synaptic plasticity is based on polymerization and depolymerization of actin in dendritic spines (55,56). Actin can be found in high amounts in dendritic spines (57) and both G- and F-actin need to be in balance in order to regulate dendritic spine morphology (55). These actin changes in polymerization are regulated by actin-binding proteins, such as drebrin, CaMKII and cofilin (56). Cofilin is an inhibitor of actin polymerization, promoting its depolymerization/treadmilling, whereas drebrin and CaMKII promote actin polymerization, stabilizing actin in dendritic spines (58). Drebrin accumulates in dendritic spines, having a

major role in spine maturation and regulates F-actin (polymerized actin). Drebrin amounts are related to the spine heads size (57).

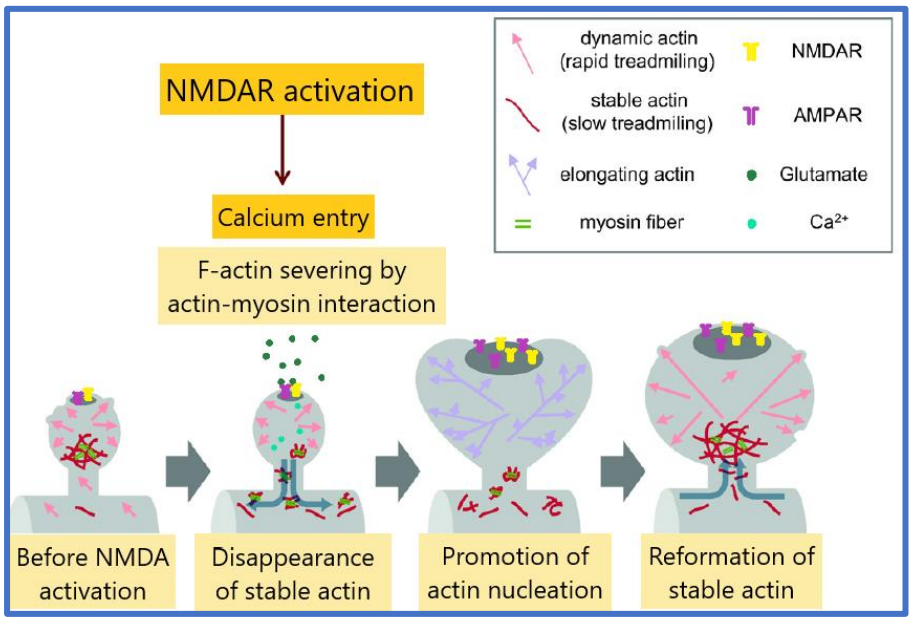

Figure 15. Representation of LTP changes in dendritic spines, caused by glutamate stimulation of NMDARs and calcium influx. Dendritic spines suffer morphologic changes due to severe actin modifications. Adapted from: (57)

In AD hippocampus and neocortex, the number of dendritic spines and synapses was found to be reduced (53). This early AD event, most probably occurring due to excessive Aβ oligomers, is caused by F-actin disassembly, which promotes synaptic impairment and consequently behavioral and cognitive changes (55). A decrease in mushroom spines is related to memory dysfunction (46). Changes in actin dynamics that lead to an increase in stubby spines and an enlargement on spines neck, cause dysfunction in LTP and LTD and may occur prior to synaptic loss and plaque formation (54,58). Neurite dystrophy was found surrounding amyloid plaques (53), so it is possible that spine increases its extension to be able to maintain working memory and new synaptic inputs surrounding plaques and NFT areas (59). Simultaneously to synaptic loss, there is an enhancement in size of postsynaptic densities (PSDs) in non-affected areas, possibly emerging as a compensatory response (54).

6.2. Mitochondrial transport and dynamics in dendrites

Due to the high energy demands, neurons require an efficient mitochondrial activity (1). Mitochondria are responsible for regulating different cellular metabolisms, signaling, coordinating stress responses and cell growth (60,61). Their biogenesis occurs in the cell body and mitochondria can move through anterograde and retrograde axonal transport (17). Mitophagy also occurs in the cell body, meaning that impaired mitochondria need to return to the cell body, via retrograde transport, in order to be degraded (60).

In early AD, energetic dyshomeostasis, due to mitochondrial impairment, can be one of the underlying pathological mechanisms, since changes in mitochondrial function are more evident in the most affected areas (31,60). In AD, several triggering factors have been described to contribute to mitochondrial dysfunction, such as aging, injury and (neuro)inflammation (50), leading to changes in mitochondrial bioenergetics, morphology and transport (62) (**Figure 16**).

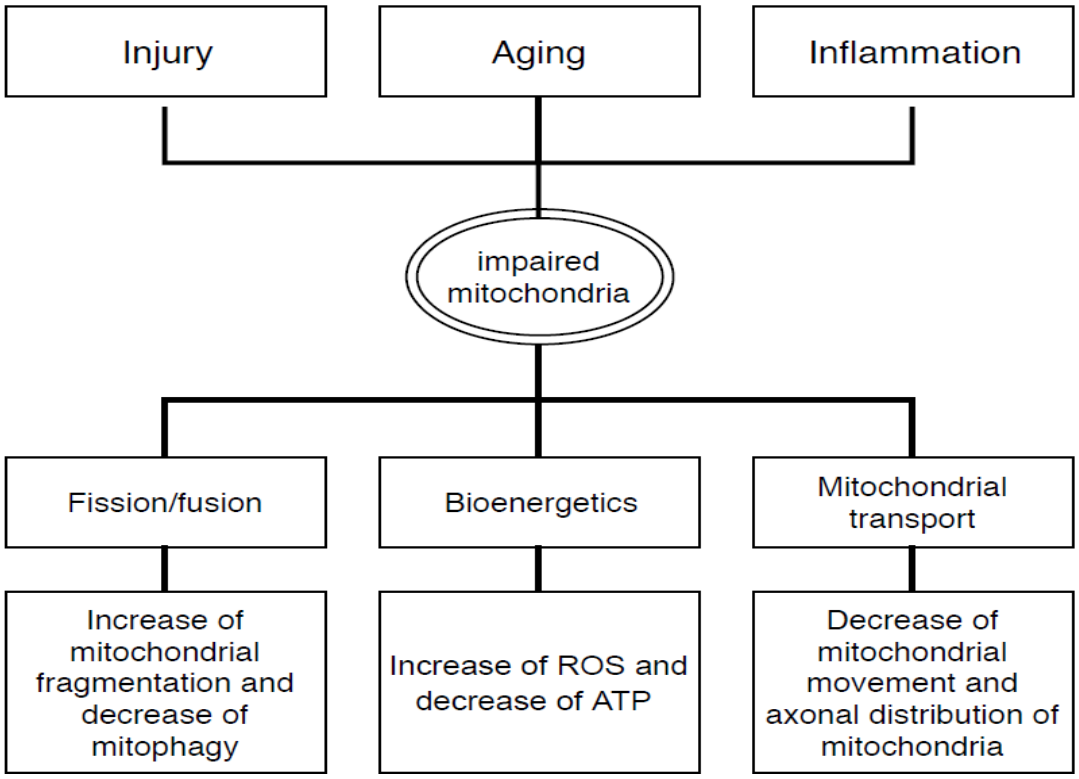

Figure 16. Different factors lead to mitochondrial impairment, involving enhanced organelle fragmentation and decreased clearance, reduced ATP levels and oxidative stress and re-distribution of axonal components, along with reduced mitochondrial transport. Adapted from: (50)

Aging is a major risk factor for AD and thus it has been implicated in mitochondrial disturbances and excessive production of ROS (3), along with decreased levels of antioxidants, culminating in the oxidation of several biomolecules, results in DNA, protein and lipid damage (24).

In order to respond to energetic demands, maintain calcium homeostasis, mitochondria rearrange into different shapes and sizes and redistributes along axons (17,50,60). Mitochondrial dynamics or structural adaptation depends on several fusion and fission cycles, that lead to morphological 'transformations' (62). Fusion results in an interconnected reticulum with inner and outer membranes being fused together, creating longer and less mitochondria, whereas fission increases mitochondrial number by generating two new mitochondria or segregate portions of the organelle for mitophagy (60,61). One of the daughter's mitochondria suffers another fusion, while the other is depolarized and is degraded by macroautophagy (50).

Mitofusins 1 and 2 (Mfn1 and 2) at outer mitochondrial membrane (OMM), and optic atrophy protein 1 (OPA-1) at inner mitochondrial membrane (IMM) are hydrolyzing GTPases responsible for regulating only fusion processes (60,62). On the other hand, dynamin-like protein 1 (DLP-1 or Drp1), which is a cytosolic protein, promotes the fragmentation, leading to fission into two daughter mitochondria, following Drp-1 interaction with Fis1 (62).

Mitochondria also move bidirectionally to be distributed axons terminals and dendrites, being crucial to have a correct mitochondrial distribution and functional regulators (61). Mitochondrial movement regulators include changes in calcium, ROS, oxygen levels and ATP (60). Mitochondrial transport depends on ATP production, meaning that a correct biogenesis is essential for mitochondrial movements. Excessive ROS production , leads to oxidative damage and changes in functional mitochondria (40). Calcium and CaMKII can both directly influence MTs stability, since MTs are highly sensitive to calcium levels and depolymerizes. This means that any change that promotes excessive calcium levels, such as Aβ oligomers through activation of NMDAR, will induce transport modifications of organelles, such as mitochondria (63).

Modified fission or fusion, cause impairment in mitochondrial motility, potentially resulting on increased mitochondrial degradation and neurodegeneration. Both hyperphosphorylated tau and Aβ have been described to cause an impairment in mitochondrial dynamic balance (61). By increasing calcium influx through NMDAR (49), increased Drp1 and decreased OPA-1 and Mfn1 and 2 (3,17,60) heightening fission processes (7,45) and causing dysfunctional axonal transport (50), less ATP generation and synaptic impairment. Intracellular Aβ can be translocated into mitochondria and enhance apoptotic pathways, cause damage in mitochondrial DNA (mtDNA) and promote mitochondrial ROS production, leading to oxidative stress (16,64), since they can easily dysregulate complex IV, disturbing electron transport chain (ETC) and ATP production (24,60), by interfering with resident proteins from the OMM, IMM or the matrix (64). Specifically from the matrix, Aβ interacts with cyclophilin D (CypD) which has a role on ETC and this complex leads to bioenergetic impairment, by increasing oxidative stress (65).

When oxidative stress becomes chronic, it causes inhibition of tau phosphatases, decreasing tau' dephosphorylation. Of relevance, changes in oxidation and neurite accumulation of damaged mitochondria start in early stages of AD. Moreover, oxidative stress promotes an inflammatory response by activating microglia, through cytokines release. This results in astrocytes invading Aβ plaques (7).

Mitochondrial axonal transport occurs through the interaction with microtubule tracks and actin filaments (60). Adaptor proteins responsible for mitochondrial transport regulation, such as Miro (mitochondrial Rho-GTPase) and Milton (or TRAK) interact with motor proteins of kinesin-

1 and 3 family, allowing anterograde axonal transport (60,62). Kinesin-1 protein (KIF5) has N-terminal motor domain with an ATPase and a C-terminal, that links the protein with the cargo. Milton protein is a KIF5 adaptor protein (50), whereas Miro is a mitochondrial calcium-binding membrane protein. Milton interacts with Miro, being indirectly involved in mitochondrial transport, as well. Milton subtypes are TRAK1 and TRAK2, being mainly in axons and dendrites, respectively, changing mitochondrial transport at on those specific areas, when damaged. Miro subtypes include Miro1 and Miro2 and when impaired lead to mitochondrial dysfunction, damaged Miro1 that alters dendritic mitochondrial transport, not axonal. Both are involved in retrograde transport and while Milton has influence in the initiation of mitochondrial movement, Miro is responsible for changing movement direction (66).

Dynein is the protein involved in retrograde transport, it has a globular motor domain, responsible for binding with microtubules acting as an ATPase. In order to function correctly, dynein needs to be coupled with dynactin complex, allowing the binding with the cargo, in this case the mitochondria. Besides, syntaphilin (SNPH), another adaptor protein is responsible for the maintenance of mitochondria in axons, acting as a ‘’static anchor’’, since neurons need stationary mitochondria in order to be able to dissociate from motor proteins and attach to the cytoskeleton. When axons have a reduced amount of SNPH, axonal degeneration increases, leading to neuronal loss (50).

It is possible that mitochondrial changes constitute early events in AD, since modifications in axonal transport seem to occur prior to NFTs formation or Aβ aggregation (50,60). On the other hand, synaptic damage, may be partially caused by a decrease in mitochondrial anterograde transport, since it reduces the number of mitochondria in the axon terminal (50).

Since microtubules are the tracks for transport along axons, changes in microtubule-associated proteins (like tau), will influence mitochondria activity (17). Since tau is mostly situated in axons (50), when it is abnormally phosphorylated, l mitochondrial distribution and dynamics will be affected (60). Besides, these changes phosphorylated tau has a negative impact on mitochondrial complex I, decreasing ATP production (3).

As described before, mitophagy is based on targeting damaged/dysfunctional mitochondria that need to be degraded. The process starts with autophagosomes that along the microtubules capture either misfolded, aggregated proteins or dysfunctional organelles. Autophagosomes will then fuse with lysosomes, having their content degraded by lysosomal enzymes. In order to mitophagy to happen normally, the transport of AVs is required from distal to somatodendritic compartments. This means that changes in retrograde transport, lead to AVs accumulation and result in impairment of mitochondrial quality control (48,60).

6.3. Impact of hyperphosphorylated tau in dendrites

Neurite disturbances in entorhinal cortex (EC) have been associated with tau; the protein then spreads to other regions of the hippocampus and consequently to inferior frontal and parietal cortex and eventually to occipital lobes (32).

Tau protein is a MAP that when impaired, causes harmful consequences on dendrites (14). As described before on this study, under normal conditions, tau has higher affinity for microtubules in axons, whereas in the presynaptic terminal tau's levels are diminished (33). When in hyperphosphorylation state, tau decreases this affinity for MTs. Under pathological conditions, hyperphosphorylated tau begins to accumulate in the soma and dendrites, mostly post-synaptic, becoming insoluble (67). There are extracellular factors that stimulate tau redistribution into dendrites, such as Aβ oligomers and chronic stress. On these compartments, tau can bind with constituents of the post-synaptic density (e.g. proteins and receptor complexes), affecting synaptic plasticity and leading to spine loss (33,36).

Fyn kinase is a member of the Src family of non-receptor tyrosine kinase and has a major influence in synaptic activity, trafficking and learning, besides being the vehicle for Aβ neurotoxicity (67). Overexpressed Fyn accelerates cognitive dysfunction and participates in tau phosphorylation (12), which has been largely correlated with cognitive deficits, when compared to Aβ (33).

Fyn suffers a recruitment through its binding with proline-rich region of tau. In physiological circumstances, this link targets Fyn to postsynaptic sites, where it modulates the function of NMDAR. Fyn can also phosphorylate tau protein, which helps with their mutual connection. Post-synaptically, tau and Fyn interact with PSD-95/NMDAR complex. Contrarily, when there is a pathological situation dendritic tau delivers more Fyn to postsynaptic sites, leading to GluN2B phosphorylation, in order to stabilize interactions between NMDARs and PSD-95. This may result in an excessive activation of NMDARs, causing neuronal excitotoxicity. Although the recruitment of Fyn is tau-dependent, Fyn is responsible for excitotoxicity caused by NMDARs (21,33).

When APP is excessively expressed, Fyn facilitates and increases the rate of synaptic dysfunction and cognitive deficits (67). High levels of soluble Aβ oligomers cause excessive activation of NMDAR, through the formation of postsynaptic complexes with PrPc in dendritic spines (24). It is well established that PrPc is a receptor for Aβ oligomers, which is highly present at postsynaptic density (12).

The connection between PrPc and Fyn tyrosine kinase is made through mGluR5 (**Figure 17**) (24) and Fyn's activation is only possible when Aβ interacts with PrPC-Fyn-mGluR5 complex (12). This connection promotes the complex stabilization through recruitment of PSD-95, leading to phosphorylation of GluN2B subunit of NMDAR. Increasing this subunit in the cell

surface, results in higher calcium influx in neurons and, consequently, in excitotoxicity. Calcium-dependent PP2B can regulate excitotoxicity caused by Aβ, by decreasing calcium influx (9) and PP2B can be activated by NMDARs activated by Aβ (68). Synaptic plasticity depends on these postsynaptic glutamate receptors, that when activated promote the redistribution of tau and Fyn kinase into the postsynaptic densities (33).

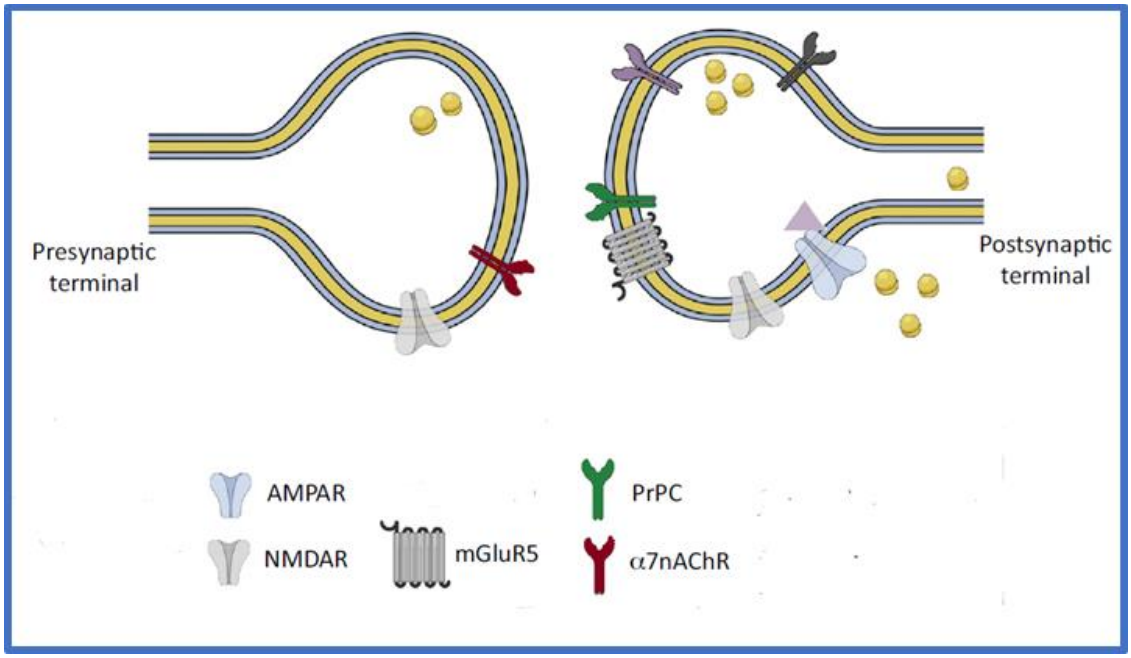

Figure 17. Presynaptic and postsynaptic receptors. Adapted from: (21)

7.Therapeutic agents that can ameliorate neurite dysfunction in AD

AD therapeutic strategies can be divided into symptomatic or disease modifying treatments (DMTs). Symptomatic agents can either have a focus on cognition improvement or be oriented to neuropsychiatric and behavioral manifestations, such as agitation, lability or delusions. On the other hand, DMTs include biologic therapies or small molecules and have different targets, such as mechanisms behind Aβ, tau protein, neuroinflammation or metabolic changes (2,69). Despite all the efforts to find an effective and safe treatment, the current approved therapies are only symptomatic and have a minor influence in slowing the rates of cognitive impairment. Nowadays, the focus is to delay the onset of AD and slowdown its progression. Unfortunately, clinical trials are not easy to develop, since disease' progression takes years (2).

Until February 2019, 132 agents were under study in 156 clinical trials, from which 28 agents were in phase 3 (**Figure 18**) and 74 in phase 2 (**Figure 19**) (69).

So far, the approved therapies to improve cognitive performance, can be divided into AChEI, which selectively inhibit the enzyme responsible for acetylcholine degradation, thus increasing the levels of the neurotransmitter in the synaptic cleft and an antagonist of extrasynaptic NMDARs, memantine (2). Accumulation of acetylcholine in the synaptic cleft facilitates neuronal transmission, since it increases synaptic activity (65). Hippocampal memory function depends on cholinergic action and, in AD, there is an evident reduction in nicotinic and muscarinic receptor levels and in choline acetyltransferase activity and acetylcholine release. AChEI, such as rivastigmine, donepezil and galantamine (2) are mainly used for palliative treatment, since they are not able to reduce disease' progression (12).

The AChEI are used in stages from mild to moderate AD, whereas memantine usage has been suggested to combat more severe disease stages and for patients intolerant to AChEI. Furthermore, there is an agent named namazaric, which combines memantine with donepezil (2,65).

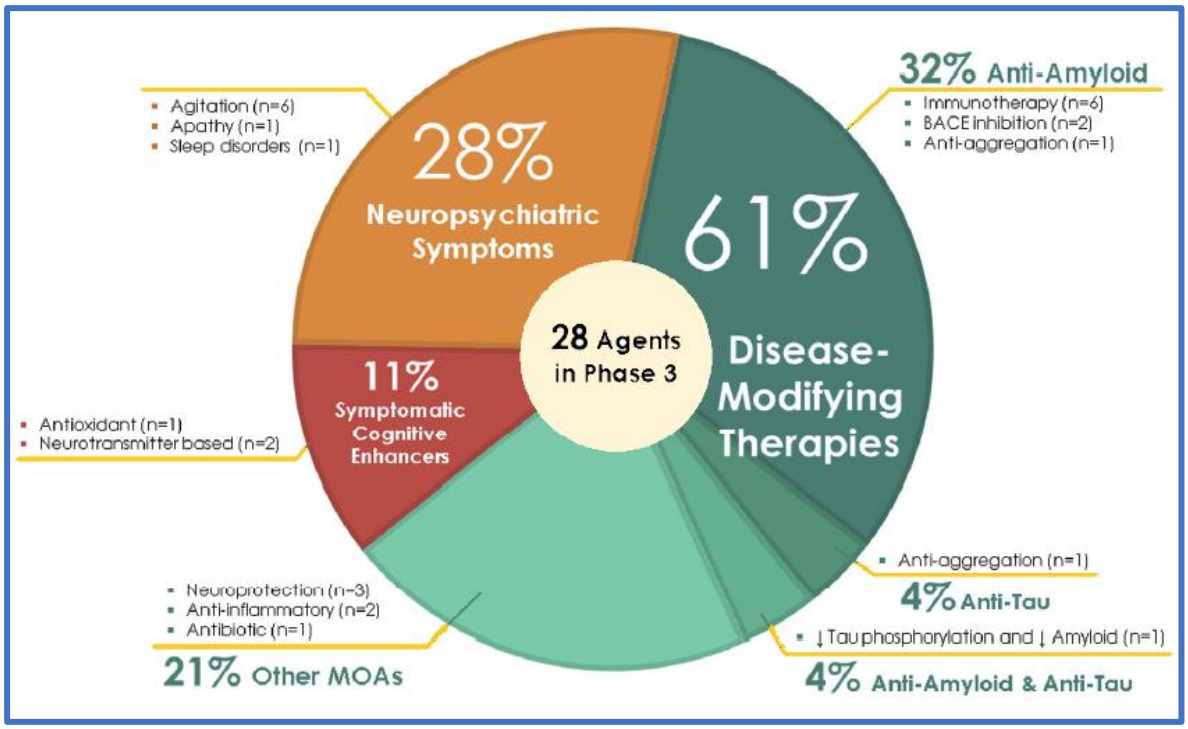

Figure 18. Mechanism of action of phase 3 pharmacological agents. Adapted from: (69)

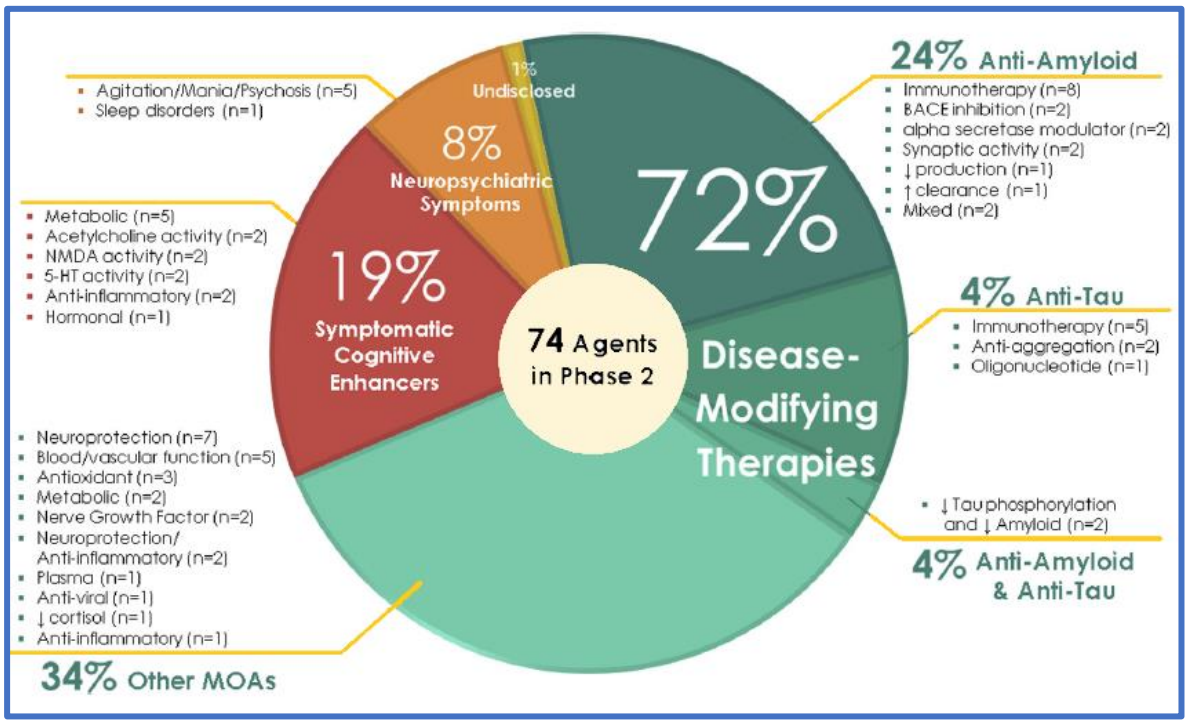

Figure 19. Mechanism of action of phase 2 pharmacological agents. Adapted from: (69)

7.1. Therapies focusing on Aβ

The first hypothesis on AD pathogenesis was based on the Aβ cascade. Accordingly, several researches were developed having the amyloidogenic pathway as a focus, even though none of them has succeeded (12). It is possible that the issues with therapeutic targeting Aβ is not with drug-target binding; instead, it seems that it would possibly be successful if the administration was prophylactic, before appearance of amyloid deposits (2). Since soluble oligomers were found to be more toxic than SPs, the treatment goal is to either remove soluble Aβ oligomers or avoid their production (24). In AD, the consequences caused by Aβ are concentration-dependent and its accumulation is due to excessive processing of APP, but also due to dysfunctional clearance mechanisms (12). Aβ monomers should be degraded by enzymes and stabilized through the interaction with small molecules, avoiding its oligomerization. This process is highly influenced by oxidative stress, which means that antioxidants could help at this stage (24).

The reason why so many clinical trials based on amyloidogenic pathway failed, might be the differences in the biology between animals and humans (70); on the other one may not forget that there is a lack of disease-related biomarkers at early stages, precluding any approval of clinical trials at presymptomatic stage. Indeed, many phase III trials were performed in advanced stages of AD, in which any therapeutic move would be useless. Still, there are researches focusing on β-secretase inhibitors and trying to increase the nonamyloidogenic route, by promoting the activity of α-secretase. Importantly, β-secretase has many more substrates that APP itself, making even more difficult to obtain a positive outcome of β-secretase (12).

The most important step in recent therapies is finding new options to eliminate excessive Aβ from the brain by immunization (24). There are two different types of immunization active and passive (70). Active immunization is based on the administration of Aβ antigens through vaccination (24). This would be a great way of fighting AD, but vaccines, that are effective in AD animal models, have not reached the expected success yet. This kind of treatment has several obstacles, due to its costs and its loss of efficacy with aging, since it depends on patient's immune system (70). Moreover, inflammation was a major side effect, which is still a challenge. On the other hand, passive immunization uses anti-Aβ antibodies. In order to clear the excessive Aβ, this mechanism includes disaggregation of Aβ deposits, inhibition of new aggregation and activation of phagocytosis by microglia (24). There is a natural immunity against AD, confirmed by treatment in early stages with monoclonal antibody (mAb) Aducanumab. This mAb recognizes Aβ oligomers, and it was found in older people with normal cognition. The goal is to mimic the natural protective immunity based on some studies, as well as using the entire Aβ as an immunogen. The need of prolonged immunizations, which could

be shorter if there is an addition of Th2 specific adjuvants, is also an obstacle for this option. Solanezumab is a humanized monoclonal Aβ antibody, that interacts only with soluble monomeric Aβ, allowing a decrease in disease' progression, by increasing Aβ's efflux from the brain to the plasma (70). Solanezumab showed benefits in patients with mild AD, but a phase III study showed no positive effect on cognition (2). Passive immunotherapy seems to be more effective and safer, without genetic limitations and does not depend on patient's immune system, since the antibodies bind to the epitopes externally to the patient (70). Other Aβ mAbs are being developed, such as Bapineuzumab, which seems to have major benefits in cognition (24). In the future, it seems that mAbs will be a possible therapy in a prodromal stage of AD. Prophylactic vaccination is still not clinically tested. All the components have their side effects and the benefits would have to be strictly measured. Moreover, active and passive immunotherapies complement each other, as if the vaccination should be administered in immunocompetent patients, in order to slow the rate of disease progression or even avoid its onset and then, as patients get older, with the appearance of immunosenescence, mAb therapy should be considered (70).

7.2. Therapies targeting on tau protein

Since all clinical trials based on targeting Aβ have failed so far, therapies based on targeting soluble forms of tau oligomers started to be more studied (14). A tau-based strategy has the goal of diminishing or slowing down the rate of progression of tauopathy. Such tau-based therapeutic strategies should focus on the reduction of tau aggregation and/or inhibit tau phosphorylation and possibly even use microtubule-stabilizing drugs (64).

Tau therapy includes an active immunization with AADvac1 vaccine, which is in phase III clinical trial. AADvac1 is based on a synthetic peptide derived from tau sequence, that can avoid β or β-helix structure of protofilaments. The goal is to prevent tau' aggregation, spreading and improve immunogenicity. This had great results, namely the absence of abnormal tau deposits in the wall of brain blood vessels (32).

Similarly, to what was described for Aβ, the hypothesis of doing passive and active immunization against tau protein ought to be considered. Both could reach goals, like reduction of tau aggregation, enhancement of tau oligomers and clearance of insoluble deposits (12). Moreover, immunization focusing on tau dimers and oligomers seems to reduce locomotor and memory dysfunction in AD mouse (14).

Depending on the study, there are several hypotheses of how immunization works against tauopathy. Antibodies may prevent tau transmission between cells. There are specific antibodies that can remove the extracellular tau aggregates avoiding its entrance and consequent effects intracellularly. This can be achieved by an anti-tau monoclonal antibody acting on the extracellular compartment. By decreasing tau extracellularly, the cell sends

intracellular tau for the extracellular milieu, reducing tau's concentration inside the neurons (3). Other studies describe that antibodies can be expressed intracellularly and target phosphorylated-tau epitope and further promote the clearance of tau aggregates (71).

GSK3β is a link between Aβ and tau protein. Besides, it also plays an essential role in the regulation of some fundamental steps in the inflammatory cascade. When GSK3β is overexpressed, it causes cognitive dysfunctions, whereas when it is diminished, it is a key factor for memory acquisition. There are inhibitors of GSK3β that will increase its phosphorylation (71) and lead to a decrease in tau' phosphorylation and tendency to aggregate (33). For instance, in some experiments lithium couples directly with GSK3β, diminishes cognitive deficits when administered in AD murine based on an injection of Aβ in the hippocampus, rescuing cognitive damages. Despite this knowledge, there are different and contradictory information about this therapeutic hypothesis in various clinical trials (71).

CDK5 inhibitors, like roscovitine and flavopiridol, are possible drugs, since they have shown positive effects by preventing neurodegeneration and excitotoxicity (12). Inhibitors for CDK5 and GSK-3β were able to reduce the levels of soluble aggregated hyperphosphorylated tau in tau transgenic mouse (71).

Targeting the autophagic lysosomal pathway might be also interesting (3). Trehaloseis able to reduce tau inclusion and improve neuronal survival (14). Besides, temsirolimus, BAG3 and NDP52 are also under current research to increase autophagic clearance of hyperphosphorylated tau (3).

The activation of tau phosphatases is also under investigation using, for instance, chronic low doses of metformin or sodium selenite, which can increase PP2A activity and inhibit tau phosphorylation (14), which results in an enhancement in cognition and a decrease in neurodegeneration (12). Metformin seems to improve learning, memory and other cognitive functions, by enhancing mitochondria and synaptic function, as well as diminishing neuroinflammation and increasing brain metabolism (60).

Targeting other post-translational modifications apart from tau phosphorylation should also constitute relevant therapeutic target. O-GlcNAcylation inhibits tau hyperphosphorylation, so its activation is a good therapeutic strategy. For instance, using Thiamet G, which is an inhibitor of the enzyme that hydrolyses this mechanism, it is possible to reduce NFTs and enhance neuronal survival. Acetylation mechanism can stimulate tau polymerization and avoid its destruction (3), so in order to reduce acetylation, it could be used HDAC6 activator (33).

Furthermore, truncated tau stimulates tau aggregation, which means that it is also a good mechanism to target, for example, with small-molecule agents, that enter the targeted tissues more easily, such as inhibitory polyphenols, phenothiazines, anthraquinones and quinoxalines (3). Tau aggregation inhibitor methylthioninum chloride or methylene blue are under

investigation because they are capable of inhibiting tau aggregation without interfering with normal binding between tau and tubulin. Nowadays, there are clinical trials to understand better the methylene blue TRx0237 which can disrupt aggregation of tau, leading to cognitive improvements. As it was previously discussed, tau oligomers might be the toxic forms of tau, which means that disaggregation higher assemblies, could be more harmful than beneficial. So, methylene blue has other targets, such as reducing Aβ levels, by enhancing its clearance or inducing autophagy, that will lead to lower levels of tau protein (12,68). Targeting tau might be a future strategy using small molecules to diminish its expression or keeping its native conformation, avoiding its aggregates into neurotoxic oligomers, without having an effect on prior aggregates (68).

7.3. Therapies targeting on axonal transport

In order to decrease disease's progression, FAT may be a therapeutic target. On one hand, targeting kinase activity may be a way of restoring FAT impairment, since phosphorylation of kinesin is a key step to cargo recruitment and dissociation. For instance, JNK when inhibited may lead to neuroprotective consequences. On the other hand, using HDAC6 inhibitors, that deacetylates microtubules, could be used. Inhibiting this molecule leads to an enhancement in microtubule acetylation, which promotes a higher affinity with both kinesin-I and dynein for linkage with microtubule. It also influences the changes caused by Aβ in tubulin acetylation and mitochondrial transport, which might be reversable (49).

Epothilone D is a small molecule MT stabilizer that sustain axonal MT and consequently axonal transport, avoiding neurodegeneration (68). MT stabilizing drugs could only be used if in small amounts and specific for the neuron or compartment, in order to avoid unwanted consequences. These agents can enhance FAT and improve cognitive functions. Besides, all the previously mentioned tau-focused therapies indirectly improve axonal transport (49,65).

7.4. Therapies targeting on mitochondria dysfunction

Since mitochondrial disturbances are an early event in AD pathogenesis, having them as a therapeutic target is a valid strategy to avoid neurodegeneration (64). Prevention of mitochondrial fragmentation, decrease of ROS levels, enhanced ATP generation and mitochondrial transport are the changes that will put us on the right direction (50).

Gene therapy is a possible therapeutic future approach on AD, such as mitochondrial gene replacement and decreasing mutations in mtDNA. Although these studies only use cellular models, since there are no suitable animal models and there is no proper pathway to therapeutic agents to reach targeted tissues. Besides, the restriction endonucleases are getting much more attention as a therapeutic agent to combat mtDNA mutations (64).

Nowadays, there are new strategies that are still under investigation, that allow antioxidants to combat oxidative damage, caused by ROS production. Some are natural, like melatonin vitamin E or C, curcumin, catalase, glutathione and *gingo biloba,* possibly decreasing Aβ harmful levels (65). Furthermore, peptide-based-multiwalled carbon nanotubes is a new developed delivery system, also under investigation, that allows oligonucleotides to get inside mitochondria, in order to treat mutations in mtDNA. Additionally, there are other compounds mitochondria-targeted antioxidant that decrease ROS production and enhance bioenergetics, dynamics and mitochondrial transport (64). Besides natural antioxidants, there are mitochondrial targeted antioxidants, such as MitoQ, Mdivi1 and SS-31 (65), under clinical trials. MitoQ is an agent that advanced into clinical trials and needs further investigation (60). MitoQ seems to inhibit Aβ's consequences, cognitive impairment, oxidative stress and loss of synapses. Other agents, like Szeto-schiller (SS), similar to small molecules and act without depending on the membrane potential, so they are like a cell-permeable antioxidant peptide and can directly target mitochondria (64). Besides, there is SS-31, a mitochondrial-targeted antioxidant, which will be better for therapeutic potential in the future, since it might help with mitochondrial transport, decreases mitochondrial fission (50) and diminishes ROS production. SS-31 has the advantage of only acting on dysfunctional mitochondria (65).

Dynamics in mitochondria are possible therapeutic targets, like increasing fusion or decreasing fission to balance the processes. Although they need more studies, peptide P110, which is a DRP1 inhibitor, can decrease mitochondrial fragmentation and hydrazine M1 can act as a fusion promotor (50). Besides, tubastatin A is a microtubule deacetylase HDAC6 inhibitor that can rescue the mitochondrial transport. Further investigations suggest that memory function can get better, when HDAC6 is not present (64). Mitochondrial division inhibitor 1 (Mdivi-1) is a reversible mitochondrial complex I inhibitor that can reduce ROS production and improve mitochondrial function. It can decrease fission, because it is a DRP1 inhibitor, promoting mitochondrial fusion. More experiments are being made to understand how safe and effective this agent is (60).

7.5. Therapies focusing on postsynaptic sites

Changes in dystrophic neurites and dendritic spine loss appear at early stages of AD (12). Nowadays, post synaptic tau protein and its mechanisms are possibly a therapeutic target, but with many obstacles to overcome. Among post synaptic related components, Fyn activity has achieved a bigger progression in clinical trials, having as a main goal to reduce post-synaptic excitotoxicity caused by tau. Saracatinib and masitinib are Fyn kinase inhibitors, which are in phase II for mild AD and III clinical trials, respectively (12,33).

8. Conclusion

Alzheimer disease is a multifactorial neurodegenerative disease, that has several underlying pathophysiological mechanisms. Disease hallmarks have a dynamic connection, being a cause and consequence of several dysfunctions as described in this review. Both amyloid and tau protein lead to synaptic dysfunction, mitochondrial modifications, axonal transport impairment, becoming a major network of ionic imbalance and neuronal instability, leading to neuronal and synaptic loss. These changes are the responsibles for the disease clinical features.

Although there are several ongoing clinical trials around the world, there are still several limitations, some of them related with the selected disease stage. Indeed, researches multiply, but the therapeutic advances did not succeed yet.

Despite some disillusion in the area, with still nothing to offer to AD patients, this is a time of opportunity for the development of novel strategies to decelerate cognitive decline.

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