UNIVERSITY OF NAPLES FEDERICO II

DOCTORATE IN

MOLECULAR MEDICINE AND MEDICAL BIOTECHNOLOGY

XXXVI CYCLE

Modulation of mitochondrial quality control pathway through inhibition of 37/67 kDa Laminin Receptor in human skin fibroblasts from genetic Alzheimer's disease

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2020-2023

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1. ABSTRACT

2. BACKGROUND

2.1. The 37/67 kDa laminin receptor: structure and function

In 1983, the 37/67 kDa laminin receptor (LR) was initially identified as a 67-kDa non-integrin cell-surface protein for laminin, the major glycoprotein of the extracellular matrix (Rao 1983). Lately, in 1988, a protein called p40, associated with 40S ribosomal particles in mouse tumour cell lines, was shown to have complete similarity with human 37/67 kDa LR (Makrides 1988).

The 37/67 kDa LR is encoded by the human ribosomal protein SA gene (RPSA) whose product consists of 295 amino acids protein that migrates with an apparent molecular mass of 37 kDa on SDS-PAGE, hence it is also defined as 37 kDa laminin receptor precursor (37 kDa LRP) (DiGiacomo and Meruelo 2016, Nelson 2008). To date, several evidence support a precursor-product relationship between 37 kDa LRP and 67 kDa LR. For instance, in radiolabelled human melanoma cells, pulse-chase experiments clearly demonstrated the transition of 37 kDa LRP into the higher molecular isoform over time (Rao 1989). Moreover, different antibodies showed cross-reactivity between the two isoforms (DiGiacomo and Meruelo 2016, Nelson 2008). Nevertheless, it still remains poorly understood what are the exact molecular mechanisms responsible for the conversion of 37 kDa LRP into the higher molecular isoform and why this protein complex would be so stable during denaturating/reducing SDS-PAGE. The most tempting hypothesis highlights acylation with fatty acids as a prerequisite to homo-dimerization or hetero-dimerization (Landowski 1995, Butò 1998).

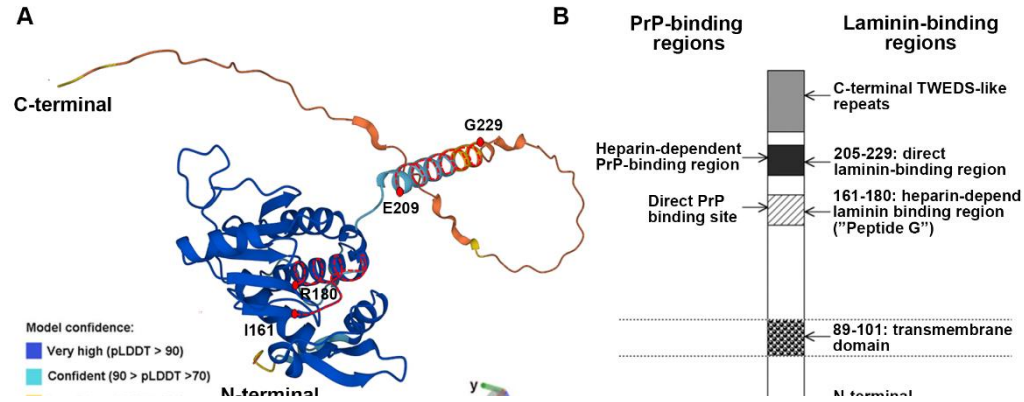

Figure 1 Structure and main functional regions of 37/67 kDa LR A) 3D models of the full length 37 kDa LRP. 37 kDa LRP consists of a globular N-terminal domain and an intrinsically disordered, unstructured C-terminal domain. The two main binding sites for PrP^C are indicated in the figure with dashed lines (in red), namely, the 161–180aa and 209–229aa region. To generate 3D models Alpha-Fold software was used producing a per-residue confidence score (pLDDT) between 0 and 100. Protein regions below 50 pLDDT may be unstructured in isolation (Adapted from Limone, Maggisano 2023). B) Schematic representation of the main functional domains of 37/67 kDa LR, PrP-binding regions and laminin-binding regions (adapted from Nelson 2008).

The 37 kDa LRP consists of a globular N-terminal domain (a.a. 1-209) and of an intrinsically disordered C-terminal domain (a.a. 210-295) (Figure 1) (Jamieson 2008). From an evolutionary point of view, the protein is likely originated as ribosomal component, indeed the N-terminus shows high homology to prokaryotic s2 family ribosomal protein, whereas the laminin-binding function in vertebrates is linked to the evolution of the C-terminal domain, which is absent in prokaryotes (Ardini 1998).

The sequence of 37 kDa LRP does not contain neither a canonical transmembrane domain, nor a N-linked carbohydrate-attachment site, thus, the membrane targeting mechanism also remains elusive. The putative membrane-spanning region resides within residues 89-101 (Rao 1989). Alternatively, another highly predicted region for membrane spanning is within residues 112-116 that forms a hydrophobic loop that could serve as membrane-inserted segment, as well as domain for protein-protein interaction (such as the interaction with PrP, prion protein, as discussed later and indicated in Figure 1).

It is likely that as consequence of unclear modifications of 37 kDa LRP, the C-terminal of the protein might be translocated through the membrane to create the extracellular laminin-binding region of the functionally active 67 kDa LR. Accordingly, in the structure of the 37 kDa LRP, the main laminin binding site, also known as peptide G, is buried within the protein, thus supporting the hypothesis that significant conformational changes would be required for membrane insertion and high-affinity laminin binding.

To date, available data do not clearly identify what are the structural changes, nor what are the regions involved in the transition of 37 kDa LRP to 67 kDa LR. However, it is tempting to speculate the hypothesis of an equilibrium between multiple conformers of the protein which could explain the diversity of 37/67 kDa LR functions. Hence, the structural versatility of 37/67 kDa LR correlates with its multifunctionality, indeed its functions are surprisingly diverse and reflect the protein's presence in the plasma membrane, ribosomes, cytosol and in the nucleus.

The most well-established function of 67 kDa LR on the plasma membrane is cell anchoring via laminin binding (DiGiacomo and Meruelo 2016, Nelson 2008). The receptor contains three laminin-binding regions: a) the C-terminal 53 amino acids containing acidic TEDWS repeats; b) the residues 205-229 (direct laminin binding-region) and c) the residues 161-180, also known as peptide G (heparin-dependent laminin binding region) that contains a palindrome (LMWWML) responsible for the laminin-binding (Figure 1). Beside cellular adhesion, laminin-binding through 67 kDa LR influences several cellular processes including migration, homing, proliferation, polarity, differentiation and tissue development.

Conversely, the cytosolic 37 kDa LRP mainly associates with the 40S small subunit of ribosomes, thus being involved in protein synthesis as well as ribosomal biogenesis and pre-rRNA processing.

Despite the absence of a clearly identifiable nuclear localization signal, nuclear functions of 37 kDa LRP have been identified. The receptor interacts

with chromatin, both directly with DNA and more tightly with histones H2A, H2B and H4 (Kinoshita 1998). Notwithstanding the consequences of this association with chromatin are not clear, although it suggests a role in chromatin maintenance or modification.

Beside its physiological functions, 37/67 kDa LR is also implicated in several pathological conditions, such as cancer, microbial and viral infections, congenital defect, and neurodegenerative disease including Alzheimer's disease (AD), as discussed in the paragraph 2.3.

2.2. Alzheimer's disease pathogenesis

Alzheimer's disease (AD) is a progressive neurodegenerative disorder, and it represents the main cause of dementia world-wide for which cure, or disease-modifying therapies are not available yet (Lane 2018).

The most characterized neuropathological hallmarks of AD are amyloid plaques and neurofibrillary tangles (NFTs) in the cerebral cortex that can coexist with other features such as neuropil threads, dystrophic neurites, astrogliosis, microglial activation, and cerebral amyloid angiopathy. The downstream consequences of these pathological aberration are synaptic and neuronal loss leading to extensive cortex atrophy and ultimately dementia.

Amyloid plaques are extracellular accumulations composed of abnormally folded amyloid-beta peptides (Aß), whereas neurofibrillary tangles consist of intracellular paired and helically wound filaments derived from the abnormal hyperphosphorylation of the microtubule-associated Tau protein (Tiwari 2019).

The toxic conformations of Aβ and Tau can induce the conversion of the corresponding normal proteins into pathological conformations, thus resembling a prion-like misfolding mechanism and the spreading of the disease across the brain (as reviewed in Sarnataro 2018).

The so-called "amyloid cascade hypothesis" is the prevalent theory to explain AD pathogenesis highlighting the accumulation of Aβ as the crucial event of the pathology (Golde 2005). Aß is generated from the proteolytic processing of amyloid precursor protein (APP), a transmembrane type I protein of 295 amino acid. APP can be alternatively processed via two independent routes (Figure 2) (Tan 2019).

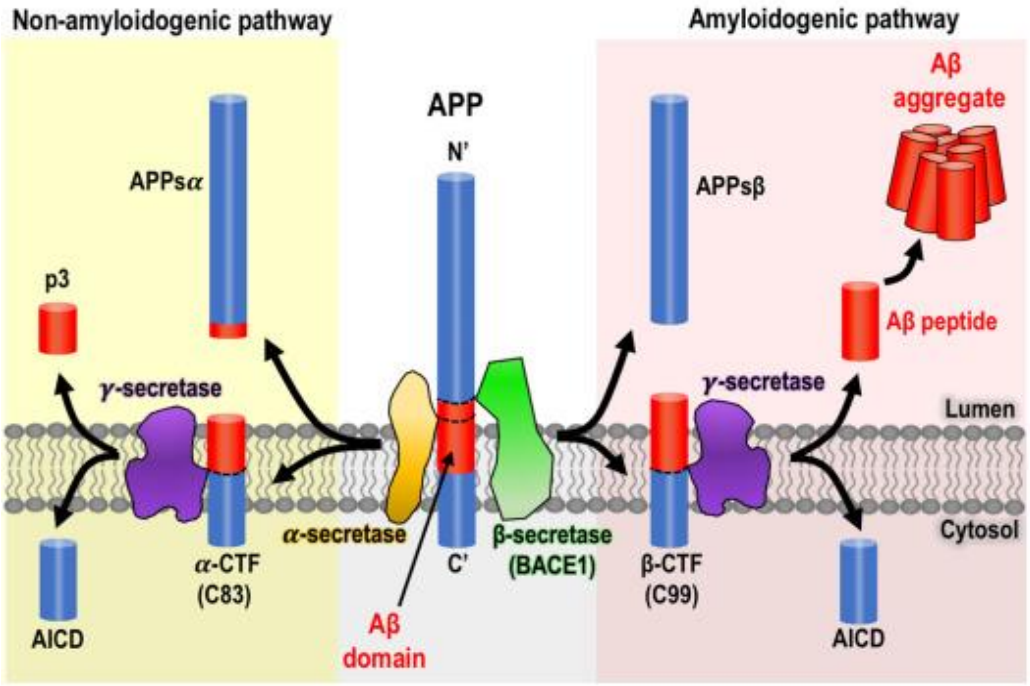

Figure 2. Alternative processing of APP in amyloidogenic and non-amyloidogenic pathways. In physiological conditions, APP is mainly processed by the non-amylodiogenic pathway through the sequential cleavage by α- and ß-secretase preventing the release of Aß peptide. Coversely, in pathological conditions, APP is processed toward the amyloidogenic pathway associated to the production of Aß by the sequential cleavage mediated by ß- and γ-secretase (Tan 2019).

In physiological conditions APP is mainly processed via the non-amyloidogenic pathway that involves the activity of α-secretase to generate a membrane-bound C-terminal fragment (α-CTF or C83) and a large secretory APPsα ectodomain. The α-CTF is subsequently cleaved by γ-secretase releasing a 3 kDa peptide called p3, and the APP intracellular domain (AICD). Since α-secretase cleaves APP within the Aß domain, the non-amyloidogenic pathway is not associated to Aß production.

Conversely, in pathological conditions APP is mainly processed through the amyloidogenic pathway by the sequential action of ß- and γ-secretase that determines Aß release. Hence, BACE1, which is the major ß-secretase in the brain, cleaves APP at the N-terminus of the Aß domain generating the membrane-bound CTF (ß-CTF/C99) and a secretory APPsß ectodomain. ß-CTF is further processed by the multi-subunit complex γ-secretase to release the Aß peptide. The processing by γ-secretase is not restricted to a single site, indeed it generates different Aβ species. The two main types of Aβ peptides consist of 40 or 42 amino acids, as consequence they are defined $Aβ_{40}$ and $Aβ_{42}$ respectively. $Aβ_{42}$ is more neurotoxic because it is highly insoluble and more aggregation prone than $Aß_{40}$.

Aß peptides aggregate to form amyloid fibrils and plaques that are considered one of the causative agents leading to neurodegeneration in AD.

The “amyloid hypothesis” is strongly supported by genetic cases of familial early-onset AD (fAD/EOAD). EOAD are rare inherited autosomal dominant forms of AD characterized by the early appearance of symptoms (between 30 and 65 years of age) (D’Argenio 2020). To date, genetic mutations responsible for EOAD have been discovered in APP, presenilin-1 and -2 (PSEN1/2) gene, even though they explain just a small percentage of all fAD cases. The majority of EOAD-related APP mutations result in enhanced Aß production or production of Aß species more prone to self-aggregate (Shao 2017, Tomiyama 2008). Similarly, the mutations identified in PSEN1/2 increase $Aß_{42}$ ratio (Bertram 2012). Altogether, the discovery of the mutations in APP, PSEN1, and PSEN2 emphasizes that the aberrant Aβ production and aggregation is a cause of AD, thus supporting the amyloid hypothesis.

The membrane trafficking of APP and of the secretases plays a crucial role in the processing of APP itself and production of Aß peptides (Tan 2019). The co-residence of APP and the secretase enzymes along their trafficking route is crucial to understand where the processing of APP itself and the production of Aβ occurs. This scenario strengths the hypothesis that the modulation of APP intracellular trafficking could be considered as therapeutic strategy to divert APP towards the non-amyloidogenic protective pathway and reduce the production of Aß.

The initial cleavage of APP by BACE1 is the rate-limiting step along the amyloidogenic pathway and therefore their convergence at the same location is essential for the biogenesis of Aβ peptides.

BACE1 is an aspartyl protease, thus it is active in acidic intracellular compartments including the endosomes and Golgi that have been demonstrated to be the main sites where Aß production occurs (Kinoshita 2003, Toh 2017) (Figure 3).

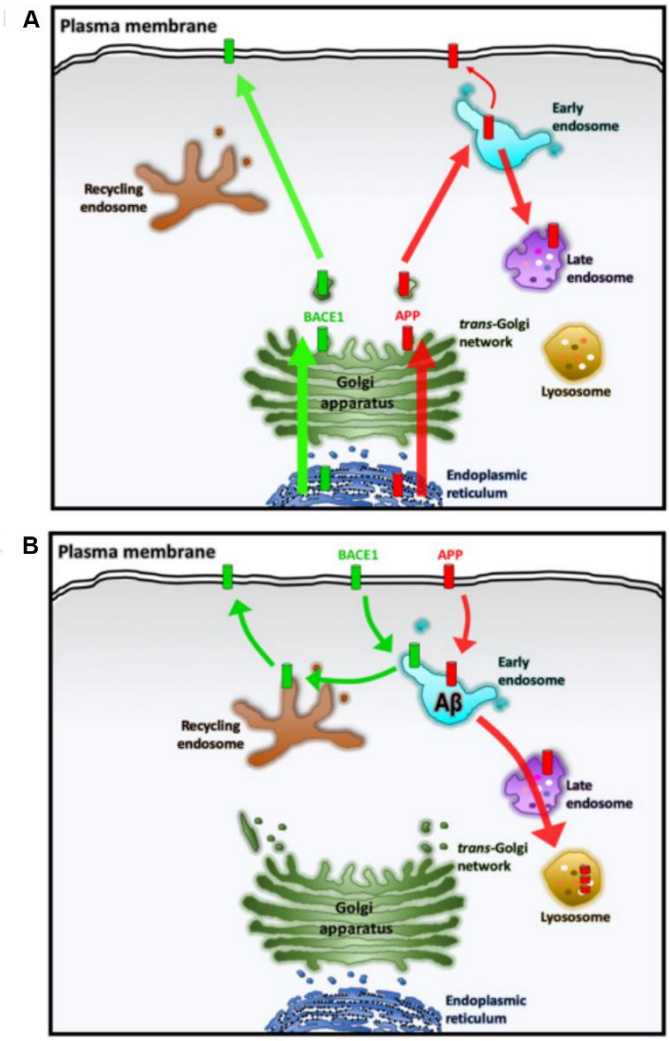

Figure 3. Intracellular trafficking of APP and BACE1. A) APP and BACE1 are synthetized in the endoplasmic reticulum and transported to the plasma membrane after acquiring the correct post-translational modifications in the Golgi apparatus. From the Golgi apparatus, APP is transported to the plasma membrane via the early endosomes, whereas BACE1 is directly transported to the plasma membrane. B) APP and BACE1 are internalized from the plasma membrane to the early endosomes that represent the major site for Aß production. From the early endosomes, APP traffics to the lysosomes for the degradation, whereas BACE1 is recycled to the plasma membrane (adapted from Tan 2019).

Conversely, it is largely assessed that α-secretase cleavage of APP mainly occurs at the plasma membrane (PM) (Sisodia 1992).
In support of this model, several studies confirmed that Aβ production occurs in the endolysosomal compartments where the acidic pH facilitates the activity of β- and γ-secretase, indeed the deacidification of these compartments through alkylating agents (Schrader-Fischer 1996), or the impairment of APP endocytosis (Koo 1994), resulted in a reduction of Aβ production.

Beside their co-residence in early endosomes (EE), APP and BACE1 also converge along the secretory pathway. Both APP and BACE1 are synthesized in the endoplasmic reticulum (ER) and undergo several post-translational modifications within the Golgi to become the mature isoform of the protein. The co-transport of newly synthesized APP and BACE1 along the secretory pathway is likely to provide an opportunity for APP processing and subsequent Aβ production (Busciglio 1993, Perez 1996).
However, it remains unclear whether the endocytic or secretory pathway plays a more significant role in Aβ production.

The relevance of intracellular trafficking for amyloidogenesis is also highlighted by recent genome-wide association studies that identified trafficking machinery genes as a class of risk-genes related to sporadic AD (as reviewed in Limone 2022b). These studies give striking support to the so-called "endolysosomal hypothesis" according to which the endolysosomal compartments may represent a "hub" that contributes to alteration of protein homeostasis and the accumulation of misfolded proteins in AD. Accordingly, several studies revealed endosome enlargement as a very early cytopathological signs in AD models (Cataldo 1997, Cataldo 2000; Nixon and Cataldo 1993, Nixon 2017).

Collectively, the dysfunction of intracellular degradative pathways, the amyloidogenesis and other emerging molecular mechanisms, may synergically contribute to cellular toxicity and neuronal death. To date, it is clear that AD is an extremely complex disease determined by multiple, simultaneous and interacting pathophysiological processes and it is not possible to reduce its pathogenesis to a single hypothesis. In this context, I would like to highlight that preliminary data from our lab in fibroblasts from genetic form of AD, strongly indicate that modulation of APP trafficking, which is altered in AD conditions, reflects on Aβ generation. However, due to space and time constraints, I will not show these results here.

2.3. The 37/67 kDa LR implication in Alzheimer's disease pathogenesis

Early version of the amyloid hypothesis sustained a crucial role for extracellular amyloid plaques as trigger of neurotoxicity in AD, however, more recent studies demonstrated that smaller and readily diffusible assemblies of pathogenic proteins represent the main cytotoxic form (reviewed in Muck and Selkoe 2012). In this scenario the plaques might be considered as local reservoir of diffusible oligomers and fibrils. The oligomeric assemblies of hydrophobic Aß bind to many molecules on the plasma membrane, e.g. proteins, lipids and extracellular matrix components, initiating multiple deleterious signalling pathway that ultimately lead to neurotoxicity (reviewed in Shrivastava 2017). This interaction promotes further pathogenic protein aggregation and abnormal redistribution of membrane components with consequences on overall membrane integrity and topology. Collectively, the effects caused by the binding

of oligomers to the plasma membrane are responsible for synaptic dysfunction, cytotoxicity and ultimately neurodegeneration.

Among the membrane proteins interacting with Aß, it has been identified the cellular prion protein (PrP^{C}) whose pathological isoform PrP^{Sc} is associated to neurodegenerative prion diseases (Aguzzi 2000). Accordingly, to Laurén (2009), who first described the interaction between PrP^{C} and Aß, PrP^{C} acts as high-affinity receptor for Aß oligomers initiating a neurotoxic signalling pathway mediated by Fyn kinase that alters both the function of NMDA receptors and neuronal synapsis (Um 2012).

PrP^{C} is a GPI-anchored protein, whose functions in both physiological and pathological conditions, strongly rely on its association with transmembrane receptors. Among these receptors, the 37/67 kDa LR has been shown to bind PrP^{C} with high affinity (DiGiacomo and Meruelo 2016, Nelson 2008). In particular, two binding regions for PrP^{C} on 37/67 kDa LR have been identified: a) 161–180aa region (Peptide G), which serves also as binding site for laminin and heparan sulphate proteoglycans (HSPG); b) 209–229aa region that similarly binds both laminin and PrP^{C} (Figure 1). On the other hand, two binding sites on PrP^{C} have been identified to interact with 37/67 kDa LR: a) the 144–179aa region (PrPLRPbd1), which mediates a direct interaction; b) the 53–93aa region, (PrPLRPbd2), mediates an indirect HSPG-dependent interaction (Sarnataro 2017, Hundt 2001).

Since the 37/67 kDa LR and Aß share several common binding partners, e.g. PrP^{C}, the hypothesis of an association between the 37/67 kDa LR and Aß warranted investigation. To date, there are studies highlighting the 37/67 kDa LR as a receptor responsible for Aβ internalization and citotoxicity. Fluorescence resonance energy transfer (FRET) and pull-down assays demonstrated the interaction between the 37/67 kDa LR and $Aß_{42}$ on cell surface of HEK293 cells (Da Costa Dias 2014). Particularly, the 37/67 kDa LR mediated $Aß_{42}$ internalization through endocytosis and contributed to the cytotoxicity of the neuropeptide. Accordingly, antibody blockade or shRNA-mediated downregulation of 37/67 kDa LR hampered Aß internalization and Aß-induced cytotoxicity in HEK293 and N2a cells (Da Costa Dias 2014, Pinnock 2016).
Although FRET assay assesses energy transfer only when donor and acceptor fluorochromes are within 10 nm from each other, to date, it is still debated whether the interaction between the 37/67 kDa LR and Aß is direct or indirectly mediated by PrP^{C}. Indeed, Pinnock (2016) demonstrated that 37/67 kDa LR and $Aß_{42}$ co-localize only on the cell surface of PrP^{C} expressing cells. In agreement, $PrP^{C\,-/-}$ cells are more resistant to cytotoxic effects of exogenous $Aß_{42}$ than PrP^{C} expressing N2a cells. The employment of anti-37/67 kDa LR specific antibody contrasted Aß-induced toxicity in PrP^{C} expressing cells. Collectively these data suggest that 37/67 kDa LR plays a crucial role in $Aß_{42}$ mediated cytotoxicity acting as receptor for the neuropeptide thanks to a likely indirect interaction mediated by PrP^{C}.

Beside its role as receptor for Aß, the 37/67 kDa LR is involved in the processing of APP, i.e. Aß production. High resolution imaging study showed a

direct interaction of 37/67 kDa LR with presenilin-1 (PS1) subunit of γ-secretase and an indirect interaction with BACE1 (Jovanovic 2014). Although, the 37/67 kDa LR and BACE1 do not directly interact, they share several common binding partners, most notably PrP^C (Griffiths 2011). The functional relevance of 37/67 LR in the amyloidogenic processing of APP is strengthened by the finding that its blockade *via* specific antibody or downregulation, strongly reduced sAPPß as well as Aß shedding in both HEK293 and SH-SY5Y cells (Jovanovic 2013). Similarly, intranasal administration of 37/67 kDa LR specific antibody to mice expressing human APP and PSEN1 transgenes with a total of five AD-linked mutations (namely 5xFAD), caused a reduction of both insoluble and soluble $Aß_{42}$ in brain homogenates, as well as a decrease in amyloid plaque formation in the hippocampus (Ferreira 2018). The blockade of 37/67 kDa LR improved short-term memory, learning and cognitive functions of 5XFAD transgenic mice. It is likely that the inhibition of 37/67 kDa LR reduced Aß generation and toxicity, thus alleviating AD symptoms.
Altogether these data sustain that 37/67 kDa LR might be involved in the regulation of amyloidogenic processing of APP.

In summary, 37/67 kDa LR is involved in AD pathogenesis acting both as a receptor for Aβ internalization, as well as regulator of APP processing and Aβ shedding. For this reason, the 37/67 kDa LR represents an appealing target for the development of new therapeutic strategies against AD.

2.4. Signalling pathways downstream 37/67 LR kDa and autophagy regulation

The interaction of 37/67 kDa LR with Aß oligomers could contribute to cytotoxicity by stimulating aberrant signalling pathways. Among the well-established signal transduction downstream 37/67 kDa LR, there are PI3K/Akt and MAPK pathways (Sun 2014). For instance, in gastric cancer cells both adhesion to laminin substrate and upregulation of 37/67 kDa LR, greatly increased Akt and ERK1/2 phosphorylation through the interaction of the receptor with phosphorylated Focal Adhesion Kinase (FAK). Conversely, silencing of 37/67 kDa LR reverted these effects and enhanced sensitivity of cells to chemotherapeutics.
Similar results were obtained by Wu and colleagues (2019), that reported the activation of PI3K/Akt and MAPK/ERK1-2 signalling pathways as consequence of the interaction between 37/67 kDa LR and Integrin alpha 6 in pancreatic cancer cell lines, influencing invasion and metastasis.
Additionally, in Merlin-deficient tumours overexpressing PrP^C, the engagement of downstream MAPK, PI3K/Akt and FAK signalling pathways occurred via 37/67 kDa LR dependent mechanism (Provenzano 2017). This study represents the first demonstration of a dependence relationship between PrP^C and 37/67 kDa LR on the stimulation of downstream signalling routes; thus, it is likely that the effects of 37/67 kDa LR on signal transduction could be mediated by its interaction with PrP^C (Limone, Maggisano 2023). Hence, PrP^C has been linked

to PI3K/Akt and MAPK pathways, however, whether these effects occur through 37/67 kDa LR needs further investigation.

Although the aforementioned studies linked the 37/67 kDa LR to the pathogenesis of cancer by the stimulation of signalling pathways related to cellular proliferation, survival and invasion, it is not possible to rule out the impact on further similarly regulated cellular mechanisms, e.g. autophagy, which is known to be compromised in neurodegenerative diseases (see next paragraph).

Autophagy is normally active at a basal level to ensure maintenance of cellular homeostasis; however, several extracellular and intracellular stimuli can boost its activity, above all nutrient starvation strongly triggers autophagy (Glick 2010). Most of the signalling pathways regulating autophagy in mammalian cells involve the serine/threonine kinase, mammalian target of rapamycin (mTOR) which is considered the master regulator of autophagy (Ravikumar 2010). mTOR can form two functional complexes based on its interacting proteins: a) a rapamycin-sensitive mTOR complex 1 (mTORC1) and b) mTOR complex 2 (mTORC2). mTORC2 activity is not related to autophagy regulation, whereas many pro-autophagic stimuli, such as nutrients availability, amino acids, growth factors, glucose, energy status and stress (hypoxia, ER stress), regulate mTORC1 activity and autophagy.

Among the most important nutrient-sensing pathways controlling mTORC1 activity there are PI3K/Akt and MAPK/ERK1-2 pathways (Figure 4).

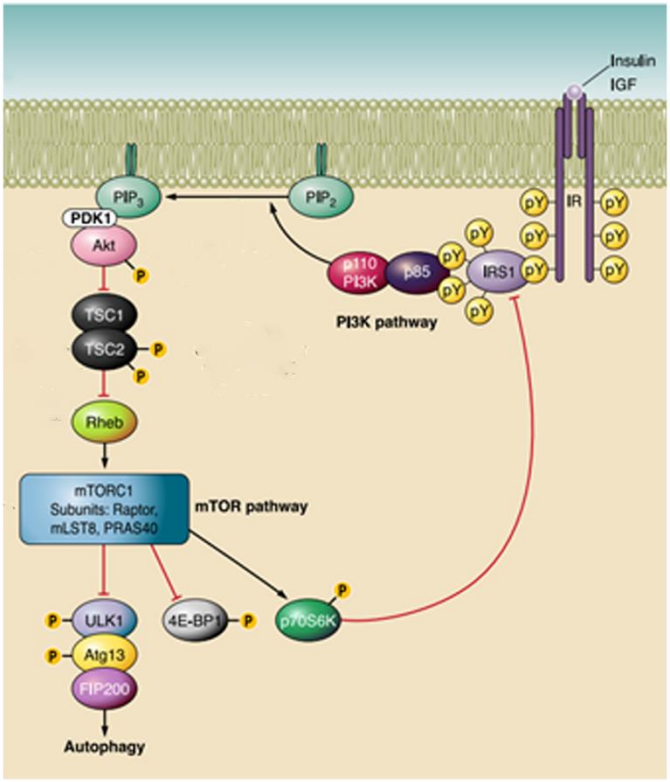

Figure 4. Main signalling pathways regulating mTORC1 activity. The binding of growth factor or insulin to cell surface receptors triggers the phosphorylation of the receptor itself and its interaction with the regulatory domain of PI3K (p85) that recognizes phosphotyrosine residues in the receptor or adaptor proteins. This interaction activates the catalytic subunit of PI3K (p110) that converts PIP_2 into PIP_3 allowing the recruitment and activation of Akt. Akt controls mTORC1 activation by inhibiting the GAP protein complex TSC1/TSC, thus favouring a GTP-bound state of Rheb and activation of mTORC1. Once activated mTORC1 complex inhibits autophagy and enhances protein synthesis by phosphorylation of specific substrates (adapted from Ravikumar 2010).

The binding of growth factors or insulin to cell surface receptors activates the class Ia PI3K which is composed of a regulatory subunit p85 and a catalytic subunit p110 (Ravikumar 2010). The regulatory subunit p85 directly interacts with phosphotyrosine residues of activated receptors or adaptor proteins and activates the catalytic subunit p110. Once activated PI3K phosphorylates plasma membrane phosphatidylinositol-4,5-bisphosphate (PIP2) converting them into phosphatidylinositol-3,4,5-trisphosphate (PIP3). PIP3, in turn, interacts with proteins containing a pleckstrin homology domain (PH) among which the serine/threonine kinase Akt that is further activated by phosphorylation at Thr308/Ser473. Among the target of Akt there is mTOR, whose Ser-2448 is a direct target site for Akt (Sekulić 2000, Rosner 2010). Moreover, to be activated mTOR needs the interaction with GTP-bound Rheb protein, whose activity is controlled by the GAP protein complex TSC1/2 (Ravikumar 2010). Akt by phosphorylating TSC1/2 complex, inhibits its action, thus favouring GTP-bound

state of Rheb and activation of mTORC1. Similarly, to Akt, also ERK1/2 regulates mTORC1 activity by phosphorylation and inhibition of TSC1/2 complex. Therefore, TSC1/2 acts as an upstream integrator of many signals, including both Akt and ERK1-2, that regulate mTORC1 activity.
Beside this, it is important to underline that further important pathways regulate mTORC1 such as amino acids availability, energy status *via* AMPK activity, Ca^{2+}/calmodulin-dependent kinase III. Furthermore, autophagy could be also controlled by mTOR-independent pathway. However, the description of these mechanisms goes beyond the interest of this overview as they are not, to date, correlated to 37/67 kDa LR.

Under nutrient-rich conditions, activated mTORC1 inhibits autophagy by phosphorylating components of the complex responsible for autophagy initiation (see next paragraph). Conversely, pro-autophagic stimuli trigger mTORC1 inactivation and autophagy initiation. Inhibition of mTORC1 and induction of autophagy is correlated with reduced phosphorylation of its downstream effectors, ribosomal protein S6 kinase (S6K) at Thr389/Thr421/Ser424 and translation initiation factor 4E binding protein-1 (4E-BP1) at Thr37/Thr46.

2.5. Mechanism of autophagy

Autophagy is a catabolic cellular pathway that allows the degradation of cytoplasmic components, e.g. proteins, lipids and organelles, via lysosomes (Mizushima 2011). To date, at least three types of autophagic pathway have been described: a) macroautophagy which is characterized by the formation of autophagosomes, b) chaperon-mediated autophagy, in which protein substrates are recognized by the chaperone HSC70 and translocated into the lysosome through LAMP2A, and c) microautophagy, in which parts of the cytoplasm are directly engulfed by the lysosomal membrane. However, macroautophagy, herein referred as autophagy, is the best characterized mechanism and the one on which the following dissertation is focused on.

Briefly, following the induction of autophagy, a portion of the cytoplasm is sequestered by a growing membrane structure, known as the "isolation membrane" or phagophore, that elongates and encloses to form a double-membrane structure, known as autophagosome. The outer membrane of autophagosome fuses with lysosomes to form the so-called autolysosome, where the hydrolases degrade the inner membrane of the autophagosome and its cargo. The autophagosome formation is a tightly regulated process controlled by the hierarchical and coordinated activity of autophagy-related protein (ATG), discovered through genetic studies in yeast. ATG proteins assemble into multimeric complexes, each of which regulates a specific step of the autophagosome formation. Highly conserved key steps of autophagosome formation include initiation, vesicle nucleation, elongation and maturation of autophagosome (Figure 4).

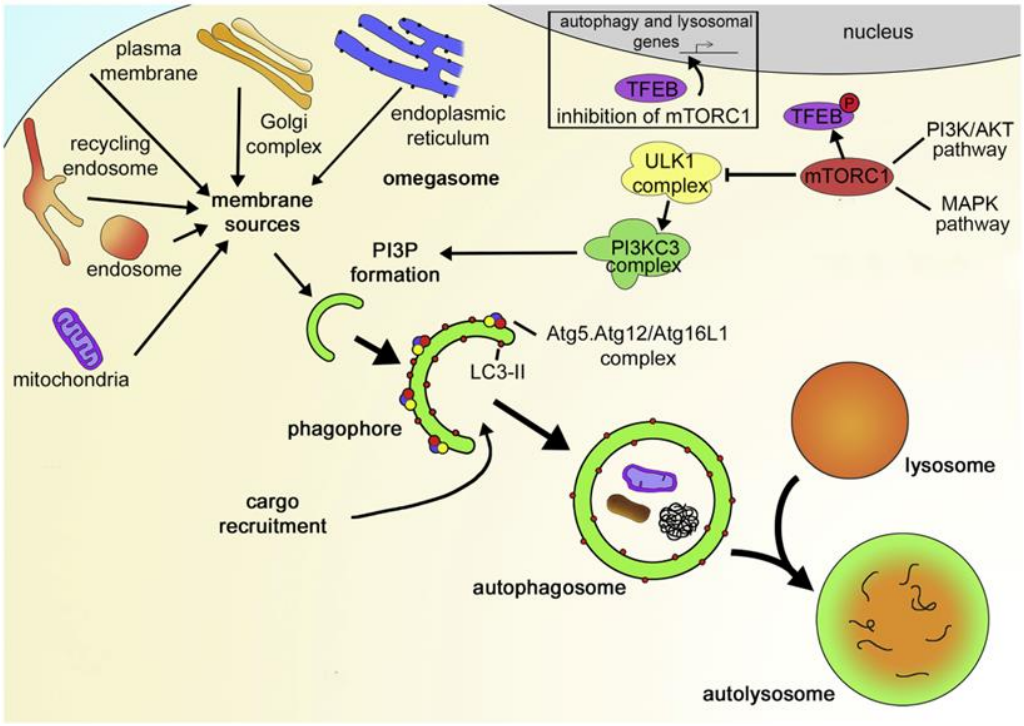

Figure 5. Autophagosome biogenesis steps. Autophagosomes formation is controlled by the activity of different ATG protein complexes that strictly regulate each step. ULK1/2 complex coordinates autophagy initiation and its activity is negatively controlled by the mTORC1. Once activated, ULK1 complex favours PI3KC3 complex activity that regulates the formation of PIP3 on specific subdomain primarily on the ER, defining the omegasome. Interestingly, the membranes that contribute to the extension of the phagophore can derive from different sources including endosomes, mitochondria, plasma membrane and Golgi complex. PIP3 allows the recruitment of downstream complexes responsible for vesicle elongation, which encloses cytoplasmic components, forming a double-membrane structure defined autophagosome that, upon fusion with lysosomes, ultimately becomes autolysosome. The maturation of phagophore into autophagosome is under the control of two ubiquitin-like conjugation systems. The first one results in the formation of ATG12-ATG5-ATG16L1 multimeric complex on the membrane of the extending phagophore, which in turn is responsible for the conjugation and correct targeting of the second ubiquitin-like molecule, namely LC3- I (adapted from Menzies 2017).

The intracellular site where the nucleation of isolation membrane occurs is still largely debated. To date, the main source of autophagosome in mammalian cells is considered to be the specialized subdomains of the ER defined omegasome, as they structurally resemble the Ω-like shape (Hayashi-Nishino 2009). However, growing evidence sustained the contribution of other organelles to autophagosome formation including Golgi membrane, endosomes, plasma membrane, nuclear envelope and mitochondria (Limone 2022, Puri 2013, Hayashi-Nishino 2010, Hailey 2010).

The initiation of autophagy and nucleation of isolation membrane is ascribed to Unc-51-Like kinase (ULK1) complex, whose activity is negatively regulated by the autophagy master regulator mTORC1 (Mizushima 2011) (Fig.4). The ULK1 complex is constitutively formed by ULK1/2 kinase, mAtg13, FIP200 and Atg101. Under nutrient-rich conditions, mTORC1 phosphorylates ULK1 and Atg13, thus exerting a suppressive effect on the complex activity (Hosokowa 2009). On the contrary, during starvation, mTORC1 is inactivated, thus releasing its inhibitory effect on ULK1 complex, that once activated, translocates from the cytosol to the ER. Subsequently to

ULK1 complex, the class III phosphatidylinositol-3-kinase (PI3K3C) complex participates to the nucleation of isolation membrane (Mizushima 2011). The PI3K3C complex consists of class III PI3K, Beclin1, Atg14L, Vps15 and AMBRA1; it uses phosphatidylinositol (PI) as substrate to generate phosphatidylinositol 3-phosphate (PI3P) at specific ER subdomain defining the omegasome region. The mechanism through which the upstream ULK1 complex regulates the recruitment and activity of PI3K3C complex is still not fully understood. One of the possible mechanisms requires the phosphorylation of AMBRA1 protein by ULK1 complex thus allowing the translocation of PI3K3C complex to ER (Di Bartolomeo 2010). Moreover, Atg14L has been discovered as the essential PI3K3C complex subunit responsible for its localization at the ER (Matsunaga 2010). PI3P enrichment on the omegasome is a crucial event for the progression of autophagosome formation as it allows the recruitment of PI3P effectors and the progression towards elongation and maturation of the phagophore into autophagosome (Mizushima 2011).

The elongation step relies on the activity of two ubiquitin-like conjugation systems. The first one is ATG12 conjugation system, and it localizes specifically to the isolation membrane. ATG12 is activated by ATG7, an E1-like enzyme, then it is transferred to Atg10 (E2-like enzyme) and finally it is conjugated to Atg15 (Mizushima 1998). The Atg12-Atg5 conjugate interacts with Atg16L to form a complex with a 2:2:2 stoichiometry via homodimerization of Atg16L. The second ubiquitin-like conjugation system is the LC3-conjugation system, which peculiarly target LC3 to a phospholipid, hence this process is also defined as LC3 lipidation (Ichimura 2000). LC3 is synthetized as a precursor, which is cleaved by Atg4 to generate a C-terminal glycine exposed form of the protein, known as LC3-I. LC3-I is activated by Atg7, transferred to Atg3 (E2-like enzyme) and finally covalently bound to phosphatidylethanolamine (PE) generating LC3-II on the growing autophagosome. Notably, the Atg12-Atg5-Atg16L complex, generated by the first ubiquitin-like system, is essential to conjugate LC3 to PE, as it acts as E3-like enzyme to promote LC3 lipidation. LC3-II is crucial for the maturation of the autophagosome, membrane tethering and hemifusion.

Extension of the phagophore is also assisted by mATG9, which is localized on trans-Golgi network and endosomal compartments. Its role in autophagy is to supply lipid bilayers to the growing phagophore (Young 2006).

Once the autophagosome completes fusion of the expanding ends of the phagophore, its outer membrane fuses with lysosomes to form autolysosomes, a process that depends on Rab7 activity (Gutierrez 2004).

2.6. A selective form of autophagy: mitophagy

Autophagy was initially recognized as a bulk degradation process induced by starvation that non-selectively transports cytoplasmic portion to the lysosomes. However, in the last decades, several studies revealed that autophagy can contribute to cellular homeostasis in non-starved cells by degrading cargos such as aggregate-prone proteins and damaged/dysfunctional organelles (Gatica

2018). The breakthrough in this scenario was the identification of ubiquitin-binding domain-containing receptors that act as a selective system to mediate the clearance of specific substates.

Mitophagy is a selective form of autophagy that specifically targets damaged mitochondria for degradation, thus it represents an essential pathway for mitochondrial quality control (Onishi 2021). The most important signal that trigger elimination of mitochondria through mitophagy is mitochondrial depolarization that activates the pathway involving the PTEN-induced putative protein kinase 1 (PINK1) and the E3 ubiquitin ligase Parkin (Narendra 2008, Narendra 2010) (Figure 6).

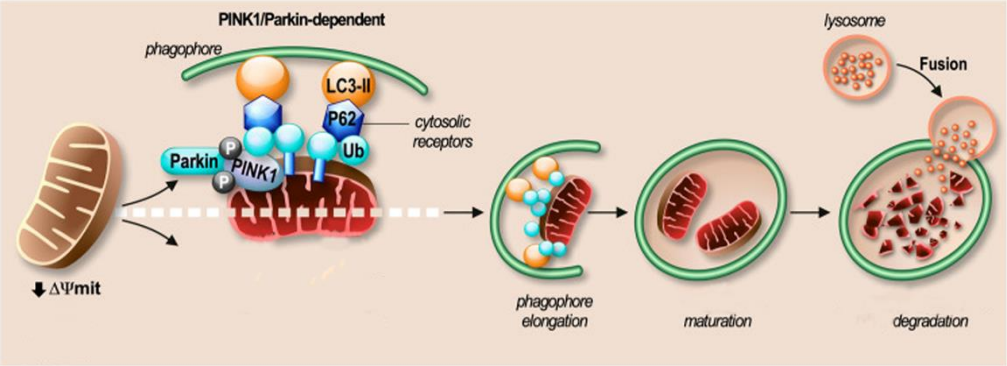

Figure 6 PINK1-Parkin mediated mitophagy. Loss of membrane potential of mitochondria triggers the activation of the PINK1-Parkin pathway. Hence, PINK1 is stabilized on the mitochondrial membrane, and it recruits and activates Parkin by Ser65 phosphorylation. Once activated Parkin mediates the poly-phospho-ubiquitination of several mitochondrial proteins that act as a "eat-me" signal for damaged mitochondria. Autophagy adaptors among which p62/SQSTM1, NDP52 and OPTN, recognize and bind to poly-ubiquitinated proteins and to LC3 on the extending phagophore. Once the phagophore is recruited, it engulfs damaged mitochondria and then, upon fusion with lysosomes, damaged mitochondria are degraded (adapted from Arnaud 2022).

PINK1 is targeted to mitochondria because of its mitochondrial targeting sequence, however, it is constitutively imported to the inner membrane and proteolytically degraded (Narendra 2010, Greene 2012). Loss of mitochondrial membrane potential precludes the import of PINK1; hence, it accumulates as dimer on the mitochondrial surface and mediates its own autophosphorylation on Ser228 and Ser402 (Okatsu 2012). Moreover, PINK1 regulates Parkin E3 activity by direct phosphorylation of Parkin on Ser65 (Kondapalli 2012). However, the Parkin phosphorylation itself is not sufficient for its activation, indeed another crucial target for the kinase activity of PINK1 is the Ser65 of ubiquitin molecules (Koyano 2014). Phospho-ubiquitin interacts with Parkin causing an intramolecular structural remodelling that accelerates E3 ligase activity. Once activated, Parkin mediates the ubiquitination of several outer mitochondrial membrane proteins on damaged mitochondrial including mitofusin, Miro, VDAC (Gegg 2010, Geisler 2010). Interestingly, Parkin seems to have low substate selectivity, instead it has evolved a spatial selectivity for depolarized mitochondria rather than substate specificity, thus allows an efficient

and quick ubiquitylation of dysfunctional mitochondria. The molecules of the core autophagic machinery work in concert with PINK1 and Parkin pathway. In selective autophagy processes, autophagy adaptors among which p62/SQSTM1, NDP52 and OPTN, have an important role in selection and uptake of the cargo (Johansen and Lamark 2011). The autophagic receptors contain both a ubiquitin-binding domain through which they recognize the cargo and an LC3-interacting region (LIR domain) that serves to recruit the phagophore membrane. Hence, these receptors work as adaptor molecule in between the cargo and the phagophore. The activity of mitophagy receptors is regulated by Tbk1-mediated phosphorylation that increase both ubiquitin chain and LC3 binding affinity (Heo 2015).

Beside their ability to recognize LC3 and the specific substrate, another crucial role of the receptors is to act as platform for the assembly and recruitment of other core ATG factors (Itakura 2012). The autophagic receptors can interact also with more upstream complexes of autophagic machinery to be recruited near damaged mitochondria. For instance, NDP52 directly binds the FIP200 subunit of ULK1 complex (Vargas 2019), whereas OPTN interacts and recruits Atg9-containg vesicles (Yamano 2020). Moreover, it has been demonstrated that PINK-Parkin pathway directly interacts with PI3K complex elements (Van Humbeeck 2011, Michiorri 2010). Parkin recruits AMBRA1 to the dysfunctional mitochondria leading to PI3K complex activation and nucleation of the isolation membrane around it. Collectively, these observations sustain that the PINK1-Parkin pathway works in concert with several components of the core autophagic machinery linking mitophagy to different steps of autophagosome formation.
Finally, the phagophore membrane extends surrounding damaged mitochondria and the formed autophagosome fuses with lysosomes to eliminate damaged mitochondria (McEwan 2015).

2.7. Autophagy impairment in Alzheimer's disease

The nervous system strongly relies on autophagic activity since, as terminally differentiated cells, neurons require an efficient autophagy to avoid the accumulation of toxic aggregates and damaged organelles that cannot be otherwise diluted through cell division. Accordingly, defects of the autophagic pathway are widely recognized as a mechanism responsible for neurodegenerative diseases pathogenesis. Therefore, autophagy induction may represent a therapeutic strategy for the neurodegenerative diseases by enhancing the removal of toxic aggregate-prone proteins.

Above all, the PI3K/Akt/mTOR signalling (described in the previous sections) is altered in neurodegenerative disorder including AD (Heras-Sandoval 2014). Dysregulation of the PI3K/Akt/mTOR pathway is commonly reported in AD brains, for instance increased Akt activation and Ser2448 mTOR phosphorylation has been observed in AD temporal cortex neurons (Griffin 2005). In addition, a significant loss and altered distribution of PTEN (the major negative regulator of Akt signalling), was also observed in AD neurons.

Furthermore, mTOR activity upregulation has been reported also in 3xTg-AD mice brains and in cell lines transfected with mutant APP (Caccamo 2011).

Beside the PI3K/Akt/mTOR pathway dysregulation, alteration of downstream autophagic machinery have been demonstrated in AD. Accumulation of autophagosomes and autolysosomes have been reported in *post mortem* brains of AD patients (Nixon 2005) and mice AD models. This indicates an unbalance between autophagosomes formation and degradation and sustains the inability of autophagic machinery to efficiently degrade toxic aggregates to guarantee the correct proteostasis.

Among the players responsible for autophagy deficiency in AD, there is the down regulation of the autophagic machinery components. In particular, Beclin1 is downregulated at both protein and mRNA levels in the neuronal population, in both human and mouse model of AD (Pickford 2008).

Another crucial step of autophagy, reported to contribute to autophagy dysregulation in AD, is autophagosome fusion with lysosome. This step is impaired in AD model, indeed the group of Tammineni et al., (2017) demonstrated defects of autophagosomes axonal transport in AD neurons.

Finally, other studies reported defects in lysosomal functions in AD pathogenesis, thus impairing the degradation step of autophagy. PS1, beside its role in APP processing, plays a crucial role in the transport and maturation of v-ATPase to lysosomes. Neurons derived from mice hypomorphic for PS1 (expressing extremely low levels of the protein, about 1% of the normal), or conditionally depleted of PS1, showed impairment of autolysosome acidification and cathepsin activation because of an altered targeting of the v-ATPase V0a1 subunit to lysosomes (Lee 2010). Accordingly, PS1 mutations causing EOAD produced a similar lysosomal/autophagy phenotype in fibroblasts from AD patients.

Interestingly, autophagy dysfunctions have been strongly related to amylogenesis, indeed Aß can be produced in autophagosomes where APP, BACE1 and γ-secretase converge (Yu 2005). Therefore, although autophagy represents an essential pathway for Aß clearance (Spilman 2010, Vingtdeux 2011), on the other hand it can also contribute to Aß generation into autophagosomes (Yu 2005). In addition, Aß itself has been reported to impair lysosomal function and autophagic clearance (Marshall 2020).

Collectively, these studies highlight a complex interplay between autophagic defects and amyloidogenesis. Indeed, alterations of autophagy cause the accumulation of autophagosomes that cannot be degraded, thus promoting Aß production that in turns further contributes to lysosomal/autophagy defects.
To date, the most reliable model proposed to explain autophagy dysregulation during the progression of AD pathogenesis, argues that at the early stage of the disease autophagy is upregulated before the deposition of Aß aggregates. Whereas in the later stage of the disease, Aß accumulation within the lysosomes impairs autophagy, and autophagosomes accumulates over the time further contributing to amyloidogenesis. In summary, initially upregulation of autophagy can sustain amyloidogenesis and Aß production that in turn

accumulates and impairs the autophagic flux, thus creating a vicious circle between autophagic flux impairment and enhanced amyloidogenesis.
Besides its link with amyloidogenesis, autophagic dysfunction in neurons can also be related to the accumulation of dysfunctional organelles such mitochondria, as described in the next paragraph.

2.8. Mitochondrial dysfunction and mitophagy impairment in Alzheimer's disease

Neurons are high-energy demanding cells; hence they are strongly dependent on an appropriate mitochondrial homeostasis to meet their energetic demand (Kann and Kovacs 2007). As consequence, mitochondrial dysfunctions are widely reported as a pathogenic mechanism causing neurodegeneration in AD.

One of the first evidence supporting the role of mitochondria dysfunction in AD is the reduced glucose and oxygen metabolism in patient's brains (Friedland 1983, Tohgi 1998).

Beyond the clinical features, mitochondrial involvement has been clearly demonstrated by studies that described structural and functional defects of this organelle.

One of the main function of mitochondria is ATP production by oxidative phosphorylation (OXPHOS). Downregulation of proteins belonging to oxidative phosphorylation complex and shortage of neuronal ATP are widely reported in AD brains (Kim 2000, Kim 2001, Liang 2008). Defective activity of mitochondrial enzymes detected in patients includes pyruvate dehydrogenase and ketoglutarate dehydrogenase (Gibson 1988, Perry 1980) and most remarkably cytochrome oxidase in the respiratory chain complex IV (Parker 1990).

Oxidative phosphorylation is strictly linked to reactive oxygen species (ROS) production; indeed, mitochondria represent the major source of ROS production (mainly superoxide anion), which derive by the electron leakage during the electron transfer along the respiratory chain (Li 2013). Oxidative stress derives from an imbalance between ROS production and antioxidant systems, thus causing excessive ROS accumulation that damages cells by oxidation of all major biomolecules (as reviewed in Misrani 2021). The brain is particularly susceptible to oxidative damage because of its high rate of oxygen consumption, elevated levels of polyunsaturated fatty acids, abundance of redox transition metal ions and low levels of antioxidant systems. Dysfunctional mitochondria and reduced oxidative phosphorylation correlate to diffuse oxidative stress reported in AD (Misrani 2021, Nunomura 2001). For instance, decreased levels of antioxidant and antioxidants enzymes have been extensively reported in AD (Omar 1999, Kim 2006, Venkateshappa 2012). Moreover, widespread increase of oxidative biomolecules products, e.g. lipid peroxidation, protein oxidation and DNA/RNA oxidation was observed in AD (Wang 2014).

Other important aspects of mitochondrial dysfunction reported in AD include calcium dis-homeostasis, mitochondrial DNA damage, altered

mitochondrial transport and altered mitochondrial dynamics (as reviewed in Misrani 2021); however, a precise description of these aspects is far from the focus of this overview.

The mitochondrial dysfunction in AD grounds its basis on structural alteration of mitochondria. Electron microscopy analyses revealed altered mitochondrial morphology in patient's brains, including, altered cristae organization, decreased mitochondrial size, accumulation of osmophilic material and lipofuscin vacuoles (Baloyannis 2006, Hirai 2001).

Interestingly, mitochondrial morphology and distribution is the resultant of dynamic processes, indeed these organelles undergo continual fission and fusion. Impaired balance between fusion and fission has been reported in AD, favouring excessive fission (Wang 2009, Manczak 2011, Wang 2008a). In support of this scenario, neurons treated with oligomeric Aß or overexpressing AD-causing mutation in APP, showed mitochondrial fragmentation and structural damage (Wang 2009, Wang 2008b). Similar results were observed also in the brain of Tg2576 and APP/PS1 mice (Trushina 2012).

In addition to mitochondrial dynamic processes, the structure and function of mitochondrial network are preserved by mitochondrial quality control mechanisms among which mitophagy that rapidly allows the degradation of dysfunctional mitochondria.

To date, impaired autophagy/mitophagy is widely recognized as an early feature of AD that, together with other molecular determinants of AD pathogenesis, is responsible for accumulation of dysfunction mitochondria (Swerdlow 2004). Besides to the alteration of the autophagic machinery already described in the previous section (e.g. downregulation of core autophagic components, alteration of autophagosomes transport and lysosomal dysfunction), alteration more specifically linked to mitophagic machinery have been identified in AD.

Impaired mitophagy is suggested by the accumulation of mitochondrial proteins in skin fibroblasts from sporadic AD patients and impaired capacity to degrade them (Martín-Maestro 2016, Drabik 2021). Similar results were reported in fAD fibroblasts and in induced pluripotent steam cell–derived neurons characterized by accumulation of mitochondrial protein TOM20 and mitophagic receptor p62 (Martín-Maestro 2017a). iPSC-derived neuronal stem cells knocked-in for PS1 (M146L) similarly showed elevated level of TOM20 and high-number of mitochondrial DNA copies together with increased expression of PINK1 and Parkin, enhanced Parkin recruitment to the mitochondria, but reduced recruitment of mitochondria in the autophagosomes (Martín-Maestro 2019).

Evidence of impaired mitophagy were also reported in AD patients' brain, that similarly displayed increased levels of mitochondrial proteins and mitochondrial DNA (Hu 2016), as well as altered PINK1 and Parkin expression accordingly to the disease stage (Martín-Maestro 2016, Du 2017). In addition, Martín-Maestro et al., also observed in AD-affected brains a downregulation of various proteins known to participate to autophagy and mitophagy processes, namely Optineurin (OPTN), ATG5, ATG12, Beclin-1, PI3K class III, ULK1, AMBRA1, BNIP3,

BNIP3L, FUNDC1, VDAC1, and VCP/P97 (Martín-Maestro 2017b). Accordingly, a reduced expression of Beclin1, ATG12, BNIP3, and PINK1, a decrease of p-S65-Ub, was also observed in the brains of APOEε4 heterozygote patients (Sohn 2021).
Moreover, the study of Fang et al. (2019), highlighted the link between mitophagy defects and accumulation of structural and functionally damaged mitochondria (reduced size, disorganized cristae and low ATP production) in post-mortem hippocampal brain samples from AD patients and in AD iPSC-derived neurons.
Another mechanism contributing to mitophagy defects in AD is linked to sirtuins, a group of proteins involved in the mitochondrial response to bioenergetic and oxidative stimuli. The downregulation of SIRT1 expression in AD patient brains, is correlated to mitochondrial dysfunction and autophagy/mitophagy impairment (Julien 2009). Indeed, SIRT1 is involved in the deacetylation and activity of several ATG proteins, as well as in the stabilization of PINK1 and upregulation of mitophagy proteins. Similarly, SIRT3, which is responsible for p62 clustering on ubiquitinated mitochondria, was found decreased in neurons of AD mice (Yang 2012).

Finally, impaired mitophagy is a very important aspect of AD pathogenesis, indeed it lies both upstream and downstream of Aß and Tau pathology in a vicious circle ultimately causing synaptic dysfunction and cognitive deficits (as reviewed in Kerr 2017).
Indeed, dysfunctional mitochondria, accumulated in neurons, promote Aß production and Tau phosphorylation. Several studies have demonstrated the link between mitochondrial dysfunction and Aß production, indeed the impairment of mitochondrial function through toxin or genetic approach accelerates Aß pathology (Esposito 2006, Chen 2015). On the other way around, Aß itself can negatively impact on mitochondrial function and integrity (Hou 2014, Casley 2002).
Collectively mitochondrial dysfunction can be an upstream inducer of Aß aggregation, but at the same time Aß can exacerbate the mitochrondial damage, thus inducing a vicious cycle in AD pathology.

The relevance of autophagy/mitophagy impairment in AD pathology and their correlation with amyloidogenesis supports the hypothesis that stimulation of these pathways represents an appealing therapeutic tool to be tested.

2.9. Small organic molecules as specific inhibitor of 37/67 kDa LR

Altogether the previous sections described the functional relevance of 37/67 kDa LR on several aspects of AD pathogenesis including its role in Aß-internalization, APP processing and Aß shedding and regulation of signalling pathway controlling autophagy. This overview reinforces the hypothesis to consider the 37/67 kDa LR as a potential target in AD to counteract several aspects of the disease and restore protein homeostasis.

The crystal structure of the human 37/67 kDa LR is an excellent platform for rational drug design. In the study from Pesapane et al. (2015), an *in silico*

screening approach, known as structural-based virtual screening (SB-VS), was applied to identify compounds from the NCI Diversity library able to selectively bind to the peptide G of 37/67 kDa LR and hamper laminin binding.
The SB-SV analysis identified 46 structurally diverse compounds to be validated in cell-based functional assay for their specificity in inhibition of cell binding to laminin-1, without altering that of other extracellular matrix proteins, such as fibronectin and vitronectin. These functional adhesion experiments of cells on laminin-coated wells led to identify a compound, NSC47924 whose IC50 was 19.35 μM.

Our previous studies demonstrated that NSC47924 is able to alter 37/67 kDa LR-PrP^C interaction both *in vitro* and in neuronal and non-neuronal cells causing the internalization of 37/67 kDa LR and its trafficking towards the endolysosomal route, whereas PrP^C was stabilized on the cell surface (Sarnataro 2016).

Moreover, as far as concern AD pathology, we found that human recombinant soluble 37LRP interacted with APP in mouse neuronal GT1 cells, and that such interaction was disrupted by a specific inhibitor of 37/67 kDa LR (Bhattacharya 2020a). Notably, the identification of APP protein binding partners and the identification of molecules able to interfere with these interactions are an important approach to be tested in pathological context. Indeed, it is tempting to speculate that the inhibitor of 37/67 kDa LR, by displacing APP from its interaction with the receptor, might control its intracellular trafficking and processing.

Finally, concerning the role of the 37/67 kDa LR in autophagic process, we demonstrated that the inhibition of the receptor inactivated Akt in human skin fibroblasts from fAD (Bhattacharya, Izzo, Mollo 2020b) and MAPK/ERK signalling in neuronal cells (Bhattacharya 2020a). Accordingly, we found that the inhibition of 37/67 kDa LR modulated the activation of LC3-assisted degradative pathway in neuronal cells (Limone 2022a).

3. AIM OF THE THESIS

4. MATERIALS AND METHODS

5. RESULTS

6. FUTURE PERSPECTIVES

7. DISCUSSION

8. CONCLUSION

9. LIST OF ABBREVIATION

37 kDa LRP	37 kDa laminin receptor precursor
37/67 kDa LR	37/67 kDa laminin receptor
AD	Alzheimer's disease
APP	amyloid precursor protein
Aß	amyloid beta
ATG	autophagy-related protein
BAF.A1	Bafilomycin A1
CQ	Chloroquine
CTF	C-terminal fragment
EE	early endosome
EOAD	early onset Alzheimer's disease
ER	Endoplasmic reticulum
fAD	familial Alzheimer's disease
LC3	Microtubule-associated proteins 1A/1B light chain 3B
LIR	LC3-interacting region
mTOR	mammalian target of rapamycin
OCR	Oxygen consumption rate
OPTN	Optineurin
OXPHOS	oxidative phosphorylation

PCC	Pearson correlation coefficient
PI3K	phosphoinositide 3-kinase
PI3K3C	phosphoinositide 3-kinase class III
PINK1	PTEN-induced putative protein kinase 1
PIP2	phosphatidylinositol-4,5-bisphosphate
PIP3	phosphatidylinositol-3,4,5-trisphosphate
PM	plasma membrane
PrP^C	cellular prion protein
PS1/2	presenilin-1/2
qRT-PCR	quantitative real time polymerase chain reaction
ROS	reactive oxygen species
rpS6	ribosomal protein S6
RPSA	ribosomal protein SA gene
TFEB	Transcription factor EB
ULK1	Unc-51-like kinase

10. LIST OF PUBLICATIONS

Bhattacharya A, Limone A, Napolitano F, et al. APP Maturation and Intracellular Localization Are Controlled by a Specific Inhibitor of 37/67 kDa Laminin-1 Receptor in Neuronal Cells. International Journal of Molecular Science. 2020;21(5):1738.

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