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Endoplasmic reticulum-Mitochondria Coupling in Alzheimer's Disease

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Abstract

Alzheimer's disease (AD) is the most common neurodegenerative disorder of the elderly and no cure is currently available, as the mechanisms leading to neuronal damage and cognitive impairments remain elusive. In the last years, accumulating evidence highlighted early perturbations of the communication between mitochondria and endoplasmic reticulum (ER) in AD models. In this short review, we summarize recent findings linking alterations of ER-mitochondria coupling with typical AD hallmarks.

Keywords

ER-mitochondria contacts, MAM, Alzheimer's disease

Introduction

In eukaryotic cells, the coordination of organelle activities is favored by the existence of specialized regions called membrane contact sites (MCSs). At MCSs, the membranes of different organelles are closely juxtaposed and this vicinity guarantees an efficient exchange of molecules, signals and information, thus allowing to match cell needs (Prinz et al., 2020; Scorrano et al., 2019).

The best-characterized MCSs are those formed between endoplasmic reticulum (ER) and mitochondria, often referred to as mitochondria-associated membranes (MAMs). Specific protein and lipid compositions control the remodeling of ER-mitochondria MCSs, which in turn regulate key cell pathways, such as calcium signaling, lipid metabolism, mitochondrial bioenergetics, autophagy and cell death. The role of MCSs and MAMs in cell physiology has been extensively reviewed elsewhere, and the interested readers are referred to some recent reviews (Csordas et al., 2018; Prinz et al., 2020; Voeltz et al., 2024). Importantly, alterations in MAM structure and/or functions have emerged as central features in different diseases (Filadi et al., 2017), including Alzheimer's disease (AD), suggesting the intriguing possibility that they might underlie pathogenesis. In this review, we summarize recent findings associating AD with perturbations of ER-mitochondria MCSs. In particular, we will focus on the links between cell alterations typically observed in AD (such as those involving Ca^{2+} homeostasis, lipid metabolism and the generation of the amyloid beta (Aβ) peptide) and MAMs.

AD and ER-Mitochondria MCSs: What is the Link?

Different hypotheses have been proposed to explain AD pathogenesis, yet in the last three decades the one which has gained more consensus is the amyloid cascade hypothesis (Goedert and Spillantini, 2006). Indeed, although AD is mostly sporadic (SAD), few cases are familial (FAD) and are linked to autosomal dominant mutations in three genes, encoding for the Amyloid Precursor Protein (APP), Presenilin 1 (PS1) and Presenilin 2 (PS2). All these genes are directly involved in the generation of the Aβ peptides, the main components of the amyloid plaques frequently observed in the brain of AD patients, hinting at an involvement of an altered Aβ processing in disease onset.

In particular, Aβ derives from the sequential cleavage of APP by β- and γ-secretase, an intramembrane aspartyl protease in which Presenilins (either PS1 or, alternatively, PS2) are the catalytic core (Goedert and Spillantini, 2006). Aβ

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generation mostly occurs at Golgi apparatus and plasma membrane (but see also below), leading to the release of Aβ peptides in the extracellular environment. However, most clinical trials specifically focused on this pathway have failed in blocking disease progression, prompting an increasing interest for the study of additional pathways that might be also involved. In this scenario, a possible link between MAM alterations and AD has been established.

The initial observation that Presenilins are enriched at MAMs (Area-Gomez et al., 2009) was closely followed by data suggesting that the expression of FAD-linked PS2, but not FAD-PS1, mutants directly strengthens ER-mitochondria physical association in different cell models, increasing the efficiency of inter-organelle Ca^{2+} transfer (Zampese et al., 2011). In parallel, an upregulation of MAM extension and activity, as measured by an increased cholesteryl ester and phospholipid synthesis, was observed in presenilin-mutant cells and in fibroblasts from both FAD and SAD patients (Area-Gomez et al., 2012), suggesting that an altered ER-mitochondria cross-talk might be a common feature in AD. A similar conclusion was also suggested by data reporting a direct effect of nanomolar Aβ concentrations in the elevation of ER-mitochondria MCSs and Inositol-1,4,5-triphosphate receptor (IP3R)-dependent ER-mitochondria Ca^{2+} transfer, as well as an upregulation of MAMs-associated proteins in brains from AD patients and $APP_{Swe/Lon}$ mouse model (Hedskog et al., 2013). In this latter model, the alterations occur before deposition of amyloid plaques (Hedskog et al., 2013).

After these pioneer works, several groups reported alterations of ER-mitochondria axis in different AD models. In most, but not all, cases, a higher physical and functional inter-organelle association has been observed (see also below), although only few studies investigated the underlying mechanism. For instance, in the presence of FAD-PS2 mutants, a stronger interaction of PS2 with Mitofusin 2 (MFN2), in turn modulating ER-mitochondria juxtaposition, has been shown (Filadi et al., 2016). In other AD cell models, elevated levels of the unprocessed APP-C99 fragments at MAMs have been associated with MAM upregulation and aberrant lipid composition (Montesinos et al., 2020). A reduction of protein phosphatase 2A (PP2A) levels and/or activity, as frequently observed in AD, has been demonstrated to favour ER-mitochondria physical tethering and Ca^{2+}-cross-talk (Chaiwijit et al., 2023). Moreover, recent evidence suggests that an altered epigenetic control of the transcription of key MAM-regulating genes, possibly triggered by Aβ peptides, might be also involved (Marinho et al., 2023).

Of note, in some models an unaltered or even lower ER-mitochondria crosstalk has been reported, such as in Purkinjie cells from FAD-PS1-E280A patients (Sepulveda-Falla et al., 2014), hippocampal neurons from APP-transgenic rats (Adami et al., 2019) and N2A cells overexpressing APP_{swe} (Fernandes et al., 2021). On the same line, decreased levels of the ER-mitochondria tethers VAPB (vesicle-associated membrane protein-associated protein B) and PTPIP51 (protein tyrosine phosphatase interacting protein-51) were observed in the temporal cortex of late stage AD patients, while a disruption of VAPB-PTPIP51 tethers was detected in pyramidal neurons in post-mortem AD cortex at a relatively early, but not late, stage of the disease (Lau et al., 2020). This suggests that tethering complexes might be a target of AD associated stressors.

Interestingly, in AD and several other neurodegenerative disorders, the hyperphosphorylation of the *tau* protein and its aggregation into the neurofibrillary tangles has been linked to disease onset. Recent reports suggest that *tau* might modulate ER-mitochondria coupling, though either increased (Cieri et al., 2018; Perreault et al., 2009) or decreased (Szabo et al., 2023) organelle juxtaposition has been observed in the presence of abnormal *tau*.

Overall, although some discrepancies are present in literature (most likely dependent on the specific AD-models under investigation), consensus has been reached suggesting that early MAM perturbations associate with AD and impinge on cell pathways which are altered in the disease, as discussed in the next sections.

ER-Mitochondria MCSs and Aβ

Recent evidence suggests that not only PSs (Area-Gomez et al., 2009), but also APP, its C99 fragment (an APP C-terminal fragment from which Aβ can be generated upon γ-secretase cleavage), β- and γ-secretase activities (Del Prete et al., 2017; Pera et al., 2017; Schreiner et al., 2015), as well as γ-secretase activating protein (GSAP) (Xu et al., 2021) are present at MAMs, implying that a considerable amount of Aβ is generated in these subcellular regions (Choi et al., 2023; Del Prete et al., 2017; Pera et al., 2017; Schreiner et al., 2015). Remarkably, the upregulation of ER-mitochondria interface by MFN2-downregulation has been shown to impair γ-secretase activity and Aβ generation in HEK293 cells stably expressing FAD-APP_{swe} (Leal et al., 2016), suggesting that MAM structure and/or protein/lipid composition might be critical for this process. Moreover, in AD cell models, an elevated concentration of C99 at MAMs, rather than of Aβ, has been linked to an altered lipid composition of both MAM and mitochondrial membranes, leading to MAM upregulation and defective mitochondrial bioenergetics (Montesinos et al., 2020; Pera et al., 2017). Increased glucocorticoid levels, an important stress-induced risk factor possibly linked to AD, have been shown to upregulate MAMs and PS1-localization at ER-mitochondria MCSs, increasing local γ-secretase activity, Aβ processing and triggering mitochondrial damage (Choi et al., 2023). In a 3D neural cell culture AD model, the enrichment of palmitoylated APP (palAPP) at MAMs has been shown to be critical for APP trafficking at cell surface and the specific Aβ generation in axons and neurites,

with MAM levels positively regulating β-secretase activity (Bhattacharyya et al., 2021). Last, in a 3D human neuronal AD model, it has been recently shown that the stabilization of tight ER-OMM contacts (membrane distance ~6 nm) by artificial linkers increases $A\beta_{40}$ and $A\beta_{42}$ levels, whereas an opposite effect is obtained upon enhancement of loose MAMs (~ 40 nm) (Zellmer et al., 2024).

Importantly, a mutual relationship between the Aβ pathway and MAMs has been highlighted by several studies (Figure 1). Indeed, ER-mitochondria MCSs are critical hubs for Aβ processing and in turn Aβ can modulate MAMs. On this line, exposure of different cell models (including primary hippocampal neurons and cortical astrocytes) to Aβ has been shown to increase ER-mitochondria association (Calvo-Rodriguez et al., 2019; Garcia-Casas et al., 2023; García Casas et al., 2024; Hedskog et al., 2013; Leal et al., 2020; Marinho et al., 2023), while APP and its catabolites interact with key MAM proteins controlling their activity (Del Prete et al., 2017). Interestingly, in *Drosophila* AD models linked to Aβ, the modulation of ER-mitochondria interaction by either the expression of artificial linkers or the downregulation of endogenous regulators triggers contradictory effects, as both the potentiation (Garrido-Maraver et al., 2020; Wilson et al., 2024) or the reduction (Hewitt et al., 2022) of organelle juxtaposition have been shown to reduce the AD-linked phenotype.

Overall, the mechanisms by which Aβ regulates MAMs have not been elucidated, yet recent evidence suggests that a Histone deacetylase-dependent control of the transcription of MAM resident proteins (in particular, those controlling Ca^{2+} shuttling) might be involved (Marinho et al., 2023).

ER-Mitochondria Ca^{2+} Transfer in AD

The efficiency of inter-organelle Ca^{2+} shuttling is very much influenced by organelle juxtaposition (Csordás et al., 2018). Accordingly, several studies associated the AD-linked MAM alterations with perturbations of ER-mitochondria Ca^{2+} exchange (Figure 1).

The exposure of hippocampal neurons or SH-SY5Y neuroblastoma cells to nanomolar Aβ concentrations has been shown to upregulate the interaction of the Voltage dependent anion channel 1 (VDAC1) with IP3Rs, favoring ER-mitochondria Ca^{2+} transfer upon IP3-dependent cell stimulation (Hedskog et al., 2013). Similarly, an increased expression of IP3R1, GRP75, VDAC1 and SigmaR1, favoring mitochondrial Ca^{2+} uptake, has been observed in HT22 cells exposed to $A\beta_{42}$ oligomers, with an upregulated IP3R and GRP75 expression also in the hippocampus of APP/PS1 mice (Marinho et al., 2023). Interestingly, exposure to Aβ has been shown to increase ER-mitochondria connection and Ca^{2+} transfer in young hippocampal neurons from rats, while triggering opposite effects in aged neurons (Calvo-Rodriguez et al., 2019). Dampened SigmaR1 levels, associated with a larger gap between ER and mitochondria and a lower IP3-dependent inter-organelle Ca^{2+} transfer, was observed in 3xTG-AD mouse brains and in primary hippocampal neurons exposed to 20 μM $A\beta_{25-35}$ by a different group (Cheng et al., 2024). These observations suggest that different Aβ species and dosages might exert opposite effects on these parameters, yet reinforcing the view that MAMs and their associated activities are particularly sensitive to AD-linked stressors.

As to the efficiency of ER-mitochondria Ca^{2+} cross-talk, a peculiar activity is that of some FAD-PS2 mutants (Figure 2). Indeed, on the one hand, FAD-PS2-N141I and FAD-PS2-T122R have been reported to increase the efficiency of this process, in accordance with their capacity to strengthen ER-mitochondria physical association (Filadi et al., 2016; Kipanyula et al., 2012; Rossini et al., 2021; Zampese et al., 2011). However, on the other hand, the same mutants dampen ER Ca^{2+} content, possibly as a compensatory mechanism. This reduces the amount of Ca^{2+} released through the IP3Rs upon sustained IP3-dependent cell stimulation and, consequently, the overall amount of Ca^{2+} taken up by mitochondria (Filadi et al., 2016; Rossini et al., 2021; Zampese et al., 2011). In turn, this lower mitochondrial Ca^{2+} uptake has been suggested to partially contribute to a defective mitochondrial bioenergetics in FAD-PS2 cell models (Rossi et al., 2020) (Figure 1).

Overall, the maintenance of mitochondrial Ca^{2+} homeostasis is critical in neuronal cells, as both aberrant increases or decreases in matrix Ca^{2+} concentrations associate with neurodegeneration (reviewed in (Filadi and Greotti, 2021; Garcia-Casas et al., 2023; Plotegher et al., 2021)). Therefore, the observed perturbations of ER-mitochondria Ca^{2+} coupling could be critical in the progressive loss of neurons in AD. Interestingly, AD-associated conditions, such as increased Aβ levels or hyperglycemia in diabetes mellitus, have been shown to increase the expression of transglutaminase type 2 (TGM2), which in turn interacts with and stabilizes the formation of IP3Rs-VDAC1 complexes, thus favoring ER to mitochondria Ca^{2+} transfer (Lee et al., 2021). This process can be reverted by treatment with Urolithin A, which could be a promising therapeutic candidate (Lee et al., 2021).

MAMs and Lipid Metabolism in AD

MAMs are cholesterol-rich lipid raft-like domains that play a critical role in the regulation of lipid metabolism. Their peculiar lipid composition, enriched in cholesterol, sphingolipids and saturated phospholipids, is not only important for the recruitment and the modulation of the activity of specific proteins (including those involved in lipid metabolism), but is also essential for non-vesicular phospholipid and cholesterol transport between ER and mitochondria (Fernandes et al., 2023; Monteiro-Cardoso and Giordano, 2024; Schenkel and Bakovic, 2014; Vance, 2014, 2020).

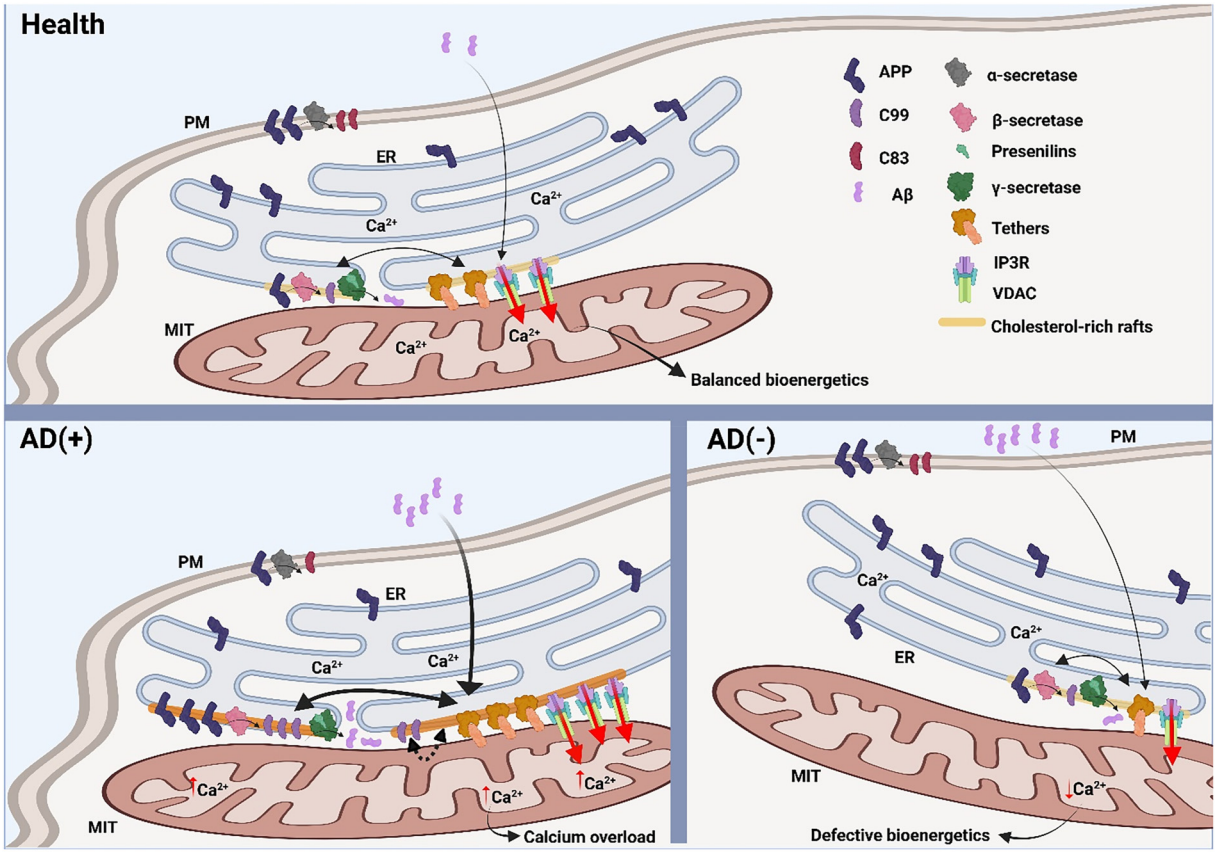

Figure 1. The Cartoon Highlights Typical Alterations in MAM Structure and/or Activity in AD, as Discussed in the Text. In Most AD Models, a Higher ER-Mitochondria Cross-Talk (Mediated by Upregulated Levels of MAM Proteins or Altered Lipid Composition) has Been Observed (AD+), Impacting on Aβ Processing. A Mutual Relationship Between Aβ (and/or the APP-Derived C99 Fragment) and MAM Lipid/Protein Composition Might Exist, as Represented by Double-Arrows. APP, C99 and Aβ Processing are Enriched at MAMs, Impinging on Their Functionality. In These Models, the Increased ER-Mitochondria Coupling Frequently Associates with an Upregulated Ca^{2+} Shuttling (red Arrows) Through the IP3R-GRP75-VDAC complex, Leading to Mitochondrial Ca^{2+} Overload. In Some AD Models (AD-), a Decreased ER-Mitochondria Juxtaposition, Possibly Mediated by Downregulation of Specific Tethering Complexes (Such as VAPB-PTPIP51), has Been Reported, in Turn Dampening Ca^{2+} Transfer to Mitochondria and Potentially Compromising Cell Bioenergetics.

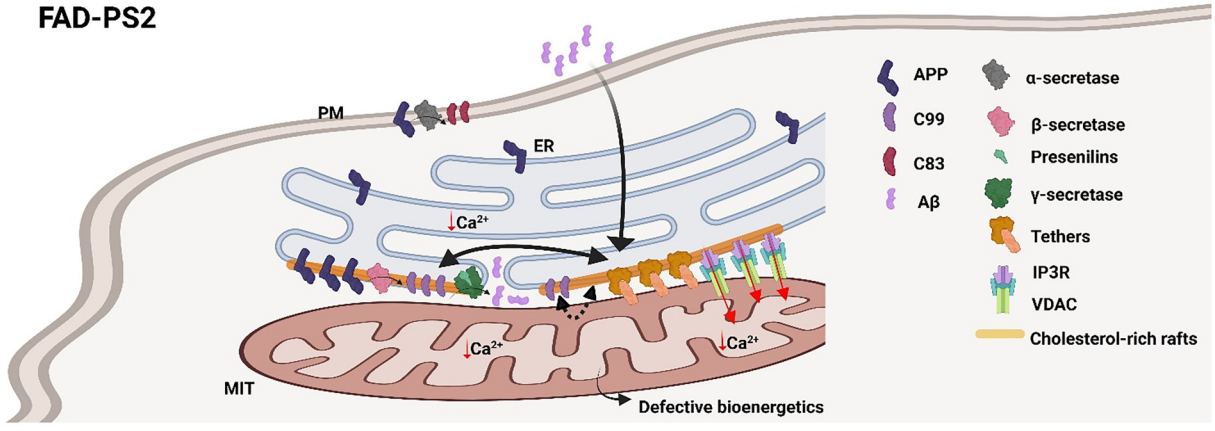

Figure 2. The cartoon represents the alterations in MAM structure/activity in FAD-PS2-N141I and FAD-PS2-T122R expressing models. In particular, ER-mitochondria interface is upregulated and might associate with an altered lipid metabolism and Aβ processing, as reported in other AD models (AD + discussed in Figure 1); nevertheless, the lower Ca^{2+} levels in the ER dampens the overall amount of Ca^{2+} shuttled to mitochondria, compromising mitochondrial and cell bioenergetics.

Remarkably, deep alterations in lipid composition, including changes in cholesterol, sphingolipids, phospholipids, glycerolipids and ceramides, have been observed in different AD cell models, as well as in plasma and frontal cortex of AD patients (Agrawal et al., 2020; Area-Gomez et al., 2012; Area-Gomez and Schon, 2024; Cutler et al., 2004; Mapstone et al., 2014; Pettegrew et al., 2001; Van Echten-Deckert and Walter, 2012; Wood et al., 2015). Hypercholesterolemia has been associated with AD (Di Paolo and Kim, 2011) and the ϵ4 variant of apolipoprotein E (APOE), a cholesterol/lipid transporter in the central nervous system, is an important risk factor for AD development (Chen et al., 2021).

In this scenario, considered the key role of MAMs in the regulation of lipid metabolism, evidence has been provided suggesting a direct link between MAMs and lipid alterations in AD. In particular, though cholesterol is present at low level in the ER membrane, it can accumulate at MAMs, where the recruitment and the activity of acyl-CoA:cholesterol acyl transferase (ACAT1) catalyses its esterification into cholesteryl esters (CE) (Rusinol et al., 1994), eventually deposited in lipid droplets (LDs) as a detoxifying mechanism from excessive cholesterol.

In different AD models, cholesterol increase has been reported (Cutler et al., 2004), as well as upregulated ACAT1 activity and CE accumulation (Area-Gomez et al., 2012; Chan et al., 2012; Puglielli et al., 2001), possibly linked with the AD-associated tau hyperphosphorilation, altered Aβ processing (van der Kant et al., 2019) and LD abundance (Area-Gomez et al., 2012; Rossini et al., 2021) (reviewed in (Area-Gomez and Schon, 2024)). Intriguingly, accumulating evidence suggests that cholesterol accumulation in AD might link the alterations of ER-mitochondria tethering and the local Aβ generation from APP. On the one hand, in fibroblasts from a FAD-PS2 patient, in which ER-mitochondria tethering is increased, the higher LD levels were rescued by genetic manipulation recovering a normal ER-mitochondria juxtaposition (Rossini et al., 2021), further suggesting that MAMs can be critical in cholesterol metabolism and ACAT1 activity. On the other hand, ACAT1-regulated cholesterol levels can control the physical association between ER-mitochondria, with higher cholesterol levels favoring the formation of inter-organelle MCSs (Harned et al., 2023). The exposure of fibroblasts and neurons to conditioned media from astrocytes expressing APOE4 increases the MAM-associated phospholipid and cholesteryl ester synthesis (Tambini et al., 2016), while the expression of a truncated form of APOE4 (Δ272–299) promotes GRP75-dependent MAM formation in hippocampal neurons and N2A cells (Liang et al., 2021). Perturbations in the transcription of genes involved in the metabolism of fatty acids, neutral lipids and cholesterol were observed in post-mortem brains of APOE4-carriers, while higher levels of triacylglycerides, associated with LD accumulation, were retrieved in astrocytes derived from APOE4-expressing human iPSC, an effect reversed by choline supplementation (Sienski et al., 2021). Recently, in APOE4/4 individuals, lipid accumulation in microglia, associated with an upregulation of genes involved in lipid synthesis, has been reported (Haney et al., 2024). Remarkably, microglia treatment with fibrillary Aβ induces triglyceride synthesis and LD accumulation, an effect potentiated in APOE4/4 microglia. The conditioned media from these high LD-containing microglia induces tau hyperphosphorylation and neurotoxicity in an APOE-dependent manner, suggesting that APOE might by critical for the transfer of neurotoxic, lipid-based species from glial cells to neurons (Haney et al., 2024).

To make the link between lipid imbalance and AD more direct, APP (in particular, its C99 fragment) has been proposed to work as a cholesterol sensor, as it is endowed with a cholesterol binding domain and might regulate the subcellular distribution of this lipid, stabilizing the formation of lipid raft at MAMs (Area-Gomez and Schon, 2024; Beel et al., 2008; Montesinos et al., 2020; Pera et al., 2017). In AD, excessive levels of C99 fragments (for instance, as a consequence of FAD-APP mutations) and/or cholesterol accumulation have been proposed to converge on MAM stabilization, altering their lipid composition and thus affecting MAM-localized Aβ generation (Area-Gomez and Schon, 2024).

Indeed, γ-secretase activity preferentially occurs at lipid raft (Urano et al., 2005) and is particularly sensitive to lipid composition (Guardia-Laguarta et al., 2009), possibly explaining the altered APP processing that leads to the aberrant formation of aggregation-prone Aβ species in AD (Figure 1). Interestingly, the lipid rafts have been shown to serve as a platform for the cleavage of palAPP by β-secretase, and MAM upregulation promotes trafficking of palAPP to cell surface, β-secretase cleavage and Aβ generation (Bhattacharyya et al., 2021).

Consistently, the regulation of MAM lipid composition, in particular of cholesterol, appears a promising therapeutic target. Different studies reported that treatments with statins (to block cholesterol synthesis) or ACAT1 inhibition might attenuate the AD phenotype in different disease models, both *in vitro* and *in vivo* (Bryleva et al., 2010; Fassbender et al., 2001; Huttunen et al., 2009; Puglielli et al., 2001). Recently, it has been shown that the increased oligomerization and accumulation at MAMs of the ATPase family AAA-domain containing protein 3A (ATAD3A) leads to cholesterol accumulation at ER-mitochondria interface, in turn triggering the AD-associated alterations of APP processing and synaptic loss. The pharmacological or genetic inhibition of ATAD3A oligomerization normalizes brain cholesterol turnover and reduces AD neuropathology in 5XFAD transgenic mice (Zhao et al., 2022), further suggesting a link between alterations of MAM-associated lipid metabolism and AD.

Conclusion

In this review, we summarized some recent findings indicating that perturbations of ER-mitochondria interface and

associated activities are critical events in AD. Although it has not been fully clarified whether these alterations underlie disease onset, or arise along with its progression, evidence has accumulated suggesting that they are endowed with the potential to interfere with AD-linked pathogenic pathways. For instance, critical steps of APP processing and Aβ peptide generation take place at MAM, and the manipulation of ER-mitochondria juxtaposition impacts on this process. Along the same line, several AD-associated alterations in lipid metabolism and Ca^{2+} homeostasis, possibly leading to the observed mitochondrial impairments, can arise from an aberrant inter-organelle communication.

Though in some cases the molecular mechanisms have not been completely elucidated, MAMs emerge as a hub in which multiple AD-associated stressors might converge (Figure 1). As an example, perturbations of the lipid (in particular cholesterol) composition of MAMs can underlie an altered APP and Aβ processing, and in turn APP and its fragments can be critical in regulating lipid raft organization/composition. Moreover, changes in both APP processing and MAM lipid profile can impinge on the efficiency of ER-mitochondria Ca^{2+} shuttling, a critical pathway regulating mitochondrial bioenergetics.

Very recently, some treatments targeting these MAM-associated pathways have been demonstrated to be beneficial in different AD models (Bhattacharyya et al., 2021; Cheng et al., 2024; Dentoni et al., 2022; Lee et al., 2021; Marinho et al., 2023; Zhao et al., 2022). We envisage that a deeper characterization of the mechanisms linking MAM alterations with AD pathogenesis will pave the way to identify modulators normalizing ER-mitochondria crosstalk in AD, aimed at recovering neuronal functionality.

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Author Contributions

MR, TF and ID prepared the figure. MR, TF, ID and RF wrote the manuscript.

Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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