Introduction

Alois Alzheimer published for the first time an article in 1898 describing patients with signs of senile dementia. However, only several years later, in 1901, the young physician encountered his most striking case of dementia, a woman just over 50 years, representing a much earlier event than his first described cases. This hallmark patient in the story of Alzheimer’s disease (AD) suffered from deteriorating memory, even of recent events, disorientation, decreasing speech abilities, and lack of judgment of the different surrounding situations. Indeed, these are some of the described clinical symptoms currently attributed to AD. In 1907 and 1911, Alzheimer published two case reports describing the clinical and histological hallmarks that characterized his demented patient [1, 2]. Ever since then, the efforts of the scientific community to gain insight into the molecular mechanisms of this disease are countless. Nevertheless, according to an Alzheimer’s Association recent report [3], AD is still the seventh leading cause of death in the United States (fifth place among individuals aged 65 and older), causing an elevated financial burden in health care.

The main histopathological features of AD are massive neuronal loss, the deposition of senile plaques, which are extracellular aberrant amyloid-β (Aβ) protein aggregates, and neurofibrillary tangles composed of intracellular hyperphosphorylated tau protein [4]. AD has either an age-associated, late-onset sporadic form, or an early-onset familial form with a genetic origin involving mutations in the amyloid-β protein precursor (AβPP) and presenilin 1 and 2 (PS1 and PS2) genes [4]. Despite this knowledge, the etiogenesis of sporadic AD remains largely unclear and several competing hypotheses have been proposed. Whereas the hypothesis that still drives the investigation of most authors is the amyloid cascade hypothesis with an added
Mitochondria: back to the basics

Mitochondria are involved in vital cellular functions that are critical for life and death and it is crucial to maintain a healthy mitochondrial population within the cells which becomes increasingly challenging when long-lived cells such as neurons and the organism as a whole ages. Mitochondrial dysfunction is one of the earliest and most prominent features of AD and recent developments in the field support an involvement of mitochondrial-dependent mechanisms in the pathogenesis of AD [15, 16]. Indeed, mitochondria have been implicated in AD pathogenesis on a multitude of levels: 1) as triggers of disease [15-19]; 2) as mediators and targets of the harmful effects of Aβ [6], causing mitochondrial dysfunction and increased reactive oxygen species (ROS) production; 3) as potential sites of Aβ production, since AβPP was found in mitochondrial membrane [20] as well as an active γ-secretase enzymatic complex [21]. In this review the physiological mitochondrial function, the importance of organelle dynamics and mitophagy will be the focus, as well as the mitochondrial pathophysiological alterations that occur in AD.

Mitochondria malfunctioning in AD

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Mitochondria are intracellular “buffers” of cytosolic Ca²⁺, internalizing it mainly via uniporter and releasing it by Na⁺/Ca²⁺ or H⁺/Ca²⁺ exchangers [36]. Abnormal cytosolic Ca²⁺ elevations trigger a rapid accumulation of the cation by mitochondria, which is particularly important in CNS given the role of Ca²⁺ in normal neurotransmission, short- and long-term plasticity and regulation of gene transcription [36-42]. Additionally, a deregulation in Ca²⁺ homeostasis can potentiate excitotoxicity, a phenomenon intimately associated with neurodegeneration [36, 43]. Decreased age-related Ca²⁺ buffering capacity has been shown in CNS, mitochondria being involved in this deregulated homeostasis [44].

A molecular link between increased ROS pro-
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duction, the deregulation of mitochondrial Ca\textsuperscript{2+} homeostasis and apoptosis induction is the opening of permeability transition pore (PTP), that enables the release of some small pro-apoptotic proteins, such as cytochrome c and apoptosis-inducing factor (AIF) to the cytosol, triggering the activation of the caspase cascade [45, 46]. The PTP is a voltage-dependent, high-conductance, non-selective pore that permeabilizes mitochondrial membranes to solutes/molecules of less than 1.5 KDa in molecular weight [47]. The exact nature of the PTP is unknown, however, currently, it is believed that it is a multiprotein complex constituted by the adenine nucleotide translocator (ANT), located in the inner mitochondrial membrane, and the voltage-dependent anion channel (VDAC, porin), located in the outer mitochondrial membrane, both constituting the pore. Several other regulatory protein components are part of the pore: a peripheral benzodiazepine receptor (PBR), which provides ligand regulation of the pore; cyclophilin D (CypD), which senses matrix Ca\textsuperscript{2+} concentration and oxidative status; mitochondrial creatine phosphokinase that monitors intermembrane high energetic status; hexokinase (HK); Bax/Bcl2 protein family that modulate pore activation through direct interactions with ANT or VDAC [48].

Mitochondrial dynamics and mitophagy

Mitochondria have long been considered dynamic organelles because they migrate within the cells and are rapidly turned over by mitophagy. Studies in the past decade added one more fascinating feature to the concept of mitochondrial dynamics: rather than being depicted as isolated, bean-shaped structures, these organelles actually continuously divide and fuse with each other and can rapidly change in number and morphology within a cell. Mitochondrial dynamics enables their prompt adaptation to changes in cellular demands either due to physiological or environmental alterations [49]. The machinery of mitochondrial fission/fusion is governed by a group of GTPases, however, the mechanisms by which they rule those processes remain to be completely elucidated. In mammals, mitochondrial fission is directed by a large cytosolic GTPase that is recruited to the mitochondrial membrane upon a fission-like stimuli, dynamin-like protein 1 (DLP1 or DRP1), and a small mitochondrial molecule located in the outer membrane, Fis1 [50-52]. Mitochondrial fusion in mammals is directed by three large GTPases, Mitofusin 1 (Mfn1) and Mitofusin 2 (Mfn2), both located in the mitochondrial outer membrane, and optic atrophy 1 (OPA1) protein, located in the inner mitochondrial membrane [53-56]. Noticeably, OPA1 has also been implicated in mitochondrial cristae structure and release of cytochrome c from OPA1-dependent subcompartments of the cristae upon an apoptotic stimulus [57].

Mitochondrial dynamics is critical for maintaining various mitochondrial functions: fusion deficient cells demonstrate greatly reduced endogenous and uncoupled respiratory rates and demonstrate reversible interorganellar heterogeneity in membrane potential and inhibition of cell growth [54, 58]. Fission deficiency also causes a reduced rate of mitochondrial ATP synthesis due to a significant decrease in complex-IV activity and an inefficient oxphos system [59]. A fragmented mitochondrial network is less efficient in mitochondrial Ca\textsuperscript{2+} uptake and mitochondrial Ca\textsuperscript{2+} diffusion, and the formation of a mitochondrial network facilitates Ca\textsuperscript{2+} propagation within interconnected mitochondria, suggesting that the balance of mitochondrial fission/fusion can significantly impact cellular Ca\textsuperscript{2+} ion homeostasis [60, 61]. Mitochondrial dynamics are also involved in apoptosis with mitochondrial fragmentation being an early event during apoptosis that precedes cytochrome c release and caspase activation [62]. Excessive mitochondrial fission is also correlated with increased ROS production [52, 63, 64].

The ability of mitochondria to move within the cells assumes its maximal importance in highly polarized cells, such as neurons, which have high energetic demands and several subcellular compartments with specialized functions [65, 66]. Defects in both fusion and fission have been shown to alter mitochondrial distribution, which is suggestive of altered mitochondrial movement [49, 52]. It is likely a size effect may play a role because neurons lacking mitochondrial fusion, also demonstrate an increased mitochondrial diameter that block efficient entry in neurites, which results in a scarcity of mitochondria in axons and dendrites leading to improperly developed neurons or neurodegeneration [67]. Similarly, reduced Drp1 expression induces a decrease in mitochondrial number in neurites, which is attributed to mitochondrial
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elongation, since large mitochondria accumulate in the base of dendritic protrusions ultimately leading to a loss of synapses and dendritic spines [68]. Accordingly, lack of mitochondrial transport results in neurotransmission defects during prolonged stimulation [69]. Recent studies demonstrated specific interactions between Mfn2 and Miro and Milton, members of the molecular complex that links mitochondria to kinesin motors, implicating mechanisms unrelated to fission/fusion may also be involved [70].

The process of mitophagy has been shown to be related to mitochondrial fission/fusion processes in mammalian cells, so that when depolarized (injured) mitochondria fail to undergo fusion and fission events, they are targeted for mitophagic clearance [71]. The mechanisms that tag mitochondria to mitochondrial elimination are not clear (for further readings, see [72]). Nevertheless it is quite accepted that fusion events exert a protective effect against mitochondrial dysfunction through the segregation of damaged components into a mitochondrion that undergoes mitophagy. Indeed, inhibition of autophagy results in decrease ΔΨm and fusion arrestment in rat myoblasts and human fibroblasts [73].

Mitochondrial dysfunction(s) in AD

It has been reported that mitochondrial abnormalities correlate with dystrophic neurites, the loss of dendritic branches and the pathological alteration of the dendritic spines present in the brains of AD cases [74]. Swerdlow and Khan [15, 16] proposed the mitochondrial cascade hypothesis to explain late-onset, sporadic AD, stating that Aβ deposition, neurofibrillary tangle formation and neurodegeneration are consequent events of mitochondria malfunctioning. This hypothesis emphasizes aging as the main risk factor for the development of the sporadic form of AD, the accumulation of Aβ being a consequence of aging [75] rather than the cause of the evolution of the neuropathology as is widely reported in the case of familial AD.

Multiple lines of studies suggest that reduced glucose metabolism is one of the best documented abnormalities in AD patients and its occurrence precedes clinical diagnosis. In longitudinal studies, the decline in mini-mental state examination (MMSE) scores in AD correlated with reduction in glucose metabolism as measured by positron emission tomography (PET) in the association areas (i.e., temporoparietal, frontal, and occipital cortices) which suggests that the clinical deterioration and metabolic impairment in AD are closely related. The analysis of the expression of 80 metabolically relevant nuclear ETC genes from laser-capture microdissected non-tangle-bearing neurons from autopsy brains of AD cases revealed that 60–70% of nuclear genes were significantly lower in those metabolically affected areas such as posterior cingulate cortex, middle temporal gyrus, and hippocampal CA1 but not in relatively spared visual cortex, suggesting that the cerebral abnormalities in metabolic rate for glucose found in fluorodeoxyglucose-positron emission tomography (FDG-PET) studies of AD may be associated with reduced neuronal expression of nuclear genes encoding subunits of the mitochondrial ETC [76]. Multiple studies suggest alterations in the activity of mitochondrial enzymes involved in tricarboxylic acid (TCA) cycle and ETC chains (Figure 1) [77, 78]. Bubber and colleagues [79] systematically examined pyruvate dehydrogenase complex (PDHC) and all the enzymes of the TCA cycle and found that there was reduced activity of decarboxylating dehydrogenases, yet a compensatory increase in dehydrogenases, alterations of which all significantly correlated with clinical state. Indeed, mitochondrial bioenergetic deficits are an early event that precedes AD pathology in animal models of AD [19, 80].

One question remains: what causes mitochondrial malfunction in sporadic AD? Based on the instability and irreparability of the mitochondrial genome due to the absence of histones and enzymatic repair systems [4, 81], it is possible that during aging, the accumulation of oxidative stress-induced mitochondrial DNA (mtDNA) damage and subsequent mitochondrial dysfunction may serve as a trigger of the appearance of the main AD histopathologic markers (Figure 1). In this regard, it is of interest to note that there was increased oxidation in both mtDNA and nuclear DNA bases in frontal, parietal, and especially, in temporal lobes of AD cases compared to age-matched controls, and that mtDNA oxidation was approximately 10-fold higher than nuclear DNA oxidation [82]. Some mtDNA mutations have been associated with increased incidence of AD [83, 84], in addition to brains having increased, unique mtDNA mutations com-
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Compared to control cases, this being further enhanced in an age-dependent fashion and preferentially in mtDNA regulatory elements, such as the control region [84]. Importantly those mutations in the control region of mtDNA could account for some of the mitochondrial defects in oxphos observed in AD, namely a reduction in the level of ND6 complex I transcript [84]. The
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impaired ophos result in an exacerbation of ROS generation, promoting the augment of the number of mtDNA mutations in a vicious positive feedback cycle (Figure 1) [85, 86].

Increased Aβ can also be involved in mitochondrial dysfunction either directly or indirectly. In this regard, it is known that Aβ is present in mitochondria. The presence of a functional γ-secretase complex [21] and AβPP in mitochondria [20] suggest that mitochondria can be themselves sites of Aβ production, although the mitochondrial presence of a functional BACE1 remains lacking. Perhaps a more likely scenario is Aβ translocation into mitochondria by the translocase of the outer membrane (TOM) complex [87] or interaction with RAGE (Figure 1) [88]. Once inside mitochondria, Aβ has the ability to complex heme groups, which constitute critical redox centers of the subunit I of cytochrome oxidase (COX) (Figure 1) [89, 90], and interacts with Aβ-binding alcohol dehydrogenase (ABAD) increasing ROS generation (via impairment of COX activity) and cytochrome c release (activating caspase 3) and decreasing ATP [91-94]. Recently it has been demonstrated that Aβ and tau exert synergistic effects in the impairment of oxidative phosphorylation system in 3xTg-AD mice [95]. Mitochondrial-associated AβPP may also exert adverse effects on mitochondrial function as it appears to block the mitochondrial import channels TOM40 and TIM23 and disables the import of the nuclear-encoded COX subunits to reach the mitochondrial interior (Figure 1) [96].

Indeed, dysfunction of mitochondrial energy metabolism also culminates in Ca2+ buffering impairment [97]. Moreira and coworkers [98-100] demonstrated that Aβ decreases the capacity of mitochondria to accumulate and retain Ca2+ promoting the PTP opening. A molecular basis to explain this phenomenon was provided by Du and coworkers [101], in which Aβ interacts with CypD, a critical molecule involved in PTP opening modulation and cell death. Consistently, CypD deficiency protects neurons from Aβ-induced PTP formation and the resultant mitochondrial/cellular stresses, while improving learning, memory and synaptic function in an AD mouse model, as well as improving Aβ-mediated reduction of long-term potentiation (LTP) [101]. This increased predisposition to PTP opening creates more chances for cell death, as discussed in the previous section (Figure 1). Furthermore, a recent study showed evidence of AβPP interaction with heat shock proteins (HSPs) and Bcl-2 (an anti-apoptotic protein), decreasing their ability to protect against insults [102].

Abnormal Mitochondrial Dynamics in AD

Mitochondrial dynamics can be affected by changes in bioenergetic status in response to physiological or environmental alterations and impact all aspects of mitochondrial functions. AD brains show ultrastructural alterations in mitochondrial morphology such as reduced number, increased size and broken internal membrane cristae [103, 104]. We determined the state of mitochondrial fission/fusion events in fibroblasts from sporadic AD patients [56, 105] and M17 neuroblastoma cells overexpressing the Swedish variant of AβPP (AβPPsw) [64]. The Aβ–induced impairment in mitochondrial fission/fusion proteins occurs both by post-translational modification, such as S-nitrosylation [106] and phosphorylation [52], and by alteration of their expression [56, 64, 105]. While it is reported that in fibroblasts from sporadic AD patients DLP1 protein levels are decreased, thus impairing fission, which is translated into the development of elongated mitochondria (Figure 1) [56, 105], at the same time it is described in M17 neuroblastoma cells overexpressing AβPPsw that besides decreased levels of DLP1, OPA1 protein levels are decreased and Fis1 levels increased (Figure 1) [64]. Indeed, AβPP overexpression or ADDL treatment induces reduced fission and fusion with a net outcome of severe mitochondrial fragmentation phenotype in both M17 and primary hippocampal neurons [52, 64]. The discrepancy of mitochondrial morphology between fibroblasts from patients and neuronal cultures subject to disease-specific insults is also seen in Parkinson-related models [107, 108] which may reflect the fact that fibroblasts are less susceptible than neurons in these disease conditions. Interestingly, AβPP-induced mitochondrial fragmentation underlies AβPP-induced deficits in mitochondrial function since overexpression of OPA1, which blocks fragmentation, could restore mitochondrial function, suggesting the fission-related structural basis for AβPP-induced functional changes.

One of the common features in all these models demonstrating abnormal mitochondrial dynam-
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ics is an abnormal mitochondrial distribution, i.e., perinuclear accumulation of mitochondria in AD fibroblasts or M17 cells overexpressing mutant AβPP or reduced mitochondrial density in neurites of primary hippocampal neurons (Figure 1). Indeed, our recent work also revealed that mitochondria accumulate in the soma and are reduced in neuronal processes in AD pyramidal neurons [52]. To explore the functional consequence of mitochondrial redistribution, we were able to demonstrate that overexpression of AβPP [64] or exposure to soluble Aβ oligomers [52] led to reduced neuritic mitochondrial density which correlated with reduced spine number and PSD95-positive puncta. More importantly, repopulation of neurites with mitochondria by overexpressing DLP1 in these cell models alleviates synaptic deficits, thus suggesting that abnormal mitochondrial localization is probably the most important contributing factor of synaptic dysfunction in the pathogenesis of AD. Such a notion is supported by the in vivo finding that in a fly model overexpressing Aβ, which demonstrates intracellular accumulation of Aβ in the soma and axon of a small group of neurons, the depletion of presynaptic and axonal mitochondria was the earliest detectable phenotype, preceding Aβ-induced presynaptic deficits in motor function [109]. Aβ-induced mitochondrial mislocalization is also confirmed in another Aβ-overexpressing fly model [110]. The depletion of mitochondria from axon or dendrite may impact synaptic function either directly through a lack of energy and calcium buffering support or indirectly through the removal of AMPA receptor [111].

Since fast axonal transport of mitochondria underlies the uniform distribution of mitochondria along the axon [65], these findings suggest that an abnormal mitochondrial transport may be involved (Figure 1). Deficits in axonal transport is implicated in AD pathogenesis since axonal swelling and reduced axonal transport were observed before apparent AD hallmarks [112]. Earlier studies demonstrated presenilin 1 mutant causes deficits in kinesin-mediated axonal transport including the transport of mitochondria through abnormal activation of GSK3β and phosphorylation of kinesin [113]. Acute treatment of Aβ monomers and fibrils induce a significant reduction in motile mitochondria [114]. We recently reported that soluble oligomers of Aβ are responsible for an abnormal axonal transport of mitochondria in primary hippocampal neurons, most likely contributing to an abnormal mitochondrial distribution [115]. Such a notion was supported by in vivo studies in one fly model [110] but not in the other similar fly model in which deficits in axonal transport occur later than the presynaptic depletion of mitochondria [109]. Therefore, more studies, especially those in mammalian systems, are still needed.

Mitophagy in AD

Evidence showing mitophagy in AD is scarce; Moreira and coworkers [77, 116] showed that there is increased mitochondrial sequestration in autophagosomes in AD. Whether these mitochondria are degraded upon their autophagosomal sequestration or remain sequestered and accumulated within autophagosomal vesicles remains to be clarified. To date some findings gave some clues about this issue. Hirai and coworkers [103] demonstrated decreased mitochondria in vulnerable neurons in AD, this observation being region-specific in brain tissue. Furthermore, the same authors also demonstrate increased cytosolic accumulation of mitochondrial markers such as mtDNA and subunit I of COX [103], which is inconsistent with an efficient autophagic-lysosomal proteolytic degradation, even suggesting a leak of sequestered material from AVs. Supporting this hypothesis, it has been reported that Aβ induces lysosomal membrane permeabilization [117, 118], very recently being reported that multiple-oligomeric aggregates of Aβ42, but not Aβ40, insert into lysosomal membrane in a pH-dependent manner, contributing to its instability [119]. Accordingly it was also demonstrated that the overexpression of Aβ42 in Drosophila neurons induced an age-dependent impairment of neuronal autophagy due to a leakage of postlysosomal autophagic vesicles (autolysosome), which caused a cytosolic acidification and damage of several cellular constituents [120]. Taken together these data suggest that an inefficient lysosomal system may be compromising the elimination of damaged mitochondria by mitophagy in AD (Figure 1). Moreover also the machinery involved in the induction of autophagy has been shown to be compromised in AD, since Beclin 1 deficiency was shown to be a feature of the brains of AD patients, a causal role being demonstrated in the exacerbation of the pathological markers of disease [121]. Another line of evidence that corroborates an impairment in efficient mito-
phagic elimination, comes from the impairment of mitochondrial fission/fusion events in AD. Since there are indications that mitochondrial fission and selective fusion tag damaged mitochondria for mitophagic elimination [71], and that mitochondrial fission/fusion events in fibroblasts from sporadic AD patients [56, 105] and M17 neuroblastoma cells overexpressing the Swedish variant of AβPP (AβPPswe) [64] is imbalanced, it is expected that mitophagic elimination of damaged mitochondria in AD brains is failing. Nevertheless, further clarification about the efficiency of the mitophagic turnover in AD brains is still needed.

Conclusion

Although AD etiogenesis is largely unknown, it is constantly growing in intricacy. Aβ and tau pathology are the most studied histopathological markers and considered by many authors as the cause of disease, particularly of genetic origin. Aβ and tau are unlikely causes of the sporadic, late onset form of AD, with mitochondria instead assuming a central stage, since these organelles are known to lose efficiency and progressively become dysfunctional with age, which is correlated with increased ROS production. Mitochondrial function has been shown to be impaired in AD in terms of metabolic energetic production and regulation of the levels of second messengers (ROS, Ca^{2+}), partially due to either accumulated damage to mtDNA or the direct harmful effects of oxidative stress or Aβ on mitochondrial components. Also mitochondrial dynamics has been documented to be altered in AD. Indeed, mitochondrial trafficking disruption in AD potentially compromises normal neurophysiological functions, such as neurotransmission. Moreover, mitochondrial fission/fusion and mitophagy disruption may have consequences on the maintenance of the homogeneity and a healthy cellular mitochondrial pool. In short, mitochondrial malfunctioning is one most likely major cause of onset and neurodegeneration in sporadic AD brains.

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