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Mitochondrial dysfunction, apoptotic cell death, and Alzheimer's disease

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Abstract

Being major sources of reactive oxygen species (ROS), mitochondrial structures are exposed to high concentrations of ROS and might therefore be particularly susceptible to oxidative injury. Mitochondrial damage may play a pivotal role in the cell death decision. Bolstered evidence indicates that mitochondrial abnormalities might be part of the spectrum of chronic oxidative stress occurring in Alzheimer's disease (AD) finally contributing to synaptic failure and neuronal degeneration. Accumulation and oligomerization of amyloid beta (Aβ) is also thought to play a central role in the pathogenesis of this disease by probably directly leading to mitochondrial dysfunction. Moreover, numerous lines of findings indicate increased susceptibility to apoptotic cell death and increased oxidative damage as common features in neurons from sporadic AD patients but also from familial AD (FAD) cases. Here we provide a summary of recent work demonstrating some key abnormalities that may initiate and promote pathological events in AD. Finally, we emphasize a hypothetical sequence of the pathogenic steps linking sporadic AD, FAD, and Aβ production with mitochondrial dysfunction, caspase pathway, and neuronal loss.

Keywords: APP mutation; β-Amyloid; Caspases; Oxidative stress; Mitochondria; Cell death

1. Pivotal role of mitochondria within the cell

Mitochondria are essential for the maintenance of cell function and viability. They are often described as the 'power house of the cell'. The primary function of mitochondria is to produce ATP through the coupling of oxidative phosphorylation with respiration. The mitochondrial respiratory chain is composed of five enzyme complexes: NADH-CoQ reductase (complex I), succinate CoQ reductase (complex II), ubiquinol-cytochrome *c* reductase (complex III), COX (complex IV), and F_1F_0-ATPase (complex V). While the respiratory enzyme complexes transfer electrons to each other and ultimately to molecular oxygen, they translocate protons across the inner mitochondrial membrane. The proton gradient set up in this way provides the energy that drives the motor of the membrane-bound enzyme ATP synthase, which catalyzes the conversion of ADP to ATP, completing the process of oxidative phosphorylation [1]. The movement of protons has two major consequences: (1) it generates a pH gradient across the inner mitochondrial membrane with the pH higher in the matrix than in the cytosol (close to pH = 7); (2) it generates a voltage gradient (transmembrane potential, $\Delta\Psi_m$) across the inner mitochondrial membrane with the inside negative and the outside positive (estimated at −150 to −180 mV negative with respect to the cytosol).

2. Mitochondria as sources and targets of ROS

The ROS category includes the superoxide anion radical as the primary product of one-electron dioxygen reduction, the extremely aggressive hydroxyl radical derived from subsequent reactions, singlet oxygen and strong non-radical oxidants such as hydrogen peroxide. Furthermore, nitric oxide and the derived peroxynitrite radical also belong to the group of ROS.

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Abbreviations: Aβ, amyloid beta; AD, Alzheimer's disease; APP, amyloid precursor protein; COX, cytochrome *c* oxidase; FAD, familial Alzheimer's disease; JNK, c-Jun N-terminal kinase; ROS, reactive oxygen species.

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Mitochondria represent the major source of ROS. One consequence of oxidative phosphorylation is the generation of unpaired electrons. The interaction of these electrons with O_2 results in the generation of superoxide anions (O_2^-), which are highly ROS. Superoxide dismutase (SOD) plays a protective role in all aerobic organisms by detoxifying the superoxide anion in a dismutase reaction producing hydrogen peroxide. Hydrogen peroxide, in turn, can be reduced to water (H_2O) by either glutathione peroxidase or catalase, or it can itself produce an even more potent radical, the hydroxyl radical ($OH^\bullet$). The hydroxyl radical is extremely reactive and can react with nearly all cellular macromolecules including DNA, protein and membrane lipids. Being the major source of ROS, mitochondria are subjected to direct attack by large amounts of ROS in the cell and might be therefore particularly susceptible to oxidative damage.

3. Mitochondria and cell death

Cell death constitutes one of the key events in cell biology. At least two models of cell death can be distinguished: apoptosis and necrosis. Apoptosis involves the regulated action of catabolic enzymes (proteases and nucleases). Characteristic features of apoptosis are changes in nuclear morphology and in chromatin biochemistry. When misregulated, apoptosis can contribute to various diseases including cancer and neurodegenerative diseases [2]. In contrast to apoptosis, necrosis does not involve any regular DNA and protein degradation pattern and is accompanied by swelling of the cytoplasm and of the mitochondrial matrix, which occur shortly before cell membrane rupture. However, it is not uncommon for one effector, such as oxidative stress to have the capacity to trigger either of these death responses, and a combination of apoptosis and necrosis may occur in the same tissue [3]. It was found that mitochondria play a central role in apoptosis, thereby exhibiting major changes in their structure and function [4,5]. The regulatory mechanisms underlying apoptosis and necrosis partially overlap in that mitochondrial membrane permeabilization may constitute a common event of both death modalities [6]. A decrease in mitochondrial membrane potential is an early universal event of apoptosis [7]. In most pathways of apoptosis, the release of mitochondrial cytochrome *c* and apoptosis-inducing factor are also key events in initiating the cascade of reactions leading to apoptotic cell death [8]. The release of cytochrome *c* is clearly regulated by the pro- and anti-apoptotic proteins of the Bcl-family (bax, bak, bad, bim, bid as pro- and bcl-2 and bcl-xl as anti-apoptotic proteins) [9]. The mechanism by which cytochrome *c* activates the apoptotic cascade remains to be fully elucidated; however, it seems to activate caspase-9 which then cleaves downstream effectors and elicits the apoptotic response.

A mismatch between the production of prooxidants and antioxidants in cells might lead to oxidative stress. As mentioned before, mitochondria are very susceptible to oxidative damage. A decrease in mitochondrial membrane potential, an impairment of the mitochondrial respiratory chain and a depletion of ATP are characteristic consequences of oxidative stress, e.g. ROS, lipid peroxidation products (such as hydroxy-2-nonenal), as well as nitric oxide [6,10]. While high levels of oxidative stress can cause necrosis, mild to moderate degrees of oxidative stress induce cell death via the apoptotic cascade. ATP deficiency further leads to a decrease of cell glutathione (GSH), which results in enhanced oxidative stress and triggers the vicious cycle of oxidative stress, mitochondrial dysfunction and apoptosis.

4. Mitochondria, oxidative stress, and AD

Mitochondrial abnormalities have been identified in a large proportion of neurodegenerative diseases [7,11,12]. AD is the most common neurodegenerative disorder marked by progressive loss of memory and impairment of cognitive ability. AD can be classified into two forms: sporadic AD, which accounts for the vast majority of AD cases and where aging represents the main risk factor, and a familial form of AD (FAD), where rare gene mutations have been identified. The latter patients suffer from an autosomal dominant inheritable variant of AD. Notably, patients with either sporadic AD or FAD share common clinical and neuropathological features including Aβ plaques, accumulation of intracellular neurofibrillary tangles, and pronounced neuronal cell loss. The amyloid plaque is composed of Aβ peptide, which is derived from the APP through an initial β-secretase cleavage followed by an intramembraneous cut of γ-secretase. Three genes are known to be causatively linked with the pathogenesis of these early onset FAD forms. Besides the genes encoding for presenilin 1 (PS1) on chromosome 14 and presenilin 2 (PS2) on chromosome 1, mutations in the APP gene on chromosome 21 account for these FAD cases. Remarkably, more than 60 FAD mutations located in these three different genes apparently result in the overproduction of Aβ providing substantial evidence that Aβ plays a central role in the pathogenesis of AD.

The free radical hypothesis of aging [13] claims that the age-related accumulation of ROS results in damage to major components of cells: nucleus, mitochondrial DNA, membranes and cytoplasmic proteins. Many authors suggest that an imbalance between the generation of free radicals and antioxidants may be involved in the pathogenesis of most neurodegenerative diseases. The fact that age is the most important risk factor of sporadic AD provides considerable support for the free radical hypothesis. Many considerations suggest that free radicals and consequently mitochondrial dysfunction are involved in age-related pathologies of AD [14–22]. It seems very likely that

oxidative damage and defective mitochondrial function are the earliest events in AD [14,23].

During the last decade considerable evidence has accumulated demonstrating oxidative stress products on certain cellular targets in AD including mitochondria, proteins, lipids, and DNA [21,24–27]. Additionally, increasing evidence suggests a diminished activity of the α-ketoglutarate dehydrogenase complex (KGDHC) in brains from AD patients [28–30].

Biochemical analysis of CNS tissue from patients with AD has yielded evidence for abnormalities of components of the electron transport chain. In many AD patients, COX activity is impaired in the CNS [31,32] and even in other tissues, including platelets [33]. This defect may contribute to impaired energy generation. Biochemically, the defect was confined to selected brain regions, such as temporal cortex and hippocampus. The respiratory chain is located in the inner mitochondrial membrane. COX is its terminal enzyme and catalyzes the transfer of electrons from its reduced substrate ferrocytochrome *c* to molecular oxygen to form water. The involvement of a nonneuronal tissue suggests that the COX defect is not simply a local consequence of the disease state, but may be genetically determined. Each enzyme complex of the mitochondrial respiratory chain consists of a varying number of subunits. Interestingly, the cellular expression of COX subunit II and IV is reduced during aging and these age-related changes are more marked in AD that might contribute to the development of the disease [34]. These subunits are encoded by both nuclear DNA and mitochondrial DNA (mtDNA). COX encompasses 13 subunits. The three largest subunits, which mainly represent the catalytic centers of COX, are encoded by mtDNA, whereas the remaining are derived from the nuclear genome [35,36]. Thus, low COX activity may result from changes in nuclear DNA, in mtDNA or in the recognition or the import of proteins through the mitochondrial membrane. Mutations in mtDNA, which may be maternally inherited or acquired with aging, can cause a variety of progressive, chronic degenerative diseases [37]. Normal aging is accompanied by the accumulation of multiple point mutations in the control region for replication of mtDNA [38]. Mutations in mtDNA mostly cause central nervous system abnormalities as well as mitochondrial myopathy. It can be speculated that a reduced $\Delta\Psi_m$, impaired ATP generation or an increased generation of ROS resulting from mutations of mtDNA may activate mitochondrial membrane permeabilization and initiate cell death. Point mutations of mtDNA were reported in AD involving complex I and COX [39–46]. Notably, COX activity correlated negatively with increasing mutational burden of mtDNA [41]. Cells depleted of their mitochondria and then fused with mitochondria isolated from AD patients showed a significantly reduced COX activity [47].

Synaptic compartments (presynaptic terminals and postsynaptic dentritic spines) are regions of neurons that are exposed to high levels of oxidative and metabolic stress. This is the case largely because glutamate receptors and calcium channels are concentrated in synaptic compartments, and the membrane depolarization and calcium influx resulting from activation of these ion channels results in oxidative stress and a high energy (ATP) demand. In order to provide high levels of ATP, mitochondria are concentrated in synaptic terminals and play a pivotal role in the regulation of synaptic function. There are many reasons to believe that synapses are the sites where the neurodegenerative process begins in AD (Fig. 1) [15,17,48]. Recent studies have shown that apoptotic biochemical cascades are activated in vulnerable neuronal populations in AD and can also be activated locally following exposure to Aβ: caspase activation, mitochondrial membrane depolarization, generation of ROS and

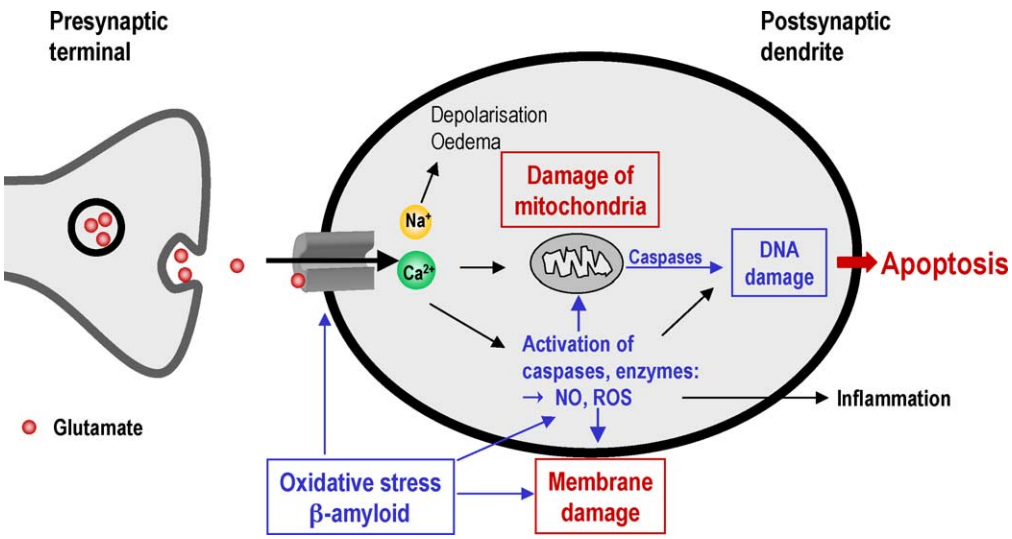

Fig. 1. Pathological processes finally leading to neuronal cell death: mitochondrial dysfunction as a key event in synaptic degeneration. See text for discussion.

nitric oxide, dysregulation of the cytosolic and mitochondrial calcium homeostasis, chromatin condensation, nuclear damage and fragmentation (Fig. 1).

5. Effects of Aβ peptide on mitochondria and pathways associated with apoptosis

Aβ, a 40–42 amino acid peptide that principally constitutes senile plaques, is thought to play a crucial role in the pathogenesis of AD. Supporting this hypothesis, Aβ has been demonstrated to be directly toxic to cultured neurons and aggregation of Aβ into fibrils is apparently required for its cytotoxic effect [49]. Recently, it has been shown that Aβ can be formed intracellularly in neurons [50]. Moreover, several findings indicate that neuronal cell death associated with Aβ peptide is apoptotic in nature [51–55]. Indeed, examination of post-mortem tissue has implicated caspases (caspase 3, 8, and 9) in sporadic AD [56–59]. One possible mechanism for initiating apoptosis could be the generation of free radicals by the peptide [60] leading to lipid peroxidation and oxidative stress. Furthermore, oxidative stress induces intracellular accumulation of Aβ [61]. Nevertheless, the biochemical mechanisms underlying Aβ toxicity remain largely unknown. One consistent observation on Aβ cytotoxicity is the rapid inhibition of cellular 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reduction to formazan [62]. Both oxidative stress-dependent and -independent effects of Aβ, such as membrane-destabilizing properties [63] seem to be detected using MTT reduction [62,64].

Several studies have suggested that Aβ may be directly toxic to isolated mitochondria [65]. Interestingly, the activities of those enzymes, which are reduced in the brains from AD patients, such as COX, α-ketoglutarate dehydrogenase and pyruvate dehydrogenase, were inhibited by Aβ [65]. Increased intracellular Aβ levels can further exacerbate the genetically driven COX defect in sporadic AD and may facilitate mitochondrial permeability transition opening [66]. These findings indicate that Aβ can directly disrupt mitochondrial function and may contribute to the deficiency of energy metabolism seen in AD. Moreover, recent data indicate that Aβ induces neuronal apoptosis via mechanisms involving JNK pathway, induction of Fas ligand, and the release of Smac via AP-1/Bim activation from mitochondria [67–69].

Mounting evidences indicate increased susceptibility to cell death and increased oxidative damage as common features in neurons from sporadic AD patients but also from FAD cases. All known mutations in APP, PS1 and PS2 apparently result in the overproduction of Aβ providing substantial evidence that Aβ plays a central role in the pathogenesis of AD probably by increasing oxidative stress. Recent findings further indicate that these mutations affect mitochondrial function linked to pathological cell death. Thus, an increased superoxide production seems to mediate the cell death-enhancing action of PS1 mutations [70]. Furthermore, PS1 and PS2 interact with members of the bcl-2 family [71] and, in turn, bcl-2 protects mitochondria against lipid peroxidation induced by oxidative stress and/or Aβ [54]. In addition, several recent findings have linked Aβ toxicity and increased Aβ production due to FAD-related APP or PS1 mutations with cell death [72–74]. Recent findings indicate that the expression of mutant PS1 or mutant APP in PC12 cells sensitises cells to apoptosis [75–77]. In addition, cultures of primary neurons from PS1 mutant knock-in mice (PS1 M146 KI) and from transgenic mice bearing human mutant PS1 M146L show increased vulnerability to cell death [78,79]. In addition, lymphocytes from FAD patients bearing PS1 mutations [80] show similar results in regard to increased vulnerability to DNA damage finally leading to accelerated cell death. Moreover, enhanced vulnerability to cell death has also been described in peripheral lymphocytes from AD patients [80–82], but not in patients with vascular dementia [82]. Specifically, oxidative stress mechanisms seem to be involved in this process [82–84].

To further elucidate the biochemical pathways induced by Aβ, we investigated the effect of the Swedish APP double mutation (APPsw) on oxidative stress-induced cell death mechanisms in PC12 cells. This mutation results in from 3- to 6-fold increased Aβ production compared to wild-type APP (APPwt). Our cell model offers two important advantages. First, compared to experiments using high concentrations of Aβ at micromolar levels applied extracellularly to cells, APPsw cells secret low Aβ levels similar to the situation in FAD brains. Thus, our cell model represents a very suitable approach to elucidate the AD-specific cell death pathways mimicking physiological conditions. Second, compared to PS1 mutant cells, these two cell lines—APPwt and APPsw—with different production levels of Aβ may additionally allow to study dose-dependent effects of Aβ. Expression of APPsw rendered PC12 cells vulnerable to the induction of cell death after exposure to oxidative stress (hydrogen peroxide) [76,85]. One potential mechanism by which APP mutations enhance this process could be the increased production of Aβ, which is able to induce apoptotic cell death. However, in the absence of apoptosis-inducing treatments, we found no evidence for increased apoptosis *per se*. Therefore, it seems rather likely that increased production of Aβ at physiological levels somehow primes APPsw cells to undergo cell death, leading to increased cell death only after additional stress, e.g. oxidative stress, a scenario which has also been suggested to occur in AD brain. Moreover, we could show that in PC12 cells bearing APPsw caspase 3 activity was significantly elevated compared to APPwt and vector-transfected control cells [76]. Interestingly, APPwt also showed an increased activity of caspase 3 compared to empty vector control cells even though to a lesser extent than APPsw cells. Our data

suggest that already very low Aβ levels in APPwt cells are probably sufficient to prime cells to undergo apoptosis and increasing Aβ levels, as in the case of APPsw cells, even strengthen the effects on caspase 3 activation. This is confirmed by findings that caspase 9 is increased and at the same time ATP levels and mitochondrial membrane potential are lowered in APPwt as well as in APPsw cells compared to vector (vct)-transfected control cells [86].

Interestingly, we could also show that the respiratory chain complexes II, III, IV and F_0F_1-ATPase in APPsw cells are more vulnerable against mitochondrial membrane potential changes than vct cells. Additionally, the complexes IV and F_0F_1-ATPase of APPwt cells are more sensitive than vct cells but as sensitive as APPsw cells [86]. Possibly, the increased Aβ production by APP-transfected cells might trigger the dysfunction of the respiratory chain complexes perhaps by a direct inhibition of the mitochondrial respiration. The inhibition of the respiratory chain by Aβ could be one explanation for decreased ATP levels and the enhanced cell death in APPsw cells. An important mediation of stress signaling in cells occurs by the stress-activated protein kinase JNK/SAPK. In APPsw cells JNK pathway activation is enhanced. The importance of the JNK pathway becomes explicit, since we observed attenuation of apoptosis by SP600125, a JNK inhibitor, through protection of mitochondrial dysfunction and reduction of caspase 9 activity [87]. In addition, we found that in APPsw cells activation of caspase 2 and caspase 8 is enhanced after exposure to oxidative stress [87,88].

6. A hypothetical sequence of the pathogenic steps of AD

On the basis of these findings, we propose a hypothetical sequence of events linking sporadic AD, FAD, Aβ production, and mitochondrial dysfunction with caspase pathway and neuronal cell loss. Two parallel pathways may occur in APP transfected PC12 cells under oxidative stress conditions (Fig. 2). Already at low Aβ levels, the intrinsic pathway is activated leading to mitochondrial dysfunction, e.g. decrease in mitochondrial membrane potential and depletion in ATP. Cytochrome *c* is released by dysfunctional mitochondria and activates caspase 9. Interestingly, it was recently discovered that intrinsic pathways, such as those initiated by cell stress, induce activation of caspase 2,

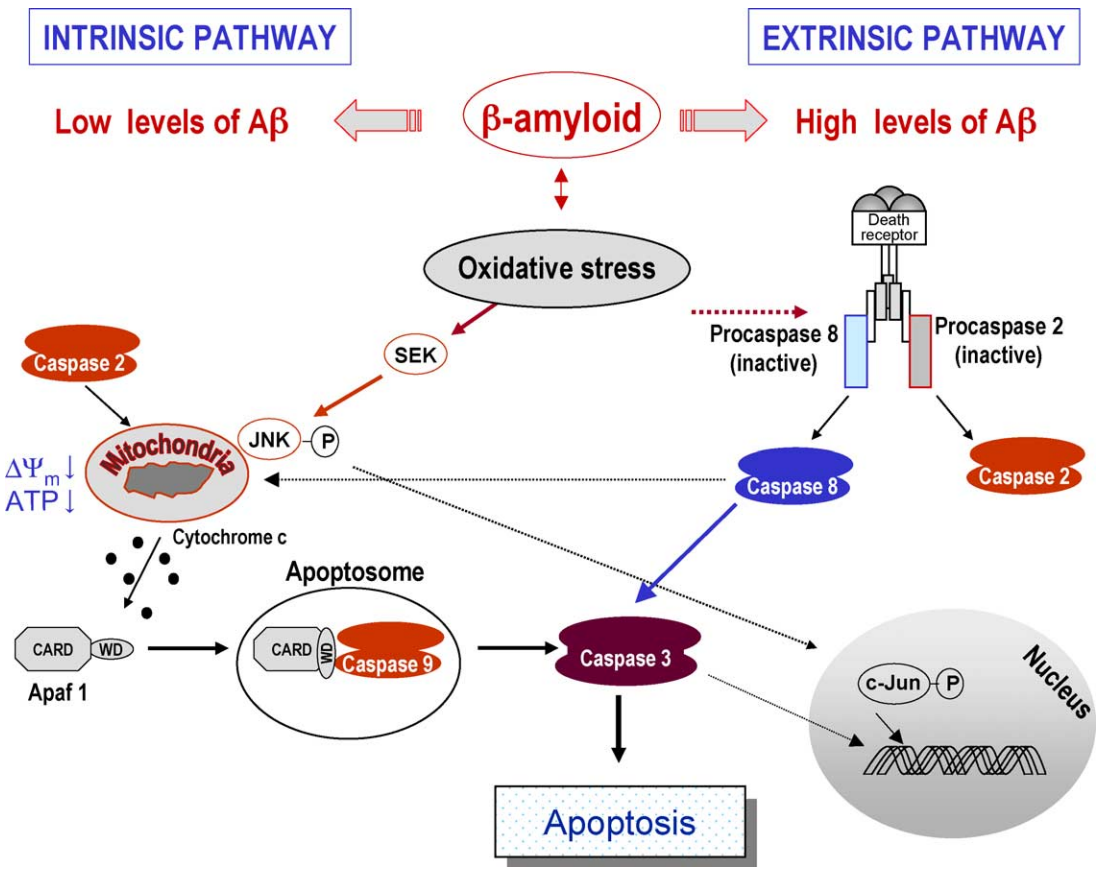

Fig. 2. Working model of apoptotic biochemical cascades occurring in AD: a hypothetical sequence of events leading to caspase activation. Two parallel pathways, activated either by already low concentrations of Aβ or in addition by high levels of Aβ, may operate within individual neurons. Oxidative stress induced or enhanced by Aβ leads to mitochondrial dysfunction. Cytochrome *c* is released by dysfunctional mitochondria and activates caspase 9. An additional mechanism is that the accumulation of high amounts of Aβ may lead to the cross-linking and activation of receptors resulting in the activation of the extrinsic apoptotic pathway. Both pathways then converge by activating the effector enzyme, caspase 3 (in accordance with [59]).

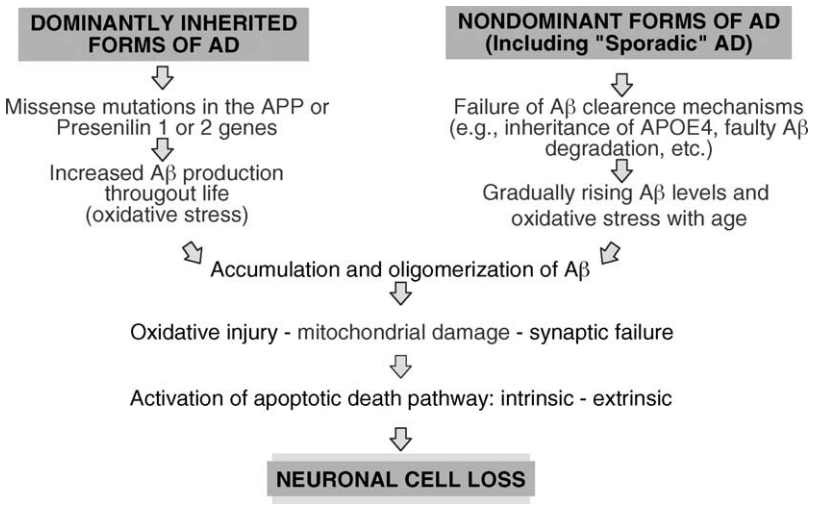

Fig. 3. A hypothetical sequence of the pathogenic steps linking sporadic AD, familial AD, and Aβ production with mitochondrial dysfunction, caspase pathway, and neuronal cell loss (in accordance with [48]).

which is required for permeabilization of mitochondria [89]. Caspase 2 activation was strongly and specifically increased in APPsw PC12 cells suggesting that this caspase is acting upstream of mitochondria and forces the intrinsic pathway when Aβ levels increase. Moreover, it has been reported that Aβ may lead to the cross-linking and activation of receptors resulting in caspase 8 activation [58]. Indeed, in our cell model, caspase 8 activation was increased in the presence of high Aβ concentration produced by APPsw cells. Whether this increase is only due to effects of extracellular Aβ on cell death receptors or can also be mediated by intracellularly accumulated Aβ is not clear. Both pathways may then converge by activating the effector enzyme, caspase 3, and the execution of cell death (Fig. 2). Nondominant forms of AD including sporadic AD, in which Aβ and oxidative stress levels gradually rise with age, and dominantly inherited forms of AD, in which high Aβ production increases throughout life, converge at a final common pathway of an increased vulnerability to apoptotic cell death (Fig. 3). Recent evidence suggests that synaptic dysfunction, which seems to occur prior to obvious neuronal degeneration, is caused by diffusible oligomeric assemblies of Aβ peptide. Within this cascade, oxidative injury and mitochondrial damage play a central role.

Mitochondria may act as amplifiers rather than initiators of caspase activity. Efforts to block the activity of post-mitochondrial caspase 9 and caspase 3 may possibly only inhibit the amplification loop, but not prevent, cell death. Based on this assumption, strategies involving efforts to protect cells at the mitochondrial level by stabilizing or restoring mitochondrial function or to target events upstream of mitochondria appear to be promising. Interestingly, perfectly in line with this hypothesis, numerous lines of evidences indicate stabilization of impaired mitochondrial function by *Ginkgo biloba* extract EGb 761 and lipoic acid [64,90]. Provided that mitochondrial dysfunction does have a causative role in disease pathogenesis, then the development of a number of novel therapeutic targets is implicated. These approaches could result in novel treatments for AD.

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