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Review

The Mitochondrial Secret(ase) of Alzheimer's Disease

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Abstract. Alzheimer's disease (AD) is a neurodegenerative disorder characterized clinically by progressive decline in memory and cognition and pathologically by extracellular amyloid-β (Aβ) deposits and intraneuronal aggregates of hyperphosphorylated tau. Since its proposal in 1992, the amyloid cascade hypothesis implicates Aβ overproduction as a causative event in disease pathogenesis, and this thinking has predominated the field's understanding of AD pathogenesis and the development of potential therapeutics (i.e., Aβ-reducing agents). Though Aβ has been shown to induce AD pathology, unanswered questions for sporadic AD development suggests this hypothesis is best applied to familial disease only. The more recent mitochondrial cascade hypothesis is supported by data showing that early impairments of mitochondrial dysfunction and oxidative stress may precede Aβ overproduction and deposition. However, the development of Aβ-reducing agents continues. Unfortunately, these agents have not been efficiently tested for their effect on one of the earliest AD pathologies, i.e., mitochondrial dysfunction. This paper will review supporting data for the amyloid and mitochondrial cascade hypotheses, reports of the effects of secretase inhibitors on AD-phenotypic cells and animals, and begin to look at a potential role for γ-secretase, which is localized to mitochondria, in AD-related mitochondrial dysfunction.

Keywords: Alzheimer's disease, amyloid-β, γ-secretase, mitochondria, mitochondria-respiration

INTRODUCTION

A disease of progressive deterioration in memory and cognitive function, Alzheimer's disease (AD) is a neurodegenerative disorder of older age, which initially reduces ability to recollect recent events and later progresses to problems with language, visuospatial skills, judgment, and problem solving [1]. Affected individuals may also experience personality changes, sleep disturbances, and disorientation to time and place [1, 2]. A clinical diagnosis of probable AD depends on the presence of impairments in memory and a second cognitive area affecting an individual's activities of daily living, without evidence of other systemic or neurological disorders that may mimic the same symptoms. Currently, a definitive diagnosis of AD can only be made with postmortem findings of two pathognomic features: extracellular deposits of amyloid-β (Aβ)-containing plaques and intraneuronal aggregates of hyperphosphorylated tau. Depending on the dementia stage, there is also neuronal loss in the hippocampus and frontal cortex. Displaying significant atrophy of the hippocampus and frontal cortex seen on CT scan and on autopsy, AD brains also show significant oxidative stress, inflammation, cholinergic dysfunction, and synaptic degeneration [3–8]. Which pathological le-

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sion is the earliest marker of the disease's etiology has remained controversial, since each is associated with other aspects of disease pathology and can ultimately contribute to neuronal death. Among the list, the roles of Aβ overproduction and mitochondrial dysfunction in AD etiology have been under investigation for some time and have led to the development of two theories for AD development: the amyloid cascade hypothesis and the mitochondrial cascade hypothesis. In addition to their individual hypothesized roles in AD pathogenesis, mitochondrial and Aβ abnormalities appear to have a circular cause-and-effect relationship to one another. This review will discuss Aβ and mitochondria as they relate to what is currently understood about AD pathogenesis, as well as how they and their related proteins may work for or against one another in disease development and therapy.

AMYLOID CASCADE HYPOTHESIS: MECHANISMS AND THERAPEUTICS FOR Aβ PATHOLOGY

First described by Hardy and Higgins in 1992, the amyloid cascade hypothesis pinpoints the overproduction of Aβ, the major component of amyloid plaques, as the initiating event in AD pathology, occurring upstream to the development of tau tangles, neuronal death, and declining cognitive function [9]. The strength of this theory lies in the following evidence: 1) in familial and Down syndrome cases of AD, point mutations and duplications in Aβ's parent amyloid-β protein precursor (AβPP) lead to early onset AD [10–13]; 2) *in vitro* and *in vivo* studies have connected Aβ generation and oligomerization with the reproduction of AD-related pathologies: tau hyperphosphorylation, inflammation, oxidative stress, synaptic dysfunction, and memory deficits in cell and animal models [14–22]; and 3) early observations of reductions in Aβ levels [23,24] appear to rescue AD-related pathologies in cell and animal models [25,26].

Aβ is a cleavage product of AβPP, a type 1 integral membrane glycoprotein encoded for on chromosome 21, ubiquitously expressed in human tissues, and localized to the plasma membrane, endoplasmic reticulum, Golgi apparatus, and mitochondria [27,28]. To date, the function of AβPP is not completely understood, but is suggested to have roles in cell adhesion, synaptic function, or neural plasticity [29,30]. AβPP's intracellular and extracellular domains are conserved across other species' protein types (i.e., APL-1, APPL, APLP1, and APLP2); however, the Aβ peptide domain is distinctive to mammalian AβPP and is not necessary for the physiological function of the AβPP species [29,30]. Several isoforms of AβPP exist due to alternative splicing, with AβPP695 predominately expressed in neurons. However, in sporadic AD (sAD), two reports reveal a ratio shift towards the neuronal expression of AβPP isoforms other than AβPP695 [31, 32], and additional reports indicate alterations in AβPP expression in platelets of AD individuals [33–35]. In addition to alternative splicing, AβPP may be truncated on the carboxyl end to form smaller and less amyloidogenic fragments. Based on suggestions that non-neuronal isoforms and truncated forms may differ in amyloidogenicity, mRNA transcription alterations and posttranslational processing may serve as important determinants for increased Aβ production in sAD, which makes up more than 90% of all AD cases compared to familial AD (fAD).

AβPP is sequentially cleaved by two of three enzymes: α-, β-, and γ-secretases. AβPP is initially cleaved by α- or β-secretases at its N-terminus, producing either soluble AβPPα or AβPPβ, respectively. α-secretase cleaves within the Aβ domain, precluding the generation of Aβ peptides. The remaining peptide (C83 after α-secretase cleavage or C99 after β-secretase cleavage) is then cleaved by γ-secretase to produce the non-amyloidogenic p3 fragment (after α-secretase) or amyloidogenic (fibril-forming) Aβ peptide (after β-secretase) [36,37]. This naturally occurring cleavage sequence differs from AD pathogenesis only in that Aβ production and deposition are increased and accelerated in the disease. Aβ peptides can be 39–46 amino acids in length depending on the site of γ-secretase cleavage, with Aβ_{40} and Aβ_{42} being the most abundant and relevant to AD pathogenesis. Secreted Aβ_{40} is more abundant overall, but Aβ_{42} production increases and is more prevalent in amyloid plaques and cerebral vasculature in familial and sporadic forms of AD [38,39]. In fAD, AβPP mutations located at or near secretase cleavage sites exist and decrease the Aβ_{40}/Aβ_{42} ratio [10,11,38], probably by altering the conformation of γ-secretase when it binds to mutated AβPP [40]. But so far, no mechanism for regulating γ-secretase specificity at the Aβ_{42}-generating site of C99 has been identified in sAD. This mechanism, once identified, could serve as a potential target in AD drug development according to the amyloid cascade hypothesis. Once generated, Aβ_{40} and Aβ_{42} readily aggregate, first forming small and then larger oligomers, and then producing fibrils that can be deposited into the

extraneuronal space. Aβ_{42} overproduction is studied as the causal event in the amyloid cascade theory because it demonstrates greater aggregation and stabilization than Aβ_{40}, as well as increased neurotoxicity and synaptotoxicity [41–45]. Early in the discovery of Aβ toxicity in AD, it was thought that the fibrillar form found within amyloid plaques was the most detrimental to neurons and synapses. However, the inability to correlate amyloid plaque levels with level and distribution of neurodegeneration and cognitive decline weakened this case [46,47]. Later, evidence for the toxicity by the soluble, oligomeric form of Aβ_{42} emerged, with specific evidence citing its role in synaptic plasticity disruption, calcium dysregulation, tau hyperphosphorylation, reduced mitochondrial respiratory protein activity, increased oxidative stress, and neuronal death [15, 17,20,22,48–52].

The amyloid cascade theory is highly reliant on a combination of data from Down syndrome, fAD, and transgenic mouse models, where the overexpression or mutation of proteins responsible for Aβ production demonstrates Aβ as the causative factor in the initiation and progression of tau hyperphosphorylation, mitochondrial and synaptic dysfunction, and apoptotic cell death. Inhibition of β- or γ-secretase or upregulation of α-secretase have been proposed as potential therapeutic targets by the amyloid cascade hypothesis, which suggests that targeting the generation, oligomerization, or clearance of Aβ could lead to a recovery or slowed decline of neurodegeneration. This hypothesis and the upregulation of β- and γ-secretase activity in the brains of AD individuals make the targeting of these enzymes a highly probable therapy. The major class of drugs currently under investigation for Aβ-reducing therapy is traditional β- and γ-secretase inhibitors, but enhancers of α-secretase activity have been proposed as well. In an effort to identify the most effective therapy, it is crucial to understand how the characteristics of each enzyme relate to AD pathogenesis.

The β-site AβPP cleaving enzyme (BACE1 and its homologue BACE2) is the aspartyl protease responsible for β-secretase activity and the production of sAβPP and C99 peptide [53], which is subsequently cleaved by γ-secretase. The brains of individuals with sAD display increased BACE1 protein and activity levels, in addition to increased levels of its cleavage product AβPP [54,55]. This increase in β-secretase activity is also apparent in mild cognitive impairment (MCI), a prodrome to AD, making it feasible that early increases in activity provide large amounts of Aβ that can exert pathological effects on mitochondria, tau protein, and synapses. In accordance with the amyloid cascade hypothesis, homogeneous and heterogeneous BACE1 knockouts eliminate or reduce Aβ generation, decrease plaque formation, and improve synaptic plasticity and memory of transgenic AD mice [56–58]. This improvement in synaptic plasticity and memory associated with reduced Aβ levels strengthens the case for using BACE1 as a therapeutic target. β-secretase inhibitors are still in early stages of development and clinical trial testing [59,60]. Therefore information on the efficacy is very limited at this time.

Since the function of AβPP and its cleavage products are not well understood, another emerging theory is that increased production of Aβ coincides with reduced levels of AβPP and other potentially beneficial cleavage products [61,62]. The enzyme responsible for the production of non-amyloidogenic AβPP products is α-secretase, a zinc metalloproteinase (ADAM9, ADAM10, and ADAM17/TACE) that releases soluble sAβPPα, a peptide suggested to have neuroprotective properties [63,64]. ADAM10 is necessary to reduce AD plaques and improve learning and memory in mouse models [65,66]. Overexpression or upregulation of α-secretase activity by agents like copper [67], muscarinic agonists, anti-cholesterol agents, and nonsteroidal anti-inflammatory drugs (NSAIDs) [63] has been proposed as a potential AD therapeutic because: 1) α-secretase activity precludes the generation of Aβ by β-secretase cleavage and would therefore increase production of the non-amyloidogenic, potentially neuroprotective cleavage products of AβPP [68] and 2) reports of reduced levels of the α-secretase and its cleavage product (sAβPPα) in platelets and cerebrospinal fluid of AD subjects have been made [69–71]. This reduction in an α-secretase cleavage product raises questions of whether Aβ overproduction is due directly to rises in β-secretase activity or indirectly through depressed α-secretase activity that makes AβPP more available for Aβ-generating cleavage, and it has potential as a potential biomarker because of its correlation with pre-clinical and clinical stages in patients with Swedish mutation AD [71]. Whether this reduction in α-secretase cleavage product is a response to increased β-secretase cleavage and reduced availability of the α-secretase substrate [55] or an otherwise unknown cause of reduced α-secretase activity with a subsequent response in increased β-secretase activity still remains to be understood, but the identification of a direct relationship in the activities of α- and β-secretase proteins would assist in determining which enzyme's activity is affected first by the inciting event for AD pathogenesis.

Table 1
γ-secretase targets and their cleavage sites. γ-secretase cleaves a number of transmembrane proteins at the sites indicated. For some proteins (AβPP and Notch), presenilin demonstrates the ability to cleave at multiple sites, resulting in products of varying lengths. The peptide sequence of Aβ is bolded

Known Target	Size	Cleavage Site
AβPP770 [37] **AβPP751** **AβPP695 (neuronal isoform)**	770aa 751aa 695aa	SGLTNIKTEEISEVKM**DAEFRHDSGYEVHHQKLVF** **FAEDVGSNKGAIIGLMVGGV IA** TVIVITLVML
Notch 1 [195]	2555aa	VQSETVEPPPPAQLHFMYVAA AA FVLLFFVGC GV L LSRKRRRQHGQLWFPEG
ErbB4 [193]		PLIAAGVIGGLFILVIVGLTFA VYVRRKSIKKKRALR
E-cadherin [82]	901aa	EAGLQIPAILGILGGILALLILILLLLLFL RRRAVVKE
p75NTR [80]	427aa	PVVTRGTTDNLIPVYCSILAAVV VGLVAYI AFKRWN

EMBL/GenBank/DDBJ databases.

Results from Kim and colleagues [72] suggest AβPP cleavage activity by α-secretase and β-secretase were not directly linked when inhibitors against TACE or BACE were used and the β- and α-secretase cleavage products were measured. Though brain β-secretase levels have been reported as increased, to our knowledge, no reports of alterations in brain α-secretase levels in AD individuals have been made. Therefore, with the data presently available to us, we know that Aβ overproduction could be due to independent decreases or increases in α- or β-secretase activity.

γ-secretase is a protein complex made up of enzymatically-active presenilin 1 or 2 (PS1, PS2), nicastrin (NCT), presenilin enhancer 2 (PEN2), and anterior pharynx-defective phenotype (APH-1). Discovery of PS1 as the catalytic enzyme responsible for Aβ generation occurred when PS1-/- mice were found to have decreased Aβ levels, in addition to altered skeletal and brain development [73]. It was further supported by evidence of γ-secretase deficiency in models of PS1 and PS2 mutations [74,75]. PS1 and PS2 are homologous aspartyl proteases whose genes are mapped to chromosomes 1 and 14, respectively [76–78]. In addition to AβPP cleavage, the γ-secretase complex is responsible for processing of Notch, E-cadherin, ErbB4, and p75NTR (Table 1) [74,79–83], and loss of function in one enzyme can be partially compensated by the other PS, though activity is usually reduced. Substrate recognition is dependent on NCT function, while assembly of the complex occurs via the scaffolding function of APH-1 [84]. APH-1a, which has two splice variants (APH-1aS and APH-1aL), and APH-1b are homologous proteins that together with PS1 and PS2 can form up to six distinct γ-secretase complexes [85]. After assembly of PS1/PS2, NCT, and APH-1aS/APH-1aL/APH-1b, PEN2 binds to activate and stabilize the complex [84,86]. The ability of the four components to form up to six distinct complexes suggests each may possess different substrate affinities and serve importance in specific environments. Some authors have also suggested that other proteins associated with the γ-secretase complex, like TMP21, may assist in determining the cleavage specificity of the enzyme with its substrates [87–90], further suggesting a physiological regulation of substrate recognition and target processing. Mutations in PS1 are responsible for over 50% of fAD cases and are highly penetrant before the age of 65 [88–90]. These PS mutations tend to shift the cleavage specificity of C99 to increase generation of $A\beta_{42}$ [91], possibly by altering γ-secretase conformation to decrease or increase binding of the enzyme with the Aβ residues responsible for forming $A\beta_{40}$ or $A\beta_{42}$, respectively [92]. PS2 mutations are less abundant, but also lead to increased $A\beta_{42}$ production. Additional genetic and environmental factors appear to be responsible for variations in penetrance because PS2 mutations tend to have variations in age of onset and symptom severity, even within the same pedigree [93]. These variations were not accounted for by apolipoprotein E (ApoE) polymorphisms [93,94], of which APOE ε4 is associated with increased risk for AD [95].

Intramembranous cleavage of AβPP is dependent on aspartate (Asp) residues in the transmembrane domain of PS1, leading to a loss or gain of γ-secretase function when Asp257 or Asp385 are deleted or mutated [79]. In addition, these Asp residues affect the ability of PS1 to cleave its other transmembrane protein targets [79,96–98]. However, interactions with an important nucleotide-binding site on PS1 can preferentially block or increase AβPP cleavage, while sparing alterations to Notch processing [99]. The specificity of γ-secretase for AβPP versus Notch can also be con-

trolled by Rac1, a G-protein suggested to play a role in AD because it mediates platelet-derived growth factor-induced cleavage of AβPP by β- and γ-secretase [100]. It was also found that the Rac1 inhibitor, EHT 1864, reduces the Aβ-generating activity of γ-secretase without affecting its role in Notch1 cleavage [101]. Therapeutic targeting of γ-secretase specificity for its targets (or certain residues as is the case with PS1 mutations on AβPP processing) would be beneficial for inhibiting the Aβ-generating γ-secretase action, without unwanted side effects caused by inhibition of Notch or E-cadherin. The potential for multiple mechanisms that control substrate and residue recognition for the complex strongly supports its use in multiple pathways. Based on such observations, one could also speculate that additional pathways may be present in which γ-secretase works. The ability for presenilins to serve in multiple cleavage roles is of great importance because potential AD treatments must consider the impact of non-specific γ-secretase inhibition on the complex's other targets.

Non-specific γ-secretase inhibitors (GSIs) are effective in reducing Aβ production [23,102] in brain, plasma, and cerebrospinal fluid, and the inhibitor DAPT (N-[N-(3.5-difluorophenacetyl)-l-alanyl]-S-phenylglycine -t-butylester) demonstrates the ability to improve fear conditioning responses in a transgenic mouse model with the Swedish AβPP mutation [25]. Though DAPT is a nonspecific inhibitor, its effect on cognition appears to be related to amyloid generation, as no effect on control animals was detected. In addition, results of reduced Aβ production and oxidative stress, improved mitochondrial membrane potential, and lessened vulnerability to apoptosis in a loss-of-function mutant of PS1 were replicated with use of DAPT [26]. These discoveries support the use of γ-secretase inhibitors in clinical trials, but non-specific γ-secretase inhibitors also inhibit Notch cleavage and signaling, resulting in side effects related to the gastrointestinal tract, thymus, and spleen. Therefore, the development of Notch-1 sparing inhibitors that selectively reduce Aβ has also become a major focus. The identification of a useful γ-secretase inhibitor must balance the concentration needed for Aβ-lowering and cognition-enhancing effects with Notch-inhibiting effects. Notch-1-sparing GSI begacestat demonstrated selective inhibition of AβPP cleavage over Notch cleavage in cells, a 52% reduction in brain Aβ_{42} in Tg2576 mice, and reversal of impaired contextual fear conditioning after oral dosing [103]. Currently in Phase III clinical trials, the notch-1-sparing GSI semagacestat (LY450139) was reported to reduce CNS Aβ levels by 47–84% (depending on dose) in healthy men ages 21–50 years of age when given orally [104]. However, it still remains to be seen if these inhibitors protect or enhance cognition in human subjects.

While we have much evidence for potential improvements by γ-secretase inhibitors, we must also acknowledge some conflicting evidence for the use of γ-secretase as a therapeutic target. While the amyloid hypothesis posits that presenilin mutations are involved in familial AD pathogenesis through the overproduction and accumulation of Aβ_{42}, the newly discussed presenilin hypothesis suggests instead that presenilin mutations may result in a loss of physiologically important γ-secretase functions [105]. Saura and colleagues reported a conditional presenilin double knockout that recapitulates features of AD, specifically impairments in synaptic plasticity and increased tau hyperphosphorylation and neuronal cell death [105], further raising questions for other physiological roles of this protein. Shen and Kelleher [91] thus proposed a hypothesis where loss of presenilin function leads to synaptic dysfunction, resulting in the tau hyperphosphorylation and neurodegeneration. In addition, they reviewed a number of PS1 and PS2 mutations that result in decreased Aβ_{40} production, reduced proteolysis of Notch and E-cadherin, and impaired γ-secretase-independent functions, such as the destabilization of β-catenin responsible for the downregulation of the *Wnt* signaling pathway. Though these reports of loss of function for γ-secretase may only represent an etiology for familial AD, additional investigations may grant us further understanding of how important γ-secretase functions are altered in sporadic AD. In addition, while this recent data conflicts with the widely acknowledged role of γ-secretase in AβPP processing and amyloid-induced neurodegeneration, understanding all of the potential roles of γ-secretase is crucial because an effect of γ-secretase inhibitors on oxidative phosphorylation and ATP production has not yet been reported. If mitochondrial bioenergetics can be pharmacologically improved, early pathogenesis of the disease may be halted and prevent disease progression.

Currently, some therapies originally developed for other AD pathologies as their targets (i.e., cholinergic dysfunction and inflammation) have come under investigation again for their potential role in AβPP processing. The cholinergic system plays an important role in memory function, and acetylcholine (ACh) levels are reduced in AD-affected brain regions [6]. In clinical trials, some cholinergic agonists and acetyl-

cholinesterase (AChE) inhibitors have led to improvements in cognition and activities of daily living [106, 107], making these drugs one of today's first-line treatments. Cholinesterases can also cleave AβPP and promote Aβ aggregation; therefore, besides their primary effect in increasing ACh levels, some inhibitors (e.g., rivastigmine) reduce Aβ generation and/or aggregation [108–111], while others (e.g., tacrine) reduce Aβ by inhibiting BACE activity [112]. Because Aβ can result in the loss of cholinergic neurons [14], this dual function could serve a synergistic function, first reducing Aβ levels and preventing cholinergic neuronal loss and second by increasing AChE present in the synaptic cleft to preserve and enhance memory. Initial efficacy by cholinesterase inhibitors in clinical trials has been documented, but unless cholinergic dysfunction is the major contributor to and/or early deficit in AD pathology, they have yet to demonstrate long-term efficacy and currently remain for symptomatic use only.

Epidemiological evidence and clinical trials suggest use of nonsteroidal anti-inflammatory drugs (NSAIDs) lowers AD risk [113,114]. Their anti-inflammatory nature makes NSAIDS a potential therapeutic against the AD-associated elevations in activated microglia and inflammatory cytokines. Some NSAIDs (e.g., ibuprofen, fluriprofen, and indomethacin) also possess the ability to reduce Aβ_{42} levels by modulating γ-secretase activity to preferentially generate the shorter, less amyloidogenic peptide Aβ_{38} [115–118]. The γ-secretase modulating feature of NSAIDs does not affect other targets of γ-secretase activity (i.e., Notch and other AβPP cleavage products) and is thought to act synergistically with its anti-inflammatory effects; therefore, this was a promising step for AD therapy. However, Aβ_{42}-suppressing NSAIDs do not appear to provide additional protection against AD development in non-diagnosed individuals compared to non-Aβ_{42}-suppressing drugs [114,119], therefore debunking the hypothesis that its positive effects are mediated partially through lowering Aβ. In addition, whatever the mechanism for NSAID effectiveness in preventative therapy in the short-term is, the data for their usefulness in already-diagnosed individuals is inconsistent. Aβ-lowering NSAIDs indomethacin and the R-enantiomer of fluriprofen appear to reduce the progression of cognitive decline during short-term use (6–24 months) [4, 120]. However, for long-term therapy, the clinical trials for therapeutic use of NSAIDs in individuals already diagnosed with AD have not shown similar degrees of protection against advancing disease [24,121, 122], suggesting NSAIDs are unable to serve beyond a preventative capacity.

Although the amyloid cascade hypothesis still remains the leading theory in the field, the link in sporadic AD is weakened by a lack of evidence for a relationship between the pattern of amyloid deposition and cognitive dysfunction. It is also compounded by the difficulty in identifying correlative biomarkers based on systemic Aβ concentrations, and the high dependence on transgenic animal and familial models for developing therapeutics. Though the amyloid cascade hypothesis has been useful in identifying downstream effects of Aβ overproduction, its usefulness is limited to familial cases. These models are solely based on Aβ overproduction as the initiating event in AD pathogenesis, and therefore lack an explanation for the genesis event for Aβ overproduction in sporadic cases of AD. Therefore, a closer look at other, potentially earlier, forms of AD pathology is warranted. If identified, a genesis event triggering AD pathogenesis may be linked to Aβ overproduction.

Since the early classification and descriptions of AD and its pathologies, many hypotheses have been suggested; unfortunately, none fully explain the early events that trigger metabolic and cellular alterations in neuronal degeneration. Once fibrillar Aβ, which makes up plaques deposits, was overshadowed by oligomeric Aβ as a causative neurotoxic agent towards neurons and synapses, it has brought back into question a long-time theory behind AD pathogenesis. Correlations between cognitive function and tau, synaptic dysfunction, and cerebral atrophy exist, but the role of overproduction and aggregation of Aβ is still perplexing because it appears to be involved in all aspects of AD pathogenesis and progression. If Aβ is the major initiating event for AD pathology, then its overproduction should show a relationship with pathology. In an effort to further elucidate an initiation event in AD, it is important to consider and evaluate all hypotheses, as well as consider relationships between each that may make neurons more susceptible to neurodegeneration.

Two pieces of evidence missing from the preclinical drug investigations of prospective disease modifying anti-Alzheimer's drugs (DMAADs) include: 1) selectivity for non-AβPP/Notch γ-secretase substrates/activities and 2) a positive effect on mitochondrial function, already shown to be impaired early in the disease. The ubiquitous presence of γ-secretase also warrants further study for additional physiological roles because even Notch-sparing molecules may not avoid adverse effects of inhibiting other physiological functions, especially those related to mitochondria. There is also an increased need for the identification of

therapeutic targets that are affected early on in disease pathogenesis. Therefore, the next step in AD research is to study the effect of these drugs on mitochondrial respiration. Since mitochondria are important for energy homeostasis, and impaired function is evident in many neurodegenerative diseases, this review will now delve into the relationship between AD and mitochondrial function, while also relating back to the contributions of other areas of AD pathology in the initiation or progression of impaired mitochondria function.

MITOCHONDRIAL CASCADE HYPOTHESIS

The mitochondrial cascade hypothesis was proposed by Swerdlow and Khan in 2004 as a suggestion for the development of sporadic AD in an aging population [123]. Based on emerging data for a significant role of mitochondrial dysfunction in AD, the hypothesis states mitochondrial DNA (mtDNA) or protein damage may be responsible for declining mitochondrial function and increasing toxic side-products. Mitochondria, the "powerhouses" of the cell, are specifically known for their roles in cell bioenergetics and programmed cell death. For its role in bioenergetics, pyruvate from the cytoplasmic process of glycolysis is transported into mitochondria, which contain the Krebs cycle and the electron transport chain. The electron transport chain (ETC) consists of complexes I to V, which sequentially oxidize NADH and $FADH_2$, reduce O_2 to H_2O, and pump protons across the inner membrane of the mitochondria, creating a proton-electrochemical gradient to produce adenosine triphosphate (ATP) from adenosine diphosphate (ADP) in a process known as oxidative phosphorylation [124]. AD-related impairments in mitochondrial function are most often described as decreases in the activity of electron transport chain enzymes, increases in reactive oxygen species (ROS), reductions in mitochondrial membrane potential, or reductions in ATP production. A decline in glucose metabolism in AD-associated brain regions occurs early in the transition from cognitively normal to MCI to AD and correlates with tau tangle pathology dementia severity [125]. The reduction in glucose metabolism may reflect a decline in mitochondrial electron and substrate transport, which is confirmed by the presence of neurons deficient in important ETC proteins and a rise in oxidative damage in AD and its prodrome MCI [126–128]. It may also suggest a shift towards oxidation-reduction reactions in other organelles, specifically the plasma membrane oxidoreductase system, which is known to be upregulated in cells lacking a functional mitochondrial respiratory system [129]. To date, increases in non-mitochondrial redox reactions have not been reported.

Though the primary event for mitochondrial dysfunction is not yet clear, according to the mitochondrial cascade hypothesis, declining mitochondrial function and increasing oxidative stress as a function of age precede and cause AD pathology, i.e., Aβ overproduction and deposition, synaptic degeneration and apoptosis, and tau hyperphosphorylation and aggregation [123]. Mitochondria serve as a normal and the major contributor of cellular oxidative stress, predominately through the electron transport occurring at complexes I and III [130,131]. During the transfer of electrons down the chain, electrons sometimes end up incompletely reducing oxygen, creating ROS like superoxide and hydroxyl radicals. Superoxide may also react with nitric oxide to form reactive nitrogen species, another source of cellular damage. Normal ROS levels serve a functional purpose, are generated in response to hypoxia, and trigger important processes that will increase cell and tissue survival [131], such as the stimulation of cell proliferation through the induction of protooncogenes *c-fos, c-jun,* and c-*myc* [132,133]. ROS production must maintain a fine balance because at high levels, it is also responsible for initiating apoptotic pathways [132]. Besides reaching levels needed for the initiation of programmed cell death, excess ROS oxidizes lipids, proteins, and DNA in the cellular or organelle wall, with the mitochondrial enzymes and mtDNA being highly accessible and their damage highly likely. The mitochondrial genome is particularly susceptible to oxidative damage because it is not protected by histones, has a lower ability to correct mistakes compared to the nuclear genome, and the proofreading subunit of its replicating polymerase (gamma) is itself a target of oxidative attack. Damage to mitochondrial enzymes and mtDNA can lead to additional mitochondrial problems that may result in greater ROS production, creating an ongoing circular chain of events (mtDNA alterations $\leftrightarrow$ ROS production). Constant exposure of mtDNA to normal and abnormal levels of ROS throughout the lifespan of an individual is a likely explanation of the accumulation of new mitochondrial mutations and deletions [134,135].

Thirteen proteins of the ETC are encoded for in mitochondria's 16.6 kB circular, double-stranded DNA [136,137], and the occurrence of spontaneous or inherited mutations and deletions in mitochondrial DNA (mtDNA) can lead to severe diseases in neurons

and muscles. Maintenance of normal mtDNA expression and bioenergetics are especially important in neurons because they are highly reliant on mitochondria-produced ATP for energy and are non-regenerating cells; therefore, any pathological developments can remain, progress, and cause neurons to die. In an attempt to determine if neurodegenerative diseases process a large degree of mtDNA alterations similar to traditional mitochondrial diseases, a number of laboratories have studied and reported high levels of mtDNA mutations in AD individuals [138]. Though the specific mutations identified and their levels have been inconsistent across studies, an increase in mtDNA alterations is still thought to play a significant role in the development of neurodegenerative diseases like AD [138] because mtDNA mutations and deletions are known to accumulate with age [139,140] and have the potential to reach a threshold at which mitochondrial function is affected. Neurons in aged and Parkinson's disease individuals demonstrate higher levels of mitochondrial alterations [139,141]. When mtDNA alterations are present, cytochrome oxidase, the enzymatically active component of complex IV, is predominately affected. Hippocampal neurons from AD brains have increased cytochrome oxidase (COX) deficiency compared to normal, aged individuals [126,142]. Loss of COX expression and activity of any ETC enzymes greatly contributes to reductions in ATP production and increases in oxidative stress, since the O_2 reduced in the ETC would now be more accessible for oxidation by leaking electrons. Evidence for high levels of lipid peroxidation have been measured in the cerebrospinal fluid of AD individuals compared to non-demented elderly individuals [143].

The mitochondrial cascade hypothesis challenges the amyloid cascade hypothesis as an explanation for the onset of AD (Fig. 1). Supporters of the amyloid cascade hypothesis have used it to explain AD-related mitochondrial pathology because: 1) AβPP and Aβ accumulates in mitochondria from brains of transgenic mice and AD subjects [144–147]; 2) binding of Aβ to the mitochondrial proteins Aβ-binding alcohol dehydrogenase and cyclophilin D is associated with increased oxidative stress, cytochrome c release, reduced mitochondrial membrane potential, and neuronal cell death [146,148]; 3) features of reduced Krebs cycle and ETC enzymatic activity, impaired state 3 and state 4 respiration, and *in vivo* elevation of oxidative stress demonstrated in AD and transgenic mouse brains were associated with Aβ [126,149–155]; and 4) the direct reduction of cytochrome oxidase expression and activity by Aβ_{42} and Aβ_{25-35} (a toxic Aβ fragment) supported this hypothesis [48,156,157]. Though evidence for Aβ-induced pathology is plentiful, PET-scan measured reductions in brain glucose metabolism in MCI and AD individuals [158–161] warrant a further look at the role mitochondrial bioenergetics play. In addition, it was recently reported that this deficit in mitochondrial bioenergy precludes an increase in Aβ production in transgenic mice and correlates well with clinical severity in humans [125,145], further supporting the claim that oxidative stress occurs upstream to Aβ overproduction, which, according to the amyloid cascade hypothesis, was a primary event in AD-related mitochondrial dysfunction. Though evidence for mitochondrial dysfunction induced by Aβ exposure is useful in determining the progression of AD once induced, this latest discovery severely weakens the amyloid cascade hypothesis as a prominent theory for sporadic cases of AD.

Secretase enzyme and mitochondrial dysfunction

In accordance with the mitochondrial cascade hypothesis, the ability of Aβ to promote a decline in respiratory capacity and the existence of mitochondrial impairments prior to Aβ deposition suggests a link between the Aβ-generating secretase enzymes, oxidative phosphorylation, and oxidative stress. First, increased BACE protein levels and activity in postmortem AD brain [54,162] are linked to increased γ-secretase cleavage of AβPP [163] and correlate with oxidative stress levels [164]. Endogenously and exogenously-induced ROS overproduction increases both BACE1 and PS1 expression and activity [165–168], which are mediated through the activation of the JNK pathway [169–171] and results in increased Aβ production [161,172]. In addition, Jo and colleagues report γ-secretase is responsible for ROS-induced increases in β-secretase activity, since pharmacological or genetic loss of γ-secretase function ameliorates an increase in β-secretase [173]. This upregulation of γ-secretase activity in oxidatively-stressed environments further supports a link between the protein and mitochondrial function. The ability of mitochondrial dysfunction to lead to the production of a damage-promoting enzyme (i.e., Aβ) raises important questions for what evolutionary advantage an increase in γ-secretase activity would provide for mitochondria. If other responses to oxidative stress include the triggering of pathways that increase survival of the cell, why would a cell decide to upregulate the activity of an enzyme that produces a protein that is

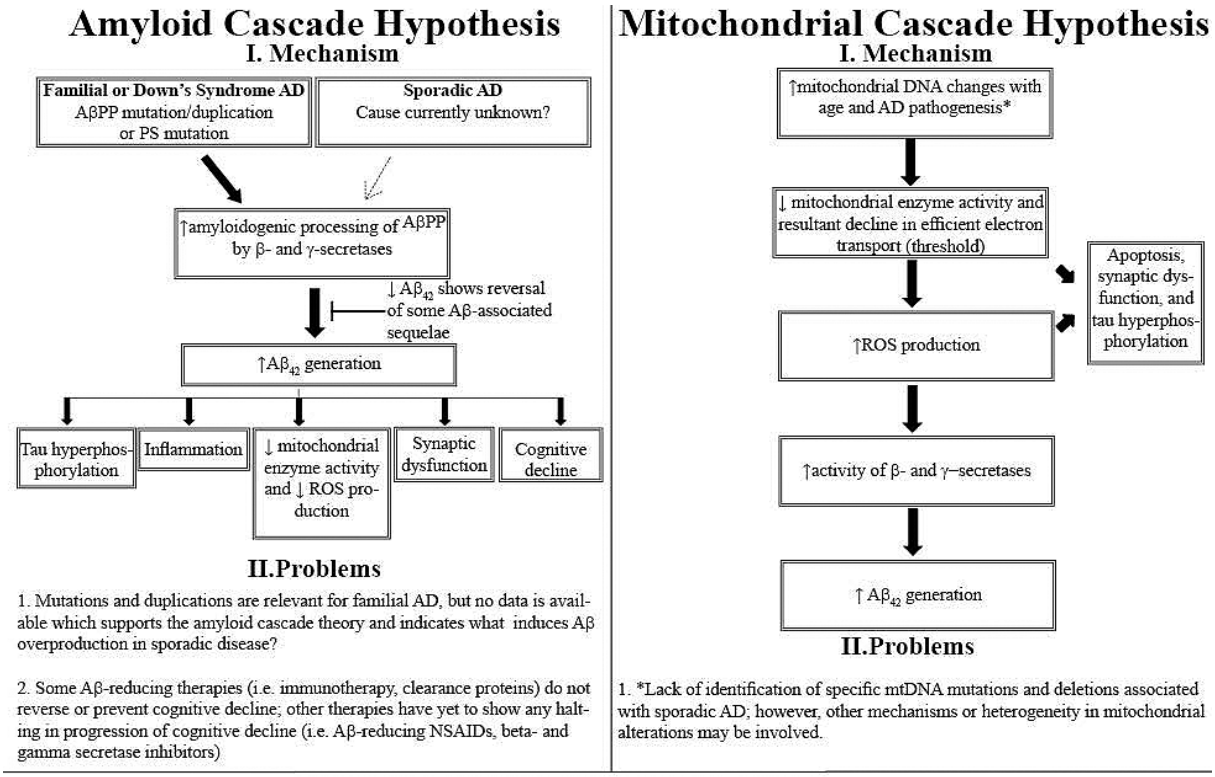

Fig. 1. Amyloid Cascade Hypothesis versus Mitochondrial Cascade Hypothesis. Hardy and Higgins [9] proposed increased Aβ as the causative agent for AD pathogenesis; supporting data from overexpression or mutation models in animals and cells supports that Aβ can induce these pathologies. However, though the expression of mutated proteins only proves Aβ can cause these sequelae, it does not prove that it is the underlying cause in the majority of cases (i.e., sporadic disease). Swerdlow and Khan [123,196] proposed mitochondrial inheritance determines mitochondrial function and response to aging. The mitochondrial cascade hypothesis suggests that in sporadic AD, mitochondrial alterations acquired over time reach a disease "threshold," at which mitochondrial dysfunction ensues, oxidative stress increases, and other AD pathology develops (i.e., Aβ overproduction, tau hyperphosphorylation/aggregation, synaptic dysfunction, and cell death).

toxic to an already-damaged organelle? γ-secretase is known to cleave a number of transmembrane proteins, so its rise in activity could easily be due to its roles in the cleavage of another protein. In a seemingly unrelated disease CADASIL (cerebral autosomal dominate arteriopathy with subcortical infarcts and leukoencephalopathy), mutations in NOTCH3 appear to have a negative effect on mitochondrial function, causing structural abnormalities and decrease complex I and V activities [174,175]. These changes in mitochondrial function may be secondary to another feature of the disease, which has not been uncovered yet. The likelihood that Notch processing is altered in AD is unlikely at this time because a role for Notch has not been identified, and Notch has been localized only to the plasma membrane, not the mitochondria. However, the heterogeneity of γ-secretase in processing proteins for physiological functions increases the likelihood that additional targets other than AβPP, Notch, and E-cadherin may exist that could be related to mitochondrial structure and function.

γ-secretase in mitochondria

To begin considering the relationship of secretase enzymes to mitochondrial function, increases in oxidative stress, and their AD-relevant target protein AβPP, it is important to review what is already known about γ-secretase in mitochondria and how it relates to both the amyloid and mitochondrial hypotheses. Understanding how secretase enzymes are related to mitochondrial dysfunction in AD will provide greater understanding towards their potential use as therapeutic targets. The catalytically active components of γ-secretase have been identified in the plasma membrane, Golgi, endoplasmic reticulum, and mitochondria [176, 177]. The presence of γ-secretase within mitochondria suggests one or more its targets must be also present

there. To date, its other known targets (i.e., Notch, p75NTR, ErbB-4, and E-cadherin) have not been reported in the mitochondria, but full-length AβPP has been shown to accumulate in the import channels of the inner and outer mitochondrial membranes in AD brains only and is associated with increased oxidative stress and reduced cytochrome oxidase activity [147, 178]. This AD-related reduction in mitochondrial function is presumed to be due to the inhibition of the entry of nuclear-encoded ETC proteins. Studies also allude to direct access of the mitochondrial compartment to mitochondria-generated Aβ. AβPP accumulation in the mitochondria may be increased to generate higher levels of Aβ_{40}, which evolving evidence suggests may have neuroprotective properties [179]. Provided Aβ_{40} does have a neuroprotective role, then mitochondrial γ-secretase activity may be triggered to increase the cells' Aβ_{40} store, but not selective enough to prevent increases in levels Aβ_{42} in mitochondria. The mechanism for increasing Aβ_{42} levels in familial cases with AβPP mutations is understood to be through an altered conformation of PS1 and PS2 when binding to AβPP. If AβPP processing within mitochondria is increased to provide Aβ_{40}-dependent neuroprotection to an ailing organelle, understanding how γ-secretase cleavage specificity of AβPP in sAD occurs is important to identifying why AβPP accumulation and γ-secretase activity increases in mitochondria go awry and lead to toxic products or identifying effective therapies for increasing neuroprotective Aβ_{40} versus neurotoxic Aβ_{42} [27,180]. This inability of γ-secretase cleavage to be selective for production of a neuroprotective amyloid peptide suggests an alternative purpose for γ-secretase activity within mitochondria: whether this enzyme is present simply for the generation of Aβ, which is shown to be extremely toxic to mitochondrial proteins, or for another physiological role within mitochondria? To date, no evidence of β-secretase activity within the mitochondrial compartment has been reported. Instead, β-secretase activity appears to be confined to lipid rafts within the plasma membrane [181], suggesting Aβ generation may be confined to this area of the cell or sAβPPβ may be imported into mitochondria to be further cleaved by γ-secretase. In the meantime, this current lack of evidence for β-secretase activity within the mitochondria leaves an unanswered question of whether Aβ is generated within mitochondria and what is the purpose of ubiquitous expression of γ-secretase, specifically for the mitochondria. In addition, it is unknown whether any mitochondria-related events are affected by mutations or deletions in γ-secretase components, just as alterations of enzyme activity may affect Notch and E-cadherin cleavage [182].

In line with the amyloid cascade hypothesis is the theory that Aβ through unknown, but highly debated, mechanisms accumulates in the mitochondria, increasing the possibility of direct contact with mitochondrial enzymes [146,148,180] as a competitive or noncompetitive inhibitor of enzyme assembly and/or function. However, the existence of mitochondrial dysfunction at an early stage of AD development in mouse models poses some questions as to whether or not this is the main route of mitochondrial toxicity in sAD. While the initiating event for AD pathology is unclear, it is evident that mtDNA mutations, Aβ overproduction, protein misassembly/reduced activity, and mitochondrial dysfunction may all play a role, either independent or dependent of one another. Regardless of the mechanism for mitochondrial dysfunction in AD, it is clear that AD pathogenesis is in part caused by dramatic increases in oxidative stress, which further deteriorates mitochondrial function and provides increases in Aβ generation.

Disease-modifying anti-Alzheimer's drugs (*DMAAD*) *and mitochondria*

To date, the development of DMAADs for sAD relies heavily on the amyloid cascade hypothesis and little work has been published as to the effect of these Aβ-reducing agents on mitochondrial respiratory function. Since there is much evidence for the development of sAD from an etiology based mostly on mitochondrial dysfunction, it is imperative that we determine what therapies may have a positive effect on mitochondrial function.

The finding of increased oxidative stress in AD brains has certainly been an enigma in the study of AD. On one hand, oxidative stress leads to downstream increases in Aβ-producing enzyme activity, specifically through increased BACE1 activity and presenilin 1 levels [166,167]. On the other hand, the presence of Aβ leads to increased generation of ROS, therefore puzzling researchers with the question of which pathology arose first in cases of sporadic AD. There has been much evidence for increased ROS due to age-related problems with bioenergetics, specifically reductions in glucose metabolism and ETC enzyme activity. Clinical relevance for targeting reduced mitochondrial function and increased oxidative stress is stronger than that for blocking Aβ generation or increasing its clearance because of the existence of data depicting potential

signs of dysfunction at a preclinical stage. The logical step for therapeutics is to identify drugs that prevent ROS production or scavenge ROS, in hopes of halting the progression of the disease. It is thought that the scavenging of free radicals by antioxidants could decrease some of the resultant pathology and prevent further decline in cognitive function. The antioxidant tricyclodecan-9-yl xanthogenate demonstrated the ability to protect against oxidative stress induced by Aβ [183]. In addition, Jayaprakasam and others reported the protection of rat neuronal cells from cell death by withanamides [184]. The antioxidant properties of these drugs were demonstrated in conjunction with Aβ_{42} toxicity, but they may prove useful in counteracting the endogenous oxidative stress generated in elderly non-demented and AD individuals. Since oxidative stress can lead to apoptosis and exists in MCI and AD, the use of antioxidants as a therapy is warranted. However, at this time, the effects of long-term antioxidant use in AD patients still remains to be known [147] and may later be overcome by the mechanism responsible for overproducing ROS.

Since Aβ is known to lead to a number of AD pathologies, i.e., tau hyperphosphorylation, synaptic and cholinergic dysfunction, and oxidative stress, many continue to study therapies that can affect AD pathogenesis at this level. Since the earliest time-point is still debated, the major target is at the level of Aβ production through the inhibition of AβPP-cleavage enzymes β- and γ-secretases. Though these may prove promising by halting the development of tau tangles and synaptic dysfunction, a major area of investigation left unstudied is whether the mitochondrial impairments caused by Aβ overproduction, aggregation, and accumulation are reversible. In addition, though currently available data shows some promise for secretase-inhibiting therapy, it is important to consider a report of immunotherapy-associated clearance of amyloid plaques without improvement in disease progression or survival [185], even though immune therapy was previously shown to reduce Aβ levels [33,186–189]. Additionally, while the overexpression of neprilysin, an Aβ-degrading enzyme, reduces Aβ levels by 50%, it did not improve impairments in spatial learning and memory in transgenic mice [190]. As previously stated, NSAIDs with Aβ-reducing ability do not demonstrate greater protective ability compared to NSAIDs without Aβ-reducing ability [114]. In addition, AD subjects treated with NSAIDs and cholinesterase inhibitors appear to only have short-term protection from the development of AD, thereby suggesting these drugs only serve a symptomatic purpose, which may be unrelated to their Aβ reducing activities. In context with these recent reports, this places further doubt that lowering Aβ actually prevents progressive neurodegeneration. These studies suggest that Aβ overproduction is not directly responsible for cognitive decline as the amyloid cascade theory states and indicates that additional studies for a pathological marker of cognitive decline are needed.

Clinical trials for β- and γ-secretase inhibitors are next in line for data showing a positive effect on cognitive function. To date, no final reports of their long-term efficacy have been provided. Currently, the animal data suggests cognition will benefit from GSI use [25], but the immune therapy also demonstrated similar results when working with mouse models. Another pitfall of the pre-clinical work from these studies is a lack of observation for their effects on mitochondrial function. Since data supporting the mitochondrial cascade theory shows us that mitochondrial impairments and oxidative stress occur earlier than Aβ deposition; γ-secretase and AβPP within the mitochondria provide direct access for generated Aβ; γ-secretase and β-secretase activities are linked to oxidative stress levels; and Aβ generated outside the mitochondria may be transported into the organelle, data for the effect of secretase inhibitors on mitochondria is absolutely necessary. Those studies that do report improvements in mitochondrial function (i.e., mitochondrial membrane potential) have only been performed in cell or animal models with overexpressed or mutated proteins [26], which is representative of familial AD and not sporadic. In an attempt to determine if secretase inhibitors could in fact recover mitochondrial function as the amyloid cascade theory suggests, we treated a sAD-phenotypic cell line with commercially available β- and γ-secretase inhibitors and measured mitochondrial function. When we began our work, we speculated based on the available data and the amyloid cascade theory that secretase inhibitors would either restore proper mitochondrial function or have no effect if Aβ was not directly responsible for this drop in mitochondrial function (or its effects were irreversible). Surprisingly, initial results from our laboratory demonstrated additional impairments in mitochondrial bioenergetics after γ-secretase inhibitor treatment of a cell line with an established AD phenotype. This preliminary data raises questions as to the effectiveness of all γ-secretase inhibitors and whether or not this decline in mitochondrial function would extend to clinical side effects, especially in a population already observed to have reduced mitochondrial function.

We hypothesize that the decline in mitochondrial function could be due to one of two possibilities. First, some Aβ peptides, which are all reduced with non-specific γ-secretase treatment, may actually prove critical to normal mitochondrial and neuronal function; and secondly, since this decline was also observed in the control lines under the same treatment conditions, γ-secretase may serve a role in regulating mitochondrial respiration, which is inhibited with the use of GSIs. Evidence supporting the first possibility includes the ability of Aβ_{40} to rescue neuronal cells lines from secretase inhibitor-induced cell death [179]. Only this size peptide was useful in rescuing the viability phenotype. However, it is still necessary to determine what other alterations in cellular or mitochondrial function are observed with a decline in viability. If a decline in mitochondrial function is associated with the cell death observed, improvement of mitochondrial function or no change could be obtained by treating cells with Aβ_{40} along with the inhibitor therapy. However, additional data from our laboratory (Young and Bennett, unpublished data) treating cells and isolated brain mitochondria suggests that the inhibition of γ-secretase further deteriorates mitochondrial function in a non-Aβ-dependent manner. Acute γ-secretase treatment was able to replicate the decline in mitochondrial respiration over 30–40 min, and this abrupt change cannot be accounted for by the absence of AβPP, Notch, or E-cadherin cleavage (unpublished data). This supports the second possibility: γ-secretase may have a role in mitochondrial function.

Supporting evidence for a secondary γ-secretase role comes from conflicting ideas about the relationship between γ-secretase and the neurodegeneration seen in AD. According to the amyloid cascade hypothesis, as previously mentioned, γ-secretase mutations lead a gain in function that leads to increased Aβ production and downstream neurodegeneration. However, loss of PS function can also result in hyperphosphorylated tau, synaptic dysfunction, neurodegeneration, and memory impairments [105]. As would be expected in cells lacking PS activity, no Aβ should be produced, generating additional questions about how an alteration of γ-secretase activity relates to other pathological features of AD.

CONCLUSION AND FUTURE PERSPECTIVES

Moving forward, the field would benefit from an identification of which forms of the γ-secretase complexes are in greatest abundance in mitochondria and for which substrates they have a greater affinity. It is possible that the ratio of γ-secretase complex makeup within the mitochondria differs from that at the level of the plasma membrane and may mediate different processes other than cleavage of the previously mentioned γ-secretase targets. The secretase enzymes appear to have numerous protein targets, such as with BACE1 and BACE2's recent identification in cleavage of pancreatic enteropeptidases and in mediating increased anxiety in mice [191,192]. However, the location of γ-secretase is specifically important to identifying additional protein targets, since no targets other than AβPP have been identified in this organelle, and its role in AβPP cleavage appears to be additive in AD development. Our preliminary data suggests a role in respiration, and we support the further study of this enzyme in maintaining normal mitochondrial function and alleviating problems. Based on the preliminary data in our laboratory, γ-secretase may likely have a role in mitochondrial respiration. This idea is not far-fetched, since a link between γ-secretase expression/activity and oxidative stress has already been established. One possibility is that γ-secretase may exert a protective effect for mitochondrial bioenergetics, leading to upregulation of the enzyme when the organelle is under greater oxidative stress. Inhibition of this enzyme could remove the normal regulatory ability γ-secretase has in respiration or may be toxic to an organelle already being damaged by the initiating event that has led to Aβ overproduction, mitochondrial dysfunction, and oxidative stress. The sequence requirement for and site of substrate cleavage differs for each substrate, suggesting other members of the complex or other cellular components are responsible for regulating which substrates are cleaved by PS. The ability of the γ-secretase complex to arrange in six distinct varieties might facilitate this regulation in different environments. In addition, the promiscuous nature of γ-secretase and the growing number of transmembrane proteins targeted by γ-secretase suggest that additional substrates may exist. In line with our unpublished data, we propose an additional substrate(s) may exist within the mitochondrial compartment and be involved in the regulation of normal mitochondrial respiration (Fig. 2). The identification of a mitochondrial protein cleaved by γ-secretase may possess cleavage features similar to those of AβPP, Notch, ErbB4, E-cadherin, and p75NTR: 1) prior cleavage by ADAM10 or another α-secretase [193]; 2) dependency of pre-α-secretase cleavage on distance of protein residues from membrane; and 3) intramembraneous cleavage by

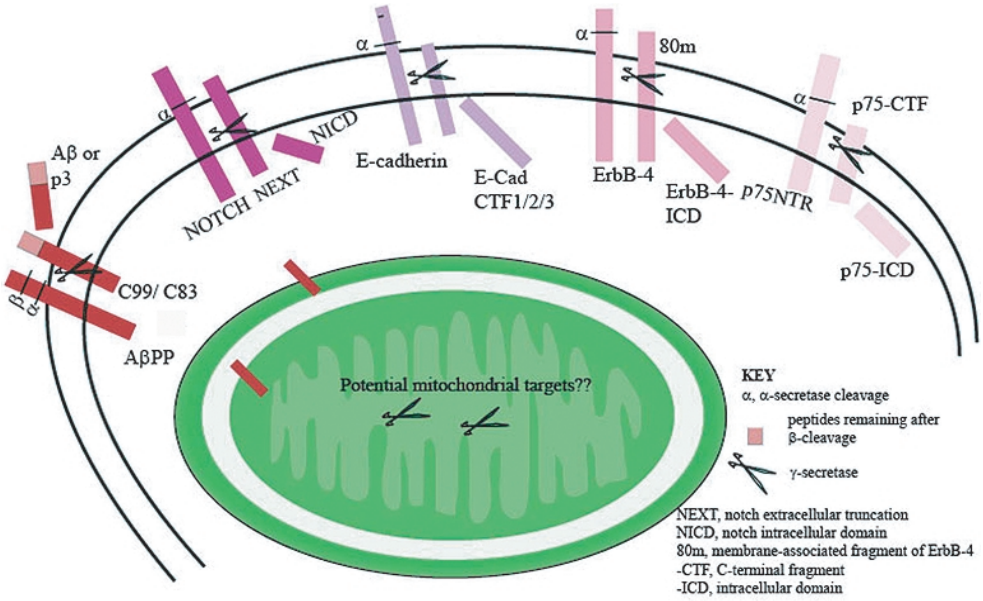

Fig. 2. γ-secretase targets in plasma membrane and potential role in mitochondria. The transmembrane proteins AβPP, Notch, E-cadherin, ErbB-4, and p75NTR [80,194,197] are targets of γ-secretase cleavage. Each protein first undergoes α-secretase cleavage of their ectodomain by a metalloprotease (e.g., ADAM10, TACE), followed by γ-secretase-mediated cleavage at multiple sites within the transmembrane domain. These products of γ-secretase cleavage are usually targeted to the nucleus. Currently, AβPP is the only known target that is also localized to the mitochondria. Enzymatically active γ-secretase is found within the mitochondrial matrix, suggesting that γ-secretase may lead to cleavage of mitochondrial AβPP, though no data implicating β-secretase activity within mitochondria has been reported. However, unpublished data from our laboratory suggests γ-secretase could also serve additional, but currently unknown roles in mitochondrial function.

γ-secretase, though in this case, it would be within a mitochondrial membrane instead of the plasma membrane [80,82,83,193–195].

There is no doubt that Aβ can cause AD-related impairments, including reductions in ETC protein activity. On the same note, it is also apparent that reductions in Aβ can rescue AD-related pathologies in cell or animal models with pathologies due to this source. Animal models have been used extensively in AD research, specifically in the recapitulation of AD pathology and rescue through overexpression and mutation models. Though no one will argue that Aβ overproduction will cause most pathologies already discussed, as in familial AD and animal models, there is still doubt that Aβ is the main cause or initiating event of the same pathologies observed in sporadic cases. Therefore, in sporadic AD, where Aβ does not appear to be the underlying cause, preventing or reversing these impairments may prove much more difficult without a greater understanding of the etiology of the impairment, the impairments themselves, and the degree of reversibility that exists. Mitochondrial impairment appears to be a more probable cause for the AD pathogenesis, through the mechanisms proposed in the mitochondrial cascade theory. In addition, it appears that though γ-secretase is traditionally studied for its role in Aβ generation, it may prove useful to understand its additional and more AD-relevant roles in mitochondrial function.

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