Review

Molecular Basis of Alzheimer’s Disease: Focus on Mitochondria

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Abstract. Alzheimer’s disease (AD) is a progressive neurodegenerative disease, characterized by memory loss and multiple cognitive impairments. With the increased aging population, AD is a major health concern in society. Morphological and pathological studies revealed that AD is associated with the loss of synapses, defective mitochondria, and the proliferation of reactive astrocytes and microglia, in addition to the presence amyloid-β and phosphorylated tau in learning and memory regions of the brain in AD patients. AD occurs in two forms: early-onset familial and late-onset sporadic. Genetic mutations in APP, PS1, and PS2 loci cause familial AD. Multiple factors are reported to be involved in late-onset AD, including APOE4 genotype, polymorphisms in several gene loci and type 2 diabetes, traumatic brain injury, stroke, and age-related factors, including increased reactive oxygen species production and dysfunction in mitochondria. It is widely accepted that synaptic damage and mitochondrial dysfunction are early events in disease process. The purpose of this article is to highlight molecular triggers to the disease process. This article also reviews factors, including age, gender, lifestyle, epigenetic factors, and type 2 diabetes, that are involved in late-onset AD. This article also discusses recent developments in research of mitochondrial structure, function, physiology, dynamics, biogenesis, mitophagy, and mitochondrial DNA changes in healthy and diseased states.

Keywords: Alzheimer’s disease, familial Alzheimer’s disease, mitochondria, mitochondrial biogenesis, mitophagy, reactive oxygen species

INTRODUCTION

Alzheimer’s disease (AD) is a progressive neurodegenerative disease characterized by memory loss, multiple cognitive impairments, and changes in personality. Dementia affects over 50 million people throughout the world today, and AD is the number one
cause for dementia, accounting for 60–80% of cases [1, 2]. This number is expected to double approximately every 20 years and estimated to increase to 75.6 million by 2030 and 131.5 million by 2050 [3]. The worldwide economic impact was over $800 billion in 2015 alone [2]. Currently, there are no drugs that can delay and/or prevent the disease process.

Histopathological and morphological examination of postmortem AD brains reveal accumulation of intraneuronal hyperphosphorylated tau and neurofibrillary tangles and extracellular amyloid-β (Aβ) deposits in the brain, as well as accumulation of dysfunctional mitochondria in nerve cells [4]. AD is also associated with the loss of synapses and cholinergic fibers, and the proliferation of reactive astrocytes and microglia [3, 4]. Soluble Aβ and phosphorylated tau accumulation in synaptic clefts and synaptic mitochondria hinder neurotransmission, causing dysfunction in neurons and effectively slow cognition in AD patients [3, 4]. The accumulation of dysfunctional mitochondria can cause or magnify pathology of Aβ plaques and tau tangles and are believed to play a central role in AD pathogenesis. Aβ accumulation is also shown to lead to inflammation due to activation of microglia and astrocytes [3, 5, 6].

AD occurs in two forms: early-onset familial AD and late-onset sporadic AD. Pathological investigations of autopsy AD brains revealed that there are no clear-cut differences between early on-set familial and late-onset sporadic AD [6]. Mutations in the amyloid precursor protein (APP), presenilin 1 (PS1), and presenilin 2 (PS2) loci, cause early-onset familial AD [6]. These genetic alterations are believed to predispose to greater mitochondrial dysfunction [3, 4]. With these genetic precursors, a person is more likely to develop AD in their 50s rather than their 70s or later [3, 4].

Further, the presence of the e4 allele of apolipoprotein E (APOE4) predisposes people to late-onset sporadic AD, as it is involved with amyloid protein deposition [4]. Furthermore, age-related factors, including reactive oxygen species (ROS), dysfunction in mitochondria, and phosphorylation of tau are responsible for development of both forms of AD [3, 4, 9].

The purpose of this article is to review molecular triggers to AD. This paper also highlights recent developments in research of mitochondrial structure, function, physiology, dynamics, biogenesis, mitophagy, and mitochondrial DNA changes in healthy and AD states.

**Mitochondrial cascade hypothesis**

The core principal behind research for mitochondria targeted molecules is the mitochondrial cascade hypothesis (MCH). Firstly, it is believed that all persons have a genetically inherited predisposition for mitochondrial function and dysfunction [9]. As shown in Fig. 1, mutant proteins association induce
mitochondrial dysfunction. Important to note is that both parents will contribute nuclear DNA (nDNA) (which codes for cyclophilin D in the mitochondrial matrix and Complex II in the mitochondrial inner membrane), but the mitochondrial DNA (mtDNA) believed to be mostly contributed from the mother will contribute greater risk to mitochondrial dysfunction [10–12]. Recently, Luo and colleagues reported that mtDNA inheritance occurs by both parents, indicating that both parents contribute mtDNA to their offspring [13]. These recent findings are in disagreement with original findings of maternal inheritance of mtDNA [11]. However, research is needed to understand the molecular mechanism for this unusual mode of paternal inheritance, how mtDNA is passed on from parent to offspring and may lead to the development of new avenues for the therapeutic treatment for pathogenic mtDNA transmission.

Secondly, how these variances in genetics are expressed is determined by interaction between each person’s inherited characteristics and the environment [10]. Overtime, according to the MCH, an observable correlation between mitochondrial age-related dysfunction and brain aging should develop, should the person be genetically susceptible to developing mitochondrial dysfunction [10]. Lastly these interactions are the basis for development of neurodegenerative diseases and determine the variant of the disease, such as early and late onset AD [10]. The MCH thus explains that AD is initiated by mitochondria, and the change in mitochondrial function determines how fast and how severe the disease will be. The MCH provides the principles for researchers to analyze biomarkers that indicate neuronal aging and AD development, and it is with these biomarkers that researchers assure the effectiveness of therapeutic development of new avenues for the therapeutic treatment for pathogenic mtDNA transmission.

It is important to note that in geriatric populations, more persons have AD than do not have AD, but researchers believe that AD is separate from the normal aging process [10]. AD is not the norm, and everyone will not eventually develop AD should they live long enough [10]. Instead, brain aging may be subdivided into compensated and uncompensated aging [10]. AD would fall under uncompensated aging, where aging effects are not mitigated [10].

Furthermore, research with cytoplasmic hybrid (Cybrid) cell lines contributes a large amount of evidence toward the MCH [10]. Cybrids are eukaryotic cells produced by the fusion of whole cells with cytoplasts without nuclei [10]. Using this technology, with two different neuronal cell lines, SH-SY5Y neuroblastoma and NT2 teratocarcinoma cells, Swerdlow and team members were able to demonstrate significant differences in cytochrome c oxidase (COX, aka ETC complex IV) activity between those of AD and non-AD cells [14]. Since COX is encoded by mtDNA, and not nDNA, a conclusion could be made that mtDNA does play a role in biogenesis of AD cells [14]. Similar differences were noted in cybrid lines with cytoplasm from healthy and mild cognitive impairment (MCI) cells, where molecular differences continued after culturing [15]. MCI is considered an early stage of AD.

Swerdlow and other researchers also noted differences in several other biomarkers that show correlation, but are not necessarily conclusive of AD [10]. These include transcription factor A of mitochondria (TFAM), and AD biomarkers FDG PET, structural MRI, amyloid PET, CSF Aβ, tau, and phosphorylated tau levels [10]. These biomarkers establish that biogenesis, and toxin levels vary with age, and that mitochondrial function plays a central role. These biomarkers also serve as tools for researchers to measure pathogenesis of AD in experimentation, and development of therapeutic drugs [16–18].

MITOCHONDRIAL DNA

Mitochondria have their own DNA, called mtDNA, which encodes for polypeptides, including complexes I, III, IV, and V of the electron transport chain-complex II, succinate dehydrogenase, is encoded by nDNA [11] (Fig. 2). Mitochondria regularly have multiple copies, 2 to 10 copies of mtDNA and the copy number also correlates with health of the mitochondrion [11]. MtDNA lack histones and tend to be coiled up into nucleoid like structures with a core, like that of bacterial DNA [11]. Unlike bacte-
Fig. 2. Mitochondrial DNA. The human mitochondrial DNA is circular, highly conserved, double stranded genetic material located in the mitochondrion. Damage at particular regions are key to the propagation of AD pathology.

ria, mitochondria are unlikely to incorporate foreign genetic material via transformation, and in experiments have shown to isolate mutant mtDNA and keep it separated from further mitochondrial fission/fusion processes [11, 19]. This response to mutant mtDNA also ensures that it remains highly conserved, but also determines rate of fission and mitophagy to maintain healthy mitochondrial dynamics.

Types of mtDNA damage

mtDNA is susceptible to damage, especially from ROS. Alkylation damage is common, and often due to endogenous S-adenosylmethionine (about 30% of which is located in cellular mitochondria) methylation of DNA, and may occur due to exogenous compounds, such as chemotherapeutic agents [11]. Mitochondrial DNA is susceptible to hydrolytic damage which may cause hydrolysis of glycosidic bonds and result in a-basic sites, or may result in hydrolytic deamination of bases, most often with cytosine [11]. Mitochondria may be more susceptible than other portions of the cell to damage from exogenous sources such as tobacco smoke and other chemicals, which may form carcinogen adducts [11]. mtDNA is also susceptible to endogenous sources of adducts such as estrogens [11]. Mismatched bases may result from replication errors from DNA polymerase γ (polymerase responsible for transcription and damage repair of mtDNA in mitochondria) or even oxidation of bases [11]. DNA strand breaks, which may be caused by noxious stimuli, can cause single or double stranded breaks, and determine the type of DNA repair pathway which may lead to additional excision of bases [11]. Oxidative damage, with which most scientific research is done, often is the result of ROS, which are mostly produced by mitochondria [11].

Collectively, strand breaks, a-basic sites, and oxidative DNA adducts are also polymerase-blocking lesions, which more than likely reduce mtDNA copy number [11]. mtDNA is actually more vulnerable to polymerase blocking lesions than nDNA [11]. Some mtDNA base damage is more susceptible to mutation than others. Among the most common is thymine glycol, a product of oxidative thymine base damage, which due to relatively efficient base excision repair excision has low mutagenicity, but temporarily inhibits polymerase-γ’s transcription ability [11]. Another is 7,8-dihydro-8-oxo-2′-deoxyguanosine, or 8-oxodG, which results from oxidative damage, and is mutagenic, causing
G:T transversions, though less likely to block polymerase [11]. These differences, along with cytosine’s susceptibility to hydrolytic deamination alludes to differences in oxidative damage leading to mutation between persons with higher versus lower Guanosine-Cytosine base pair content [11].

Oxidative damage of mtDNA

As damage to mtDNA may result from AD pathology, it is very important to understand the mechanism by which mtDNA damage is done. For instance, ROS species are products of nine, or possibly more, enzyme systems in mitochondria, but researchers tend to focus on those in the electron transport chain (ETC) [11]. In addition, oxygen may be converted to hydrogen peroxide via reverse electron transport from complex II to complex I, though this is unlikely [11]. Commonly, electrons may escape the ETC and induce superoxide production, and likely may be exacerbated by mitochondrial permeability transition (MPT) pores induced by Aβ peptides [11]. Normally, superoxide formed outside the mitochondrion prohibits it from crossing its membrane, and damage from superoxide is also limited when its presence inactivates mitochondrial aconitase [11]. When superoxide inactivates mitochondrial aconitase the ETC is slowed down, due to decrease in complex I and III activity [11]. Thus, it may be likely that AD induced reduction in biogenesis may be a healthy reaction to excessive superoxide production.

The superoxide produced by the ETC normally is converted to peroxide, which is the most efficient molecule in inducing oxidative stress because it has a relatively stable structure, and may cross lipid membranes [11]. Peroxide actually does not directly attack mtDNA, but when in contact with a metal cation such as Fe^{2+}, may proceed with a Fenton chemical reaction, producing \( \cdot \text{OH} \) radical, which may rapidly cause mtDNA damage [11]. Researchers have found that damage caused by \( \text{H}_2\text{O}_2 \) was even more frequent in mtDNA than in nDNA, of which the most predominant form of oxidative damage was to the mtDNA sugar-phosphate backbone [11].

Mitochondrial DNA damage repair systems

There are several base repair pathways possible for mtDNA damage that are very similar to those of nDNA. With mtDNA mismatches caused by polymerase-\( \gamma \) slippage error, mismatch repair (MMR) exchanges the mismatched base with the correct base. The repair process depends on the Y-box binding protein (YB-1) and not MSH used in nDNA MMR [11]. It should be noted that in vitro study has shown that polymerase-\( \gamma \) transcription fidelity is reduced when oxidized dGTP content is low (0.06–0.6%), but in vivo mitochondrial oxidized guanine content may be up to 10%, which is indicative of guanine’s susceptibility for oxidation, as a major cause of mtDNA mutation [11].

A DNA strand break can be single stranded, which constitutes loss of one nucleotide, and warrants single stranded base repair, or double stranded, which constitutes a break in the phosphate backbone in both strands and often accompanied by the loss of one or more nucleotide warranting double stranded base repair. In both types of repair, there is damage at either the 5’ terminus or 3’ terminus [11]. In single strand breaks (SSB), \( \text{H}_2\text{O}_2 \) oxidative damage can cause the abortion of topoisomerase I activity, even more often than with double strand breaks (DSB) [11]. SSB repair is very similar to base excision repair and differs with enzymes such as aprataxin, TDPI, and APE, and does not use DNA glycosylases [11].

In repair of DSB, there may be homologous recombination (HR) or non-homologous end joining (NHEJ). Recombination of mtDNA is infrequent as it may result in mutations, and the resultant mutant mtDNA must then be isolated [11]. HR is dependent on mitochondrial copy number, which would allow for a template for a successful repair of the DSB. HR is also able to be conducted between one individual mitochondrial and another, due to the network of mitochondria which at times may have multiple mitochondrial recombination junctions and catenation [11]. Mammalian mtDNA may even use recombination dependent initiation for replication, which is evidence that the balance mitochondrial dynamics (fission/fusion/mitophagy) greatly affects mtDNA copy number, especially non-mutated mtDNA copy number [11]. Notable as well is the fact that there is a lack of extensive recombination junctions in human infant mtDNA [11]. This suggests that recombination likely increases with age, and that the success of a healthy network of mitochondria is dependent on this process [11]. How Aβ peptides and phosphorylated tau fibrils affect this process is likely key for the healthy mitochondrial bioenergetics and dynamics.

With NHEJ, mitochondrial enzymes may join blunt-end DNA fragments, and the accuracy of preserving functionality of the genes is low [11]. Restriction enzymes that cut around the DSB of the damaged mtDNA target preferential end points, and...
sometimes the majority of the damaged mtDNA is degraded altogether [11]. If excessive damage is done to mtDNA within a mitochondrial network, resultant cells may lack mtDNA altogether, which denotes and means by which AD pathology may lead to degeneracy of a mitochondrial network within a neuron [11].

The leading mtDNA damage repair system is base excision repair (BER) and is the most studied process thus far [11]. Most oxidative damage of mtDNA is repaired via BER, where damaged base(s) are excised and replaced with proper base sequence [11]. This is important as the most common product of mtDNA damage is 8-oxodG, which suggests that malfunctioning of BER systems in mitochondria with high Guanine mtDNA content may be more susceptible to mutation, fragmentation, and/or mitophagy [11]. Short-patch BER is initiated by DNA glycosylase which recognizes the damaged base, whereas Long-patch BER is initiated by an AP site (absence of purine or pyrimidine) [11]. In BER, once a damaged base is removed, polymerase-γ and DNA ligase III (Lig 3) fill the gap with the correct base and seal the gap between the phosphate backbones [11]. It is important to note that experimental knockout of Ligase 3 has been shown to result in lower mtDNA count, and that researchers have successfully introduced exogenous mitochondrial targeted Ligase 3 to cells, which was shown to restore viability [11]. Thus, where AD pathology induces damage of endogenous Ligase 3, introduction of exogenous mitochondrial targeted Ligase 3 may be a plausible means for future restoration of mitochondrial viability in AD cells.

Degradation of mtDNA

Extensive research has been conducted on the efficacy of BER systems, and due to the excessive copies of mtDNA, research has strayed away from determining how mtDNA damage, leading to loss of mtDNA copy number may affect mitochondrial health and dynamics [11]. Loss of mtDNA copy number may be prevented by antioxidants such as coenzyme Q, vitamin E, melatonin, and mtDNA re-synthesis processes [11]. In an experiment with intra-gastric introduction of ethanol, approximately half of mtDNA copies in all organs sampled were lost, including heart, liver, brain, and skeletal muscles of mice; meanwhile antioxidant administration was able to prevent and loss [20]. This indicates that given the right toxic substance, loss of mtDNA copy number may be a legitimate threat to mitochondrial and cell viability, though antioxidants may succeed in restoration. Crawford et al. were also able to show that oxidative stress initiated by H₂O₂, propagated the influx of Ca²⁺ and resulted in subsequent degradation of mtDNA, reducing copy number [21]. These studies highlight two needed areas of future study, especially longitudinal study, analyzing the effects of oxidative stress induced by AD pathology, and mitochondrial degeneracy due to mtDNA degradation and reduction of copy number.

In experiments conducted by Alexeyev et al. when BER system was inhibited, excessive damaged mtDNA which exceeded a particular threshold level were degraded [11]. Their conformation in fact was altered to become linear, which likely exposed them to exonucleolytic degradation [11].

Mitochondrial DNA peroxidation damage in Alzheimer’s disease

Peroxidation

Damage of mtDNA is believed to originate with hydrogen peroxide [5, 22]. With respect to healthy mitochondria, the mtDNA of mitochondria in AD cells have greater numbers of sporadic mutations, including the most common 5-kb deletion mutation [23]. ROS, especially those formed from lipid peroxidation, are prone to attack mtDNA, and the relative scarcity of an mtDNA repair system, and the mtDNA proximity to ROS generation makes mtDNA especially vulnerable to mutations [23]. There are indeed many mtDNA mutations that are unique to AD patients, and the noted three-fold increase in ROS damage oxidative damage is likely a major contributor [23]. The mutations of mtDNA in AD patients are not secluded to brain tissues, but are also found in blood samples and lymphoblastoid lines of blood samples [23]. What is known to cause mitochondrial dysfunction is the fact the mtDNA mutations and deletions occur at portions of the mtDNA necessary for proteins, and thus result in inhibition of proper mitochondrial functionality [23].

Lipid peroxidation

Lipid peroxidation levels in controlled health subjects and AD patients both lead to mtDNA damage [23]. ROS species attack fats and lipids, particularly hydrogens of aliphatic tails, via a chain of reactions initiated by free radicals, generating an ongoing series of lipid peroxidation products [23]. Lipid peroxides and lipid peroxy radicals pose risks to the cell, espe-
cially to the cell and organelle membranes, which is composed mostly of lipids [24]. Products of lipid peroxidation may lead to apoptosis, mutation, and malignant growth when they interact with DNA [24].

With respect to AD, the majority of lipid peroxidation byproducts studied include: reactive aldehydes, including 4-hydroxynonal, malondialdehyde (MDA), and 2-propenal (acrolein); and chemically and metabolically stable isoprostanoids, including F2-isoprostanes and F4-neuroprostanes [23]. Wang et al. [23] reported that 4-hydroxynonal concentrations were considerably high in the hippocampus, entorhinal cortex, temporal cortex, amygdala, parahippocampal gyrus, ventricular fluid, and plasma of the brain in AD patients with respect to healthy patients of comparable age [23]. The lipid peroxidation byproduct MDA was significantly high in the hippocampus, pyriform cortex, temporal cortices, and erythrocytes of several AD patients [23]. Studies also reported no significant difference in basal MDA but did find higher concentrations of stimulated MDA generation in several portions of the brain with AD pathology [23]. Furthermore, in portions of the AD brain’s hippocampus/parahippocampal gyrus, amygdala, superior and middle temporal gyrus, and cerebellum had elevated concentrations of acrolein, which is noted to be the most reactive of the $\alpha,\beta$-unsaturated aldehydes [23]. Additional lipid peroxidation products, the F2-isoprostanes and F4-neuroprostanes were found to be of higher than normal concentrations in AD patients’ brains, cerebrospinal fluid, urine, and plasma [23]. Notable is the fact that F2-isoprostanes and F4-neuroprostanes, formed from the peroxidation of arachidonic acids and docosahexaenoic acid respectively, are relatively stable products [23].

**mtDNA peroxidation damage**

The mtDNA damage caused by peroxidation and lipid peroxidation initiate inflammatory signaling in astrocytes, and causes cascade reactions resultant in AD [5, 22]. Some molecules causing inflammation include: mtDNA, ATP, cardiolipin, TFAM, cytochrome c, formyl peptides, and RNA [5, 22]. As inflammation increases with age, diet, changes in testosterone and estrogen levels, associated diseases such as diabetes and metabolic syndrome, susceptibility for peroxidation damage also increases [5, 22]. Thus, mitochondrial dysfunction, inflammation, and resultant AD pathology, are interdependent [5, 22].

**AGE, GENDER, AND ALZHEIMER’S DISEASE**

**Aging**

In AD, in addition to mtDNA damage, nDNA damage results from oxidative stress. Considerable changes include the acceleration of the aging process, which manifests in several ways. Increased somatic mtDNA mutations, gene regulation, via methylation, which may result from oxidative stress, as well as resultant altered microRNA production, can affect the course of disease in AD [11, 25].

Experts estimate that more than 70% of miRNA transcribed in the body are in the brain, and under stressful conditions, these miRNA may upregulate p53 production [25]. The p53 protein is not only responsible for tumor suppression, but also expedient of the aging process which consequently provides the environment for AD development [25]. In addition, miR-26a regulates GSK-3β, which also expedites the aging process via gene regulation [25]. When mitochondrial antioxidants and cellular antioxidants are unable to fully detox their environment from ROS, oxidative stress results, and this is followed by aging and mitochondrial dysfunction [25].

Aging is a part of the normal human life cycle and is often coupled with gradual age-related disability [9]. These age-related disabilities include worsening muscle, skeletal, cardiovascular, and neuronal conditions, and diseases such as diabetes, cancer, and neurodegenerative disease [9]. The two most common neurodegenerative diseases, AD and Parkinson’s disease, occur mostly sporadically and susceptibility increases with age [9]. Thus, age is a strong correlative factor when considering the development of late-onset AD [26]. It is hypothesized that a main reason for late-onset AD is the dysfunction in ability for clearance of ROS species, which increases with age, damages mitochondria, reduces bioenergetics, and has been shown to reduce lifespan across many species [26]. Furthermore, phosphorylation of tau particles is known to increase with age, and thus age is strongly correlated with the development of debilitating neurofibrillary tangles in the brain [1]. It should also be noted that DNA damage repair systems’ efficiency wanes with age, and thus possibility of mutation of key genes, especially the AβPP gene, increases with age [9].
Gender

AD is two times more prevalent in women than men [27]. Menopause could be a factor and studies have shown a correlation between decline in estrogen levels and memory decline [27]. In laboratory studies estrogen reduces Aβ formation, inhibits phosphorylation of tau protein, and increases apolipoprotein expression, which suggests that postmenopausal estrogen replacement therapy may be beneficial for treatment of cognitive impairment [27].

Low testosterone levels also show a strong correlation with the development of AD [28, 29]. With men, andropause, a condition where there is a decline in testosterone most often with age, likely increases the risk for AD [28, 29]. In many cases, men with AD, have been documented to have metabolic syndrome up to a decade after first signs of low-testosterone [28, 29]. Even more so, a progression to T2D increases the loss of testosterone, which then in a cyclical fashion intensifies the effects of T2D [28, 29]. Studies have shown that testosterone therapy may alleviate and improve conditions stemming from T2D, metabolic syndrome, especially insulin resistance, excessive adipose tissue, and high cholesterol [28, 29]. Meanwhile, testosterone depletion therapy, in response to prostate cancer, has been shown to multiply the incidence and prevalence of T2D [28].

ENVIRONMENTAL AND CONTROLLABLE FACTORS OF ALZHEIMER’S DISEASE

Rodent models and clinical studies have indicated that there are risk factors that inhibit resistance to AD, and may aid neurodegeneration with respect to AD. Progress in this field of research is limited, though suggestions are made to keep mentally stimulated and maintain regular physical activity [30]. In addition, a high fat, high calorie diet combined with a sedentary lifestyle increases chances of developing AD [30]. In addition, diets with low dietary folate intake resulting in high homocysteine levels as well as high copper and iron intake increase chances of developing AD [30]. A history of head trauma, particularly brain trauma, predisposes people to developing AD [30]. Furthermore, stroke, diet, and type 2 diabetes (T2D) are additional risk factors [3].

There is a strong correlation between obesity, T2D, metabolic syndrome, and AD, which similarly owe their pathogeneses to decreasing metabolic rates [25, 28]. T2D pathogenesis is not fully understood, but characteristics include hyperglycemia or high blood sugar and insulin resistance [31]. Obesity results from excessive weight gain, where the caloric intake exceeds the caloric expense, and strongly correlates with metabolic syndrome and T2D [32]. Metabolic syndrome may be characterized as a combination of interrelated conditions including high blood pressure, high blood sugar, and excessive abdominal/midsection/subcutaneous body fat, abnormal triglyceride and cholesterol levels, and increased risk of heart disease [28].

Obesity correlates with cognitive impairment, but more so with middle age, than advanced age, increasing susceptibility to AD [25, 28]. Obesity’s ability to proliferate inflammatory cytokines, such as TNF-α and IL-1β, has an agonistic effect on neurodegeneration, as it causes inflammation in adipose tissue and neuronal tissue (especially the cerebral cortex and the hypothalamus) [28]. Cytokines and ROS (such as superoxides, hydroxyl radicals, hydrogen peroxide, and nitric oxide) are both products of activated microglia, particularly present as a result of obesity [33].

The hyperglycemia resultant of diabetes, as well as the over nutrition of obesity may overpower mitochondrial Kreb’s cycle and ETC, leading to mitochondrial dysfunction and excessive ROS production [31]. In T2D, mitochondrial dysfunction may result in its characteristic insulin resistance, evidenced by preceding decreases in mtDNA density in hyperglycemic patients [32]. With metabolic syndrome, there are similar increases in ROS production, decrease in mtDNA density, and mitochondrial dysfunction, meanwhile metabolic syndrome is also associated with mtDNA polymorphisms [32]. T2D pathology also increases uncoupling protein activity, and reduces coenzyme Q concentration and activity in mitochondria [31].

There is a strong correlation between T2D and AD pathologies. In fact, AD is also considered the type 3 diabetes [34]. The PI3K-GSK3β signaling, neuronal stress signaling, and inflammatory pathways of AD are similar to those in T2D [34]. Additional factors that intensify the correlation between T2D and AD are impediments to vasculature, including: inhibition of lipoprotein receptors and oxidative stress (factors that increase probability of membrane and DNA damage), hypertension, cerebrovascular disease (disease of cerebral blood vessels likely to cause stroke), and high cholesterol [28, 34]. Lipoproteins and lipoprotein receptor-related protein 1 (LRP-1)
are especially useful to the liver and brain in the removal of Aβ, while LRP-1 is likely to induce recovery of memory and learning abilities, when used to combat Aβ-related neuronal damage [28]. Meanwhile T2D, obesity, and metabolic syndrome inhibit this natural defense against AD [28].

Advanced glycation end products, created from advanced glycation resultant of T2D and metabolic syndrome, are proteins and/or lipids which can initiate the propagation of oxidative stress [28]. Furthermore, the receptor for advanced glycation end products has the ability to bind to Aβ fibrils as a ligand, effectively supporting the aggregation of Aβ fibrils in neurons and microglia [28].

There are several factors that increase the susceptibility to mitochondrial dysfunction, pertaining to T2D, obesity, and metabolic syndrome. mtDNA haplogroups, such as N9a, have been shown to be resistant to T2D, while mtDNA haplogroups B and F are at higher susceptibility to T2D [32]. There is variation across the sexes, but studies show that among patients with metabolic syndrome, women are more susceptible to cognitive degeneration [28]. In addition to weight gain and excessive caloric intake indicative of metabolic syndrome, mitochondrial dysfunction is exacerbated by mitochondrial toxins, and industrial products indicative of westernization, such as pesticides, rodenticides, insecticides, industrial toxic wastes, and a variety of industrial products like persistent organic pollutants and polychlorinated biphenyls [32]. Astonishingly, residents of the ‘Corn Belt’ have a high prevalence of obese BMI of 30 kg/m², which correlates with high concentrations of atrazine measured in the ground water, an herbicide used regularly since the 1960s [32]. In cultured cell and rat studies, the chemical was shown to cause mitochondrial dysfunction and insulin resistance [32]. Longitudinal studies show that with worsening of metabolic performances, comes worsening of some CNS function, including working memory [28].

Overall, there is a strong correlation linking obesity to inflammation, T2D, metabolic syndrome, mitochondrial dysfunction, and AD [28, 31, 32]. With the current healthcare crisis surrounding the “big three”, hypertension, elevated cholesterol, and diabetes (which also correlate strongly with metabolic syndrome and obesity), it will be important to address the greater susceptibility to mitochondrial dysfunction and AD [28, 32, 35].

The aforementioned mitochondrial dysfunction, mtDNA damage, oxidative stress, age, gender, dietary, and environmental factors all create an environment to induce proliferation of toxic molecules; most notably Aβ and phosphorylated tau. Their accumulation, circulation, and deposition throughout the CNS completes the cycle for AD pathology, especially with regards to mitochondrial dysfunction.

Amyloid-β association with mitochondria

The Reddy laboratory [35] and others [36–38] have established an Aβ association with mitochondria. This abnormal association induces free radical production, reduces mitochondrial ATP and mitochondrial enzyme activities, and increases in ROS production [35, 39, 40].

Shi Du Yan’s group reported Aβ binds to the mitochondrial enzyme amyloid binding alcohol dehydrogenase (ABAD), inducing a perturbation of the enzyme, prohibiting ABAD from binding the coenzyme NAD⁺ and effectively inhibiting its enzymatic activity [37, 41]. The loss of enzymatic activity of ABAD leads to the loss of ability to remove toxic aldehydes, and alters mitochondrial membrane structural integrity, changing its permeability and compromising respiratory enzymes [2, 37].

Furthermore, it was found that Aβ interaction with mitochondrial matrix protein cyclophilin D (CypD) interferes with several mitochondrial pathways, including components of the ETC, and are directly responsible for reduction in bioenergetics [42]. CypD is an enzyme with peptidyl-prolyl isomerase (PPI) activity, able to change the conformation of peptide bonds around the rigid amino acid proline between cis and trans conformations [12]. These PPIs are able to augment phosphorylation of proteins as well, which likely affects tau function and structure [12]. The AβPP contains proline, but Aβ does not. It is believed that PPI phosphorylation at the Thr668 site of AβPP, and increases the production of Aβ [12]. CypD, aka Cyp40, encoded by PPID gene on chromosome 4 and located in the matrices of mitochondria, is believed to regulate the MPT pore opening and closing [12, 43]. When CypD, which associates strongly with Aβ, forms a CypD-Aβ complex, there is an increase in cellular ROS [12]. The resultant CypD-Aβ initiated oxidative damage is believed to strongly contribute to mitochondrial dysfunction in AD [12].

Aβ also exhibits its toxic effects by physically preventing the cytochrome c from affixing itself to cytochrome c oxidase [2, 42, 44, 45]. This prevents the cristae of mitochondria from folding to contain...
the complexes, and inhibits proper electron transport in the ETC [2, 42, 44, 45].

**Phosphorylated tau interaction with mitochondria**

Recently, the Reddy laboratory reported abnormal interaction of phosphorylated tau with Drp1, leading to increased Drp1 GTPase enzymatic activity and excessive mitochondrial fragmentation in AD [46].

Tau particles associate with and bind to microtubules within the cytosol of the cell [40]. These microtubules are necessary to give the cytoskeleton and the cell its structure, and are vehicles for mitochondria and other organelles within the cell to move about [40]. The tau particles normally provide stability to the microtubules but are regularly phosphorylated via oxidative phosphorylation during neural activity [3]. When they are hyperphosphorylated, they help to break up and depolymerize microtubules [8, 40]. There is a strong correlation between this process and neuron degeneration in AD [40]. Interaction between neurons of the hippocampus and glutamate result in sustained increases in Ca\(^{2+}\) which cause hyperphosphorylation of tau, and damage mitochondria [8]. Lipid peroxidation and ROS from mitochondria also phosphorylate tau, which may be exacerbated by SOD deficiency [8].

**MITOCHONDRIAL FUNCTION AND DYSFUNCTION**

Mitochondrial dysfunction is extensively reported in both early-onset familial and late-onset sporadic AD [9, 39]. In familial AD, genetic mutations in APP, PS1, and PS2 loci induce mitochondrial dysfunction whereas in late-onset sporadic AD, mitochondrial dysfunction is caused by multiple variables, including age, age-related accumulation of oxidative stress and lifestyle activities [9, 39].

The mitochondrion serves many purposes in the eukaryotic cell. It is believed that the mitochondrion once was a separate prokaryotic cell (likely an aerobic alpha proteobacteria), which was engulfed by distant relative of modern eukaryotic cells to share a synergistic relationship. Within the cell, it moves freely using dynamin motor GTPases [48]. Within the cell, it serves as the greatest producer of energy, packaged in the molecule ATP. In addition to providing the energy to sustain the life of the cell, the mitochondrion also supplies the signaling factors necessary for programmed cell death or apoptosis, autophagy, and necrosis [48]. Given the mitochondria’s center at cell bioenergetics, including neurocellular bioenergetics, it serves a primary role in the development of AD pathology. During the progression of AD pathology, glucose uptake is reduced and biogenesis is impaired, oxidative phosphorylation enzyme and TCA cycle processes are impaired and reduced, and peroxisome proliferator-activated receptor-y coactivator 1α (PGC-1α) levels are reduced [8]. The mitochondrial dysfunction which ensues upsets mitochondrial dynamics and contributes to proliferation of Aβ and phosphorylated tau, synaptic dysfunction, and impaired cognitive function.

**Mitochondrial dynamics**

Fission enables mitochondria to remain a favorably small size for better mobility within the cell [49] (Fig. 5). Fusion enables mitochondria to attach to intracellular structures such as the endoplasmic reticulum [49]. Mitochondrial fusion also allows the mixture of the mitochondrial contents that dilute possible damaged components. Fission increases the surface area of the mitochondrial network, effectively augmenting the capability of aerobic glycolysis, while fusion provides more intracellular surface area for oxidative phosphorylation and fatty acid oxidation with the addition of more cristae on the inner mitochondrial membrane (IMM) [49].

Mitochondria are not static organelles. They have motility within the cell, powered by GTPases, and may change their structure and shape as well, via mitofusin proteins. These morphology changes are summarized as either fusion or fission [48]. Fission is the ability for a mitochondrion to divide into two or more independent mitochondria, while fusion is the ability for two or more mitochondria to fuse and become one [50, 51]. Fission and fusion are likely vestigial processes of the mitochondrion when its ancestral form was a separate organism, and these processes hold a similar purpose of reproduction of mitochondria today [11]. Throughout the lifespan of the mitochondrion, there is a build-up of damage. The network of mitochondria within a cell work together to salvage viable portions of a mitochondrion to form a separate independent mitochondrion via fission or fusion [48]. Meanwhile, the damaged portion is engulfed and discarded via mitochondrial autophagy, or mitophagy. Dysfunction in mitochondrial fusion or fission is
also strongly connected to cancer, cardiovascular diseases, and neurodegenerative disease, such as AD [48].

The structure and function the IMM and outer mitochondrial membrane (OMM) of the mitochondrion are very different and require different mechanics and pathways for fission and fusion [1, 50]. Included among the proteins responsible for OMM fission are: the cytosolic GTPase Dynamin-related protein-1 (Drp1; also known as dynamin-like protein 1 or DLP1), Fission-1 (Fis1), Mitochondrial fission factor (MFF), two homologous proteins Mid49 and Mid51 (aka MIEF1) function as Drp1 receptors which recruit and assemble Drp1 on the OMM and TBC1D15 [1, 50]. Less is known about IMM fission factors [50]. Mitochondrial Protein 18 (MTP 18), is an 18kDa protein that likely interacts with Drp1, which produced in excess causes mitochondrial fragmentation, but diminution results in fusion [50, 51]. The short optic dominant atrophy 1 (S-OPA 1), protein, produced from the proteolysis of long OPA1 (L-OPA1) supports IMM fusion, also is regulated for IMM fission [1].

Inclusive of the fusion proteins are the outer mitochondrial membrane’s GTPase mitofusins (Mfn1 and Mfn2) and the optic atrophy 1 (OPA1) protein of the inner mitochondrial membrane, all these proteins are involved in mitochondrial fusion machinery [52]. Mfn1 and Mfn2 have catalytic domains that bind GTP at their N-terminals and transmembrane domains at the C terminals [52]. Loss of either Mfn1 or Mfn2 causes the mitochondrion to fragment [52]. The GTPase OPA1 may be spliced to form L-OPA1, and evidence shows that the regulation of the balance between L-OPA1 and OPA1 is used to maintain the balance between fission and fusion [52]. There are other proteins in addition to the aforementioned proteins necessary for fission/fusion, though many are still not fully understood [1].

When AD impairs mitochondrial dynamics, there is dysfunction in glucose utilization, ATP production, Ca\(^{2+}\) regulation, growth and differentiation of neuronal axons, and frequency of cellular apoptosis [8]. This dysregulation results in upregulation of fission factors, downregulation of fusion factors, as well as reduction in mitophagy [8].

\(\text{Aβ and phosphorylated tau interaction with Drp1: impaired mitochondrial dynamics}\)

Recently, the Reddy laboratory reported abnormal interaction of phosphorylated tau with Drp1, leads to increased Drp1 GTPase enzymatic activity and excessive mitochondrial fragmentation in AD [46] (Fig. 5). Reddy’s group also reported that Aβ and phosphorylated tau interaction with Drp1, causes additional mitochondrial dysfunction and synaptic damage in AD neurons [46, 47]. These mechanistic insights are well received and much appreciated in AD and mitochondrial fields.

The excessive fission disrupts the inter-mitochondrial connectivity, and disrupts axonal transport of mitochondria. The successive bioenergetics reduction due to this fragmentation then leads to synapse inhibition, neuronal damage, and cognitive deficiency in AD [2, 3]. It is thus strongly believed that the essential source of mitochondrial dysfunction in AD is the interaction between Aβ and Drp1 [3]. Aβ particles also stimulate release of ROS species, which in turn damage mtDNA, which induces the creation of additional Aβ particles [45].

**Mitochondrial fragmentation**

Fragmentation has numerous causes, including: 1) ROS interaction with fission proteins, especially GTPase Drp1 enzyme inducing mitochondrial fission; 2) interaction between Drp1 and mutant Htt, Aβ or DJ1/LRKK2 proteins which induce upsurge of GTPase Drp1 activities leading to fragmentation; 3) S-Nitrosylation of nitric oxide to cysteine thiol changes conformation of Drp1 enzyme to improve GTPase Drp1 activity, inducing mitochondrial fission; and 4) phosphorylation of Serine amino acids at 616, 585, and 637 sites within Drp1 changes GTPase activity, resulting in fission dysfunction [46, 47, 50, 52, 53]. In AD, there is upregulation of Drp1, Fis1, MiD51, MiD49, and Mff which causes excessive division of mitochondria into fragments [8].

Coupled with the upregulation of fission factors is the downregulation of fusion factors such as Mfn1, Mfn2, and OPA-1 [8]. This inhibits mitochondria from forming larger mitochondria, creating bioenergetically inefficient mitochondria, and rendering the mitochondrial network incapable of recovering from fragmentation [8].

**Mitophagy and Alzheimer’s disease**

In addition to fission and fusion, damaged mitochondrial dynamics are mitigated with mitophagy. It is believed to occur at fertilization, throughout gestation, through the infant stage and the entirety of human life [8]. Mitophagy, which is autophagy of
the mitochondrion, involves the engulfment by a vesicle and fusion with lysosomes for destruction of the mitochondrion. [8, 49, 50, 53]. The autophagy process is regulated by highly evolutionary conserved encoding and may be categorized as macroautophagy, chaperone-mediated autophagy, or microautophagy [8]. Macroautophagy is the autophagy of focus in mitophagy [8].

Mitophagy is governed by PTEN-induced putative kinase 1 or PINK1 and the ubiquitin ligase parkin [8, 49, 50, 53]. When damaged mitochondria experience depolarization of the IMM, the mitochondrial surface receptor protein PTEN-induced kinase 1 (PINK1) is triggered to change into its stable conformation, which then adheres to the OMM [8]. PINK1 recruits parkin to the OMM, and indicates to parkin to begin E3 ligase activity in the cytoplasm [8, 53]. Parkin in turn induces ubiquitination conformational changes of proteins in the OMM [8, 53]. These proteins are marked for recognition and bound by optineurin (OPTN), p62, NDP52, and NBR1 that usher the mitochondrion towards the mitophagy pathway [8]. Finally, this pathway recruits an autophagosome to engulf the mitochondrion, which will then fuse membranes with a lysosome, effectively destroying it and its contents [8, 49, 53].

Neuroprotective behaviors, such as fasting caloric restriction and exercise, which also increase bioenergetics increase autophagy and mitophagy, whereas the converse, such as poor diet, excessive eating, and lack of exercise, reduce mitophagy [8]. When resources such as ATP are low, a mitochondrion is less likely to be targeted for mitophagy, and mitochondrial fusion is upregulated [8, 49, 50]. In fact, reduction in mitophagy is a sign of aging, and combined with Aβ amyloidosis and phosphorylated tau particles, reduced mitophagy is a sign for AD [8].

Mitophagy is further inhibited by Aβ and phosphorylated tau, which induce oxidative damage and cellular deficits that result in reduction of Parkin and PINK1 proteins [8]. The reduction of Parkin and PINK1 reduces the number of completed mitophagy processes, which in turn contributes to increased Aβ and tau particles [8]. This AD induced impairment of mitophagy is also characterized with accumulation of dysfunctional lysosomes and autophagosomal vacuoles unable to recruit proteins to complete mitophagy, and accumulation of dysfunctional mitochondria in the neuronal soma, worsened by hyperphosphorylation of tau which inhibits mitochondrial axonal transport [8].

Mitochondrial membrane and membrane potential

The OMM contains many pores for proteins and ions to pass through. It surrounds the IMM, and is separated from it via the intermembrane space. The IMM contains high levels of cardiolipin, but lacks cholesterol, unlike other membranes, and thus is very rigid and difficult to permeate. Its surface contains many folds, compressed closely to form cristae [1, 49, 50, 54]. Within the cristae are the many transmembrane, peripheral, and integral proteins, which are particularly necessary for the ETC and oxidative phosphorylation [1, 49, 50, 54]. Contained within the IMM is the matrix, where the Kreb’s cycle takes place [1, 49, 54].

The mitochondrion is the site for two important processes, the Kreb’s cycle (Citric Acid Cycle), and the ETC, coupled with oxidative phosphorylation by complex V or ATP synthase, to create ATP [1]. In the mitochondrial matrix, the Kreb’s cycle churns acetyl coenzyme A (produced from pyruvate product of glycolysis in the cytosol) coupled with H2O, and oxaloacetate through a process of addition and removal of carbon backbones to phosphorylate GDP (guanosine diphosphate) to GTP (guanosine triphosphate); and reduce NAD+ (nicotinamide adenine dinucleotide) to NADH, ubiquinone to ubiquinol, and flavin adenine dinucleotide (FAD) to the semiquinone, FADH2 [49]. The aforementioned biomolecules are then oxidized by complexes I, III, and IV, of IMM [49, 50, 54]. The complexes, along with cytochrome c, and quinone, operate the ETC along the IMM. Complex I or Complex II transfers two electrons to quinone, reducing it to ubiquinol, which in turn transfers electrons to complex III [26].

Amid transferring of electrons, the complexes pump the hydrogen ions (or protons) into the intermembrane space, creating a high proton gradient between the matrix and the intermembrane space [49]. This gradient, creates a strong negative potential across the IMM from 150 to 180 mV, and is the driving force for transmembrane enzyme ATP synthase to phosphorylate ADP to ATP [26]. The phosphorylation of ADP to ATP by ATP synthase is known as oxidative phosphorylation, and it is coupled by the reduction of O2 to H2O [26]. The following diagrams provide a general schematic for the Kreb’s cycle, ETC, and oxidative phosphorylation [49]. A side effect of mitochondria’s role to generate energy is the production of ROS in the pro-
cess, which in excess have the potential to damage mitochondria and the cell [1]. Mitochondrial dysfunction from ROS damage is followed up with loss of membrane potential [54]. UCP2 reduces mitochondrial ROS by shifting the movement of protons into the matrix rather than the intermembrane space, effectively behaving like an antioxidant [49, 55]. Antioxidants such as superoxide dismutase, coenzyme Q, and vitamin E also aid to reduce damage by ROS [11].

Mitochondrial dysfunction

At the heart of disruption of mitochondrial respiration is the opening of the mitochondrial permeability transition pore [55]. The translocase of the OMM permits the Aβ peptide to enter the mitochondria, where it then travels to the IMM [56] (Fig. 3). Aβ polypeptide induces MPT pore widening when oxidized cardiolipin (oxidized by mitochondrial hydrogen peroxide, which is enhanced by the presence of cytochrome c) synergizes with calcium ion [55]. As the MPT pore widens, it disrupts the permeability barrier of the IMM and leads to drop in intermembrane proton gradient, intermembrane potential, and uncoupling of oxidative phosphorylation [55]. This MPT pore also allows cytochrome c and other pro-apoptotic proteins to escape into the cytosol and trigger the caspase cascade and cell death by apoptosis [8, 55]. Aβ manipulates enzyme activity of complexes I, II, and IV [55] (Fig. 4). Thus, disruption of mitochondrial membrane by Aβ interaction disrupts Ca^{2+} levels, mitochondrial dynamics, leading to fragmentation, dysfunction oxidative damage, and thus degradation of AD neuron structure and function [3, 8].

Studies show that the loss of phospholipids in the IMM may be the starting point for defective complex IV, and reduction of mitochondrial bioenergetics [55]. Experimentally adding phospholipids to the IMM, after there has been a reduction in cytochrome C, reverses defectiveness of complex IV [55]. Out of the three main phospholipids in the IMM (phosphatidylethanolamine, phosphatidylcholine, and cardiolipin), cardiolipin is the only one not present in the other layers of the mitochondria [55]. Due to cardiolipin’s conical shape, it does not form a bilayer, but instead interacts with other lipids on the membrane, causing bends in the membrane’s shape, curving into cristae [55].

Cardiolipin plays a role in the management of the supercomplexes on the IMM, which are essential components of the ETC. If there is disorder in the supercomplex formation, then there will likely be an increase in ROS production from complex I [53, 55]. Cardiolipin is especially vulnerable to oxidative damage, and release of ROS from complex I would only lead to more reduction in cardiolipin in the IMM [53, 55]. Cardiolipin also helps to affix cytochrome c to the IMM, and aids electron transport between complexes III and IV [55].

Restoration of bioenergetics of mitochondria (particularly restoration of cytochrome c, and complexes I, III, IV; structure of mitochondrial cristae; flow of electrons through the ETC) will depend on restoration of cardiolipin in the IMM [55].

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Fig. 3. Aβ interaction with cellular membrane. The change in Ca^{2+} gradient caused by the amyloid beta pore formation inhibits neurotransmission, and subsequently reduced cognition. The influx of Ca^{2+} also causes mitochondrial dysfunction, inducing the production of ROS, creating mPTP, disrupting the ETC, causing the outflow of ROS, which leads to neuronal apoptosis and cell death [2, 25].
Fig. 4. Alzheimer’s disease mitochondrion. AD initiation within the mitochondrion may be attributed to toxic protein interaction (Aβ and phosphorylated tau), chemical imbalance (reactive oxygen species overproduction, calcium ion imbalance) leading to membrane potential loss, and structural damage (lipid peroxidation, mitochondrial DNA damage, and cardiolipin loss).

MITOCHONDRIAL BIOENERGETICS

ATP levels

Neurons have a high demand for ATP, and a dormant neuron alone requires about 4.7 billion ATP molecules per second [1]. Glycolysis alone would not suffice for ATP production, so the network of mitochondria within neuronal cells is the main powerhouse for neuronal activities, including the synapses and maintenance of healthy cognition [1]. ATP production is a sign of mitochondrial health. ROS species not only damage mtDNA and alter mitochondrial membrane potential, but also may decrease ATP levels due to mutation of the ATP6 gene resulting in a dysfunctional Complex V or ATP synthase [49, 57].

As the functionality of the mitochondrion deteriorates, the efficiency at which it may produce ATP, the main biomolecule for storage and delivery of energy will decline. There are several causes, including lipid peroxidation of membranes, which causes pores to open up in the IMM, reducing the proton gradient, and ROS that manipulate mtDNA coding for ATP synthase [54]. Reduced ATP production leads to inadequate supply of energy to sustain the cell [54, 57]. A substantial reduction in ATP production is a tell-tale sign of AD pathology [54, 57].

Essential to ATP production is gene expression of cellular and mitochondrial DNA. The amino acid lysine (Lys or K) is an essential amino acid, and a component of many proteins in the body. Lysine’s positioning on histones of cellular DNA, sets it up for acetylation and/or deacetylation, a biomolecular mechanism for gene expression control [58]. The acetylation, addition of a negatively charged acetyl group to lysine’s amine moiety at the end of its R-group (ε, Epsilon amino group) effectively loosens up tightly wound, also negatively charged DNA via repulsion, readying it for transcription [58]. This is an essential step for the transcription of proteins functionality for metabolic processes, cellular transport, control of gene transcription, and signal transduction [58]. The lysine acetyltransferases (KATs) and lysine deacetylases (KDACs) work together to conceal the ε-amine’s positive charge (acetylation) or expose it (deacetylation) [58]. The overall effect on the protein is a slight conformational change that reduces or increases the protein’s activity, affinity, structural integrity, or interaction with other proteins [58].

The most recognizable KAT/KDAC combination is of histone acetyltransferases (HAT) and histone deacetylases (HDACs) [58]. Sirtuins comprise a class of KDACs, are abbreviated SIRT and are numbered 1 through 7. Sirtuins 4 and 6 regulate apoptosis and DNA repair via catabolism of
the coenzyme nicotinamide adenine dinucleotide (NAD\(^+\)) into nicotinamide and 2'-O-acetyl-ADP-ribose and/or 3'-O-acetyl-ADP-ribose and function as ADP-ribosyl transferases to proteins [58]. SIRT4 and SIRT5 function mostly in the mitochondria of the cell, and it should be noted that balance between NAD\(^+\) and NADH during bioenergetics pathways affects SIRT4 activity [56]. SIRT4 and SIRT5 present minimal deacetylase activity [59]. SIRT5, performs demalonylation (particularly of lysine malonylation), desuccinylation (leading to activation of Cu/Zn SOD1), and glutarylation of mitochondrial proteins 1000 times greater of a ratio to its deacetylation activity [59]. Its desuccinylation activity in particular moderates mitochondrial physiology and biogenesis via promotion of respiration and ATP synthesis and its desuccinylation of SOD1 eliminates ROS [59]. SIRT4 monitors ATP balance and mitochondrial biogenesis [60] (Fig. 7).

Sirtuins 1, 2, 3, 5, and 7 function as deacetylases and can serve as transcription regulators that may cause histones to adhere tightly to DNA [58]. When ROS damage leads to DNA DSBs, SIRT1 and SIRT6 are activated for DNA repair [59]. SIRT1 is present mostly in the cytosol of cells in the brain, and functions to deacetylate PGC-1\(\alpha\), conjugating it into its active form [58]. PGC-1\(\alpha\), a regulator of mitochondrial biogenesis, is activated by brain-derived neurotrophic factor (BDNF), and operates as a coactivator varying signals with biological responses, allowing the cell to adapt to adjustments in energy demand [8, 58]. PGC-1\(\alpha\), also via coactivation, regulates nuclear respiratory factors (NRFs) which moderate transcription of nuclear-encoded mitochondrial structural protein, which are transported to a mitochondrion, and processed from their pre-protein form to final protein form within the mitochondrion [58]. These nuclear respiratory factors coactivated by PGC-1\(\alpha\) also moderate the transcription of TFAM which is necessary for replication, transcription, and maintenance of health mtDNA [58]. BDNF is responsible for hippocampal synaptic plasticity, learning, memory, and neuronal stress resistance and impairment of its function is a marker of AD [8]. Studies have shown that dysfunction in BDNF signaling affects a cascade of dysfunction culminating in the synapse and causing neuronal degeneration in AD [8].

**MITOCHONDRIAL BIOENERGETICS DISRUPTION IN ALZHEIMER’S DISEASE**

\(\beta\) and sirtuin interaction

Limited interaction with transient \(\beta\) results in the transcription of mitochondrial SIRT4 and SIRT5 [59]. Nicotinamide functions as an inhibitor of all sirtuins, while EX527 and AGK2 are inhibitors of SIRT1 and SIRT2, respectively [58]. Exercise, however, which stimulates neuronal activity may upregulate SIRT3, which induces neuroplasticity and cellular stress resistance [8] (Fig. 6).

Researchers have found that \(\beta\) peptides may induce overexpression of PARP1 and cause neuronal death [59]. The PARP1 overexpression in particular leads to NAD\(^+\) depletion, which inhibits activity of
Fig. 6. SIRT4 ADP-ribosyl transfer and inhibition of glutamine dehydrogenase (GDH). A) Multiple transaminase convert α-ketoglutarate to glutamate and GDH converts glutamate to α-ketoglutarate, while reducing NAD$^+$ to NADH. B) Aβ upregulates SIRT4 activity, which converts NAD$^+$ via ADP ribosylation to nicotinamide and 2-O-acetyl-ADP ribose and 3-O-acetyl-ADP ribose, reducing NAD$^+$ availability for GDH. C) ADP ribosylation inhibits GDH, and reduces α-ketoglutarate necessary for citric acid cycle and bioenergetics of mitochondria.

SIRT1, particularly its ADP-ribosyltransferase activity [59].

It has been shown that inhibition of PARP1 may conversely lead to expression of SIRT1, which has been shown to promote expression of α-secretase, downregulation of BACE1, and reduced Aβ generation [59]. Furthermore, γ-secretase may also be regulated by SIRTs [59].

Free radical production

ROS species must be kept in balance, and their byproducts are often used as a sign of a healthy functioning cell [23]. Mitochondrial derived ROS initiate propagation of cytokines for inflammatory response and aid the abstraction of bacteria [47]. Hydrogen peroxide (H$_2$O$_2$) derived from the mitochondria suppresses M1 expression of macrophages and upregulates M2 activity of macrophages, supporting macrophage polarization [9, 53, 57]. Mitochondrial ROS are needed for initiating NLRP3 inflammasome innate immunity response, particularly propagation of cytokines interleukin-1β and –18 [49]. Mitochondrial ROS from complex III of the ETC activates CD4$^+$ of T Cells [48]. ROS from mitochondrial glycerol-3-phosphate dehydrogenase 2 form ubiquinol from ubiquinone in the IMM, which in turn propagates the production of ROS from complex I in the ETC, which also is important for T Cell stimulation [49, 53]. Mitochondrial ROS also augments B Cell activity through heme amalgamation, changing immunoglobulin function [49]. Nonetheless, imbalances in ROS caused by mitochondrial damage, and/or reduction in antioxidants...
or their related enzymes can cause overproduction of ROS [23, 53, 57]. Overproduction of ROS and their byproducts is a sign of AD and is often coupled by energy deficiency [23, 53, 57]. This reduction in bioenergetics is exacerbated by reduced levels of mitochondrial complex V, or ATP synthase in brain regions affected by phosphorylated tau [8].

As discussed, complexes I and III of the ETC are a normal source of ROS production, but with aging of the body, and deficiency in the ETC, there is an increase in electron leaking and ROS generation [49, 55]. Damage from ROS is difficult to avoid, as they are necessary for signaling within the body and reacting with biomolecules, but also damage living tissue, and thus are a catch-22 [23, 53]. Their reactivity allows them to oxidize biomolecules, especially nucleic acids, proteins, and lipids [23]. The brain is especially susceptible, given the high demand on energy, high demand on oxygen, high supply of peroxidizable polyunsaturated fatty acids (the brain also being 60% fat), high concentration of iron capable of ROS generation, and scarcity of antioxidants and enzymes with antioxidant functionality [23]. The mitochondrion is a source of ROS, and is also highly susceptible to damage from ROS [23]. This is strong evidence for the compound damaging effects that lead to AD [23, 53, 57].

Furthermore, a mitochondrion in a neuron typically has a longer half-life than one of another bodily tissue, and is more susceptible to long-term nitrooxidative damage due to this and neurons’ low concentrations of glutathione which serves as an antioxidant [1]. This sustained mitochondrial dysfunction is worsened by reduction in mitophagy in AD cells, where damaged mitochondria persist much longer than they would in healthy cells [8].

Several researchers have proven the damage caused by lipid peroxidation and H₂O₂. In a study conducted by Reddy’s group in 2010, mouse neuroblastoma (N2a) cells incubated with Aβ treatment there was greater H₂O₂ production and lipid peroxidation, reduced ATP production, cytochrome oxidase activity, and cell viability, along with impaired mitochondrial membrane potential [44]. In fact, the IMM of cells incubated with Aβ were seldom visible or highly damaged, and was coupled by reduction in neurite outgrowth [44]. Similar effects were noted by Reddy’s group again with N2a cells transfected with mutant cDNA expressing mutant AβPP, where the N2a cells demonstrated AD pathology with greater H₂O₂ and lipid peroxidation production, reduced ATP production and cytochrome oxidase activity [62]. In addition, there was reduced cell viability and a reduction in mtDNA copy number, as well as increased GTPase Drp1 enzymatic activity [62]. In another study by Reddy’s group, N2a were inoculated with Aβ toxicity to induce AD pathology, which resulted in increased H₂O₂ and lipid peroxidation, reduced mitochondrial ATP and cytochrome oxidase activity [63]. These effects also markedly reduced cell viability [63].

In vivo studies with mice grown to exhibit mutant AβPP AD pathology, greater lipid peroxidation and H₂O₂ production was exhibited, combined with evidence of reduced mitochondrial fusion, cytochrome oxidase activity and ATP production [64]. These damaging effects were proven to be reversed by SS-31 administration as well [64] (Fig. 8).

In another transgenic tau mice study, Kandimalla and colleagues [65] found mitochondrial dysfunction, including increased H₂O₂ and lipid peroxidation levels, reduced cytochrome oxidase activity, and mitochondrial ATP levels, primarily from phosphorylated tau impairment [65]. Furthermore, fusion proteins were decreased, mitochondrial length was reduced and mitochondrial number was increased in these mice [65]. These findings indicate that phosphorylated tau induces mitochondrial abnormalities [65].

Furthermore, it should be noted that in postmortem human AD brains, mitochondria have a notable deficit of oxidative phosphorylation, especially a great reduction in cytochrome oxidase activity [66]. The reduction in cytochrome oxidase activity was most profound in the hippocampus and temporal cortex of the AD brain, which is believed to be the main contribution to the reduction in bioenergetics in the AD brain [66]. This is likely owed to oxidative stress noted above.
Fig. 8. Mitochondrial axonal transport. Loss of mitochondrial axonal transport is the major cause for cognitive decline in AD. The AD neuron has greatly reduced neurotransmission at the synapse in comparison to healthy neurons.

Mitochondrial oxidative stress

Oxidative stress is one of the major sources of initial pathogenesis of AD, and the mitochondria plays a critical role as the source of 90% of endogenous ROS [23]. Via the ETC, there is inevitable leakage of electrons from the IMM, which despite checks and balances from antioxidant enzymes, mitochondrial ROS provides a constant source of superoxide anion [23, 61]. The ROS species produced also aid to inhibit productivity of the mitochondria, and evidence shows that dysfunctional mitochondria are inefficient at generating ATP, and simultaneously are excessive producers of ROS [23, 61]. In this light, the mitochondrion of AD cells becomes self-destructive. The majority, if not all mitochondrial functionality is impaired in AD tissue [23].

There is evidence that superoxide and the radicals formed via lipid peroxidation are products of the same oxidative damage pathway which activate uncoupling proteins [67]. It should thus be duly noted that ROS are an agent of damage and degeneration in cells, particularly when it comes to nucleotide damage, propagation of free radicals, membrane damage, and protein denaturation. However, the cell maintains a balance between ROS produced and mitigating pathways, including those conducted by enzymes. When ROS levels are too low in a cell, cells are vulnerable to promoting mutation proliferation, cancer and tumor growth, since phagocytosis and apoptosis of damaged cells and organelles, and processes such as mitophagy which excise damaged mitochondria, are necessary for healthy cells and tissues [51, 68].

SYNAPTIC DYSFUNCTION AND COGNITIVE DECLINE

Obstruction of the synapse with Aβ peptides inhibits neurotransmission, and Aβ uptake within the cell induces mitochondrial fragmentation, oxidative damage, reduction in biogenesis as well as inhibition of mitochondrial axonal motility [53]. Hyperphosphorylation of tau produces neurofilibrillary tangles responsible for inhibition of mitochondrial axonal motility as well as degeneration of neuronal cells [53]. The overall impact leads to synaptic dysfunction, cognitive decline, neuronal loss, as well as memory loss [3].

The origin of synaptic dysfunction is the second layer of the entorhinal cortex, where degeneracy progresses to the hippocampus, temporal cortex, frontoparietal cortex, and subsequently the subcortical nuclei) [3, 61]. Evidence has shown that as AD worsens, the entorhinal cortex and the hippocampal dentate gyrus of the brain progressively become more disconnected meanwhile the hippocampal regions CA3, CA1, and subiculum too are further dissociated [3].

Inability to form new memories is one of the various symptoms of AD, and degeneration within the hippocampus is one of the root causes [61]. The hippocampus is responsible for formation, organization, and storage of new memories as well as the association of emotions and senses to these new [3, 61]. Available for cell culturing are HT22 cells, sub-cloned from the mouse HT-4 hippocampal cell line, which allows researchers study pharmacological effects of drugs targeted to mitigating AD pathology in vitro [3].

A recent study led by Reddy et al. [3] quantitatively and qualitatively measured degeneracy of HT22 cells transfected with mutant AβPP via cDNA, and were able to demonstrate how AβPP and Aβ polypeptide contributed to memory loss [3]. Overall, the AβPP and Aβ reduced MAP2 and thus inhibited neuronal ability to form dendrites, reduced synaptic proteins necessary for effective neurotransmission, increased mitochondrial fission as well as ROS production [3]. Furthermore, AβPP and Aβ inhibited mitophagy and autophagy proteins, necessary for maintenance of mitochondrial dynamics, mitochondrial network, and neuron network fidelity and vitality [3, 61]. Over-
all obstruction of synaptic protein and inhibition of mitochondrial dynamics including axonal motility demonstrated impedance of cognition, and inhibition of dendrite formation necessary for formulation of new synapses; which inhibits new memory formulation [3]. HT22 cells transfected with AβPP are thus prime for development of assays for drug development for mitigation of AD.

Important to note are the limitations of studying cells in vitro, outside of interactions associated with human bodily functions. This is further accentuated by the length of experiment, of 48vhours, to note the degeneracy of neuronal cells. This again is indicative of the need for longitudinal study with additional environmental variables to further mimic in vivo AD pathology.

CONCLUDING REMARKS

AD is a major health concern in today’s society. Although tremendous progress has been made in understanding the biology of the disease and development of therapeutics, we still do not have a molecule that can delay and/or prevent the disease process in elderly individuals and patients with AD. Synaptic damage and mitochondrial dysfunction are early events/triggers of disease process. Mitochondria, the powerhouses of the cell, are responsible for the majority of the metabolic processes that keep humans alive. Unfortunately, dysfunction in these super organelles renders us susceptible to critical, debilitating diseases. Mapping the pathogenesis of these diseases is at the center of developing mitigating strategies for AD and similar metabolic and neurodegenerative diseases. Today’s medical research has made considerable headway in common illnesses which share similar origins, such as metabolic syndrome, obesity, and T2D; accordingly, treatment for prevention should strongly consider these therapeutic strategies as a foundation for treatment. Due to the similarity of AD with T2D, strong consideration should be made for means for improving insulin regulation, where defective brain insulin signaling occurs, in order to alleviate AD [25].

Another hope for future research is to increase mitochondrial resistance to degeneracy. The mtDNA copy number reduction with age, relative increase in mtDNA recombination repair with age accompanied with recombination repair fidelity decline with age, and the mtDNA repair system decline with age are all areas to address in future studies. The eventual loss of mtDNA copy number which affects mitochondrial dynamics contributes to increased fission and mitochondophagy to maintain the fidelity of mtDNA in the mitochondrial network, but AD pathology which upregulates Drp1 results in a devastating impact causing fragmentation, eventual wide spread neuronal loss, brain shrinkage and cognitive degeneracy, combined with reduction in mitochondophagy. Understanding mechanisms by which mtDNA copy number may be increased is thus a plausible means for effective AD pathology resistance.

The cascade of debility from mitochondrial dysfunction, to Aβ accumulation and neurofibrillary tangles at the tail end also illuminate the necessity for therapeutic efforts to be AD specific. Future research should consider the effect of Aβ on Sirtuin regulation, which play a key role in mitochondrial bioenergetics regulation. The ability to prevent Aβ’s ability to manipulate Sirtuin activity would expectantly increase mitochondrial bioenergetics.

As AD pathology persists in neurons, the reduction in bioenergetics, results in reduction in phosphorylation necessary for DNA repair processes such as kinase 3’-phosphatase (PNKP) in short patch BER. Inefficient amounts of antioxidants leading to oxidative damage of mtDNA os worsened by inefficient DNA repair. The damage mtDNA is isolated by mitochondria, to maintain mtDNA fidelity; however, the accumulation of damaged mtDNA leads to mitochondrial dysfunction. Thus, there is also room for research drawing connections between bioenergetics and maintenance of mtDNA fidelity.

Key components of the mtDNA repair processes are also plausible subjects for future research. For example, reduction in Lig 3 may lead to reduction in mitochondrial viability. Persons with higher content of Guanine and Cytosine bases may be more susceptible to oxidative mtDNA damage as mtDNA repair systems and antioxidants become deficient with age. In fact, it was noted by Suter and Richter [69] that healthy mtDNA contain low levels of 8-oxodG, demonstrating correctly functioning mtDNA repair. Nonetheless, damaged and eventually degraded mtDNA had high concentration of 8-oxodG, accentuating failure of mtDNA repair [11]. Furthermore, in experiments where BER the system is inhibited, mtDNA is degraded and thus copy number is reduced [11]. Researchers [11] have also noted that oxidative stress induces more damage within cells to mtDNA than nDNA, particularly polymerase-blocking strand breaks and a-basic sites, which further reduces mtDNA copy number.
Studies would also benefit from focus on pharmaceuticals that trigger ATP reduction, and induce AD-like reduction in biogenesis. There are common pharmaceuticals that induce mitochondrial damage by reducing ATP production. Examples include doxorubicin (a form of chemotherapy) and nucleoside reverse-transcriptase inhibitors such as zidovudine (an antiretroviral to deter and or alleviate the onset of HIV/AIDS, particularly for pregnant mothers) which include side effects of toxicity of mitochondria [54]. There have been recalls of pharmaceuticals such as troglitazone, which caused hepatotoxicity because of their ability to cause mitochondrial dysfunction [54].

Furthermore, future studies of pharmaceuticals that target the IMM would greatly improve biogenesis. There are difficulties that are inherent to creating a drug that aims to improve ATP synthesis. There are many components that are necessary to have a healthy functioning ETC, and it would be ineffective to target one particular protein, lipid or cofactor, as they would all need to be regulated as well, but improvement of the integrity of the IMM would likely produce the strongest results [54]. Nonetheless, even if a drug were to be discovered or synthesized, transportation of this pharmaceutical through the body to specifically target the IMM without causing detriment to the cell or mitochondrion would be very challenging [54].

Future, studies targeted to the early detection of AD pathology will also need to establish healthy ROS baseline levels. As ROS are essential for cellular pathways, and mitochondrial dynamics, a longitudinal study will need to be conducted to establish baseline and fluctuation ROS concentration for in vitro cell cultures. An essential dynamic to consider is the mitochondrial response to accumulation of superoxide leads to the reduction of bioenergetics of the ETC, which mitochondria do to reduce the overall number of ROS including $O_2^-$ produced. Thus, low bioenergetics often results in self-preservation, by effectively reducing ROS production.

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REFERENCES


