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Review

# Aging and amyloid beta-induced oxidative DNA damage and mitochondrial dysfunction in Alzheimer's disease: Implications for early intervention and therapeutics

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## ARTICLE INFO

## ABSTRACT

Alzheimer's disease (AD) is an age-related progressive neurodegenerative disease affecting thousands of people in the world and effective treatment is still not available. Over two decades of intense research using AD *postmortem* brains, transgenic mouse and cell models of amyloid precursor protein and tau revealed that amyloid beta (Aβ) and hyperphosphorylated tau are synergistically involved in triggering disease progression. Accumulating evidence also revealed that aging and amyloid beta-induced oxidative DNA damage and mitochondrial dysfunction initiate and contributes to the development and progression of the disease. The purpose of this article is to summarize the latest progress in aging and AD, with a special emphasis on the mitochondria, oxidative DNA damage including methods of its measurement. It also discusses the therapeutic approaches against oxidative DNA damage and treatment strategies in AD.

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## Contents

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## 1. Introduction

Alzheimer's disease (AD) is the most common progressive neurodegenerative disorder and the major cause for dementia [1]. As people age, memory and the ability to carry out tasks often decline and their risk for neuronal damage increases. AD is strongly an age-associated disease. An estimated 5.4 million Americans have AD; approximately 200,000 people aged <65 years with AD comprise the younger-onset AD population, and over 50% of individuals that are 85 years old will be affected by AD [2]. Pathologically, AD is associated with extracellular amyloid-beta (Aβ) deposits and intracellular aggregates of hyperphosphorylated tau, and neurofibrillary tangles, and also accompanied by oxidative stress/mitochondrial dysfunction and synaptic damage [1,3–7] (Fig. 1). Aging is considered as a major risk factor in AD. However, the mechanisms that underlie aging process in AD progression are not fully understood.

Several hypotheses have been proposed to explain the causes of aging. Mechanisms that govern genome integrity and stability are major guarantors of viability and longevity. 1) Telomere-shortening and its relationship with aging is extensively investigated and reported that changes in telomere structure and function are linked to aging [8–10]. 2) Activation of p53 is emerged as prime factor of a functional decline of a tissue in aging [11]. 3) Signaling that control energy production and metabolism, including insulin/IGF pathway is another important mechanism that contributes to aging [12,13]. 4) The mitochondrial oxidative damage is the strong cellular event involved in aging process [6,14–16]. 5) The latest evidence suggests a unifying mechanism that involves a combination of factors in aging, including telomere shortening, and mitochondrial oxidative damage, p53 activation, and reduced peroxisome proliferator-activated receptor gamma, coactivator 1 alpha and beta (PGC-1α and PGC-1β) [17,18].

Among these, mitochondrial oxidative damage has been found excessively in the brains of aged people, especially AD patients and also AD-like transgenic animal models. The purpose of this article is to summarize the recent progress of aging and Aβ-induced oxidative DNA damage and related pathways in the pathogenesis of AD. Further, this article also discusses therapeutic approaches of oxidative DNA damage in treating patients with AD.

## 2. Cellular changes of aging and oxidative DNA damage

Aging is a complex process that has been linked to accumulation of DNA damage. In mammals, including humans, an accumulation of oxidative DNA damage in different tissues including brain is observed during aging [19–21]. Recent observation from young, middle and old age mice groups further indicate that oxidative DNA damage is increased with aging (Mao and Reddy, unpublished observations). Telomeres, the ends of linear chromosomes, repetitive DNA regions of hexanucleotide repeats, protect chromosomal ends from deterioration during DNA replication. Telomerase (TERT), a reverse transcriptase, forms a complex with an RNA template and cofactors to extend telomeres. The shelterin protein complex then binds the 3′ single-stranded end of telomeric DNA, protecting it from DNA damage responses [22,23]. Interestingly, TERT is also localized in mitochondria and regulates/modulates oxidative damage on the mtDNA [24].

Inhibition of TERT function and related process leads to short telomeres and premature age-related diseases. In fact, telomere shortening represents a cell-intrinsic mechanism leading to DNA damage accumulation and activation of DNA damage at checkpoints in aged cells. Activation of DNA damage checkpoints in response to telomere malfunction results in induction of cellular senescence — a permanent cell cycle arrest. Senescence represents a tumor suppressor mechanism protecting cells from evolution of genomic instability and transformation [25]. As a drawback, telomere shortening may also limit tissue renewal and regenerative capacity of tissues in response to aging and chronic disease.

Short telomeres are a hallmark of dyskeratosis congenita, which is caused by mutations in genes (including TERT) controlling telomere homeostasis. Induced pluripotent stem cells from dyskeratosis congenita patients demonstrate that telomere shortening, induced by mutations of TERT and other related genes, eventually results in loss of self-renewal [26]. In aged organs, telomere shortening may also increase the risk of age-related diseases, including neurodegenerative diseases and cancer, by initiation of chromosomal instability, loss of proliferative competition of aging stem cells, and selection of aberrant growing clones. Consequently, aged individuals are more susceptible and vulnerable to various diseases. Recently, proteins were found in human serum, which are induced by telomere dysfunction and DNA damage. It was shown that these proteins (CRAMP, EF-1alpha, stathmin, n-acetyl-glucosaminidase and chitinase) increase in aged people and are further elevated in geriatric patients, representing new biomarkers of human aging and disease [21,27].

Interestingly, lifestyle factors (such as exercise, smoking, body mass) also affect on the expression of these serum markers of DNA damage. The expression of biomarkers of DNA damage correlated positively with other cellular aging marker p16 (INK4a) expression and negatively with telomere length in peripheral blood T-lymphocytes. Together, these data provide experimental evidence that both aging and lifestyle impact on the accumulation of DNA damage during human's aging [28].

Emerging evidence suggests that telomere shortening can limit the function and maintenance of adult stem cells, which are of utmost importance for organ maintenance and regeneration. In mouse models, telomere dysfunction leads to a depletion of adult stem cell compartments suggesting that stem cells are very sensitive to DNA damage [29].
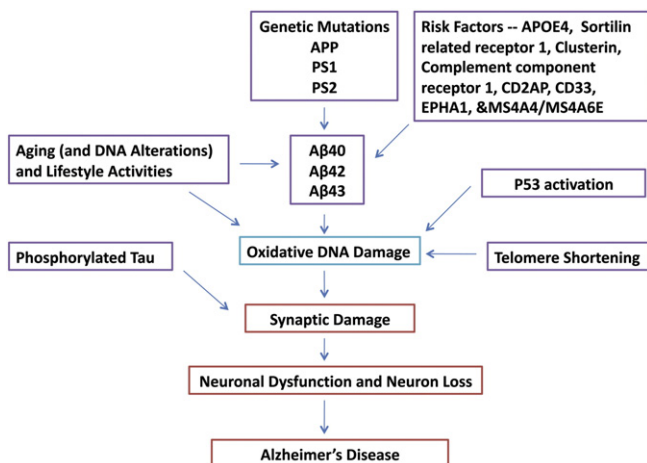


**Fig. 1.** Factors that are responsible for causing Alzheimer's disease (AD). Genetic mutations in amyloid precursor protein, presenilin 1 and presenilin 2 cause early-onset familial AD. Genetic variants in sortilin related receptor 1, clusterin, complement component receptor 1, CD2AP, CD33, EPHA1, and MS4A4/MS4A6E genes contribute to late-onset AD, in addition to ApoE 4/4 genotype. Aging, lifestyle activities and oxidative DNA damage are major contributing factors for the development of AD.

Recent work reveals that additional effects of telomere shortening on neuronal differentiation, as adult multipotent progenitors with critically short telomeres yield reduced numbers of neurons that, furthermore, exhibit underdeveloped neuritic arbors. Genetic data indicate that the tumor suppressor protein p53 not only mediates the adverse effects of telomere attrition on proliferation and self-renewal but it is also involved in preventing normal neuronal differentiation of adult progenitors with dysfunctional telomeres. The effect of p53 on neuritogenesis is mechanistically linked to its cooperation with the Notch pathway in the upregulation of small GTPase RhoA kinases, Rock1 and Rock2, suggesting a potential link between DNA damage and the Notch signaling pathway in the control of neuritogenesis [30]. It has also been shown that telomerase expression is downregulated in the subependymal zone of aging mice leading to telomere length reductions in neurosphere-forming cells and deficient neurogenesis and neuritogenesis, it suggests that age-related deficits could be caused partly by dysfunctional telomeres and demonstrates that p53 is a central modulator of adult neurogenesis, regulating both the production and differentiation of postnatally generated olfactory neurons [30].

In addition, the transcription factor p53 protects neurons from transformation and DNA damage through the induction of cell-cycle arrest, DNA repair and apoptosis in a range of *in vitro* and *in vivo* conditions. Indeed, p53 has a crucial role in eliciting neuronal cell death during development and in adult organisms after exposure to a range of stressors and/or DNA damage [31].

p53 takes a critical part in a number of positive and negative feedback loops to regulate aging, carcinogenesis and other biological processes. Uncapped or dysfunctional telomeres are an endogenous DNA damage that activates ATM kinase and then p53 to induce cellular senescence or apoptosis. Recent studies have shown that p53, a downstream effector of the telomere damage signaling, also functions upstream of the telomere-capping protein complex by inhibiting one of its components, TRF2 (telomeric repeat binding factor 2). Since TRF2 inhibition leads to ATM activation, a novel positive feedback loop exists to amplify uncapped telomere-induced, p53-mediated cellular responses. Siah1 (seven in absentia homolog 1), a p53-inducible E3 ubiquitin ligase, plays a key role in this feedback regulation by targeting TRF2 for ubiquitination and proteasomal degradation [32].

In addition to telomere shortening mechanism, ROS and mitochondrial dysfunction as further described below, and environmental factors (*e.g.* toxic chemicals and irradiation), as well as DNA repair defects also contribute to accumulating DNA damage during aging [21,33,34] (Fig. 1). Interestingly, recent findings suggest environmental genotoxins, specifically methylazoxymethanol (MAM), the genotoxic metabolite of the cycad azoxyglucoside cycasin, target common pathways involved in neurodegeneration and cancer, the outcome depending on whether the cell can divide (cancer) or not (neurodegeneration). Exposure to MAM-related environmental genotoxins may have relevance to the etiology of related tauopathies, notably, AD [35]. It also has been indicated that DNA damage is an initial and critical contributor for aging, and various factors that affect aging may all function ultimately through the accumulation of persistent DNA lesions containing unrepairable DNA double-strand breaks [20,36].

Mice deficient in the Polycomb transcriptional repressor Bmi1 develop numerous abnormalities including a severe defect in stem cell self-renewal, severe neurological abnormalities, alterations in thymocyte maturation and a shortened lifespan [37,38]. Cells derived from $Bmi1^{-/-}$ mice have impaired mitochondrial function, a marked increase in the intracellular levels of ROS and DNA damage, as well as subsequent engagement of the DNA damage response pathway. More interestingly, the antioxidant N-acetylcysteine or genetic disruption of the DNA damage response pathway by Checkpoint kinase (Chk) 2 deletion can rescue many deficits, and eventually elongates significantly lifespan [38]. These observations further indicate the important role of mitochondria, ROS and DNA damage in aging and neurodegenerative diseases.

Therefore oxidative DNA damage may be the center of aging and age-related diseases such as AD. Hence, protection from DNA damage presents a basic approach for elongation of healthy age and treatment of such age-related diseases.

## 3. Amyloid beta, particularly Aβ42 in Alzheimer's disease

Amyloid-beta peptide (Aβ) appears to play a pivotal role in the development of AD and amyloid plaques as the most important hallmark is routinely used for diagnosing AD in brain tissue [5,39,40]. Increasing studies demonstrated the toxicity of diverse Aβ species *in vitro* and *in vivo*, confirming the importance of age-dependent Aβ accumulation in AD pathogenesis [41–44]. A recent study revealed that area-specific vulnerability to Aβ deposition is associated with local levels of brain metabolism and neuronal activity, indicating that Aβ and glucose metabolism are linked to AD [45]. Transgenic mice (at least APP23 line), overexpressing mutant human amyloid precursor protein (APP), exhibit selective neuronal death in the brain regions such as hippocampus and cortex that are most affected in AD, suggesting that amyloid plaque formation is directly involved in AD neuron loss [46]. In APP/PS1 transgenic mice, global neocortical neuron loss is not apparent up to 8 months of age, but local neuron loss in the dentate gyrus is observed [47]. Recent stereological analysis of neocortical neuron number in subregions with high neuron density such as the granule cell layer of the dentate gyrus, modest but significant neuron loss is also found [48]. In addition, parallel studies using multiphoton microscopy and *in vivo* microdialysis revealed that pharmacological reduction of soluble extracellular Aβ by as little as 20–25% was associated with a dramatic decrease in plaque formation and growth. Interestingly this small reduction in Aβ synthesis was sufficient to reduce amyloid plaque load in 6-month-old but not 10-month-old mice [49]. These observations demonstrate that amyloid plaque effects first on some critical regions and vulnerable neurons in brain, and early interference that reduces the Aβ level and the plaques prior to neurons die is the key for treatment of this disease.

Furthermore, microinjection of plaque-equivalent concentrations of fibrillar Aβ in the aged rhesus monkey cerebral cortex results in profound neuronal loss, tau phosphorylation and microglial proliferation [50], indicating the both direct and indirect toxic roles to neuron of Aβ plaques. Recent evidence further indicates that the fibrillar conformation of Aβ deposited in compact plaques is associated with the pathologies observed in AD. Quantitative analysis revealed that the area adjacent to fibrillar Aβ, containing compact but not diffuse plaques in aged rhesus, aged human, and AD cortex, displays significant loss of neurons and small but statistically significant reduction in the density of cholinergic axons [51].

The Aβ peptide is a 38–43 amino acid in length and is generated by proteolytic processing of the APP, a cell-surface receptor *via* β-secretase (β-amyloid cleavage enzyme, BACE1) and γ-secretase complex containing presenilins consequently [1,40,52–54]. Although several small Aβ molecules exist in the AD brain, the most common species are Aβ40 and Aβ42. Aβ40 is the major species in soluble Aβ, and is constitutively secreted into culture medium and cerebrospinal fluids [55,56]. However a number of studies have shown that Aβ42 is in fact the predominant species in the parenchymal amyloid deposits in AD brain, and it is an initially deposited species; furthermore, elevated Aβ42 levels, as well as particularly the elevation of the ratio of Aβ42 to the shorter major form Aβ40, has been identified as a key feature in early events in the pathogenesis of AD [57]. The specific pathological importance of Aβ42 has drawn attention to seeking drugs that will selectively lower the levels of this peptide through reduced production or increased clearance while allowing normal protein processing to remain substantially intact [52,57–60]. Furthermore mutations in APP and presenilin (PS1 and PS2) genes have been shown to increase the production of the longer Aβ form Aβ42 relative to Aβ40, and to cause the familial autosomal dominant form of AD [56,61–63]. Notably it has been shown that expression levels

of BACE1, the limiting enzyme of Aβ production, are increased by oxidative stress, ischemia and hypoxia [64,65].

Interestingly transgenic mice expressing high levels of Aβ40 do not develop overt amyloid pathology. However, mice expressing lower levels of Aβ42 accumulate insoluble Aβ1–42 and develop compact amyloid plaques, congophilic amyloid angiopathy, and diffuse Aβ deposits. Further, when mice expressing Aβ42 are crossed with mutant APP (Tg2576) mice, there is also a massive increase in amyloid deposition. These data further establish that Aβ42 is essential for amyloid deposition in the parenchyma and also in vessels [66].

Plasma Aβ levels are elevated in early-onset AD caused by autosomal dominant mutations. Recent findings showed that similar genetic elevations exist in late-onset AD (LOAD), providing strong evidence for the existence of novel, as yet unknown genetic factors that affect LOAD by increasing Aβ [67]. Further, higher plasma Aβ42 at baseline was a significant predictor for the development of probable or possible AD at 5 years [68]. Notably, in recent report of early AD (predementia) trial design, it has been proposed that CSF Aβ42 or amyloid PET imaging may be optimal biomarkers for selecting subjects for anti-amyloid interventions [69].

Based on these fundamental observations, immunization of AD patients with synthetic full length Aβ42 (AN1792, Elan Pharmaceuticals) has been studied in a randomized, double-blind, placebo-controlled phase 2a clinical trial [70,71]. Treatment was discontinued following reports of encephalitis, and in the 1 year analysis of the AN1792 data, vaccinated subjects did not show improvement and no single test revealed benefits of vaccination, although the Z-test was consistent with a potential slowing of decline. However, approximately 4.6 years after immunization with AN1792, patients defined as responders in the phase 2a study maintained low but detectable, sustained anti-AN1792 antibody titers and demonstrated significantly reduced functional decline compared with placebo-treated patients [70]. These data support that Aβ immunotherapy, even though some patients suffered side effects, may have long-term functional benefits, and further support the Aβ cascade hypothesis of AD. In addition, another anti-amyloid therapy that reduces amyloid accumulation as measured by PIB-PET in a recent clinical trial also supports the Aβ hypothesis as well as the rationale for treatment with an anti-amyloid intervention [72].

Overall, increasing evidence from human and animal models demonstrates that amyloid plaque, especially derived from toxic Aβ42, is the main hallmark of the AD, it is also a predictive marker for the progression of preclinical to symptomatic AD, therefore treatments especially early treatments that reduce Aβ production or increase its clearance will have great promise as a potential therapeutic agent.

## 4. Hyperphosphorylated tau in Alzheimer's disease

The other hallmark of AD is intracellular neurofibrillary tangles, aggregated from hyper-phosphorylation of tau. Tau is a neuron-specific microtubule-associated protein and a critical component of the neuronal cytoskeleton. As one of the main microtubule-associated proteins, tau will lose the ability to bind microtubules when the homeostasis of phosphorylation and dephosphorylation is disturbed in neurons. Then hyperphosphorylated tau eventually induces neurodegeneration and neuron loss by disrupting neuronal cytoskeleton. It has been shown that DNA damage-activated kinases such as Chk1 and Chk2 may be involved in tau phosphorylation and toxicity in the pathogenesis of AD [73]. Tau may also be a crucial partner of Aβ, enhance the pathology of Aβ, as well as Tau-Aβ interplay a role in AD. Research on the pathological changes in AD indicates that accumulated Aβ in vivo may initiate the hyperphosphorylation of tau. Also, the signal transduction pathways of tau hyperphosphorylation may be related to accumulated Aβ [74–77]. Fibrillar Aβ containing compact but not diffuse plaques in the aged rhesus cortex also contained activated microglia and clusters of phosphorylated tau-positive

swollen neuritis [51]. On the other hand, recent study showed that peptides from structure-based designs can disrupt the Aβ fibril formation of original proteins, including those, such as tau protein, that lack fully ordered native structures. The specifically inhibiting peptides have been designed on structures of dual-β-sheet 'steric zippers', the successful inhibition of amyloid fibril formation not only shed light on new strategy of the development of therapeutics but also strengthens the hypothesis that amyloid spines contain steric zippers [78].

Tau, also known as axonal protein, has a dendritic function in postsynaptic targeting of the Src kinase Fyn, a substrate of which is the NMDA receptor (NR). Missorting of tau in transgenic mice expressing truncated tau (Delta-tau) and absence of tau in tau (−/−) mice both disrupt postsynaptic targeting of Fyn. This uncouples NR-mediated excitotoxicity and hence mitigates Aβ toxicity. Delta-tau expression and tau deficiency prevent memory deficits and improve survival in Aβ-forming APP23 mice, a model of AD. These deficits are also fully rescued with a peptide that uncouples the Fyn-mediated interaction of NR and PSD-95 in vivo. These findings suggest that this dendritic role of tau confers Aβ toxicity at the postsynapse with direct implications for pathogenesis and treatment of AD [77].

Furthermore, recent studies in several APP and tau transgenic mouse models revealed that mitochondrial dysfunction might be a possible link between Aβ and tau in AD pathology in vivo [79]. In a triple AD mouse model, a massive deregulation of 24 proteins, of which one-third were mitochondrial proteins mainly related to complexes I and IV of the oxidative phosphorylation (OXPHOS). Notably, deregulation of complex I was tau dependent, whereas deregulation of complex IV was Aβ dependent. Synergistic effects of Aβ and tau were evident in 8-month-old AD mice as only they showed a reduction of the mitochondrial membrane potential at this early age. At the age of 12 months, the strongest defects on OXPHOS, synthesis of ATP, and reactive oxygen species (ROS) were exhibited in the AD mice, again emphasizing synergistic, age-associated effects of Aβ and tau in perishing mitochondria [79].

Overall, these observations indicate the involvement of tau and Aβ synergistically, as well as the fundamental role of mitochondria in the AD pathology.

## 5. Mitochondria in Alzheimer's disease

### 5.1. Mitochondria are the main source of ROS

Mitochondria are the cytoplasmic organelles essential for life and death. Mitochondria perform several cellular functions, including production of major part of cellular ATP, regulation of intracellular calcium, the release of proteins that activate the caspase family of proteases and free radical production and scavenging. The mitochondria contain the respiratory chain or electron transport chain (ETC) that is located in the inner mitochondrial membrane and consists of five complexes (complexes I–V); the fifth complex is directly involved in ATP synthesis. The complexes of the mitochondrial respiratory chain are made up of multiple subunits, and all contain proteins encoded by nuclear DNA and mtDNA, except for complex II, which is entirely encoded by nuclear DNA [16,80–82]. The mitochondria, also called the powerhouses, are the chief energy-producing organelles in the most cells, which provide most energy for our normal life. Usually, energy in the form of ATP is efficiently produced via OXPHOS in the mitochondrial respiratory chain. Several decades of research have firmly established that ROS production is inherent to mitochondrial oxidative metabolism, and mitochondria are believed to be the major intracellular source of ROS. Several years of research revealed that free radicals are produced at multiple sites in the mitochondria: Complexes I and III produce superoxide radicals via electron leaks, these radicals are dismutated by manganese superoxide dismutase, generating $H_2O_2$ and oxygen. $H_2O_2$ is converted into $H_2O$ by either

glutathione peroxidase or catalase [83]. Complex II also produces ROS conditionally [82]. Components of tricarboxylic acid, including α-ketodehydrogenase and pyruvate dehydrogenase generate superoxide radicals in the matrix. In addition, mitochondrial outer membrane also produces free radicals *via* monoamine oxidase (localized on the outer mitochondrial membrane), by catalyzing the oxidative deamination of primary aromatic amines. This deamination is a quantitatively large source of $H_2O_2$.

A little ROS may not be toxic to cells, and may have some benefit roles to cells and homoeostasis. Recent data strongly suggest that ROS, and specifically mitochondria generated ROS, are involved in physiological signaling cascades regulating various cellular and organ functions [84,85]. However, excessive and/or sustained increase in ROS, may lead to oxidative stress, as aggravating or primary factors in numerous pathologies, including aging and neurodegenerative diseases, are widely recognized [6,82,85–87].

Mounting evidence suggests that Aβ cascade hypothesis remains the major cellular event in AD; increasing evidence also indicates a mitochondrial cascade hypothesis, which is for many of the biochemical, genetic, pathological as well as clinic features of AD, and mitochondrial dysfunction may initiate the disease, particularly sporadic AD [88]. This hypothesis, importantly, is supported by recent observations showing that early impairments of mitochondrial dysfunction and oxidative stress may precede Aβ overproduction and deposition [88–92].

### 5.2. Mitochondrial localization of Aβ

As described above Aβ deposit is the main hallmark of AD, and Aβ42 is the most toxic peptide and the predominant species in the parenchymal amyloid deposits in AD brain, and it is an initially deposited species [57–60]. Although the classical view is that Aβ is deposited extracellularly, both cellular and biochemical studies carried out in different models of AD and aging have provided evidence that this peptide can also accumulate inside neurons, target mitochondria, and contribute to disease progression [93–99]. By using *in vivo* and *in vitro* approaches, Hansson Peterson et al. demonstrated that Aβ is transported into rat mitochondria *via* the translocase of the outer membrane (TOM) and localizes within the mitochondrial cristae. A similar distribution pattern of Aβ in mitochondria has been shown by immunoelectron microscopy in human cortical brain biopsies [100].

It has been shown that Aβ42 can induce mitochondrial mislocalization, which contributes to Aβ42-induced neuronal dysfunction in a transgenic Drosophila model. In the Aβ42 fly brain, mitochondria were reduced in axons and dendrites, and accumulated in the somata without severe mitochondrial damage or neurodegeneration. In contrast, organization of microtubule or global axonal transport was not significantly altered at this stage. Aβ42-induced behavioral defects were exacerbated by genetic reductions in mitochondrial transport, and were modulated by cAMP levels and PKA activity. Levels of putative PKA substrate phosphoproteins were reduced in the Aβ42 fly brains. Importantly, perturbations in mitochondrial transport in neurons were sufficient to disrupt PKA signaling and induce late-onset behavioral deficits, suggesting a mechanism whereby mitochondrial mislocalization contributes to Aβ42-induced neuronal dysfunction. These results demonstrate that mislocalization of mitochondria underlies the pathogenic effects of Aβ42 *in vivo* [101].

### 5.3. Aβ induces ROS production

Oxidative stress and its sequelae are likely related to both apoptotic and necrotic mechanisms of neurotoxicity. There is evidence suggesting that tissues from both AD patients and individuals with mild cognitive impairment have elevated levels of oxidative DNA damage [33]. And *post-mortem* tissue provides strong evidence for significant increased levels of cellular oxidative stress in vulnerable regions (cortex and hippocampus) of AD brains compared to aged controls [3,33,102,103]. Importantly, Aβ peptides directly initiate free radical/ROS formation, cellular dysfunction, and subsequent neuronal death [104–107]. Further mitochondria are thought to be the central target for oxidative stress induced damage [93,108]. Recent primary culture study has shown that oligomeric Aβ42 could induce reactive ROS production from cortical neurons through activation of NADPH oxidase [109]. Notably there is a defect in the antioxidant defense system, which may lead to oxidative damage in patients with AD. It has been found that erythrocyte antioxidant enzyme activities (catalase, glutathione peroxidase GPX and superoxide dismutase SOD) were significantly lower in patients with AD compared with controls. These results suggest that alterations in these enzymes may play a role in the etiopathogenesis of AD [110]. Therefore Aβ-associated oxidative stress and related antioxidant defense system may be of fundamental importance in AD etiology and pathogenesis.

### 5.4. Mitochondrial DNA changes in AD

The Aβ cascade hypothesis remains the main pathogenic model, as suggested by familiar AD, mainly associated to mutation in APP and presenilin genes. The remaining more than 98% of AD patients are mostly sporadic late-onset cases, with a complex etiology due to interactions between environmental conditions and genetic features of the individuals. Somatic mutations in mitochondrial DNA (mtDNA) could cause energy failure, increased oxidative stress and accumulation of Aβ, which in a vicious cycle reinforces mtDNA damage and oxidative stress. However, no clear causative mutations in the mtDNA have been linked to AD, even some variations have functional consequences, including changes in enzymatic activity [111]. Indeed, results of studies on the role of mtDNA polymorphisms or haplogroups in AD are controversial [91,112].

Recently, to investigate the possible association between mtDNA-inherited sequence variation, a high resolution analysis (sequencing of displacement loop and restriction analysis of specific markers in the coding region of mtDNA) in 936 AD patients and 776 cognitively assessed normal controls from central and northern Italy was performed. Among over 40 mtDNA sub-haplogroups analyzed, sub-haplogroup H5 is a risk factor for AD in particular for females and independently from the APOE genotype. Multivariate logistic regression revealed an interaction between H5 and age. When the whole sample is considered, the H5a subgroup of molecules, harboring the 4336 transition in the tRNAGln gene, already associated to AD in early studies, was about threefold more represented in AD patients than in controls (2.0% *vs* 0.8%; p = 0.031), and it might account for the increased frequency of H5 in AD patients (4.2% *vs* 2.3%). The complete re-sequencing of the 56 mtDNAs belonging to H5 revealed that AD patients showed a trend towards a higher number of somatic mutations in tRNA and rRNA genes when compared with controls [113].

The ultrastructural features of vascular lesions and mitochondria in brain vascular wall cells from human AD, and APP transgenic positive (Tg+) mice have been studied. *In situ* hybridization using mtDNA probes for human wild and 5 kb deleted types and mouse types that was performed along with immunocytochemistry have shown that there was a higher degree of mitochondria DNA deletion along with amyloid deposition, overexpression of oxidative stress markers, and mitochondrial structural abnormality in the vascular walls of the human AD, Tg (+) mice compared to age-matched controls. Therefore, selective pharmacological intervention, directed for abolishing the chronic hypoperfusion state, would possibly change the natural course of development of dementia [114].

### 5.5. Mitochondrial dysfunction in AD

It has been shown that impairment occurs to all five of the mitochondrial OXPHOS complexes in the AD brain. Mitochondrial

dysfunction, which is associated with metabolic dyshomeostasis and reduced ATP synthesis, occurs early in AD. Additionally, mitochondrial dysfunction is proposed to link amyloid deposition and neuronal synaptic loss. Thus, the existence of mitochondrial dysfunction is important in AD [96,115].

Age-dependent accumulation of mutations in mtDNA and resulting increase in oxidative stress and impairment in mitochondrial respiratory chain, especially complex IV, gained attention as potential factors that could participate in the onset of sporadic AD [16,88]. In fact, a reduced activity of the cytochrome *c* oxidase (COX, complex IV of respiratory chain) has been reported in different brain regions of AD patients [116].

COX activity was also decreased in AD transgenic (Tg2576) mice, suggesting that mutant APP/Aβ impair mitochondrial metabolism in AD. Furthermore, an increase in hydrogen peroxide and a decrease in COX activity were found in young Tg2576 mice, prior to the appearance of Aβ plaques [93]. Also, *in vitro* study using primary neuron culture and confocal microscopy demonstrates that Aβ impairs the mitochondrial movement [117]. These findings indicate that mitochondria are the targets of Aβ, and mitochondrial dysfunction happens at early stage of the disease, suggesting that early mitochondria-targeted therapeutic interventions may be effective in delaying AD progression in treating AD patients.

The mechanism of the inhibitory potential of the Aβ42 on activity of electron transport chain enzyme complexes was investigated in human mitochondria. Synthetic Aβ1–42 specifically inhibited the terminal complex COX in a dose-dependent manner that was dependent on the presence of $Cu^{2+}$ and specific "aging" of the Aβ42 solution. Maximal COX inhibition occurred when using Aβ42 solutions aged for 3–6 h at 30 °C. The level of Aβ42-mediated COX inhibition increased with aging time up to approximately 6 h and then declined progressively with continued aging to 48 h [96]. Photo-induced cross-linking of unmodified proteins followed by SDS-PAGE analysis revealed dimeric Aβ as the only Aβ species to provide significant temporal correlation with the observed COX inhibition. Analysis of brain and liver from an AD model mouse (Tg2576) revealed abundant Aβ immunoreactivity within the brain mitochondria fraction. These data indicate that endogenous Aβ is associated with brain mitochondria and that Aβ1–42, possibly in its dimeric conformation, is a potent inhibitor of COX, but only when in the presence of $Cu^{2+}$. Therefore, $Cu^{2+}$-dependent Aβ-mediated inhibition of COX may be an important contributor to the neurodegeneration process in AD [96]. Increased copper concentration has been found in the AD brain that implies that copper may participate in the pathophysiology of AD [118]. In fact, copper can bind to APP and Aβ, then affects the structure and toxic of APP and Aβ, and then affect the formation of beta-sheet structure that is widely accepted as toxic secondary structure of Aβ [118].

To test the possibility that age-dependent decline in the mitochondrial respiratory function, especially COX activity, may participate in the formation and accumulation of Aβ, a neuron-specific COX-deficient mouse was first generated by the Cre-loxP system, in which the COX10 gene, which encodes the farnesyltransferase required for COX assembly and function, was deleted by a CamKIIα promoter-driven Cre-recombinase. These knockout (KO) mice showed an age-dependent COX deficiency in the cerebral cortex and hippocampus. Then AD-like double transgenic mice expressing mutants of APP and PS1 in a neuron-specific COX-deficient background were generated. Surprisingly, COX10 KO mice exhibited significantly fewer amyloid plaques in their brains compared with the COX-competent transgenic mice. This reduction in amyloid plaques in the KO mouse was accompanied by a reduction in Aβ42 level, BACE1 activity, and oxidative damage. Likewise, production of ROS from cells with partial COX activity was not elevated. Collectively, these results suggest that, contrary to previous models, a defect in neuronal COX does not increase oxidative damage nor predispose for the formation

of amyloidogenic APP fragments [119,120]. On the other hand, this study also indicates that genetic modification of mitochondria can inhibit ROS overproduction, eventually reduce Aβ level, prevents the development and progress of AD, suggesting a useful approach for treatment of the disease.

## 6. DNA damage in Alzheimer's disease

### 6.1. Definition of oxidative DNA damage

Increased ROS/free radical production/NO, either produced from mitochondria or from inflammation process (macrophage/microglia in brain) can cause oxidation of nucleotide acids, especially DNA. The resulting DNA damages are present in most, if not all, human diseases, including AD, and aging [15,121]. The toxic hydroxyl radical is known to react with all components of the DNA molecule, damaging both the purine and pyrimidine bases and also the deoxyribose backbone [15,85]. Therefore DNA is a particularly important target for oxidation, as damage may lead to heritable alterations. Consequently, damage to DNA has been well studied, and several classes of product identified: base oxidation and fragmentation products (*e.g.* single- and double-strand breaks); inter/intra-strand cross-links; DNA–protein cross-links; and sugar fragmentation products [15,122]. The hydroxyl radical is perhaps the most frequently form of ROS, with over twenty different products formed from OH* attack on the bases in DNA. The principal oxidative DNA damage products include 8-hydroxyadenine (8-OH-Ade), 8-hydroxyguanine (8-OH-Gua; and its deoxynucleoside equivalent, 8-OH-dG), 5,6-dihydroxy-5,6-dihydrothymine (thymine glycol, Tg) and ring-opened lesions: 4,6-diamino-5-formamidopyrimidine (FapyAde) and 2,6-diamino-4-hydroxy-5-formamidopyrimidine (FapyGua). Overall, the most extensively studied DNA lesion is the formation of 8-OH-G and 8-OH-dG, which is widely accepted and used as an oxidative DNA biomarker. Permanent modification of genetic material resulting from these oxidative damage incidents represents the first step involved in mutagenesis, aging and age-related diseases, including cancer and neurodegenerative diseases including AD.

### 6.2. Methods for detection of oxidative DNA damage

Oxidized DNA can be detected by several ways: 1) high pressure liquid chromatography (HPLC) analysis for oxidized DNA from pure genomic DNA; 2) immunohistochemistry analysis using brain sections and antibody that is specific for 8-OHdG or 8-OHG; and 3) comet assay that detects DNA damage in a single cell.

#### 6.2.1. HPLC analysis using genomic DNA

DNA can be isolated from tissues or blood either by traditional phenol extraction/ethanol precipitation (multiple) or a convenient kit, the DNA purity and concentration are determined spectrophotometrically. An OD of 1 at 260 nm corresponded to ~50 μg/ml DNA. Only samples with a high degree of DNA purity (such as OD 260/230 ratio above 2.0), could be used for HPLC analysis. To avoid any artificial DNA damage from phenol extraction, the salt methods have been developed [123–125]. It has been shown that the sodium iodide (NaI) isolation method is a more sensitive and reliable assessment of 8-oxo-2-deoxyguanosine levels than the classical phenol method [126]. Relative commercial kits are also developed by companies such as Qiagen, which may significantly save the timing course.

The measurement of 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxo-dG) (also called 8-hydroxy-2′-deoxyguanosine, 8-OHdG) is widely used method for determining oxidative DNA in DNA sample [127,128]. In brief, DNA will be digested and dephosphorylated by nuclease P1 followed by alkaline phosphatase. Digested DNA is analyzed by HPLC equipped with a reverse-phase analytical column and coupled with a photodiode array detector followed by an electrochemical (EC) detector.

The quantification of nucleosides will be monitored by UV (at 254 nm) while the measurement of 8-oxo-dG was by EC detector (at 290 mV). On the other hand, 8OHdG can also be determined *in situ* (brain or other organs, different region and sub-region) locally using immunohisto-chemistry with a specific 8OHdG antibody, following quantification with Stereologer software, Image J or other software analysis.

### 6.2.2. Immunostaining analysis is a qualitative method to measure 8-hydroxyguanosine (8-OHG), a marker for oxidative DNA damage in brain sections of aging, AD mouse models and AD brains [93,129–131]

This method determines the accumulated oxidative DNA in cells. There are several good quality antibodies are available to detect DNA damage in the brain sections. Several studies quantified 8-OHG in the brains of neurodegenerative diseases [132].

### 6.2.3. The comet assay is another useful genotoxicity test for DNA damage [33,133,134]

In addition, purified DNA repair enzymes, applied to DNA during the course of the comet assay procedure, greatly increase the sensitivity and specificity of the assay. The kinetics of cellular repair after low doses of oxidative damage have been followed with this modified comet assay [135]. Therefore, the successful measurement of biomarkers of oxidative damage in human populations establishes the comet assay as a valuable tool in molecular epidemiology.

Overall these are useful methods to detect and quantify oxidized DNA in cells. However, HPLC based method yields more accurate oxidized DNA in cells.

### 6.3. Oxidative DNA damage in AD and therapeutic applications

In addition to Aβ stimulation of ROS production, which directly causes DNA damage, it has been shown that Aβ42 has DNA nicking activities similar to nucleases. Further, magnesium ion was shown to enhance the DNA nicking activity of Aβ, and Aβ oligomers showed more DNA nicking activity compared to monomers and fibrillar forms. These data support a role for Aβ in causing direct DNA damage [33,136].

In the Tg2576 mouse model of AD, at the age of three months the mice show no apparent signs of behavioral, neuroanatomical, cytological, or biochemical alterations. However, recent biochemical and morphological studies demonstrated that significant alterations in several antioxidant proteins are already detectable at this early stage of the disease and protein composition change concomitantly with early oxidative stress. Interestingly, the neocortex shows a compensatory response, consisting in an increase of ROS scavenging enzymes, while the hippocampus appears more prone to the oxidative insult [137].

The accumulation of DNA damage can be particularly deleterious in postmitotic cells such as neurons, which are not self-renewed through cell proliferation. Therefore, the accumulation of oxidative DNA damage in nuclear DNA (nDNA) is believed to result in a slow build-up of DNA adducts in the genome able to trigger neuronal death, whereas its accumulation in the mitochondrial DNA (mtDNA) can result in base substitutions and deletions leading to the erroneous transcription of genes encoding important subunits of the electron transport chain, with subsequent mitochondrial dysfunction, increased oxidative damage, and neuronal death [33]. It has been shown that mtDNA is particularly sensitive to oxidative damage. In *postmortem* brain tissue there was a significant threefold increase in the amount of 8OHdG in mtDNA in parietal cortex of AD patients compared with controls. In the entire group of samples there was a small but significant increase in oxidative damage to nDNA and a highly significant threefold increase in oxidative damage to mtDNA in AD compared with age-matched controls [138]. Comparing levels of lipid peroxidation in any of the brain regions no differences were found, and a significant difference was found only in the parietal lobe,

increased levels of 8-hydroxyadenine, 8-hydroxyguanine, thymine glycol, Fapy-guanine, 5-hydroxyuracil, and Fapy-adenine, oxidized DNA bases were observed in parietal, temporal, occipital, and frontal lobe, superior temporal gyrus, and hippocampus. The baseline level of oxidative DNA damage in the temporal lobe was higher than in other brain regions in both control and AD brain [139]. Similarly, levels of multiple oxidized bases in AD brain specimens were significantly higher in frontal, parietal, and temporal lobes compared to control subjects, whereas cerebellum was only slightly affected in AD brains; mtDNA had approximately 10-fold higher levels of oxidized bases than nDNA. Oxidative DNA damage was also found in mild cognitive impairment (MCI), the phase between normal aging and early dementia [140–142]. These data are consistent with higher levels of oxidative stress in mitochondria, suggesting that oxidative damage to mitochondrial DNA may contribute to the neurodegeneration of AD, including the earliest detectable phase of AD. Interestingly, the fact that mtDNA has more oxidative damage also confirmed by the reliable assessment using the NaI isolation method, which showed that the levels of 8oxodG in mtDNA isolated from mouse liver, heart and brain were 6-, 16- and 23-fold higher than nDNA from these tissues. The steady-state levels of oxo8dG in mouse tissues range from 180 to 360 lesions in the nuclear genome and from one to two lesions in 100 mitochondrial genomes [19].

Interestingly, point mutation frequencies in mtDNA were 2 to 3-fold higher in the parietal gyrus, hippocampus, and cerebellum from AD subjects compared to normal controls. In contrast, levels of a commonly studied deletion mutation, mtDNA (4977), were not elevated in AD. The frequency of point mutations did not vary significantly among the three brain areas, whereas the frequency of mtDNA (4977) was 15- to 25-fold lower in the cerebellum in comparison to the cortex; this regional variation was seen in both the normal and AD brain [143]. It has also been shown that somatic mtDNA control region (CR) mutations accumulate with age in post-mitotic tissues including the brain and that the level of mtDNA mutations is markedly elevated in the brains of AD patients [144]. The elevated mtDNA CR mutations in AD brains are associated with a reduction in the mtDNA copy number and in the mtDNA L-strand transcript levels. Further, mtDNA CR mutations increase with age in control brains; that they are markedly elevated in the brains of AD, Down syndrome (DS) and dementia (DSAD) patients. The increased mtDNA CR mutation rate is also seen in peripheral blood DNA and in lymphoblastoid cell DNAs of AD and DSAD patients, and distinctive somatic mtDNA mutations, often at high heteroplasmy levels, are seen in AD and DSAD brain and blood cells DNA. In aging, DS, and DSAD, the mtDNA mutation level is positively correlated with BACE1 activity and mtDNA copy number is inversely correlated with insoluble Aβ40 and Aβ42 levels [145]. These data further indicate that increased Aβ, which produced by BACE1, causes the significant elevation of mtDNA mutation, and this mtDNA mutation may be responsible for both age-related dementia and the associated neuropathological changes observed in AD and DSAD.

Overall oxidative DNA damage, especially mitochondria DNA damage in AD brain is a basic event during the initiation and development of the disease. Interestingly, increasing data including ours have shown that antioxidants such as CoQ and its variants, and that antioxidant enzymes and transcription factors such as NrF2, can significantly reduce ROS production and tissue damage, eventually improve cell survival and cellular function *in vitro* and *in vivo* [146–149] (Fig. 2). Some of traditional Chinese medicines/herbal medicines may also works as antioxidants, neuroprotectants and/or mitochondrial boosters, such as *Ginkgo biloba* and *Ginseng*, therefore more studies in humans with strong methodology may provide useful information or effective medicine in the field [148,150].

Recently we crossed APP transgenic mouse with mitochondria-targeted catalase mouse (MCAT) and produced MCAT/APP double transgenic mice. We found that MCAT not only significantly reduces
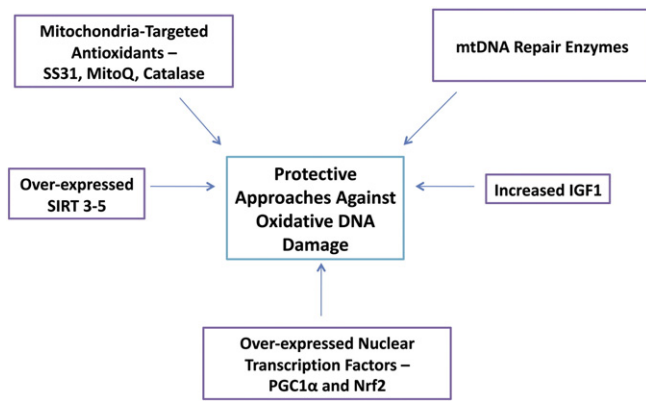
**Fig. 2.** Protective therapeutic approaches that decrease oxidative DNA damage in aging and AD. 1. Mitochondria-targeted antioxidants, 2. overexpression of mitochondria-localized sirtuins 3–5, 3. mitochondria DNA repair enzymes, 4. increased levels of insulin-like growth factor 1 and 5. overexpression of nuclear transcription factors.

DNA oxidative damage, but also dramatically inhibits Aβ deposits, eventually elongates the AD mouse lifespan (Mao and Reddy, unpublished results). These observations suggest that mitochondria-targeted antioxidants may be a potential therapeutic approach for AD.

Notably, a placebo-controlled, double-blind, randomized trial showed that a safe and effective medicine, huperzine A (400 μg/day for 12 weeks), which was originally isolated from the Chinese herb *Lycopodium serratum*, also known in China as Qian Ceng Ta or Jin Bu Huan, remarkably improves the cognition, behavior, activity of daily life, and mood of AD patients [151], confirmed early report, which concluded that huperzine A is a promising drug for symptomatic treatment of AD [152]. In a phase II trial of huperzine A in mild to moderate AD in the US, huperzine A 200 μg BID did not influence change in ADAS-Cog at 16 weeks. In secondary analyses, huperzine A 400 μg BID showed a 2.27-point improvement in ADAS-Cog at 11 weeks *vs* 0.29-point decline in the placebo group (p = 0.001), and a 1.92-point improvement *vs* 0.34-point improvement in the placebo arm (p = 0.07) at week 16 [153]. Therefore, overall Huperzine A is a well-tolerated drug that could significantly improve cognitive performance in patients with AD, and it appears to be more effective than FDA approved acetylcholinesterase inhibitors, AChEIs [150,154–156]. Considering the study design was not strong, sample sizes were still small, as well as individuals vary for an optimal effective dose, rigorous design, randomized, multi-center, large-sample trials of huperzine A and its derivatives for AD are needed to further assess the effects.

## 7. DNA repair as a therapeutic strategy in Alzheimer's disease

Despite that oxidative DNA damage plays an important role in aging and diseases, including AD, as there are multiple pathways for its repair, clearly the cell does not want this damage to persist. Terminally differentiated neurons in the adult brain are able to re-enter the cell-division cycle under certain circumstances [157]. In humans, circumstantial evidence comes from diseases with DNA repair defects, such as Xeroderma Pigmentosum, which shows an accumulation of oxidative DNA damage and increased frequency of skin cancers and, in certain cases, characteristic neurological degeneration. There are several reviews summarized the recent progress on DNA repair, especially in neurodegenerative diseases, in both nDNA and mtDNA [33,158–161].

Among many repair pathways, the base excision repair (BER) pathway is the most important cellular protection mechanism responding to oxidative DNA damage. The BER pathway has been extensively studied in AD brains, and it is of particular importance in post mitotic tissues such as brain tissues, where simple base modifications are more likely to occur than major DNA damage. The key enzymes in the BER process are DNA glycosylases, which remove different damaged bases by cleavage of the N-glycosylic bonds between the bases and the deoxyribose moieties of the nucleotide residues. To complete the repair after glycosylase action, the apurinic/apyrimidinic (AP) site is further processed by an incision step, DNA synthesis, an excision step, and DNA ligation through two alternative pathways. The short-patch BER (1-nucleotide patch size) and long-patch BER (2-6-nucleotide patch size) pathways need AP endonuclease to generate a 3′ hydroxyl group but require different sets of enzymes for DNA synthesis and ligation. Protein–protein interactions have been reported among the enzymes involved in BER. It is proposed that the successive players in the repair pathway are assembled in a complex to perform concerted actions [33,158]. It has been shown that the activity of most enzymes/proteins involved in DNA repair were down-regulated in AD brains [33,162], indicating a deficiency of the DNA repair system in such degenerative diseases.

For many years, the repair of most damage in mtDNA was thought limited to short-patch base excision repair (SP-BER), which replaces a single nucleotide by the sequential action of DNA glycosylases, an AP endonuclease, the mtDNA polymerase gamma, an abasic lyase activity, and mtDNA ligase. However, recent studies have considerably expanded our knowledge of mtDNA repair to include many mechanisms seen in nDNA repair, such as long-patch base excision repair (LP-BER), mismatch repair, and homologous recombination and nonhomologous end-joining [161,163]. In addition, elimination of mutagenic 8-oxodeoxyguanosine triphosphate helps prevent cell death due to the accumulation of this oxidation product in mtDNA. Also, recent evidence provided that irreparably damaged mtDNA might be targeted for degradation. Therefore, multiple DNA repair pathways and controlled degradation of mtDNA function together to maintain the integrity of mitochondrial genome [163].

The mammalian mitochondrial protein TFAM was originally identified as a transcription factor (A) but also appears to play a direct role in DNA repair. TFAM binds stronger to oxidatively damaged DNA than to intact DNA and shows higher affinity for 8-oxoG-containing base pairs than the relevant OGG1 and MYH DNA glycosylases [164,165]. Recent data suggest that TFAM indeed modulates the BER pathway in mitochondria by virtue of its DNA-binding activity and protein interactions [166]. TFAM also binds to p53, a tumor suppressor which localizes to mitochondria in response to death signals [167]. On the other hand, the 3′–5′ exonuclease activity of p53 can hydrolyze oxidized nucleotides like 8-oxodG at DNA 3′-ends, a reaction which is enhanced upon interaction with the mitochondrial single-stranded DNA-binding protein (mtSSB) [168].

In mammalian cells, oxidative damage to the mitochondrial DNA also correlates with the translocation of the tumor suppressor p53 to the organelles, which introduces a further regulation level, as p53 is thought to modulate DNA repair. Furthermore, p53 stimulates both removal of damaged bases and nucleotide re-insertion [169].

Although aging is clearly associated with modifications in mitochondrial DNA repair capacity, the situation is contrasted. This may also reflect a balance between DNA damage and repair, as mitochondrial DNA oxidative damage is inversely correlated with maximum life span in mammals [169] (Fig. 3). On the other hand, changes in mitochondrial BER play a role in DNA damage accumulation and age-related functional decline [34]. In mammals, variations in organelle BER capacity with age appear to be organ and tissue-specific [170], for example, increased BER enzyme activities in mitochondria can be found in liver [158] or gradual decline in brain [171–173].

Base excision repair is thought to be the primary DNA repair pathway for small base modifications such as oxidation. Bohr's group employed a set of functional assays to measure BER activities in brain
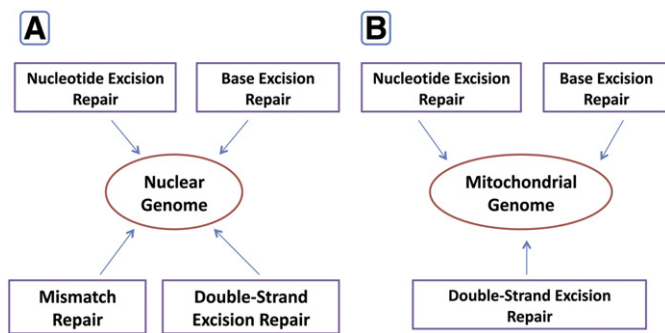
**Fig. 3.** DNA repair mechanisms of mitochondrial and nuclear genomes.

tissue from short *post-mortem* interval autopsies of sporadic AD patients and age-matched controls, as well as amnestic mild cognitive impairment (MCI) subjects. They found significant BER deficiencies in brains of AD patients due to limited DNA base damage processing by DNA glycosylases and reduced DNA synthesis capacity by DNA polymerase β. Interestingly the BER impairment was not restricted to damaged brain regions and was also detected in the brains of amnestic MCI patients, where it correlated with the abundance of neurofibrillary tangles. These findings suggest that BER dysfunction is a general feature of AD brains that could occur at the earliest stages of the disease. The results support that defective BER may play an important role in the progression of AD, and that mitochondrial BER might be critical in the development and maintenance of the central nervous system during aging [174].

As summarized in Fig. 3, overall, these studies indicate that DNA repair in both mitochondrial and nuclear genomes are involved in AD, and recent research advancements may help develop strategies to repair DNA changes that are caused by aging, Aβ, hyperphosphorylated and other oxidative insults.

## 8. Conclusions and future directions

Increasing evidence suggests that mitochondrial oxidative damage is an early event and plays a key role in the progression and pathogenesis of AD. Recent AD *postmortem* brains, transgenic mouse models and cell models of AD revealed that Aβ significantly induces DNA damage in age-dependent manner in neurons from patients with AD or AD-like animal models. Aβ also induces oxidative mtDNA damage, in turn, generates excessive free radicals, and causes more DNA damage, it likely forms a vicious cycle. On the other hand, antioxidant system, DNA repair and neurogenesis (including adult stem cells in brain) are functionally going down in aged humans and AD patients. All these factors and pathways likely work together to induce synapse and neuron damage, eventually neuron loss in AD patients.

Given the complexities of AD, therapies that target the causes and different mechanisms simultaneously would be effective. A strategy using a multifaceted therapy that targets Aβ, hyperphosphorylated tau, and mitochondrial oxidative damage may be an ideal approach. More importantly, evidence from clinical trials and studies of experimental therapeutics of AD mouse models indicate that early treatment is the key for cure or effective treatment of the disease. Similarly, prevention of the AD from happening in our populations is another best way to fight this number one of the common progressive neurodegenerative diseases.

## Acknowledgements

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