Oxidative Stress Events and Neuronal Dysfunction in Alzheimer's Disease: Focus on APE1/Ref-1-Mediated Survival Strategies

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Abstract

Alzheimer's disease (AD) is an important public health problem which affects millions of people worldwide. The major pathological hallmarks associated with AD are the accumulation of amyloid beta (Aβ) in senile plaques and neurofibrillary tangles (NFT) made up of hyperphosphorylated tau proteins. New findings suggest that oligomeric Aβ is a more toxic species than fibrillar Aβ relevant to AD pathology. Although the molecular mechanism(s) underlying the disease is not identified completely, various factors have been implicated in the development of AD. Accumulating evidences point towards the role of oxidative stress and mitochondrial dysfunction in the pathogenesis of AD and recognise them as an early event in AD development. Ageing is considered the greatest risk factor for AD and is linked to oxidative stress which causes accumulation of somatic mutations in mitochondrial DNA (mtDNA) over time and leads to genome

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instability and mitochondrial dysfunction. Recent studies on AD patients and transgenic mouse models suggest that amyloid precursor protein (APP) and Aβ localise to mitochondria, interact with mitochondrial proteins, disrupt electron transport chain (ETC), increases reactive oxygen species (ROS) level, impair axonal mitochondrial trafficking, thus leading to synaptic damage and cognitive decline associated with AD. It is not known whether accumulation of Aβ is the cause or outcome of declining mitochondrial function in AD. In order to counteract oxidative stress and maintain genome integrity, various DNA repair pathways exist, base excision repair (BER) pathway being the predominant pathway for repairing oxidised base lesions in neuronal cells. APE1 is the central enzyme of the BER pathway, having both repair and redox activities and shown to enhance neuronal survival after oxidative stress. Newer studies are revealing the role of APE1 in maintenance of mitochondrial genome repair and function. In this scenario, antioxidant-based therapy, which could reduce oxidative stress and modulate the activities of APE1, can serve as effective treatment providing neuroprotection in AD. This book chapter summarises some recent developments in understanding the pathogenesis of AD linking Aβ-induced oxidative stress, mitochondrial dysfunction, role of APE1 and phytochemicals toward AD therapeutics.

Keywords
Alzheimer's disease • Oxidative stress • Amyloid beta • Mitochondria • APE1/Ref-1 • Phytochemicals

1 Introduction

In 1907, Alois Alzheimer, a German psychiatrist and neuropathologist, described the hallmark lesions associated with 'presenile dementia', which later came to be known as Alzheimer's disease AD [1], a progressive and always fatal disorder characterised clinically by memory loss and behavioural abnormalities. The hallmarks described were extracellular plaques composed of amyloid beta (Aβ) and intracellular neurofibrillary tangles (NFTs) made up of a protein called tau (τ). Since then, it has been more than 100 years that the neuropathological hallmarks of the disease have been described, the underlying molecular mechanism(s) of the pathogenesis of AD are yet to be elucidated. Since 1992, the 'Aβ cascade hypothesis' has been the main model describing the pathogenesis of AD. According to it, accumulation of Aβ, owing to increased processing of amyloid precursor protein (APP), induces biochemical, histological and clinical changes associated with the disease [2]. Various modifications of the model have taken place over the time and currently, oligomers, the soluble form of Aβ, rather than insoluble fibrillar Aβ, are considered to be the more toxic species and relevant to AD pathogenesis [3]. Many studies have proposed that oxidative stress plays an important role in the pathogenesis of AD and consider it to be one of the early changes associated with AD [4]. Also, mitochondrial dysfunction is seen as an early event in the pathogenesis of AD [5]. Thus, these studies have led to the formulation of a new hypothesis, viz. 'mitochondrial cascade hypothesis', indicating the role of the mitochondria and its dysfunction initiating late-onset AD pathologies particularly in relation to sporadic AD [6].

Ageing is considered to be the greatest risk factor for AD. Linking ageing with neurodegenerative diseases, the free radical theory of ageing

suggests that oxidative imbalance, i.e. elevated levels of reactive oxygen species (ROS), has a role in the pathogenesis of many neurodegenerative diseases like AD [7]. The brain is particularly vulnerable to oxidative stress due to a low level of antioxidant system and high consumption of oxygen [7]. There are a number of exogenous and endogenous sources of ROS, which increase the oxidative stress and lead to genome instability. Mitochondria are considered to be the major internal source of ROS. One study has showed that overexpression of antioxidant catalase, targeted to the mitochondria reduces oxidative damage, thus highlighting the role of the mitochondria as a source of these radicals [8]. In line with this evidence, many studies have shown the mitochondria as a source as well as a target of ROS. The link between Aβ and free radical generation ultimately leading to increased ROS in neuronal cells has been well established [9–12]. There is an emerging body of evidence revealing that Aβ enters the mitochondria and induces generation of free radicals causing oxidative damage, thus providing a link between Aβ and mitochondrial dysfunction in the pathogenesis of AD [13].

Postmitotic cells like neurons are terminally differentiated cells which cannot be replaced. Neurons are particularly sensitive to oxidative damage. Accumulation of DNA base modifications especially 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-OHdG) are the major factors leading to genome instability [14, 15]. Base excision repair (BER) is the predominant pathway in the nucleus as well as in the mitochondria that removes these oxidised base lesions [16, 17]. Amongst the BER enzymes, apurinic/apyrimidinic endonuclease (APE1) is a multifunctional enzyme involved in DNA repair and redox regulation of various transcription factors (TFs), thus is also known as redox effector factor 1, Ref-1 [17–19]. Elevated nuclear expression of APE1/Ref-1 in cerebral cortical regions of AD patients highlights the role of APE1 in neurons in response to oxidative stress [20]. But mitochondrial APE1's function is still not clear. Recently, it was shown that nuclear APE1 regulates nuclear-encoded mitochondrial-related genes by modulating the DNA-binding activity of nuclear respiratory factor-1 (NRF1) in a redox-dependent manner [21]. This indicates that APE1 regulates the expression of some nuclear-encoded mitochondrial constitutive genes and consequently modulates mitochondrial functions in response to oxidative stress.

There are a number of phytochemicals which have shown their neuroprotective abilities in rescuing the Aβ-induced oxidative stress in vitro and in vivo. Some of them have also shown modulation of APE1/Ref-1's repair and redox activities, making them effective molecules for prevention and treatment of cancers and degenerative diseases [22–24]. Thus, phytochemicals may serve as effective treatments providing neuroprotection in AD. This book chapter summarises the available literature in the field and suggests a link between oxidative stress, mitochondrial dysfunction, APE1 and phytochemical-based interventions towards AD therapeutics.

2 Neurodegenerative Diseases

Neurodegenerative diseases represent a range of diseases affecting the central nervous system (CNS) characterised by selective neuronal vulnerability and degeneration in specific regions of the brain. This causes disabling and debilitating conditions involving either impairment of memory (dementia) or movement-related disabilities (ataxia), ultimately leading to death. The degeneration is caused due to abnormal accumulation and aggregation of proteins in specific parts of the brain intracellular or extracellular as insoluble or soluble forms. These diseases are also known as protein conformational diseases as the aggregated proteins bear a β-sheet conformation that aid in protein aggregation and fibril formation [25, 26] which are the pathological hallmarks associated with these diseases. The exact reason as to why these proteins begin to accumulate is not known but research has revealed that this may happen due to a number of reasons, majorly due to disruption of ubiquitin-proteosome machinery, autophagy failure and oxidative stress. Some of the common neurodegenerative

diseases include AD, Parkinson's disease (PD), Huntington's disease (HD) and amyotropic lateral sclerosis (ALS). Although the mechanism of the pathogenesis of these diseases is not understood to date, some genetic and environmental factors have been implicated which confer susceptibility to these diseases.

2.1 Alzheimer's Disease

AD is the most common neurodegenerative disease and leading cause of dementia in people above 65 years of age. It is a progressive brain disorder in which a person's memory, thinking ability and behaviour is affected with the deterioration worsening with time. Around 27 million people are estimated to be affected by this disease [27]. AD is characterised by the presence of two major neuropathological hallmarks which are extracellular Aβ plaques and intracellular NFTs composed of tau causing neuronal dysfunction [28]. Ageing is the greatest risk factor for AD with majority of patients above 65 years of age [29]. But cases of early-onset of AD are also present in those 35–60 years of age [30].

Genetic mutations are the known cause of early-onset familial AD with a prevalence of less than 1 % [29]. Thus, three forms of early-onset AD which are inherited as autosomal dominant traits involving three genes, viz. APP on chromosome 21, presenilin 1 (PS1) on chromosome 14 and presenilin 2 (PS2) on chromosome 1 [30], are recognised. Mutations in any of these three genes can make an individual susceptible to AD. Apolipoprotein E, type ϵ4 (APO ϵ4), is a risk factor for late-onset familial and sporadic AD, which accounts for majority (>99 %) of the AD cases [31]. Several genome-wide association studies (GWAS) have identified and implicated many genes in the aetiology of AD [32–34].

Accumulating evidences have shown that oxidative stress and mitochondrial dysfunction are important events occurring during the development of AD. In relation to this, decline in mitochondrial function with advancing age owing to accumulation of somatic mutations in mtDNA is reported [35]. Aβ is also known to induce free radicals [10, 11, 36] and cause decline in mitochondrial function. Oligomeric Aβ is considered to be more toxic than the insoluble fibrillar Aβ [3], which localises to the mitochondria and interacts with a number of mitochondrial proteins, causing synaptic damage and leading to memory impairment and cognitive decline, associated with AD [37, 38]. But the underlying mechanism(s) of the pathogenesis of AD is not known as yet. At present, there is no cure for AD but symptomatic treatments are available to relieve the symptoms and slowdown the impairment of memory associated with AD.

2.2 Factors Governing/ Responsible for AD Pathology

AD, a disabling and fatal disease, has become an important public health problem and poses an enormous economic and social burden for affected individuals, their caregivers and society. Though the neuropathological hallmarks of AD are known, the etiological factors involved in the pathogenesis of the disease are unknown. A number of studies have indicated some risk factors for onset of disease, advancing age being the most important risk factor. Other potential risk factors for AD are described in the following sections.

2.2.1 Genetic Factors

First, an individual having a family history of AD, is susceptible to develop it, especially if he is a first-degree relative of an affected person [29]. In comparison to males, females are more prone to develop AD. Persons with Down's syndrome (trisomy 21) are at a greater risk of developing AD [39]. Additionally, an individual's chance for developing AD increases if he inherits an *APOE* ϵ4 allele, one of the three common alleles (ϵ2, ϵ3, ϵ4) of *APOE* gene, from his parents and has an increased risk if two *APOE* ϵ4 alleles are inherited [29]. Mutations in *APP*, *PS1* and *PS2* genes are recognised to make a person susceptible to early-onset of familial AD [30]. Apart from the *APOE* gene, a number of new genes are implicated in the pathogenesis of late-onset AD

(LOAD). Bridging integrator 1 (BIN1), also known as amphiphysin 2, is now recognised as an important genetic risk factor after *APOE4* for LOAD [40]. BIN1 transcripts levels were observed to be elevated in AD brains, showing BIN1 as a genetic susceptibility locus in AD [32]. Also, decreased expression of drosophila BIN1 ortholog *Amph* suppressed tau-mediated neurotoxicity, highlighting the role of BIN1 in mediating AD risk and its role in modulating AD pathogenesis at the level of the tau pathway [32]. Thus, BIN1 can be thought of as a target for treatment of AD. In addition to *APOE* and *BIN1*, other susceptibility gene loci identified include phosphatidylinositol-binding clathrin assembly protein (*PICALM*), ATP-binding cassette transporter (*ABCA7*), CD2-associated protein (*CD2AP*), clusterin (*CLU*), complement receptor 1 (*CR1*), CD33 antigen (*CD33*), ephrin receptor Eph-A1 (*EPHA1*) and a cluster of membrane-spanning 4A (*MS4A*) genes [33, 34, 40, 41].

2.2.2 Vascular Factors

Drinking alcohol poses a threat of developing AD at a later life. Heavy drinkers who are *APOE4* ϵ4 allele carriers are at a greater risk of developing AD at a later life than moderate alcohol drinkers [42]. Many studies have found association between AD and cigarette smoking. For example, *APOE4* noncarriers are at a greater risk of developing AD [43]. People with high blood pressure in middle age are more susceptible to develop AD at a later stage in their life [44]. Obesity is also a risk factor for AD [45]. Higher body mass index (BMI) or obesity in middle age can increase the risk of developing AD at a later life [46]. Higher serum cholesterol levels are also associated with development of AD at a later stage [47, 48]. Use of statins, i.e. cholesterol lowering drugs, poses a lower risk of AD [49]. In line with this, it was seen that people having a diet rich in polyunsaturated fatty acids and fish have a lower risk of AD [50] as compared to those consuming saturated fatty acids in their diet [51, 52]. An association between diabetes and AD has been observed. Diabetic people are more prone to develop AD, with an increased risk if diabetes occurs in middle age [53]. All these factors are modifiable risk factors that can be modulated by adopting healthy eating habits and an active life thus, lowering the risk of dementia and AD.

2.2.3 Head Trauma and Head Injury

Increased risk of AD development is associated with head injury and head trauma [29]. People with moderate head injuries are at an increased risk of developing dementia than those without any head injuries. Those with severe head injuries are at the greatest risk of developing dementia at a later life. Susceptibility of developing AD increases if a person carries an APOE ϵ4 allele and has suffered any head injury [54]. Thus, boxers and football players are at greater risk of AD at a later life [29].

2.2.4 Mild Cognitive Impairment

Mild cognitive impairment (MCI) is said to be a transitional stage between ageing and development of AD [55]. It is a condition in which a person has memory impairment though it doesn't affect a person's daily activity. It is shown that those who have MCI are at a greater risk of developing AD at a later life [55].

2.2.5 Autophagy Failure

Accumulation of Aβ plaques and tau protein in neurons shows that the neuronal housekeeping and protein quality control systems are impaired in AD. One of these is autophagy, which is a lysosomal degradative process involving removal of toxic proteins and preventing protein aggregation. Recent studies have indicated beclin-1 to be involved in autophagy regulation and modulation of APP metabolism [56]. Beclin-1 expression was also seen to be reduced in the AD brain [57]. Rapamycin is emerging as a potential neuroprotective agent for AD and functions via enhancement of autophagy [58]. A recent study has shown that Aβ(1-42)-induced beclin-1 expression was upregulated by rapamycin and that the beclin-1-dependent autophagy can prevent neuronal cell death before occurrence of AD in PC12 cells [59]. Inhibition of beclin-1-dependent autophagy was shown to speed up neuronal cell death. Thus, beclin-1 dysfunction is associated with

AD. Therefore, autophagy failure is a potential factor leading to accumulation of toxic Aβ plaques and tau in AD.

2.2.6 Brain Inflammation

Chronic brain inflammation is associated with AD. Activated microglia and reactive astrocytes are seen in close proximity to neuritic Aβ plaques in the AD brain [60]. Studies have shown that complement system is activated in the AD brains [61, 62]. The microglia, astrocytes along with complement system components, cytokines and chemokines are involved in inflammatory responses against Aβ. Microglias are cells of CNS involved in the protection of the brain as first-line defence against any invading pathogen and pathological conditions. On the other hand, astrocytes provide trophic support to neurons and are involved in Aβ clearance [63]. In order to clear Aβ, the activated microglia and astrocytes secrete ROS, nitric oxide (NO) and proteolytic components which further increase APP production and proteolytic processing of APP, thus causing neuronal dysfunction [63]. β-site APP-cleaving enzyme 1 (BACE1), a membrane-bound aspartic proteinase, is primarily expressed by the neurons and is associated with the generation of Aβ peptides from APP owing to its β-secretase activity [64]. BACE1 expression has been observed in reactive astrocytes in the AD brains while resting astrocytes do not display BACE1 at detectable levels, thus showing that activation of astrocytes may lead to the development of AD [65]. Another study showed that the TF NF-κB acts as a repressor in neuronal and nonactivated astrocytes, while NF-κB acts as activator of BACE1 transcription in activated astrocytes and Aβ-exposed neuronal cells. Also, the presence of increased level of activated astrocytes with ageing is well demonstrated, which may lead to increased processing of BACE1 causing increased Aβ resulting into chronic inflammation and subsequently astrocyte activation ending up forming a feedback loop [66]. Thus, inflammation plays a major role in AD pathology.

2.2.7 Hormones

Levels of reproductive hormones change with advancing age and are considered as risk factors for AD. Studies have shown that an elevated level of luteinising hormone (LH) increases the risk for developing AD [67]. Subsequently, using a transgenic mouse model of AD, it was shown that leuprolide {a gonadotropin-releasing hormone (GnRH) agonist} lowered serum LH levels, improved working memory and decreased Aβ deposition [68]. A study has also suggested that spatial memory impairment observed in postmenopausal women or female rats after ovariectomy is attributed to high LH levels [69]. Thus, reduction in LH levels may serve as potential therapeutics for AD. Also, in males there is a significant reduction in testosterone and elevation in LH levels with ageing. This is a potential risk factor for development of AD in males. A study showed that gonadectomised (GDX) mice had increased levels of Aβ, and the levels of Aβ were significantly lowered on treatment with testosterone [70]. This suggests potential of androgen therapy for treating AD in hypogonadal men.

2.2.8 Pathogens

Intracellular bacterial pathogen *Chlamydophila* (*Chlamydia*) *pneumoniae* is a risk factor for AD. Studies have shown presence of *Chlamydia* in the brains of LOAD patients [71]. Also, the presence of herpes simplex virus type 1 (HSV1) in the brain of *APOE4*-ε4 carriers is a risk factor for development of AD [72]. In a recent finding, association between periodontal disease and AD was established by the presence of elevated serum antibodies against periodontal infection, caused by bacteria, in individuals who later developed AD as compared to serum antibody levels in control individuals [73]. The mechanisms leading to the pathogenesis of AD due to the presence of these pathogens in the brain need to be understood to a greater extent.

2.2.9 Metal Exposure

Exposure to metals has been linked to AD from a very long time. A recent study has shown that elevated magnesium (Mg^{2+}) in the brain has a synapto-protective effect and improves cognition deficits by reducing Aβ plaques and stabilising BACE1 expression in a transgenic mouse model of AD [74]. An earlier study had shown that reduced Mg^{2+} levels are present in AD patients

[75]. This shows that restoring brain Mg^{2+} levels has a potential to treat AD. Elevated levels of aluminium (Al) are seen in the serum of AD patients [76]. In line with this study, recently it was shown that Al may mediate liver toxicity in AD patients and lead to free copper (Cu^{2+}) in serum, as seen in AD patients [77]. This study has put forward a likely mechanism showing that Al toxicity to liver leads to abnormal synthesis of ceruloplasmin and ATPase7B causing increase in Cu^{2+} levels in serum, and these free Cu^{2+} may cause accumulation of Aβ and neuronal dysfunction associated with AD [77]. Thus, reducing Al levels and reviving normal liver function can be thought of as a treatment for AD. Another significant study has showed that drinking silicon (Si)-rich mineral water reduced Al levels, leading to the removal of Al via urine and thus improved cognition in AD patients [78]. Lead (Pb) exposure has also been linked to the pathogenesis of AD. Pb is shown to increase APP expression and change methylation patterns of *APP* gene, making it hypomethylated in PC12 cells [79]. Another study showed that Pb exposure facilitates Aβ fibril formation and increases Aβ deposition in a transgenic mouse brain [80]. Another evidence supporting the role of Pb in the pathogenesis of AD showed increased fibrillisation of tau protein on exposure to Pb via interaction with His330 and His362, the His mutants of wild-type tau [81]. Inorganic Cu^{2+}, present in drinking water and Cu^{2+} supplements, has also been linked to AD and cognitive impairment in the elderly [82]. A recent study has demonstrated that exposure to iron ($Fe^{2+)}$ leads to upregulation of a disintegrin and metalloproteinase 10 (ADAM10) and increased transcription levels of BACE1, and these were reported to be associated with increased expression of APP-α-CTF and APP-β-CTF, respectively, in PC12 cells, suggesting that Fe^{2+} induces enhanced expression of ADAM10/BACE1 leading to altered APP carboxyl-terminal processing [83]. Increased intracellular calcium (Ca^{2+}) levels brought about by oligomeric Aβ are associated with impaired synaptic plasticity. A study pointed towards the synaptic loss of phosphorylated (active) Ca^{2+}/calmodulin-dependent protein kinase II-α[p(Thr286)CaMKII], a critical enzyme mediating synaptic events, in the MCI and AD hippocampal regions. The loss of p(Thr286) CaMKII was also observed in mice hippocampal regions on treatment with oligomeric Aβ. This was shown to be prevented by inhibiting the phosphatase calcineurin (CaN). Thus, dysregulated Ca^{2+} signaling is associated with AD and MCI [84]. Altered Zinc (Zn^{2+}) homeostasis is implicated in AD. Higher concentrations of releasable Zn^{2+} are present in synaptic vesicles as compared to extracellular fractions in AD hippocampus [85]. In line with this, Bjorklund et al. also showed that in the case of non-demented with AD neuropathology (NDAN) individuals, Aβ oligomers are absent in hippocampal postsynapses along with lower total Zn^{2+} levels, thus leading to intact cognitive function in NDAN individuals [86]. Excess exposure to Zn^{2+} leads to enhanced APP processing, Aβ accumulation and memory impairment as seen in transgenic mice and SH-SY5Y human neuroblastoma cells overexpressing the Swedish mutant form of human *APP* (*APPsw*). Thus, Zn^{2+} overload has a toxic role in AD pathogenesis [87].

2.2.10 Air Pollution

There is a possible association between air pollution and development of AD. Studies have shown that air pollution causes accumulation of Aβ(1-42), increase in cyclooxigenase-2 (COX-2) expression and brain inflammation that cause neuronal dysfunction leading to pathological hallmarks that are associated with AD [88]. Damage to olfactory bulb, olfactory mucosa and frontal regions of the brain, as observed in AD brains, is associated with exposure to air pollution [89]. Ozone (O_3) is a powerful gaseous air pollutant and oxidising agent. It is shown that memory impairment, motor deficiency and lipid peroxidation are caused by exposure to different doses of O_3 in rats [90]. Dysregulation of inflammatory processes, progressive neurodegeneration, chronic loss of brain repair in the hippocampus and brain plasticity changes occurred in rats on exposure to low doses of O_3 [91]. Thus, air pollution is an important environmental hazard which may play a role in development of AD. Additional studies are needed at the

population level to clearly understand the role of air pollution in pathophysiology of AD.

2.3 Amyloid Proteins (Oligomers and Fibrils)

The Aβ protein is central to the amyloid cascade hypothesis, the prevailing hypothesis for more than 20 years, which explains the pathogenesis of AD. Though it does not pinpoint towards a specific Aβ species in the aetiology of AD, several studies have implicated soluble oligomers of Aβ, rather than monomers or insoluble amyloid fibrils, in causing neuronal dysfunction in AD [2, 92, 93].

Abnormal processing of APP in the neurons leads to generation and accumulation of Aβ. APP is a type I, single-pass transmembrane protein with large extracellular domains and belongs to a family of proteins which include amyloid precursor-like proteins (APLP1 and APLP2) in mammals and the amyloid precursor protein-like (APPL) in drosophila [94]. APP contains a 40- or 42-amino acid sequence, i.e. Aβ(1-40) or Aβ(1-42), and 3 sites for cleavage by various proteinases, which are designated as α, β and γ secretases [95]. APP is expressed in all tissues ubiquitously and present on the plasma membrane, majority of which localises to endoplasmic reticulum (ER), Golgi apparatus (GA) and mitochondria [96]. Two pathways exist for processing of APP, viz. amyloidogenic pathway and non-amyloidogenic pathway as illustrated in Fig. 1. In the amyloidogenic pathway, the activity of β-secretase (also known as BACE1) at the beginning of Aβ domain of APP results into a soluble N-terminal fragment (sAPPβ) and an amyloidogenic C-terminal fragment of 99 amino acids (CTF99). Further γ-secretase cleaves this C-terminal fragment to mainly Aβ(1-40) and Aβ(1-42), which may accumulate in the mitochondria and other cellular compartments affecting the cellular functions and leading to mitochondrial dysfunction and hyperphosphorylation of tau. Non-amyloidogenic pathway is the major APP processing pathway in which the activity of α-secretase generates a large soluble N-terminal fragment (sAPPβ) and a non-amyloidogenic C-terminal fragment of 83 residues (CTF83) owing to the cleavage within the Aβ domain. This C-terminal fragment is then cleaved by γ-secretase resulting into non-amyloidogenic peptide (P3) and APP intracellular domain (AICD) which are non-toxic and degraded rapidly and occurs in majority of individuals including non-demented and healthy individuals [97]. In case of early-onset AD, mutations in *APP, PS1* and *PS2* are known to activate β- and γ-secretases leading to generation of Aβ, but in case of sporadic AD, it is proposed that oxidative stress activates β-secretase which increases the production of Aβ [97]. Although the mechanism of APP trafficking is not known, APP is said to be axonally transported, endocytosed and sorted to different compartments of the cell, thus leading to Aβ generation and accumulation in different cellular compartments thus, impairing normal cellular function. A recent study pointed towards the role of huntingtin-associated protein 1 in regulating APP trafficking to the non-amyloidogenic pathway resulting into reduced Aβ levels [98]. As excessive BACE1 expression and APP processing can lead to uncontrolled production of Aβ, different regulatory mechanisms are present [99, 100]. Apart from the transcriptional control, a complex network of neurotransmitter systems and translational regulation is present. Amongst the different systems, viz. glutamatergic, adrenergic, serotonergic, cholinergic and dopaminergic systems, cholinergic system is known to be affected in the early stages of AD. A study showed that downregulation of M2 acetylcholine receptor in the brains of AD patients affects a number of AD-relevant genes including BACE1 [99].

In the amyloidogenic pathway, different lengths of Aβ are produced ranging from 37 to 43 amino acids. Aβ(1-40) is the most dominant species produced from the processing of APP. In comparison to Aβ(1-40), only a small amount of Aβ(1-42) is produced in the human brain in the ratio of approximately 99 to 1. But Aβ(1-42) is the main component of the amyloid deposits associated with AD and has a tendency to form aggregates spontaneously and, thus, may form oligomers. Therefore, Aβ(1-42) is considered more neurotoxic than Aβ(1-40) [101]. While both Aβ(1-40) and Aβ(1-42) are capable of forming

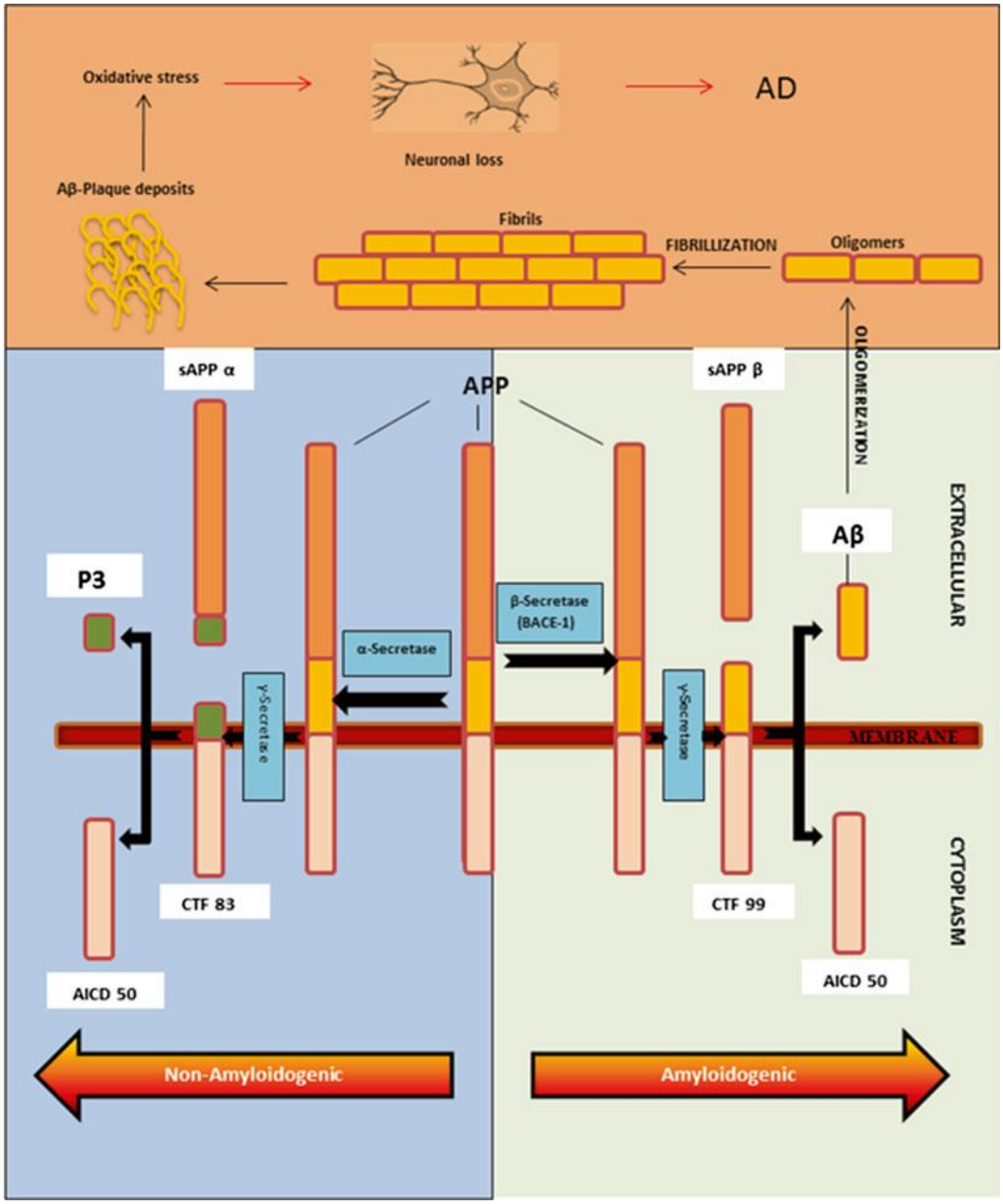

Fig. 1 APP processing leads to the generation of cleavage products via the amyloidogenic and non-amyloidogenic pathways. Both oligomerisation and aggregation of Aβ lead to the formation of senile plaques, a hallmark of AD phenotype. APP processing occurs via two pathways: amyloidogenic and non-amyloidogenic pathway. In the amyloidogenic pathway, β-secretase cleaves APP at the beginning of Aβ domain generating a soluble N-terminus fragment (*sAPPβ*) and amyloidogenic C-terminal fragment of 99 residues (*CTF 99*). Next, γ-secretase acts on CTF 99 and generates Aβ and APP intracellular domain (*AICD 50*). While in the α-secretase-based non-amyloidogenic pathway, α-secretase cleaves within the Aβ domain of APP and generates a soluble N-terminal fragment (*sAPPα*) and a non-amyloidogenic C-terminal fragment of 83 amino acid residues (*CTF 83*). This C-terminal fragment, i.e. CTF 83, is further cleaved by γ-secretase and generates a non-amyloidogenic peptide (*P3*) and APP intracellular domain (*AICD 50*). The amyloidogenic pathway, which generates the toxic Aβ, occurs in 10 % of individuals who have chances of developing AD in the later life. The generated Aβ(1-40) or Aβ(1-42) may further undergo different conformations including oligomerisation resulting into generation of oligomers which are considered to be the most toxic species of Aβ implicated in AD. Further fibrillisation may take place leading to fibril formation and deposition in the neurons, a characteristic feature of AD

amyloid fibrils, Aβ(1-42) is said to make the fibrils much faster than Aβ(1-40). Using ion-mobility spectrometry, it was shown that when Aβ(1-40) and Aβ(1-42) are present together in a solution, the Aβ(1-40) and Aβ(1-42) monomers form dimers, trimers and tetramers. But, when present alone, Aβ(1-42) tends to form pentamers and hexamers (paranuclei) which on self-association form dodecamers, protofibrils and fibrils. Aβ(1-40) alone produces oligomer distribution up to tetramer level only. This pointed out that when present together, Aβ(1-40) inhibits the oligomerisation of Aβ(1-42) and inhibits protofibril and fibril formation by Aβ(1-42). Thus, as Aβ(1-40) is the predominant species present in the human brain in ~10 times the level of Aβ(1-42) in a healthy human brain, the former inhibits the oligomerisation of Aβ(1-42) and prevents the development of AD [102]. Studies have shown that mutations in *APP*, *PS1* and *PS2* may lead to enhanced accumulation of the more toxic Aβ(1-42) species [101, 103–105].

It is reported that some NDAN individuals have significant amounts of amyloid plaques without displaying cognitive decline. A study pointed towards a resistance mechanism contributing towards the maintenance of cognitive function in these individuals. This study showed that oligomeric Aβ is absent in hippocampal postsynapses in NDAN brains. In addition, normal levels of phosphorylated (active) CREB, a transcription factor important for synaptic plasticity, are present in NDAN individuals advocating for the normal functionality of synapses in these in these individuals [86]. A number of studies have indicated that amyloid plaque formation does not correlate with AD pathogenesis [106]. Studies have also shown that deleterious changes associated with AD occur very early before the accumulation of amyloid plaques [107]. Accumulating evidences reveal that oligomers, the soluble form of Aβ, are the more toxic species and associated with cognitive decline in AD, rather than the insoluble fibrillar deposits [108]. Further, different approaches have been employed which have supported the above findings and implicated soluble Aβ in AD. Hippocampal long-term potentiation (LTP) is one such approach, which is correlated with learning and memory, and is shown to be inhibited by synthetic and naturally secreted human Aβ oligomers. Behavioural studies performed on living wild-type rats showed that rats developed learning and memory deficiency after human oligomers were infused in the hippocampus, which were readily observed in morris water maze models [101]. Recently, a possible role of cellular prion protein (PrPc) acting as a neuronal receptor for oligomeric Aβ was speculated. It was shown that there was no inhibition of LTP after treatment with oligomeric Aβ in mice lacking PrPc, thus pointing towards the role of PrPc in Aβ neurotoxicity [109].

Oligomeric Aβ(1-42) is also demonstrated to be involved in inducing toxicity in cholinergic neurons leading to cholinergic dysfunction and progressive basal forebrain cell loss, assumed to be an early event in the pathogenesis of AD. Heinitz et al. showed that on treating SN56.B5.G4 cells with oligomeric Aβ(1-42), many genes of the ER and GA involved in protein modification and degradation were affected. This indicated a possible role of ER-mediated stress in oligomeric Aβ(1-42) toxicity in cholinergic neurons and leading to cholinergic dysfunction in AD [110]. In line with this, another study by Joerchel et al. showed that SN56.B5.G4 cells when treated with oligomeric Aβ(1-42) affects the expression of a number of proteins, viz. calreticulin, MAPK kinase 6c, γ-actin, Rho-GDP dissociation inhibitor (Rho-GDI), ubiquitin carboxyl-terminal hydrolase-1 (UCHL-1) and α6-tubulin, which are known to be affected in the brains of AD patients, thus pointing towards the role of Aβ in affecting the integrity of the proteome in AD [111].

The amyloid cascade hypothesis has reached another level of complexity with recent studies revealing the appearance of multiple types of Aβ oligomers and their role in the pathogenesis of AD [2, 112, 113]. Various studies have recognised four endogenously produced Aβ oligomeric assemblies, viz. dimers, trimers, Aβ*56 and APFs (annular protofibrils), which may alter neuronal function in human and transgenic mice and have different consequences on neuronal survival [114]. In case of AD, soluble Aβ

monomers may form higher-order assemblies ranging from low-molecular weight oligomers (dimers and trimers) to Aβ*56, then to APFs and fibrils, which are the primary components of amyloid plaques, characteristic of AD.

Taken together, recent studies provide the evidence that Aβ oligomers are the more toxic species than insoluble fibrillar deposits relevant to AD pathology (Fig. 1). Also, soluble monomers can form higher-order assemblies finally leading to fibril formation which is the characteristic component of amyloid plaques. Thus, development of antibodies against specific oligomeric species can be an effective approach for treating AD.

2.4 Role of Mitochondria in AD Pathology

The 'Mitochondrial cascade hypothesis' was formulated in 2004, which provides an explanation of the aetiology of sporadic AD [115]. According to the 'mitochondrial cascade hypothesis', ageing and sporadic AD are two convergent events, and the etiological factors for autosomal dominant and sporadic AD are not the same. Also, mitochondrial dysfunction has been viewed as the common element between autosomal dominant and sporadic AD forms. In addition to sporadic AD, this hypothesis also predicts the aetiology of autosomal dominant AD forms. In case of autosomal dominant forms, it points out that excessive Aβ causes mitochondrial dysfunction and this Aβ-induced dysfunction further initiates histopathologies associated with AD. For sporadic AD cases, it is believed that age-related mitochondrial changes cause mitochondrial dysfunction and activate downstream cellular changes as observed in sporadic AD which include processing of APP to Aβ, tau phosphorylation, synaptic loss and finally neurodegeneration [6]. Thus, mitochondrial cascade believes Aβ accumulation as a downstream event in the cascade.

Mounting evidences are present which point to an association between ageing and mitochondrial dysfunction. An earlier study pointed out that somatic mutations in mtDNA can lead to premature onset of ageing phenotypes [116]. But it is not known whether the decline in mitochondrial function is a cause or outcome of ageing.

Of particular interest, a large number of evidences from studies involving experimental models and human samples suggest an association of APP and Aβ with the mitochondria. In particular, a study involving a mouse model showed that Aβ, particularly Aβ (1-42), accumulates in the mitochondria in the presence of a mutant *APP* gene and there is a decline in the activity of respiratory complexes III and IV. Also, Aβ was shown to accumulate in the mitochondria very early before the extensive extracellular deposition takes place [117]. These point towards the involvement of the mitochondria in the pathogenesis of AD (Fig. 2).

In addition, it is reported that age-related accumulation of somatic mutations in mtDNA causes an increase in ROS production in the mitochondria [118, 119]. It is known that oxidative stress induces an increased expression of BACE1 [120, 121]. Thus, it is speculated that with advanced age, an increase in ROS levels occur which increases Aβ levels owing to increased processing of APP by BACE1. Also, this generated Aβ induces an increased production of free radicals (ROS and RNS), causing decline in mitochondrial function. Thus, a feedback loop exists in which age-related ROS levels cause increased production of Aβ, and this Aβ further leads to increased production of ROS, resulting in memory impairment and cognitive decline associated with AD [36].

Several evidences point out that Aβ interacts with mitochondrial proteins and increase ROS levels, finally leading to synaptic damage. Mounting evidences show that Aβ, in its soluble oligomeric form, disrupts the axonal mitochondrial transport and disturbs the mitochondrial fission-fusion balance [122, 123]. Mitochondrial trafficking/transport is a phenomenon in which synaptic mitochondria, synthesised in the cell body, are transported down the axon/dendrite to areas of high energy demand, thus serving cellular energy demands [97]. During the transport, the

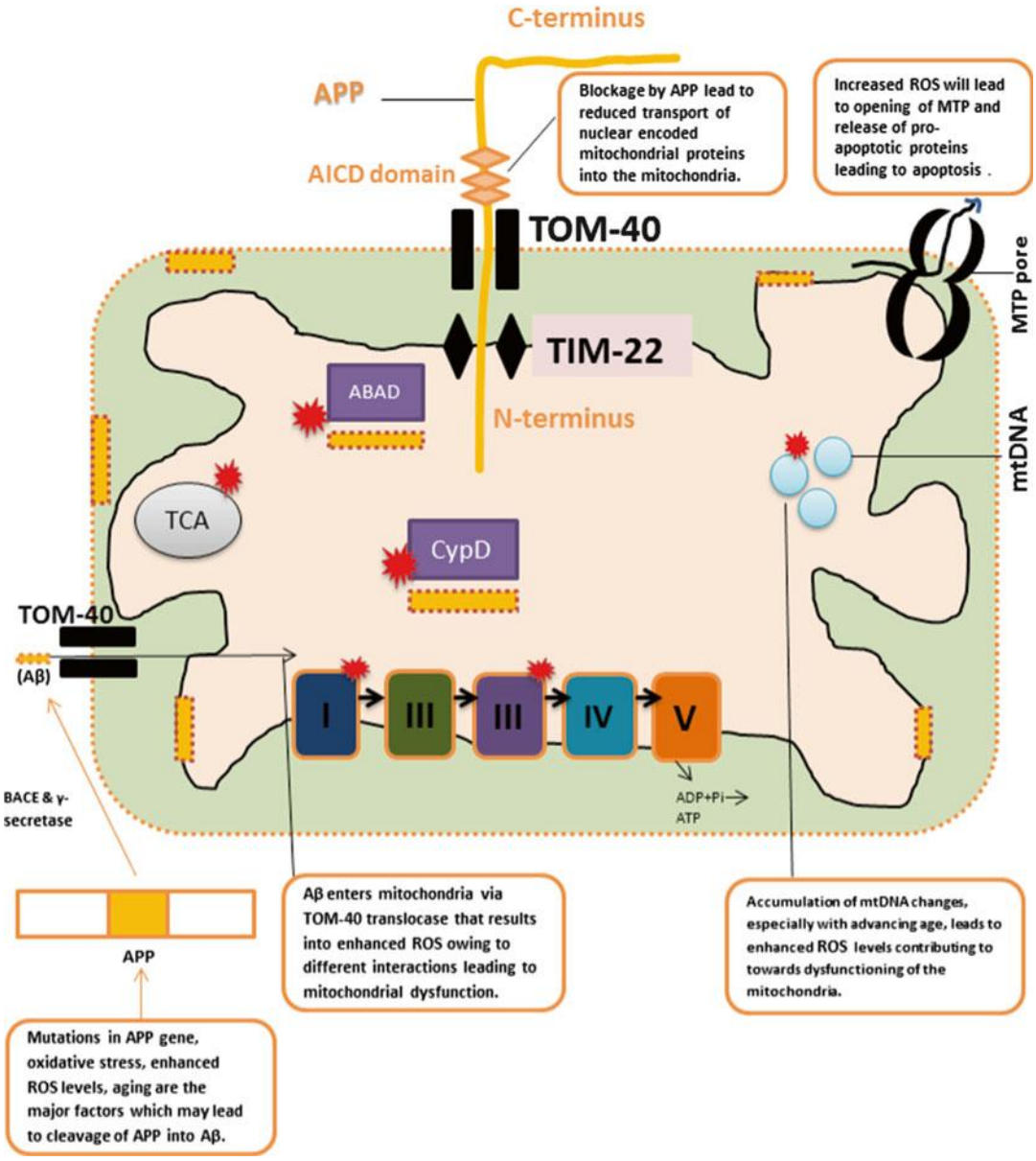

Fig. 2 Aβ induces mitochondrial dysfunction that results in neurodegeneration and AD. A mitochondrion is surrounded by two lipid membranes: outer membrane and inner membrane. The outer membrane is porous, while the inner membrane restricts ionic flow and harbours the electron transport chain (*ETC*). ETC is composed of complexes I–V and is responsible for the generation of mitochondrial ATP via oxidative phosphorylation. Electron leaks in complexes I and III are responsible for the generation of free radicals in the mitochondria. Components of tricarboxylic acid cycle (*TCA*), viz. α-ketoglutarate dehydrogenase and beta oxidation, are present in the mitochondrial matrix and generates superoxide radicals. These generated radicals thus cause lipid peroxidation and protein and DNA oxidation. In case of early-onset AD, genetic mutations in *APP*, *PS 1* and *PS 2* activate beta- and gamma-secretase and lead to increased processing of APP to Aβ. This Aβ is found to accumulate in the outer, inner mitochondrial membrane and the matrix. When associated with the outer membrane, it causes blockage of the entry of nuclear-encoded mitochondrial proteins. On being localised to inner membrane, it directly induces free radical production, decreases cytochrome oxidase activity, interferes with complex activities and impairs ATP production. Once inside, Aβ interacts with mitochondrial matrix proteins (ABAD and cyclophilin D) which increases the oxidative stress and worsens the mitochondrial damage. In case of late-onset AD, ageing is one of the major factors contributing to increased free radical production owing to mutations in mtDNA. APP is also said to be localised to mitochondrial outer membrane and forms complexes with the translocases of outer (*TOM*) and inner membrane (*TIM*). But the importation of C-terminus of APP is blocked due to the AICD domain of APP and thus blocks the mitochondrial pores. This prevents the entry of nuclear-encoded mitochondrial proteins causing impairment of mitochondrial enzyme activities, increased oxidative stress, neuronal damage and cognitive decline

mitochondria can encounter each other, leading to fusion (aided by fusion proteins) and subsequently resulting in exchange of mitochondrial content. This is necessary to maintain genome stability as it allows exchange of highly pathogenic mtDNA and helps to maintain mitochondrial function [124]. Mitochondrial fission plays an important role in apoptosis. Unbalanced fission and fusion results in disruption of mitochondrial dynamics, causing mitochondrial fragmentation and contributing to mitochondrial dysfunction [125], which has been speculated to be the underlying mechanism(s) causing synaptic degeneration in AD. Recently, it was shown that both monomers and oligomers of Aβ interact with mitochondrial fission protein, dynamin-related protein 1 (Drp1) and may be involved in abnormal mitochondrial dynamics causing mitochondrial fragmentation leading to neuronal dysfunction in AD. Also, expression levels of mitochondrial fission genes *Drp1* and *Fis1* (Fission 1) were increased and those of mitochondrial fusion genes *Mfn1* (mitofusin 1), *Mfn2* (mitofusin 2), *Opa1* (optic atrophy 1) and *Tom40* were decreased in AD patients [37]. This study also provided evidence that mitochondrial fragmentation may be initiated by interaction of Aβ with Drp1 causing abnormal mitochondrial dynamics, which increases as AD progresses. Thus, targeting these abnormal interactions can serve to minimise the neuronal damage caused by AD.

It has been shown that interaction of Aβ with Aβ-binding alcohol dehydrogenase (ABAD) in the mitochondria causes enhanced ROS production and apoptosis in AD patients and transgenic mice [126]. Some recent studies have implicated mitochondrial permeability transition pore (mPTP) formation in Aβ-mediated mitochondrial dysfunction [127]. The mPTP consists of cyclophilin D (CypD) in the mitochondrial matrix, voltage-dependent anion channel (VDAC) in outer mitochondrial membrane and adenine nucleotide translocase (ANT) in inner mitochondrial membrane. CypD has a role in opening of mPTP by binding with ANT and VDAC after its release from the matrix [38]. In relation to VDAC1, a recent study has shown that interaction of Aβ and phosphorylated tau with VDAC1 blocks mitochondrial pore leading to mitochondrial dysfunction in AD patients and transgenic APP mice [12]. Another mitochondrial protein CypD is shown to enhance mitochondrial and neuronal stress by interacting with Aβ. This interaction promotes ROS which leads to recruitment of CypD to the inner mitochondrial membrane and results in opening of mPTP causing cell death [128]. In addition, this study showed that CypD deficiency improved learning and memory in a mouse model of AD. A recent study has provided a new insight into the role of CypD in the disruption of axonal mitochondrial transport [38]. It was shown that depletion of CypD protects the Aβ-induced axonal mitochondrial transport damage and improves mitochondrial motility and dynamics. An increase in axonal mitochondrial density and bidirectional transport of the axonal mitochondria was observed on CypD depletion. CypD in the presence of Aβ promoted the opening of mPTP and, thus, disrupted Ca^{2+} homeostasis and increased accumulation of ROS, further activating P38 MAPK signal transduction pathway, causing synaptic injury. It was also demonstrated that CypD-mediated mPTP blockade improved synaptic function against Aβ toxicity using CypD-deficient mice. This study suggests a relation between CypD-mediated disruption of axonal mitochondrial trafficking and Aβ-induced mitochondrial dysfunction leading to synaptic injury, thus speculates a mechanism of mitochondrial dysfunction involved in the pathogenesis of AD (Fig. 2).

Another study has shown that age-related accumulation of Aβ occurs to a larger extent in the synaptic mitochondria as compared to the non-synaptic mitochondria and this accumulation in the synaptic mitochondria occurs very early as compared to its accumulation in the non-synaptic mitochondria. This led to altered mitochondrial transport in murine primary axons [129]. Thus, it can be ascertained that the synaptic mitochondria are more probable targets of Aβ-induced oxidative stress. Sirtuin 3 (SIRT3), a deacetylase has an essential role in maintaining mitochondrial function. Studies have highlighted its neuroprotective role and its role in energy homeostasis by maintaining basal ATP levels for survival of the cells [130, 131]. A recent study has shown upregulation of SIRT3 in relation to Aβ-accumulation in AD patients and transgenic AD mouse model. Also, it was shown that mitochondrial ROS levels regulated SIRT3 expression

[132]. This speculates that upregulation of SIRT3, seen in AD patients and mouse model, may be due to Aβ-induced mitochondrial oxidative stress. This suggests that in response to Aβ-induced oxidative stress in the mitochondria, upregulation of SIRT3 occurs to promote neuronal survival.

Recently, the RanBP9-cofilin pathway has been implicated in AD when it was shown that RanBP9, a scaffolding protein, generates Aβ and promotes Aβ-induced neurotoxity along with activating cofilin, having a key role in regulating actin dynamics and mitochondria-mediated apoptosis in a mouse model of AD pathology [133]. Also, suppression of Aβ and RanBP9-induced apoptosis by siRNA knockdown of cofilin confirmed the role of RanBP9-cofilin pathway in AD and can be the probable therapeutic target for lowering Aβ-induced neurotoxicity.

A study by Kopeikina et al. showed that soluble tau species are more toxic than tau aggregates and cause mitochondrial distribution deficiencies in a mouse model of tautopathy and in the human AD brains, possibly due to axonal transport deficiencies resulting in mitochondrial and neuronal dysfunction [134]. This suggests that soluble tau like the oligomeric Aβ are more toxic than aggregated form and involved in neuronal dysfunction associated with AD.

Poly (ADP-ribose) polymerase-1 (PARP-1), a predominantly nuclear enzyme responsible for genome stability and transcriptional regulation, is thought to be involved in the pathogenesis of AD. Activation of PARP-1 by oxidative stress (induced by Aβ) is believed to be an early event in the pathogenesis of AD. Enhancement of PARP-1 activity and accumulation of PAR is observed in the brains of AD patients [135]. A recent study has established the role of PARP-1 in microglial activation by its interaction with NF-kB [136]. Recently, the mitochondrial localisation of PARP-1 and its interaction with mitochondrial protein, mitofilin, has been established [137]. It was shown that in the absence of PARP-1, there is an accumulation of mtDNA damage, suggesting that mitochondrial PARP-1 has a role in mtDNA damage repair/signaling. Overexpression of PARP-1, Bax and p53 and altered mitochondrial function in the presence of oxidative stress induced by Aβ has also been shown recently [138]. Additional studies are needed to ascertain the role of PARP-1 and mitochondrial dysfunction in AD pathogenesis.

Taken together, these studies suggest that mitochondrial dysfunction is associated with ageing and AD. Along with Aβ, age-related accumulation of somatic mutations in mtDNA increases mitochondrial ROS levels leading to a decline in mitochondrial function. Recent studies have pointed out the role of oligomeric Aβ in the pathogenesis of AD. Oligomeric Aβ and APP are said to localise to mitochondria, mainly synaptic mitochondria, interact with mitochondrial proteins and disrupt axonal mitochondrial trafficking, causing synaptic injury and cognitive impairment. In relation to this, the use of mitochondria-targeted antioxidants can be seen as important approaches to treat AD.

3 Apurinic/Apyrimidinic Endonuclease (APE1): An Emerging Neuroprotective Enzyme

There are many external and internal agents, which bring human genome under stress and finally bring modification in the genomic stability. These threats are mainly produced internally from mitochondrial electron transport chain (ETC) or externally by different biological, chemical and physical agents like ultraviolet (UV) rays, ionising radiation (IR), chemotherapeutic agents, pollutants and heavy metals [139]. ROS attacks DNA readily and generates a variety of DNA base lesions [140]. DNA damage is a continuous process and ~10^4 DNA lesions are estimated to be produced in a mammalian genome each day as a result of spontaneous decay, errors in replication and cellular metabolism. To maintain genomic integrity, a cell has an internal regulatory mechanism which maintains DNA damage and repair in a balanced condition. There are two pathways by which cells maintain genome integrity: (i) antioxidants which quench the ROS/RNS, nonenzymatic antioxidants (e.g. α-tocopherol, β-carotene,

lycopene and ascorbic acid) and enzymatic antioxidants (e.g. superoxide dismutase (SOD), glutathione (GSH), peroxidases and catalase) and (ii) DNA repair by different processes. The DNA repair system comprises of base excision repair (BER), transcription-coupled repair (TCR), global genome repair (GGR), mismatch repair (MMR), homologous recombination (HR) and nonhomologous end joining [NHEJ] [141].

In mammals and higher organisms, different organs consist of various cell types; some of them are dividing while others are nondividing. In adults, cell types such as myocytes, adipocytes, skin cells and neurons are nondividing cells, i.e. terminally differentiated [142, 143]. BER is the major pathway for oxidative DNA base damage caused by ROS/RNS as well as for abasic (AP) sites and single-strand breaks (SSBs). Apurinic/apyrimidinic endonuclease (APE1) is a primary BER enzyme and responsible for repair and removal of AP sites and strand breaks [16, 17, 144].

Human *APE gene* (~3 kb in size) is localised on chromosome 14q11.2-12 and consists of four introns and five exons [145]. The human APE *cDNA* is about 1.4 kb in length and encompasses a coding region of 954 nucleotides and encodes a protein comprising of 318 amino acids. APE1 is abundant (~10^5 copies per cell) in eukaryotic cells and has a relatively long half-life [~8 h] [146]. APE1 is a dual function protein. Its C-terminus displays repair activity and its N-terminal contains a bipartite nuclear localisation signal, NLS [18, 147, 148] and displays redox activity responsible for transcriptional regulation through redox-based mechanisms [18, 149, 150].

3.1 Role of APE1 in Oxidative DNA Damage Repair

The ROS-induced damage to the DNA is implicated in a number of human diseases including neurodegenerative diseases like AD, PD, HD and cancers [16, 17, 151]. It is thus very important to repair the ROS-induced DNA damage in order to maintain the genomic integrity. BER, an evolutionarily conserved process, is responsible for repairing most endogenous lesions like oxidised bases, AP sites and SSBs in both nuclear DNA and mitochondrial DNA. The basic BER pathway involves enzymes, viz. DNA glycosylase, APE1, DNA polymerase and DNA ligase. APE1 is involved in the repair of oxidised base lesions generated in the DNA as a result of oxidative damage. Attempts to generate APE1-null mice were not successful and lead to an early embryonic death [152, 153]. Further attempts to generate cell lines from APE1-null embryos failed, showing the essentiality of APE1 in maintaining cell viability. A study pointing towards the role of APE1 in neuronal cell survival showed that overexpression of APE1 in hippocampal and sensory cells exposed to H_2O_2 lead to an increase in cell viability [154]. Upregulation of APE1 in cerebral cortical region of AD patients was also seen [155]. An immunohistochemical study pointing towards the role of APE1/Ref-1 in regulating cellular response towards oxidative stress showed that increased nuclear expression of APE1/Ref-1 is present in cerebral cortical regions of AD patients [20]. Another study showed the colocalisation of APE1/Ref-1 with Aβ in the senile plaques in AD hippocampus [156]. This study also showed that varying concentrations of Aβ(1-42) regulates APE1/Ref-1 expression, thus pointing towards the neuroprotective role of APE1/Ref-1 in response to oxidative stress. A number of evidences point towards the role of cyclin-dependent kinase 5 (Cdk5) in mediating neuronal loss. In line with this, it was shown that Cdk5 complexes with p35 and phosphorylates APE1 at Thr232, causing reduction in APE1's endonuclease activity and leading to accumulation of DNA damage and neuronal loss [157]. It can be interpreted that APE1 has a major role in overcoming the oxidative stress and maintaining neuronal cell viability and integrity.

3.1.1 Nuclear BER Pathway

A number of DNA repair pathways operate in the nucleus. Amongst them, BER pathway is the most

versatile repair pathway operating in the nucleus in response to oxidative damage for repairing alkylated and oxidised DNA lesions, AP sites and SSBs. Two models of BER are present: short-patch BER (SN-BER) and long-patch BER (LP-BER). SN-BER involves removal of a DNA lesion and incorporation of a single nucleotide, while a patch size of 2–8 nucleotides is associated with LP-BER [17, 144, 158]. The choice of the pathway depends on factors like type of lesions, AP sites and nature of 5′ terminus. The first step of the BER pathway is recognition and excision of a damaged base by DNA glycosylase. Two types of DNA glycosylases are present: monofunctional and bifunctional. Monofunctional DNA glycosylases (M-DG) include thymine DNA glycosylase (TDG), uracil-DNA glycosylase 1 (UDG1) and MutY homolog (MUTYH) and excise the substrate base, e.g. alkylated bases and uracil, generating an AP site which is later processed by APE1. Bifunctional DNA glycosylases (B-DG) which include 8-oxoguanine DNA glycosylase (OGG1) and *Nei*-like-1 (NEIL1), *Nei*-like-2 (NEIL2) and endonuclease III-like 1 (NTH1) have an additional lyase activity specific for oxidised bases and incise the DNA backbone 3′ to the AP site via β or β,γ elimination [144, 159–161]. The second step of BER involves processing of the generated AP site by APE1 that generates a nick containing a 3′OH residue and dRP at 5′ end, by cleaving the phosphodiester bond 5′ to the AP site. During the third step of the BER pathway, repair of the AP site is catalysed by polymerase (pol) β. If an unaltered group in deoxyribose is present, then pol β owing to its dRP lyase activity can carry SN-BER. The LP-BER occurs when AP sites are further oxidised by ROS and pol β cannot remove the 5′ blocking groups. 5′ flap-endonuclease-1 (FEN1), part of DNA replication machinery, is shown to displace and cleave this 5′ blocking group along with 4–6 nucleotides as a single-stranded DNA flap. PCNA also has a role to play by acting as a sliding clamp in LP-BER. The last step of BER involves nick sealing by DNA ligase which in case of LP-BER involves DNA pol ϵ/δ and DNA ligase I, and in case of SN-BER involves DNA ligase IIIα/XRCC1 complex [144, 160]. PARP-1 is known to modulate the capacity of BER and efficiently recognise and repair SSBs, thus acts as a DNA damage sensor and signal transducer [17, 162]. It was shown that Aβ is involved in the activation of PARP-1 through NO cascade in adult hippocampus [163]. Another protein XRCC1 also acts a SSB sensor protein and acts as a scaffold for BER proteins for SSB repair [144]. XRCC1 also interacts and stimulates APE1 [164]. Thus, it can be said that BER pathway is indeed a versatile pathway involved in maintaining genome integrity and involving a number of enzymes and interactions.

3.1.2 Mitochondrial BER Pathway

It is well known that the mitochondria have a role in the ageing process. Mitochondrial DNA (mtDNA) is more prone to oxidative DNA damage due to its close proximity to the site of ATP production in the inner mitochondrial membrane [165]. Thus, DNA damage repair in the mitochondria appears to be very important for maintaining proper functioning of the cell, especially during ageing. Various studies have shown that DNA repair actively takes place in the mitochondria, which was earlier thought to be present only in the nucleus. Recent studies have identified new DNA repair enzymes that participate in the DNA repair pathways operating in the mitochondria. Amongst the different repair pathways, BER is considered to be the major DNA repair pathway taking place in the mitochondria (Fig. 3). BER in the mitochondria helps to cope up with the oxidised DNA lesions generated due to the presence of free radicals and thus maintains mtDNA stability. The basic mechanism by which mitochondrial BER acts remains the same as the nuclear BER, but some specific BER enzymes are present in the mitochondria and these are coded by nuclear genes [166]. For a very long time, it was considered that only SN-BER occurs in the mitochondria which include removal of a DNA lesion and incorporation of a single nucleotide. But now it is believed that owing to the rate at which oxidised base lesions are generated in mtDNA, LP-BER may also take place in the mitochondria [17, 160, 167, 168]. The first step of the mtBER pathway involving recognition of a damaged base is

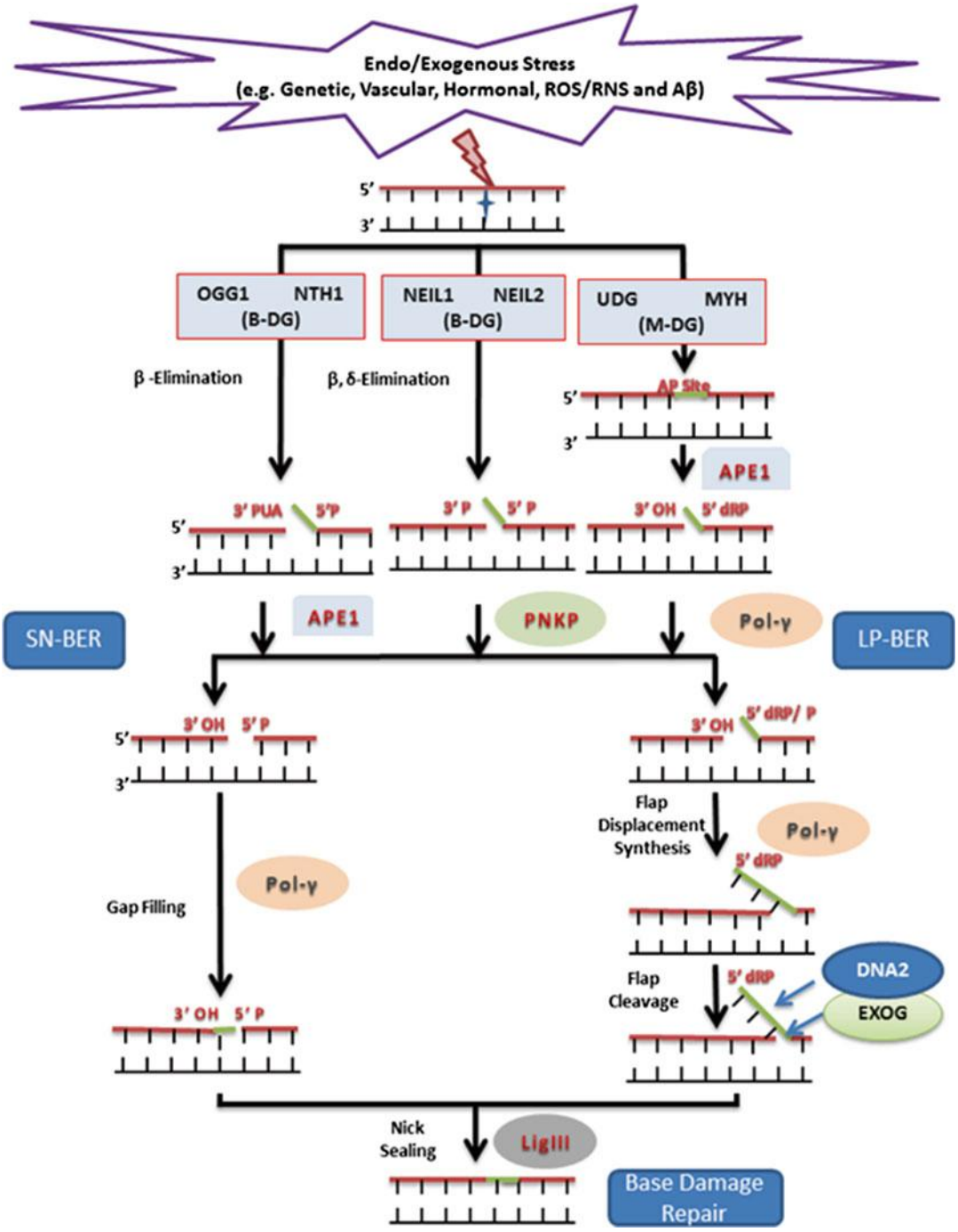

Fig. 3 Mitochondrial BER has more or less similar repair machinery as nuclear BER. DNA damage repair in the mitochondria is believed to be important in maintaining genomic integrity, especially during ageing. The basic mechanism of mitochondrial BER (*mtBER*) remains the same as nuclear-BER pathway, but some specific enzymes are present in the mtBER pathway. Both SN-BER and LP-BER are believed to take place in the mitochondria. Two M-DG, i.e. UDG1 and MYH, and four B-DG, viz. OGG1, NTH1, NEIL1 and NEIL2, are present in the mitochondria. APE1 in the mitochondria is found to be an N-terminal truncated product of APE1. In the mitochondria, DNA polγ is the only polymerase both in LP-BER and SN-BER pathways. Ligation of single-strand nick is performed by DNA ligase III. In addition, EXOG is an essential component of BER/SSBR pathway unique to the mitochondria and forms complex with APE1, DNA polγ and DNA ligase III and is involved in repairing endogenous SSBs in the mtDNA

performed by DNA glycosylase. Two M-DG, i.e. UDG1 and MYH, and four B-DG, viz. OGG1, NTH1, NEIL1 and NEIL2 are present in the mitochondria [169, 170]. Next step of the mtBER pathway involves processing of the AP site by APE1, which is the main AP endonuclease of the mammalian cell, and this mtAPE1 is believed to be an N-terminal truncated product of APE1. It is also shown that deletion of the 33 N-terminal residues increases the specific activity of mtAPE1 by threefold [171]. The next step involves insertion of correct nucleotides by DNA polγ which is followed by ligation of single-strand nick by DNA ligase III (Fig. 3). This mitochondrial ligase III is derived from *LIG3* gene and is known to be independent of XRCC1 while the nuclear variant of DNA ligase III interacts with XRCC1 [165, 172]. A recent study has shown that EXOG is an essential component of BER/SSBR pathway unique to the mitochondria and forms complex with APE1, DNA polγ and DNA ligase III and is involved in repairing endogenous SSBs in the mtDNA. Also, it was shown that depletion of EXOG increases ROS levels and induces apoptosis in normal cells [173]. FEN1 is involved in repairing oxidative DNA damage via LP-BER in the mitochondria [174]. Cellular death and embryonic lethality in presence of gamma radiation-induced DNA damage was observed in *FEN1* gene knockout mice [175]. The hDNA2, possessing nuclease, helicase and ATPase activities, is also involved in DNA replication and repair in the mitochondria. The hDNA2 forms a complex with polγ and stimulates the polymerase activity. It is also involved in RNA primer removal during mtDNA replication. The hDNA2, owing to its nuclease property, can also process flap LP-BER intermediates. This points towards the synergistic roles of FEN1 and hDNA2 to process the 5′flap intermediates during DNA replication and repair in the mitochondria [176]. Thus, all through these years we have gained knowledge about some of the repair pathways occurring in the mitochondria and identified different repair enzymes present in the mitochondria but much more needs to be understood towards establishing pathophysiologies which overtakes these repair processes.

3.2 Role of APE1 in Redox and Transcriptional Regulation

Owing to APE1's N-terminal domain which contains the NLS and redox regulatory domain, APE1/Ref-1 is considered to be an important mammalian redox regulator of transcription. It is identified that Cys65 is the redox active site in APE1/Ref-1 and that Cys93 interacts with Cys65 via disulphide bond formation and thus these two Cys residues contribute to alter the redox state of a number of TFs [177]. A number of studies have shown that APE1/Ref-1 modifies the DNA-binding ability of several TFs, such as activator protein-1 (AP-1), Fos and Jun, NF-κB, p53, Myb, early growth response-1 (Egr-1), polyoma virus enhancer-binding protein-2 (PEBP-2), activating transcription factor/cAMP response element-binding protein (ATF-CREB), hypoxia-inducible factor (HIF-1α) and HIF-like factor [18, 149, 178]. The reduction of Cys in the DNA-binding domains of the TFs by APE1/Ref-1 enhances the DNA-binding ability of TFs. It was earlier shown that reduction of the conserved Cys residue in the DNA-binding domain of c-Jun by APE1/Ref-1 enhances the DNA-binding activity of AP-1 in vitro [179]. Also, the ability to reactivate Fos-Jun DNA-binding declines on oxidation of APE1, which can be restored on treatment with thioredoxin, TRX [177]. Thus, it can be said that alterations in the redox state could lead to alterations in gene expression of key cellular signaling and other regulatory proteins. While APE1 is considered as a redox activator of several TFs like AP-1, p53, HIF-1α, it also acts as a trans-acting factor which causes Ca^{2+}-dependent repression of parathyroid hormone (PTH) and renin genes [180, 181]. An increase in extracellular Ca^{2+} causes binding of APE1 to negative Ca^{2+} response element (nCaRE: nCaRE-A and nCaRE-B) in the respective gene promoters causing repression of PTH and renin gene expression. Acetylation of APE1 is also shown to modulate APE1's transcriptional regulatory function. In addition, APE1 interacts stably with other trans-acting factors like HIF 1-α, STAT 3

and CBP/P300 [18, 153, 182]. Thus, it points towards the redox-independent functions of APE1 in regulating transcription.

3.3 Other Functions

Apart from being the major DNA repair enzyme of the BER pathway and redox activator of several TFs, newer studies have shown that APE1 serves some other important functions as discussed below.

3.3.1 APE1 as Endoribonuclease

For a very long time, RNA decay in eukaryotes was considered to be an exoribonucleolytic process, while in prokaryotes it was considered to be an endoribonucleolytic process. But numerous evidences in the recent past have demonstrated that endoribonucleases also have a significant role in eukaryotic RNA metabolism and contribute to RNA turnover in eukaryotes [183]. Recently, APE1 was identified as an endoribonuclease that cleaves within the UA and CA dinucleotides of *c-myc* (a proto-oncogene) mRNA and regulates the *c-myc* mRNA levels [7, 147]. It was shown that APE1 knockdown in HeLa cells led to increased *c-myc* mRNA levels and its half-life [147]. In line with this, a study identified the active site of APE1 and found that common active site is shared for endoribonuclease and nuclease activities of APE1 but the mechanisms of cleavage of RNA and DNA are not identical [184]. Thus, the role of APE1 in controlling the levels and turnover of other mRNAs in the neuronal as well as other cell types need to be understood.

3.3.2 Maintenance of Cellular Homeostasis

Maintenance of proper cellular redox balance is an essential prerequisite for proper functioning of biological systems. An increase in the ROS/RNS levels beyond the normal physiologic limits could disturb the redox homeostasis leading to cell death and disease development. To cope up with the increased oxidative stress build-up in the cell, different enzymatic/nonenzymatic antioxidant systems are present. APE1/Ref-1 is known to act as an important redox regulator of the cell which helps in maintaining proper levels of ROS/RNS for cell survival and proliferation. An earlier study showed that APE1/Ref-1 helps in regulating oxidative stress by inhibiting ROS production and NF-κB activation by modulating the activation of rac1 GTPase and inhibits apoptosis [185, 186]. Overexpression of APE1/Ref-1 was shown to increase SH-SY5Y cell viability following exposure to H_2O_2 [150]. A recent study of Mantha et al. has identified several key neuronal proteins those are involved in various cellular functions are interacting with APE1 in response to Aβ(25-35)-induced stress in PC12 and SH-SY5Y cells [187]. Thus, it can be interpreted that APE1/Ref-1 has a role in providing protection against oxidative stress and helps to maintain cellular redox balance.

4 Importance of Phytochemicals in Modulating Functions of APE1/Ref-1 Towards AD Therapeutics

Human beings have used plant extracts for centuries for treating various types of ailments. For the last few years, scientists are trying to figure out the active ingredients present in the plant extracts responsible for the specific action and decipher the molecular mechanism(s) by which these phytochemicals exert their action (Fig. 4). Phytochemicals like resveratrol, isoflavones, curcumin, decursin, EGCG, L-carnitine and *Ganoderma lucidum* extract have shown potent properties against neuronal disorders. In addition, some of them have also shown their effect on modulation of APE1/Ref-1 repair as well as redox activity in relation to cancer. Limited studies warrant additional studies to see how these phytochemicals may affect APE1/Ref-1 functions in neurodegenerative diseases like AD. The following sections describe these phytochemicals as having potential therapeutic effect against AD.

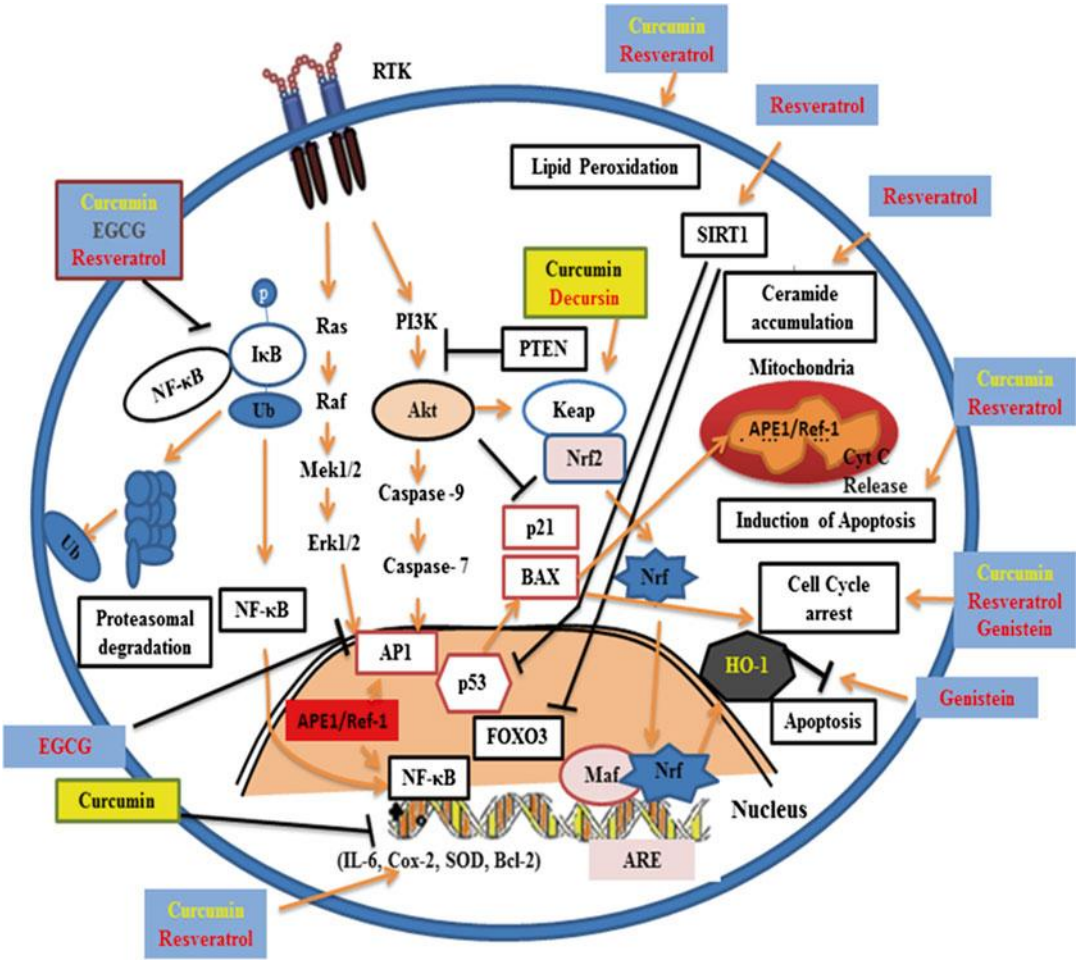

Fig. 4 Phytochemicals regulate different cellular interactions and modulates APE1/Ref-1 activity towards AD therapeutics. The phytochemical resveratrol triggers apoptosis through ceramide accumulation. Resveratrol activates SIRT1 and inhibits the transcription regulator p53 and FOXO3. Curcumin and decursin activate Nrf and rescue cell from oxidative stress. EGCG is an active polyphenol in green tea and inhibits multiple signal transduction pathways, including AP-1 and NF-κB, whereas curcumin and resveratrol activate different cellular factors like IL-6, Cox-2, SOD and Bcl-2. Curcumin, genistein and resveratrol also induce apoptosis and cell cycle arrest via functionally activated p53

4.1 Resveratrol

Resveratrol (3,5,4′-trihydroxy-*trans*-stilbene) is a phytoalexin and a polyphenolic compound found in the seeds and wine made from grape cultivars which provides natural protection to the plant against environmental stresses such as UV radiation and fungal infections. Resveratrol is the possible explanation for the French Paradox. According to French Paradox, France has a low rate of coronary heart disease (CHD) in spite of high intake of saturated fats, thus presents a situation which is paradoxical when compared with other countries having comparable diet rich in saturated fats and subsequently high CHD [188]. Wine intake is highest in France and studies have pointed out that drinking red wine confers cardioprotection and this is attributable to resveratrol present in it along with other polyphenols [189, 190], thus explaining the paradox. Therefore, resveratrol has assumed a great importance over time. The protective abilities of resveratrol have

been attributable to its antioxidant properties [191]. Several lines of studies have shown that drinking red wine also confers neuroprotection and reduces the incidence of neurological diseases like AD [192, 193]. It has been shown that resveratrol exerts protective effects against Aβ-induced neurotoxicity in rat hippocampal cells with the involvement of PKC [194]. Resveratrol showed anti-apoptotic effect and interference in cell cycle progression in SH-SY5Y neuroblastoma cells [195]. The anti-Aβ potential of resveratrol in clearing Aβ via a mechanism involving activation of proteasome was also identified [196]. In addition, resveratrol oligomers from *V. amurensis* were shown to rescue Aβ-mediated oxidative stress in PC12 cells by inhibiting ROS production [197]. Resveratrol was found to stimulate NO production and reduce the oxidative stress after a focal cerebral ischaemia (FCI) injury in rats [198]. Downregulation of iNOS and enhancement of HO-1 expression by resveratrol rescues the Aβ-induced neurotoxicity [199]. Upregulation of iNOS is associated with Aβ levels [200], indicating a connection between iNOS and Aβ in the progression of AD. Resveratrol is also shown to be a SIRT-1 activator protecting the neuroblastoma cells from oxidative damage caused by Aβ [201].

SIRT-1 has an important role in maintaining genome integrity through regulation of BER pathway. An increase in association of APE1 with XRCC1 under genotoxic stress is reported, while the knockdown of SIRT-1 decreases this association. Resveratrol has been shown to promote binding of APE1 to XRCC1 by a mechanism involving activation of SIRT-1 [202]. Resveratrol is also shown to regulate the redox activity of APE1/Ref-1 and is identified as a potent APE1/Ref-1 inhibitor [24]. In this study, an increase in expression of Ref-1 was seen in human melanoma cells which may be partly due to mitochondrial dysfunction owing to high ROS levels and presence of oxidised melanin in these cells. Overexpressing Ref-1 led to increase in basal NF-κB transcription activities. In addition, in response to APE1/Ref-1 antibody, reduced AP-1 and NF-κB DNA-binding activities were observed. Thus, resveratrol seems to act as an APE1/Ref-1 inhibitor upregulating AP-1 and NF-κB DNA-binding activities, highlighting its anti-melanoma potential [24]. In a recent finding, it was shown that resveratrol mitigates the $AlCl_3$-induced direct neuroinflammation in rats [203]. Also, an increase in APE1 level and decrease in β-secretase and Aβ levels were observed. In addition, a decrease in expression of TNF-α, IL-6 and iNOS in the rat brain was seen on treatment with resveratrol, thus revealing the anti-inflammatory effects of resveratrol [203]. Taken together, these findings suggest resveratrol as a potent phytochemical for treating oxidative stress-induced mitochondrial dysfunction and inflammation in neurodegenerative diseases like AD and which might alter the APE1/Ref-1 function to mediate neuronal cell viability to counter AD.

4.2 Curcumin

Curcumin is the main active flavonoid derived from the rhizome of *Curcuma longa* (Zingiberaceae). Curcumin has potent anti-inflammatory property due to its antioxidant activity resulting in the scavenging of the ROS generated inside the body under stress conditions [204]. Curcumin owing to its antioxidant and anti-inflammatory action suppresses the oxidative damage and decreases amyloid deposition [205].

Curcumin acts as a strong metal chelator and has the ability to repress the inhibition of DG, NEIL1 caused by divalent metals like Cu and Fe in SH-SY5Y neuroblastoma cells [206, 207]. Curcumin acts as a potential therapeutic agent owing to its two effects – reduction of oxidative stress and acting as a metal chelator [160, 207]. Curcumin is shown to increase the heme oxygenase1 (HO-1) expression in cultured hippocampal neurons in response to glucose oxidase (GO)-mediated oxidative damage [208]. Curcumin has also shown to reduce the formation of Aβ and decrease plaque burden in transgenic AD mice [209]. Moreover, curcumin has a strong ability to cross blood-brain barrier (BBB) and shown to reduce aggregation of Aβ(1-40) and cause disaggregation of Aβ(1-40). In addition, curcumin prevented Aβ(1-42) oligomer formation and toxicity,

making it an effective molecule for prevention and treatment of AD [210]. Thus, this curry spice has a great potential in alleviating oxidative stress and improving cognitive decline in AD.

4.3 Decursin

Decursin (D) and decursinol angelate (DA) are the major coumarins present in the roots of *Angelica gigas* Nakai (Umbelliferae). The roots of *Angelica gigas* Nakai have been used in traditional Korean medicine for treating anaemia and as a sedative and an anodyne agent [211]. Many reports highlight the antitumour [211], antibacterial [211], anti-nematodal [212] and antioxidant [213] properties of *Angelica gigas* Nakai, mainly due to the presence of D and DA. A study demonstrated the anti-amnesic property of D which rescued the impairment induced by scopolamine through the inhibition of acetylcholinesterase (AChE) in the hippocampus of treated mice [211]. Decursin was shown to cross the BBB [212], thus showing a potential to intervene the CNS to treat disorders like AD. The neuroprotective role of D and DA in rescuing the glutamate-induced oxidative stress in primary cortical cells was highlighted in a study [214]. Another study showed the neuroprotective ability of D and DA and its role in nuclear factor erythroid 2-related factor (Nrf2) activation and elevation of antioxidant levels in rescuing Aβ-mediated oxidative stress in PC12 cells [213]. Both D and DA were shown to inhibit Aβ fibrillation. This study indicated that D and DA can be utilised as an important antioxidant to help reduce the oxidative stress induced by Aβ in AD. In a recent finding, it was shown that in response to Aβ(23-35)-induced oxidative stress, treatment with D leads to decreased ROS levels and activation of mitogen-activated protein kinases (MAPK) signal pathways, leading to Nrf2 activation and upregulation of HO-1 expression, thus protecting the PC12 cells from Aβ-mediated neurotoxicity [215]. Taken together, these findings suggest that D and DA can protect neurons from Aβ-mediated oxidative stress. Further studies are needed to show the potential of D and DA in modulating APE1/Ref-1's functions to limit neurodegeneration and increase cell survival in AD.

4.4 Soy Isoflavones

Soy isoflavones are the major flavonoids found in soybean, which have been a traditional food in Asia for a very long time. Apart from isoflavones, soy is also rich in phytic acid, trypsin inhibitors and saponins [216]. But soy isoflavones have dragged attention in the recent past due to its numerous health benefits particularly its neuroprotective effects. Soy isoflavones are also referred to as phytoestrogens due to their beneficial effects on estrogenic problems [217]. Soy isoflavones include genistein, daidzein and glycitein [218]. An earlier study showed that genistein could attenuate the oxidative stress induced by Aβ(25-35) and reduce the ROS levels and inhibit cell apoptosis possibly through Nrf/HO-1signal pathway in PC12 cells [219]. Another study showed that genistein improves the short-term spatial memory in rats by mitigating Aβ(1-40)-induced impairment via an estrogenic pathway [219]. Soy isoflavones suppressed the production of inflammatory cytokines and downregulated NF-κB activity, which was induced by Aβ(1-42) and improved the learning and memory impairment in rats [220]. Another study showed that isoflavones, specifically genistein and glycitein, have an anti-fibrillisation, anti-oligomerisation and fibril-destabilising potential on Aβ(1-40) and Aβ(1-42) in vitro and that glycitein, in particular, binds directly to Aβ monomers, oligomers and fibrils and exhibit highest affinity for Aβ(25-35) [221]. Thus, isoflavones can be employed towards effective therapy to directly target amyloid assemblies for the treatment of AD. In addition, genistein showed neuroprotection and increased cell viability and protein kinase C (PKC) activity in PC12 cells which were treated with Aβ(25-35) and this involved PKC signaling pathway, which is known to regulate neuronal survival in AD [222]. In addition, downregulation of *PS1*, involved in Aβ generation, by treatment with genistein was shown [223]. A recent finding showed that soy

isoflavones reduced the oxidative stress in the mitochondria induced by Aβ(1-42) in the rat brain and increased the mitochondrial membrane potential (MMP) and antioxidant function [217]. As a result, isoflavones help to maintain redox balance in the brain. Together, these findings show that isoflavones with potential can improve mitochondrial function and maintain redox balance for neuronal survival.

4.5 Epigallocatechin-3-Gallate (EGCG)

Epigallocatechin-3-gallate (EGCG) isolated from the leaves of green tea (*Camellia sinensis*) and a type of catechin [224]. It has a number of beneficial health effects owing to its neuroprotective, anticarcinogenic and anti-inflammatory property [225, 226]. The consumption of green tea and incidence of dementia, AD and PD are inversely correlated [227, 228]. Numerous animal model studies have suggested that EGCG exerts neuroprotective effects against age-related cognitive decline and neurodegenerative diseases. An earlier study using in vitro and in vivo models has shown that EGCG elevates the levels of soluble APP-α (an N-terminal cleavage product) and promotes the cleavage of α-C-terminal fragment of APP. This shows that EGCG promotes α-secretase activity leading to decreased Aβ levels and plaque formation [229, 230]. EGCG is shown to bind to the β-sheet-rich aggregates and bring about a conformational change remodelling mature α-synuclein and Aβ fibrils into smaller amorphous nontoxic protein aggregates and reduce cellular toxicity [231]. Another in vivo study involving passive avoidance and water maze tests showed that EGCG reduces the Aβ(1-42)-induced memory dysfunction dose-dependently and suppresses the activities of β- and γ-secretase. In addition, an inhibition in the activation of extracellular signal-regulated protein kinase (ERK) and NF-κB by EGCG was observed in the Aβ(1-42)-injected mouse brains [232]. EGCG has also emerged as a mitochondrial restorative compound which was demonstrated to restore MMP, ROS levels and ATP levels in a double-transgenic mouse model of AD. Thus, EGCG was shown to lessen the Aβ-induced mitochondrial dysfunction, which is implicated during the onset and progression of AD [230]. These studies point out that EGCG, owing to its anti-amyloidogenic and mitochondrial restorative property, has a tremendous potential in AD therapy.

4.6 L-Carnitine

L-carnitine is a derivative of the amino acid, lysine. Its name is derived from the fact that it was first isolated from meat (*carnus*). Acetyl L-carnitine (ALCAR), an L-carnitine ester of acetic acid, crosses the BBB and modifies acetylcholine production in the brain [233]. ALCAR is involved in regulation of mitochondrial energetics and oxidative stress associated with ageing [234]. An earlier study pointed towards induction of HO-1 and upregulation of Nrf-2, a redox-sensitive TFs, on treating astrocytes with ALCAR [235]. ALCAR was also shown to increase the synthesis of nerve growth factor receptors (NGFR) in PC12 cells and thus elicits neurite outgrowth by stimulating NGF uptake in these cells [236]. ALCAR is a physiological activator of the mitochondrial fatty acid metabolism and has been reported to improve cognitive deficits in aged animals and to slow deterioration in AD patients [237]. A study showed that ALCAR promotes α-secretase activity and physiological APP metabolism by facilitating the delivery of ADAM10 to the postsynaptic compartment regulating α-secretase activity towards APP, leading to release of a non-amyloidogenic product [238]. A study by Abdul et al. showed that ALCAR displayed neuroprotective effect towards Aβ(1-42)-induced oxidative stress in cortical neurons by upregulating the levels of glutathione (GSH) and heat shock proteins [HSPs] [239]. Thus, ALCAR displays neuroprotection and modulates mitochondrial function and oxidative stress, thus has a potential and can be employed in AD therapy upon further studies.

4.7 Ganoderma Lucidum

G. lucidum is a medicinal fungus used clinically in many Asian countries for health and longevity. A study showed the neuroprotective effect of *G. lucidum* in which the extract induced the neuronal differentiation of PC12 cells and prevented NGF-dependent PC12 neurons from apoptosis. This effect was thought to be mediated by the activation of ERK and CREB signaling pathways that maintained the survival of the NGF-dependent neurons [240]. An earlier study by Pillai et al. had demonstrated that an aqueous extract of *G. lucidum* protected against the radiation-induced nuclear DNA damage [241]. *G. lucidum* polysaccharides (GLP) was shown to reduce the expression of Caspase-3 and FasL leading to improved cognition and learning ability in Aβ(25-35)-injected mice [242]. Another study provided evidence that *G. lucidum* increased the non-amyloidogenic protein secretion, i.e. sAPPα secretion, in SH-SY5Y cells involving phosphatidylinositol 3 kinase (PI3K) and ERK signaling pathways [243]. A study pointing towards the antioxidant properties of *G. lucidum* has shown that the activities of heart TCA enzymes and mitochondrial complex (I-IV) improved on treating aged mice with an ethanolic extract of *G. lucidum* [244]. In line with this, another study showed that *G. lucidum* elevated the activities of mitochondrial dehydrogenases, i.e. succinate dehydrogenase (SDH), malate dehydrogenase (MDH), α-ketoglutarate dehydrogenase (α-KGDH) and pyruvate dehydrogenase (PDH), as well as complex I and II activities in the mitochondria of aged Wistar rat brains. Also, the level of lipid peroxidation was decreased in the *G. lucidum*-treated rats [245]. A recent in vivo study involving Sprague-Dawley rats showed that *G. lucidum* spore (GLS) improved mitochondrial functioning, alleviated oxidative stress and protected the hippocampal neurons from apoptosis, improving cognition in these rats [86]. Another recent study showed that *G. lucidum* promoted neurite outgrowth in differentiating N2a cells [246]. Thus, *G. lucidum* seems to have a great therapeutic importance in reviving brain and cognitive health in AD patients.

Although some of the phytochemicals described here are not studied directly with relation to APE1/Ref-1's functions, their beneficial effects as discussed further suggest testing them to understand their role in modulating repair, redox and other newly discovered roles of APE1/Ref-1 towards neuronal cell survival. It is a prerequisite for the neuronal cell to counter the oxidative stress responses elicited by different agents and mechanisms as discussed in this review and, further, APE1-/Ref-1-mediated intervention along with phytochemicals, thus, emerges a new field of study to tackle the AD.

5 Conclusions and Future Perspectives

AD is a disabling and debilitating disease affecting millions worldwide and is projected to affect many more. The pathological hallmarks of the disease are known from a very long time, but the molecular mechanism(s) underlying the AD is not known to date. Researchers have tried to understand the various factors responsible for the progression of this fatal disease. Some risk factors have been associated with the disease. These are mutations in the *APP*, *PS1* and *PS2* genes, which are responsible for the accumulation of Aβ, the main culprit, in the neurons leading to development of early-onset AD. A significant number of studies pointing out that Aβ oligomers are the more toxic species rather than the insoluble fibrillar deposits. Other risk factors for AD include the presence of *APOE* ε4 allele. Ageing is the greatest risk factor for AD. In the recent past, studies have implicated oxidative stress and mitochondrial dysfunction in the pathogenesis of AD. It is also observed that mitochondrial dysfunction occurs very early in AD pathogenesis. Studies have pointed out that accumulation of somatic mtDNA mutations over time causes genome instability and mitochondrial dysfunction. It is well established that Aβ causes oxidative stress. Recent studies have shown that Aβ and APP localise to the mitochondria, interact with mitochondrial proteins, increase ROS/RNS levels and cause mitochondrial dysfunction. Recent studies have provided evidence that Aβ accumulates more in the synaptic terminals of neu-

rons and interferes with the axonal mitochondrial transport, leading to synaptic damage and cognitive decline associated with AD. During the mitochondrial trafficking, fusion process occurs which is essential for exchange of pathogenic mtDNA. Unbalanced fusion/fission has been implicated in various neurodegenerative diseases like AD. BER is the predominant pathway responsible for repairing oxidised base lesions in the nucleus as well as in the mitochondria, with APE1 being the central repair enzyme of this pathway. Importance of APE1/Ref-1 for the cell can be discerned by the fact that attempts to generate APE1-null mice failed as it led to their early embryonic death. APE1/Ref-1 has been shown to play a major role in overcoming the oxidative stress and maintaining neuronal cell viability and integrity owing to various roles played by it, viz. as a redox and transcriptional regulator, as an endoribonuclease and as a regulator of cellular homeostasis. Phytochemicals like soy isoflavones, resveratrol and curcumin have been shown to modulate APE1/Ref-1 activity both in vitro and in vivo. In addition to these, decursin, L-carnitine, *Ganoderma lucidum* and EGCG have shown to lower the oxidative stress induced by Aβ in various studies. Thus, these phytochemicals have a potential to reduce the oxidative stress and modulate functions of APE1/Ref-1 and can be used as an effective approach to treat AD by protecting APE1/Ref-1's functions.

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