Review Article

Nucleic acid oxidation in Alzheimer disease

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Abstract

Increasing evidence suggests that oxidative stress is intimately associated with Alzheimer disease pathophysiology. Nucleic acids (nuclear DNA, mitochondrial DNA, and RNA) are one of the several cellular macromolecules damaged by reactive oxygen species, particularly the hydroxyl radical. Because neurons are irreplaceable and survive as long as the organism does, they need elaborate defense mechanisms to ensure their longevity. In Alzheimer disease, however, an accumulation of nucleic acid oxidation is observed, indicating an increased level of oxidative stress and/or a decreased capacity to repair the nucleic acid damage. In this review, we present data supporting the notion that mitochondrial and metal abnormalities are key sources of oxidative stress in Alzheimer disease. Furthermore, we outline the mechanisms of nucleic acid oxidation and repair. Finally, evidence showing the occurrence of nucleic acid oxidation in Alzheimer disease will be discussed.
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Keywords: Alzheimer disease; DNA oxidation; DNA repair; Oxidative stress; RNA damage; RNA repair; Free radicals

Contents

Introduction

Alzheimer disease (AD) is a progressive, degenerative brain disorder resulting in cognitive and behavioral decline and is the leading cause of dementia in the Western world. Two pathological hallmarks are observed in AD patients at autopsy: intracellular neurofibrillary tangles (NFT) and extracellular senile plaques in the neocortex, hippocampus, and other subcortical regions of the brain essential for cognitive function [1]. NFT are formed from paired helical filaments composed of neurofilaments and hyperphosphorylated tau protein [2]. In turn, plaque cores are formed mostly from deposition of amyloid-β peptide that results from the cleavage of the amyloid-β protein precursor (AβPP).

Although the etiology of AD remains largely unclear, there is accumulating evidence suggesting that oxidative stress occurs before the onset of symptoms in AD and such oxidative changes are pervasive throughout the body, being detected peripherally [3–6] as well as being associated with the vulnerable regions of the brain affected in disease [7–10]. Indeed, oxidatively modified products of nucleic acids (e.g., 8-hydroxydeoxyguanosine, 8-hydroxyguanosine) and proteins (e.g., 3-nitrotyrosine, protein carbonyls), as well as products of lipid peroxidation (e.g., 4-hydroxynonenal, F2-isoprostane, malondialdehyde) and glycoxidation (e.g., carboxymethyllysine, pentosidine), are known as markers of oxidative damage. Several markers among them have been demonstrated in the affected lesions in the postmortem brain tissue or premortem cerebrospinal fluid (CSF), plasma, serum, and urine from the patients with these diseases [11,12]. Most recently there have been multiple studies showing that lipid peroxidation, protein oxidation, and nucleic acid oxidation occur in mild cognitive impairment (MCI), which possibly represents a prodromal stage of AD [13–17]. The increased levels of oxidative damage in neurodegenerative conditions are often accompanied by reduced levels of antioxidant defense mechanisms in the subjects [18,19]. Remarkably, a number of known genetic and environmental factors of the neurodegenerative diseases, namely disease-specific gene mutations, risk-modified gene polymorphisms, and risk-modified lifestyle factors, are closely associated with oxidative damage [12,18,20], which implicates a pathogenic role of oxidative damage in the process of neurodegeneration.

Chronic exposure to the damaging effects of reactive oxygen species (ROS), which are mainly formed in mitochondria during normal cellular metabolism, is considered a major contributor to the aging process [21]. The effects of ROS are believed to be especially devastating to postmitotic tissues because the damaged cells cannot be replaced by intact ones. Oxidative modifications of RNA, nuclear DNA (nDNA) and, particularly, mitochondrial DNA (mtDNA), are thought to play a key role in the selective neuronal loss associated with mammalian aging and neurodegeneration [9,22–24]. Because of the proximity to ROS, lack of protective histones, and limited repair mechanisms [21], mtDNA is highly susceptible to oxidative damage. Damage to mtDNA could potentially result in bioenergetic dysfunction and consequently to aberrant nerve functions.

Here we will present data supporting the notion that mitochondrial and metal abnormalities are key sources of oxidative stress in AD. This article also provides an overview of nucleic acid oxidation and repair mechanisms. Finally, data from the literature demonstrating the occurrence and accumulation of damaged nucleic acids in AD are discussed.

Main sources of oxidative stress in Alzheimer disease

Mitochondria

Mitochondria are essential organelles for neuronal function because the limited glycolytic capacity of these cells makes them highly dependent on aerobic oxidative phosphorylation for their energetic needs. However, oxidative phosphorylation is a major source of endogenous toxic free radicals, including hydrogen peroxide (H_2O_2), hydroxyl ($^\bullet OH$), and superoxide ($O_2^{\bullet-}$), that are products of normal cellular respiration [25]. With inhibition of the electron transport chain, electrons accumulate in complex I and coenzyme Q, from which they can be donated directly to molecular oxygen to give $O_2^{\bullet-}$ that can be detoxified by the mitochondrial manganese superoxide dismutase to give H_2O_2 that, in turn, can be converted to H_2O by glutathione peroxidase. However, $O_2^{\bullet-}$ in the presence of nitric oxide ($NO^\bullet$), formed during the conversion of arginine to citrulline by nitric oxide synthase, can generate peroxynitrite ($ONOO^-$) and lead to protein modification [26]. Furthermore, H_2O_2 in the presence of reduced transition metals can be converted to toxic $^\bullet OH$ via Fenton and/or Haber Weiss reactions, a process that we have specifically localized to neurofibrillary pathology in AD [27]. Inevitably, if the amount of free radical species overwhelms the capacity of neurons to counteract these harmful species, oxidative stress occurs, followed by mitochondrial dysfunction and neuronal damage. Reactive species generated by mitochondria have several cellular targets, including mitochondrial components themselves (lipids, proteins, and DNA).

In addition to the key role of mitochondria in the maintenance of cell energy and generation of free radicals, these organelles are also involved in cell death pathways, namely apoptosis. There are three main apoptotic pathways leading to the activation of caspases, which converge onto mitochondria and are mediated through members of the Bcl-2 family such as Bid, Bax, and Bad [28]. The end result of each pathway is the cleavage of specific cellular substrates, resulting in the morphological and biochemical changes associated with the apoptotic phenotype. The first of these depends on the participation of mitochondria (mitochondrial pathway), the second involves the interaction of

a death receptor with its ligand (death receptor pathway), and the third is triggered under conditions of endoplasmic reticulum (ER) stress (ER-specific pathway) [29]. Although a number of apoptosis-related proteins are increased in AD [30,31], it is unlikely that this leads to rapid cell death [32,33].

Previous studies from our laboratory show that the neurons showing increased oxidative damage in AD also possess striking and significant increases in mtDNA, cytochrome oxidase, and lipoic acid [34,35]. Surprisingly, much of the mtDNA, cytochrome oxidase, and lipoic acid is found in the neuronal cytoplasm and, in the case of mtDNA and lipoic acid, in vacuoles associated with lipofuscin. We also observed an overall reduction in microtubules in AD compared to controls [36]. Altogether these data indicated that the abnormal mitochondrial turnover, as indicated by increased perikaryal mtDNA and mitochondrial protein accumulation in the face of reduced numbers of mitochondria, could be due to a defective microtubule system resulting in deficient mitochondrial transport. In other studies, we analyzed the ultrastructural features of vascular lesions and mitochondria in brain vascular wall cells from human AD, YAC, and C57B6/SJL transgenic positive (Tg(+)) mice overexpressing AβPP. We observed a higher degree of amyloid-β deposition, oxidative stress markers, mtDNA deletion, and mitochondrial structural abnormalities in the vascular walls of human AD, YAC, and C57B6/SJL Tg(+) mice compared to the respective controls [37,38]. All the abnormalities observed occur before neuronal degeneration and amyloid deposition [39,40].

Overall, these results indicate a clear involvement of oxidative stress, mitochondria dysfunction, and neuronal damage/death during the evolution of AD. In fact, an intricate interorganelle cross talk was previously suggested by Ferri and Kroemer [28], who reviewed the participation of distinct organelles, namely the nuclei, lysosomes, ER, and Golgi, in the release of death signals that converged in mitochondria, the central executioners. To obtain more information on the involvement of mitochondria in AD pathophysiology please see the review articles by Moreira et al. [41,42] and Zhu et al. [43].

Redox-active metals

The loss of homeostasis of iron and copper in the brain is accompanied by severe neurological consequences. In patients with AD, overaccumulation of iron in the hippocampus, cerebral cortex, and basal nucleus of Meynert colocalizes with AD lesions, senile plaques, and NFT [27,44]. Iron is an important cause of oxidative stress in AD because it is found in considerable amounts in the AD brain [45] and, as a transition metal, is involved in the formation of ˙OH via the Fenton reaction [46]. Furthermore, it has been reported that amyloid-β itself is a substrate for ˙OH [44]. Amyloid-β extracted from postmortem AD brains presents oxidative modifications such as carbonyl adduct formation, histidine loss, and dityrosine cross-linking, making this protein less water soluble and less susceptible to degradation by the proteases [44,47,48]. Furthermore, it has been reported that amyloid-β deposition and AβPP cleavage and synthesis are promoted by the presence of iron [49], likely consequential of oxidative stress-mediated events [50,51].

Huang and collaborators [52] presented in vitro evidence that trace levels of zinc, copper, and iron are initiators of an amyloid-β_{1-42}-mediated seeding process and amyloid-β oligomerization and these effects were abolished by chelation of trace metals. Interestingly, the in vitro toxicity of amyloid-β is also abolished by pretreatment with metal chelators [53]. Data from our laboratory and others also indicate that heme oxygenase-1 (HO-1) is induced in AD brains [54,55]. HO-1 catalyzes the conversion of heme to biliverdin and iron. Biliverdin, in turn, is reduced to bilirubin, an antioxidant. Because HO-1 is induced in proportion to the level of heme [56], the induction of HO-1 suggests that there may be an abnormal turnover of heme in AD. This idea is consistent with the mitochondrial abnormalities associated with AD because it is well known that many heme-containing enzymes are found in mitochondria. As previously discussed, our ultrastructural studies suggest a high rate of mitochondrial turnover and redox activity in the residual body of lipofuscin [34,35]. In turn, the increase in heme induces synthesis of more HO-1, suggesting that mitochondrial turnover promotes oxidative stress via increase of redox-active iron [57].

Recently, we reported that ribosomal RNA (rRNA) provides a binding site for redox-active iron and serves as a redox center within the cytoplasm of vulnerable neurons in AD in advance of the appearance of morphological change indicating neurodegeneration [57]. Furthermore, we observed that oxidized nucleic acids are commonly observed in the cytoplasm of the neurons that are particularly vulnerable to degeneration in AD [10]. 8-Hydroxyguanosine (8OHG), a marker of nucleic acid oxidation, is likely to form at the site of ˙OH production, a process dependent on redox-active metal-catalyzed reduction of H_2O_2 together with cellular reductants such as ascorbate or $O_2^{˙-}$. Interestingly, the levels of 8OHG are inversely related to the amount of amyloid-β present as extracellular deposits [10] or intracellular oligomeric forms [58], suggesting a complex interplay between amyloid-β and redox metal activity that may be critical to metal dynamics within the neuronal cytoplasm [59,60]. A possible key element in these dynamics is mitochondria in the neuronal cell body.

Copper can also participate in the Fenton reaction to generate ROS [61,62]. Although conflicting results exist concerning the amount of copper and the formation of senile plaques [63,64], there is accumulating evidence that both iron and copper in their redox-competent state are bound to NFT and amyloid-β deposits [27,45,46]. However, a recent study reported that cognitive decline correlates with low plasma concentrations of copper in patients with mild to moderate AD [65]. To obtain more information on the involvement of metals in AD pathophysiology, please see the review articles by Adlard and Bush [66] and Zhu et al. [43].

DNA oxidation and repair

ROS-mediated oxidative injury can result in DNA modifications, including base alterations, single and double strand breaks, sister chromatid exchanges, and DNA–protein cross-links [67,68]. The hydroxyl radical plays a major role in DNA oxidation. Because the copper ion, through the Fenton reaction,

participates in the attachment of DNA to the nuclear matrix, it is likely that ˙OH is formed in close proximity to the DNA target [69]. Additionally, ROS attack of DNA can lead to the production of more than 20 oxidized base adducts [70,71], changes that are more subtle and require close analysis of DNA molecules to determine alterations. DNA bases may also be modified by neurotoxic markers of lipid peroxidation including 4-hydroxynonenal and acrolein, leading to formation of bulky exocyclic adducts. Formation of modified DNA bases could result in alterations in replication of DNA or inappropriate base pairing producing mutations that could lead to altered protein synthesis. Indeed, it has been reported that ˙OH reacts with purines to form mutagenic 8-hydroxypurine and putatively nonmutagenic formamidopyrimidine lesions. The formamidopyrimidine lesions inhibit DNA synthesis, likely modulating the mutagenic potential of the 8-hydroxypurine lesions, which would suggest that the ratio of these oxidized bases is biologically important [72–74]. Because it has the lowest oxidation potential of the four DNA bases, guanine is the most readily oxidized base through free radical attack of C8 leading to the formation of 8OHG under elevated oxygen tension and 2,6-diamino-4-hydroxy-5-formamidopyrimidine (fapyguanine) under conditions of reduced oxygen tension [68,70,75]. Because of the ease of oxidation and the presence of oxygen, 8OHG is the most commonly studied marker of DNA oxidation and is measured using either enzymatic digestion of DNA and high-pressure liquid chromatography or acid hydrolysis of DNA followed by derivatization and analysis using gas chromatography. Previous studies show that 8OHG is increased in the aging brain [76–78]. Although present at lower absolute concentrations compared to 8OHG, fapyguanine is of importance in that it is formed from radical attack of C8 of guanine followed by opening of the ring under conditions of low oxygen tension. Similar adducts, 8-hydroxyadenine and fapyadenine, are formed during radical attack of C8 of adenine. Measurement of both adducts for a particular base provides information regarding the conditions under which adducts were formed.

mtDNA oxidation, especially 8OHG, is of a much greater magnitude than nDNA. nDNA gains some protection from histones and morphologically is not close to large oxidant generation. In contrast, mtDNA does not have protective histones, is in close proximity to oxidant generation, may not have a rich antioxidant system, and has relatively limited DNA repair capacity, which may account for some of its vulnerability to oxidation. The accumulation of oxidatively modified nDNA and mtDNA results from an imbalance between the rate of oxidation and DNA repair mechanisms.

To counteract the multiple factors causing DNA damage, three DNA repair pathways have evolved. The most prominent repair pathways are base excision repair (BER), nucleotide excision repair, and mismatch repair. BER is characterized by the excision of nucleic acid base residues in the free form that contain lesions including oxidative damage, alkylating adducts, and deamination products. Nucleotide excision repair removes damaged nucleotides as part of fragments up to 30 bases in length. Mismatch repair corrects single misrepaired nucleotides and smaller loops.

BER is the main pathway to repair oxidative DNA damage. The first step in BER is the removal of the damaged base by substrate-specific DNA glycosylases. These enzymes catalyze the hydrolysis of the N-glycosidic bond between the modified base and the sugar moiety to release the base and generate an abasic (AP) site and this site will be cleaved by an abasic lyase or abasic endonuclease [79]. Repair can then proceed through the short- or long-patch BER. The short patch involves the incorporation of a single nucleotide into the gap by DNA polymerase followed by strand ligation by DNA ligase. The long-patch BER involves incorporation of several nucleotides, typically 2 to 7, followed by cleavage of the resulting 5′ flap and ligation. Mitochondria possess independent BER machinery, the components of which are coded by nuclear genes [79].

Several DNA ligases that have both nuclear and mitochondrial forms have been identified. Mitochondrial (UNG1) and nuclear (UNG2) forms of uracil-DNA glycosylase are generated by alternative splicing and transcription from different positions in the UNG gene [80]. Similarly, nuclear and mitochondrial adenine-DNA glycosylases are encoded by alternatively spliced forms of the MYH gene [81]. The oxoguanine-DNA glycosylase (OGG) 1 is the primary enzyme for the repair of 8-oxoguanine (8-oxoG) in both nDNA and mtDNA [82,83]. Previous studies showed that OGG1-deficient mice exhibit only a moderately elevated spontaneous mutation rate in nonproliferative tissues. $OGG^{-/-}$ mice do not develop malignancies and show no marked pathological changes. These animals are viable; however, their cells, in particular liver cells, exhibit a higher steady-state level of endogenous 8-oxoG in their genomes [82,83]. In light of the fact that it was shown that $OGG^{-/-}$ mouse embryonic fibroblasts are not deficient in the repair of 8-oxoG in the actively transcribed DNA strand, it is likely that the increase in the cellular level of 8-oxoG may be localized to the nontranscribed DNA strand or regions of the genome that are not actively transcribed [84]. In humans two distinct OGG1 isoforms are present: α-OGG1, which localizes to the nucleus and mitochondria, and β-OGG1, which localizes only to mitochondria [85].

Nei-like homologs (NEILs) are a recently identified group of bifunctional DNA glycosylases, which are mammalian orthologs of the *Escherichia coli* MutM/Nei. Both NEIL1 and NEIL2 have a broad substrate specificity in vitro, including but not limited to 8-oxoG, 2,6-diamino-4-hydroxy-5-formamidopyrimidine, 5-hydroxycytosine, and 5-hydroxyuracil. NEILs are localized in the nucleus [86,87] as well as in the mitochondria [88].

The AP generated after the removal of the damaged base by DNA glycosylase is incised by abasic endonucleases/lyases such as exonuclease III in *E. coli* or APE1 in mammalian cells. APE1 has been localized to the nucleus, the cytoplasm, and the mitochondria, despite the lack of a classical mitochondrial targeting sequence [89–91]. APE2 is also partly localized to the mitochondria in HeLa cells and a putative mitochondrial targeting sequence was found in the APE2 gene [92].

The gap generated by the cleavage of the AP is filled in by DNA polymerases: Pol β or Pol δ/ε in the nucleus and Pol γ in the mitochondria. Pol γ is the only DNA polymerase identified so far in vertebrate mitochondria and functions both as the replicative and as the repair polymerase [93].

The final step in the BER pathway is ligation of the nick left behind by DNA polymerases. In the nucleus, ligase I participates in long-patch BER and ligase III participates in short-patch BER. The human ligase III gene also encodes a mitochondrial variant [94,95]. The localization of ligase III protein to mitochondria suggests, then, that this enzyme may perform the ligation step in mitochondrial BER. Age-related changes in BER have been intensively studied using several experimental approaches. Measuring the levels and kinetics of AP sites after damage of nDNA [96] showed that senescent human fibroblasts as well as leukocytes from old donors have higher basal level of AP sites than young cells. Upon treatment with H_2O_2 the level of AP sites rose faster in young cells than in old cells, suggesting a deficiency in DNA glycosylase activity [96]. Furthermore, a significant decrease in the mitochondrial incision activity of 8-oxoG-DNA glycosylase, uracil-DNA glycosylase, and the endonuclease III homolog was found in the brains of old mice, whereas smaller changes were observed in nuclear incision activity [97–99]. Similarly, a decline in cleavage activity was observed in mitochondrial and, to a lesser extent, in nuclear extracts from senescent human fibroblasts [100]. A different in vitro assay measuring the repair of the synthetic DNA substrate containing a single G:U mismatch showed a strong decline in BER activity in brain, liver, and germ-cell nuclear extracts of old mice [101]. Germ-cell extracts from old mice were found to contain reduced levels of APE1, and supplementation with purified recombinant enzyme restored the activity to a level similar to that of the young animals [101]. Reduced abundance of DNA polymerase β in brain extracts from mice and rats has also been reported [101–103]. In addition to altered enzyme activity, the mechanism for the age-related decline of BER may lie in an altered response to DNA damage. For example, the expression of DNA polymerase β and AP endonuclease was induced by DNA damage in young mice, whereas aged mice showed a lack of inducibility [104]. Furthermore, old mice and senescent human fibroblasts were deficient in the translocation of OGG and AP endonuclease into both their nuclei and their mitochondria [105,106].

The inactivation or malfunction of DNA repair mechanisms leads to the accumulation of damaged DNA that characterizes several pathological conditions, including AD.

DNA oxidation and accumulation in Alzheimer disease

Several groups have documented the accumulation of oxidative DNA damage during aging in the brain [107–109]. Because of the critical role of DNA in cellular function, oxidative damage to DNA may be one of the most important factors in neuron degeneration in AD. Lu et al. [23] found that oxidative DNA damage accumulates preferentially in the promoter regions of several genes involved in synaptic plasticity, vesicular transport, and mitochondrial function. A differential accumulation of oxidative DNA lesions among brain regions has also been reported, suggesting that some areas of the brain are more prone to oxidative DNA modifications than others [110,111].

A substantial body of evidence indicates that oxidative DNA damage is a feature of AD in the brain as well as in peripheral tissues. Higher concentrations of oxidized pyridines and purines were detected in lymphocytes and leukocytes of AD patients compared with controls [6,112–114], and increased levels of 8-oxoG were observed in DNA from ventricular CSF of AD patients [115]. Recent findings indicate increased oxidative damage in leukocytes [6] and brain tissue [17] of subjects with MCI, which suggests that DNA oxidation may constitute an early event in the progression of AD, before cytopathological alterations, and may contribute to the pathogenesis of the disease.

Although DNA damage is elevated in both nDNA and mtDNA, the latter may be more easily oxidized than nDNA due to its proximity to free radical species. Previous studies show that there is more 8-hydroxy-2′-deoxyguanosine in mtDNA than in nDNA and there is a significant increase in aged subjects compared to younger subjects [116,117]. More recently, Wang and collaborators [118] reported that the levels of multiple oxidized bases in AD brain specimens were significantly higher in frontal, parietal, and temporal lobes compared to control subjects and that mtDNA had approximately 10-fold higher levels of oxidized bases than nDNA. These data are consistent with higher levels of oxidative stress in mitochondria. Without efficient repair ability in the brain in AD, mutations in nDNA and mtDNA may result in neuron death through defects in oxidative phosphorylation [25,115,119].

Additionally, a higher incidence of single- and double-strand breaks and alkali-labile DNA lesions has been detected in the AD cerebral cortex [120–122]. On the other hand, both increased and unaltered 8-hydroxy-2′-deoxyguanosine levels have been reported in AD brain nucleus samples [119,123,124]. A complex study has reported augmented degrees of some other (8-OH-adenine, 8-OH-guanine, and thymine glycol), but not all oxidized bases in the AD cerebral cortex [125]. The amounts of multiple oxidized bases of both nDNA and mtDNA were greater in the frontal, parietal, and temporal lobes in AD compared with the controls [118]; and, as expected, DNA from the temporal lobe displayed the most oxidative damage, whereas the cerebellum was only slightly affected, and mtDNA had 10-fold higher levels of oxidized bases. Increased mtDNA damage in AD is also associated with reduced mtDNA content, cytochrome oxidase protein levels [126], heteroplasmic mtDNA control-region mutations, and a reduction in the mtDNA L-strand transcript and in the mtDNA/nDNA ratio [127].

The accumulation of DNA damage in AD patients poses the question of whether the disease is accompanied by a deficiency in DNA repair. Several groups have tested this hypothesis, with inconclusive results. Some studies confirmed a decreased DNA repair efficiency [128–130], whereas others did not [131,132].

RNA oxidation and repair

Recent progress in genetics reveals an expanding universe of RNA beyond its classical function as intermediate for protein synthesis in the "central dogma" that describes transcription from DNA to messenger RNA (mRNA) and translation from mRNA to proteins. It is now evident that only a minority of genetic transcripts (2–3% in the human) code for proteins. Noncoding RNA (ncRNA), rather than being "junk," not only

has structural and catalytic functions but also plays a critical role in regulating the timing and rate of gene expression [133–135]. Of particular interest, the complexity of an organism is poorly correlated with its number of protein-coding genes, but highly correlated with its number of ncRNAs [136]. Furthermore, the increasing variety of ncRNAs being identified in the CNS suggests a strong connection between the biogenesis, dynamics of action, and combinational regulatory potential of ncRNAs and the complexity of the CNS [134,135]. Therefore, further advances in studies on the mechanisms and consequences of RNA damage and its surveillance may have a significant impact on the understanding of the pathophysiology of currently unresolved complex diseases including neurodegenerative and psychiatric diseases [134,136,137].

Because RNA is mostly single-stranded and its bases are not protected by hydrogen bonding and probably less protected by specific proteins, RNA may be more susceptible to oxidative insults than DNA [9,138,139]. This is a reasonable proposition given also the relative cellular abundance of RNA and the subcellular distribution of RNA that locates in the vicinity of the source of ROS, mitochondria [9]. Indeed, greater oxidation in RNA than in DNA was demonstrated in several experimental studies on isolated DNA and RNA [140] as well as on non-neuronal cell lines and tissue, namely, human leukocytes [141], human skin fibroblasts [142], and human lung epithelial cells [143].

Although more than 20 different types of oxidatively altered purine and pyrimidine bases have been detected in nucleic acids [144–147], guanine is the most reactive of the nucleic acid bases [148]. Therefore, the oxidized base 8OHG is the most abundant among the oxidized bases [138]. The 8OHG-containing nucleoside can be formed in RNA by direct oxidation of the base and also by the incorporation of the oxidized base from the cytosolic pool into RNA through the normal action of RNA polymerase [146,148]. Not only 8OHG but also 8-hydroxyadenosine, 5-hydroxycytidine, and 5-hydroxyuridine have been identified in oxidized RNA [148], which may have altered pairing capacity and thus be at the origin of erroneous production. Indeed, 8OHG can pair with both adenine and cytosine, and thus the oxidized RNA compromises the accuracy of translation [146,149].

The biological consequences of oxidatively damaged mRNA species have been investigated in vitro by expressing them in cell lines. Oxidized mRNAs lead to loss of normal protein level and protein function and potentially produce defective proteins leading to protein aggregation, a common feature of neurodegenerative diseases [150]. Recently it has been shown that the translation of oxidized mRNA in cell lines causes the accumulation of short polypeptides, which is a result of premature termination of the translation process of the oxidized mRNA and/or the proteolytic degradation of the modified protein containing the translation errors due to the oxidized mRNA [151]. Additionally, it has also been shown that the oxidative damage to E. coli 16S rRNA results in the formation of short complementary DNA (cDNA) [152]. Also the biological consequences of ribosomal oxidation have been investigated in vitro using a translation assay with oxidized ribosomes from rabbit

reticulocytes and showed a significant reduction of protein synthesis [57]. Previous studies demonstrate that the brains of subjects with AD and MCI present ribosomal dysfunction associated with oxidative RNA damage [14,153]. Isolated polyribosome complexes from AD and MCI brains show decreased rate of protein synthesis without alteration in the polyribosome content. Decreased rRNA and transfer RNA (tRNA) levels and increased 8OHG in the total RNA pool, especially in rRNA, are accompanied by the ribosomal dysfunction, whereas there is no alteration in the level of initiation factors [14]. These findings have indicated that RNA oxidation has detrimental effects on cellular function whether the damaged RNA species are coding for proteins (mRNA) or performing translation (rRNA and tRNA). Recently, Shan and collaborators [154] used cortical primary dissociated cultures to investigate the relationship between RNA oxidation and neuronal degeneration induced by various oxidative insults such as H_2O_2, glutamate, and amyloid-β peptide. The authors observed that RNA oxidation was an early event far preceding cell death, not merely a consequence of dying cells. RNA oxidation occurred primarily in a distinct group of neurons that died later. Identification of oxidized RNA species revealed that significant amounts of mRNAs were oxidized and that some mRNA species were more susceptible to oxidative damage. Consequently, the levels of proteins corresponding to the oxidized mRNA species were significantly decreased [154]. It is noteworthy that studies on some anti-cancer agents have shown that RNA damage can lead to cell-cycle arrest and cell death, much as DNA damage does. RNA damage may cause cell death via involvement of a p53-dependent mechanism associated with inhibition of protein synthesis or a p53-independent mechanism different from inhibition of protein synthesis [155].

Degradation of RNA plays a central role in RNA metabolism and damaged RNA can be removed through degradation by ribonucleases (RNases), but selective degradation activity for oxidized RNA has not been established for known RNases [139,156]. Oxidative stress induces cytoplasmic mRNA processing bodies (P-bodies), the site of active degradation of mRNA [157]. In contrast to mRNAs with rapid turnover, stable RNAs, consisting primarily of rRNAs and tRNAs and encompassing as much as 98% of total cellular RNA, may be protected against RNase action by tertiary structure, assembly into a ribonucleoprotein complex, or even blocking the RNA's 3′ terminus [156].

Until recently, it has been considered that damaged RNA may be only degraded rather than repaired. However, Aas et al. [158] have suggested that the cells have at least one specific mechanism to repair RNA damage, indicating that cells may have a greater investment in the protection of RNA than previously suspected [138,155,159]. Indeed, alkylation damage in RNA is repaired by the enzyme AlkB in E. coli and the related protein in humans [158]. AlkB and its homolog hABH3 cause hydroxylation of the methyl group on damaged RNA bases and thus directly reverse alkylation damage. AlkB and hABH3, but not hABH2, repair RNA, as AlkB and hABH3 prefer single-stranded nucleic acids, whereas hABH2 acts more efficiently on double-stranded DNA [158].

Because oxidation of nucleotides can occur in the cellular nucleotide pool and the oxidized nucleotide can be incorporated

into DNA and RNA, the mechanism avoiding such incorporation of the oxidized nucleotide is involved in coping with nucleic acid damage [138,139]. MutT protein in *E. coli* and its mammalian homolog MutT homolog 1 (MTH1) and Nudix type 5 (NUDT5) proteins participate in this error-avoiding mechanism by hydrolyzing the oxidized nucleoside diphosphates and/or triphosphates to the monophosphates [146,149,160]. Indeed, the increase in the production of erroneous proteins by oxidative damage is 28-fold over the wild-type cells in *E. coli* MutT-deficient cells, which is reduced to 1.2- or 1.4-fold by the expression of MTH1 or NUDT5, respectively [146]. Correspondingly, MTH1 deficiency augments the RNA oxidation induced by kainic acid treatment in MTH1-null mouse [161]. An increased expression of human MTH1 in the vulnerable neuronal populations has been demonstrated in the postmortem brains of AD [162], which may indicate a compensatory up-regulation of the MTH1 against oxidative stress [160]. In addition to the degradation activity of MTH1 and NUDT5, several enzymes involved in nucleotide metabolism show a discriminator activity against the oxidized nucleotides. Guanylate kinase, an enzyme that converts GMP to GDP, is inactive on 8-OH-GMP, whereas nucleotide diphosphate kinase, an enzyme that converts GDP to GTP, fails to show such discriminating function [163]. Similarly, ribonucleotide reductase, an enzyme that catalyzes reduction of four naturally occurring ribonucleoside diphosphates, is inactive on conversion of 8-OH-GDP to 8-OH-dGDP, which avoids incorporation of the oxidized nucleotide into DNA synthesis [163]. The final gatekeeper discriminating the oxidized nucleotide from normal nucleotide is RNA polymerase, which incorporates 8-OH-GTP into RNA at a much lower rate compared to the normal GTP incorporation [139,149].

Then, one important question is whether cells have machineries against oxidatively damaged nucleotides that are contained in RNA, because RNA can be directly oxidized even if the incorporation of oxidized nucleotides into RNA is blocked strictly. Recently, proteins that bind specifically to 8OHG-containing RNA have been reported, namely, *E. coli* polynucleotide phosphorylase (PNP) protein and human PNP [164,165] as well as human Y-box-binding protein 1 (YB-1) [166]. The binding of the specific protein likely makes the 8OHG-containing RNA resistant to nuclease degradation [164]. However, it has been proposed that these proteins may recognize and discriminate the oxidized RNA molecule from normal ones, thus contributing to the fidelity of translation in cells by sequestrating the damaged RNA from the translational machinery [164,165]. The human PNP protein binds to 8OHG-containing RNA preferentially and cellular amounts of human PNP protein decrease rapidly by exposure to agents inducing oxidative stress, whereas amounts of other proteins in the cells do not change after the treatments [166]. Recently, human YB-1 is demonstrated to be a component of P-bodies, where active degradation of mRNA occurs. YB-1 is translocated from P-bodies to stress granules during oxidative stress, which suggests a dynamic link between P-bodies and stress granules under oxidative stress [167].

It is possible that the RNA quality control mechanisms are defective or inefficient in AD as well as in other neurodegenerative diseases. Further elucidation of the mechanisms of repair or avoidance of RNA damage and their potential role in preventing human diseases might provide new approaches to a number of unresolved issues of life science, although it has not been the major focus in investigation for a long period [9,138].

RNA oxidation and accumulation in Alzheimer disease

Disruption of transcriptional or translational fidelity in neurons leads to the accumulation of aberrant or misfolded proteins and neuronal death [168,169], which suggests a role for RNA damage in the underlying mechanisms of neurological diseases. As previously discussed, oxidative damage to DNA has been well studied and several classes of products are identified. However, many fewer studies have focused on oxidative damage to RNA and only several kinds of oxidatively modified bases in RNA have been previously reported [144,170].

Shan et al. [150] used Northwestern blotting with a monoclonal anti-8OHG antibody to isolate and identify oxidized RNA species and showed that significant amount of poly(A)$^+$ mRNA species were oxidized in AD brain. The oxidation to mRNA was further confirmed by cDNA synthesis and Southern blotting of the immunoprecipitated mRNA species. Densitometric analysis of the Southern blot results revealed that 30–70% of the mRNAs from AD frontal cortices were oxidized, whereas only 2% of the mRNAs were oxidized in age-matched normal controls [171]. Honda et al. [57] and Ding et al. [14] reported that rRNA, extremely abundant in neurons, contained 8OHG in AD brain. Remarkably, rRNA showed higher binding capacity to redox-active iron than tRNA, and consequently the oxidation of rRNA by the Fenton reaction formed 13 times more 8OHG than tRNA [57]. Of note, both immunocytochemical studies [9,12] and biochemical studies [14,150] revealed that the regional distribution of RNA oxidation in the brain was consistent with the selective neuronal and vascular wall cells vulnerability. There were increased levels of 8OHG in the hippocampus and cerebral neocortex in AD, whereas no alteration in the 8OHG level was found in the cerebellum in AD compared with controls [9,12,14,150]. Immunocytochemical approaches further enabled confirmation that the oxidized RNAs were localized predominantly in neuronal and vascular wall (especially endothelial) cells compared with glial cells [10,58,172]. In addition, coexistence of RNA oxidation with mtDNA overproliferation and deletion in a select population of vulnerable neurons and vascular wall cells was demonstrated [173,174].

There is a considerable amount of evidence supporting early involvement of RNA oxidation in the pathological cascade of neurodegeneration, especially in AD. Namely, RNA oxidation has been observed in postmortem brains of cases with early stage AD [10,12], a presymptomatic case with familial AD mutation [58], Down syndrome cases with early stage AD pathology [175], and subjects with MCI [14,153]. Furthermore, there are increased levels of oxidized RNA in CSF in cases with shorter duration of AD as well as in AD cases with higher scores in a cognitive scale. The early involvement of RNA oxidation in disease pathophysiology suggests that oxidized RNA has a key role in neuronal degeneration and death and is not just an epiphenomenon. Oxidized RNAs are likely degraded more rapidly compared to the
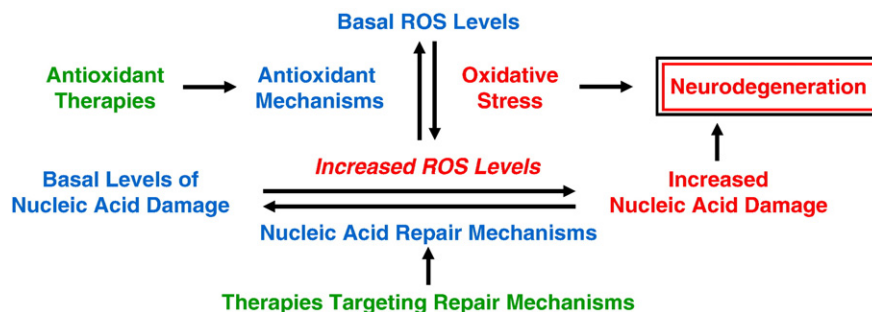
Fig. 1. Nucleic acid oxidation and repair. ROS are produced by normal metabolism. However, during aging and in age-related neurodegenerative diseases the levels of ROS increase and the repair mechanisms decline, leading to the oxidative lesion of several biomolecules including nuclear and mtDNA and RNA. The accumulation of damaged nucleic acids may lead to the development of neurodegenerative conditions such as AD. Therapeutic strategies aimed at increasing the efficiency of repair mechanisms or fighting oxidative stress could be useful in the arrest of disease progression.

other types of oxidatively modified macromolecules, whereas protein carbonyls, lipid peroxidation products, and glycoxidation products are stable at the site of generation because of a cross-link formation resistant against degradation. Therefore, when we detect the markers by in situ approach on the tissues affected by disease, protein carbonyls, lipid peroxidation products, or glycoxidation products indicate a "history" of oxidative damage, whereas RNA oxidation reflects a "steady-state balance" of the damage [9,176]. In accordance with this concept, protein carbonyls and lipid peroxidation products such as 4-hydroxynonenal and F2-isoprostane have been demonstrated in neurons of AD brains with no predilection for pathology burden [8,47], possibly reflecting the damage all through early and advanced stages of neurodegeneration ("history" of the damage). These observations contrast remarkably with the pattern of the RNA oxidation, a "steady-state" marker, which is prominent in neurons without pathology [10,175].

Conclusion

Oxidative damage of nucleic acids plays an important role in aging and neurodegenerative conditions such as AD. Indeed, AD is characterized by an accumulation of damaged DNA (nuclear and mitochondrial) and RNA, which is positively correlated with the increased levels of oxidative stress and, possibly, the decreased activity of repair mechanisms that occur in this pathology (Fig. 1). Indeed, evidence from the literature establishes that oxidative damage is an early event in the pathogenesis of AD, which can serve as a therapeutic target to slow the progression or perhaps the onset of the disease. Furthermore, particular emphasis should be placed on the early stage involvement of DNA and RNA oxidation in AD, which suggests a primary role for nucleic acid oxidation in the process of neurodegeneration. The proteins involved in nucleic acid repair pathways could represent important therapeutic targets. Indeed, some of these proteins are already being studied for cancer treatment. For example, the inhibition of various proteins in the DNA repair pathways sensitizes cancer cells to DNA-damaging agents such as chemotherapy and/or radiation [177]. In neurodegenerative conditions the goal of the therapeutic strategies must be the opposite, i.e., increase the activity of proteins involved in the repair pathway. However, before the design of new therapeutic

strategies, it is important to answer the question of how neurodegeneration is linked to deficiencies in specific DNA and/or RNA repair pathways. Although intensive research in the area of DNA repair has led to rapid advances in understanding DNA repair mechanisms and the possible role of DNA repair in preventing cellular dysfunction and human disease, the specific role of DNA repair in neuronal cells and in the central nervous system is still unclear. Multidisciplinary efforts, encompassing molecular biology, physiology, stem cell biology, and imaging and clinical/translational approaches, are being combined to understand how genome instability and DNA repair contribute to neurodegenerative conditions (for more information, see [178]). Bioinformatics can be used to analyze single-nucleotide polymorphisms and identify gene constellations represented in patients with specific clinical syndromes (for more information, see [178]). Eventually, this approach might be developed as a prognostic or diagnostic tool for neurodegenerative disease.

References

[1] Mattson, M. P. Pathways towards and away from Alzheimer's disease. *Nature* **430:**631–639; 2004.

[2] Perry, G.; Rizzuto, N.; Autilio-Gambetti, L.; Gambetti, P. Paired helical filaments from Alzheimer disease patients contain cytoskeletal components. *Proc. Natl. Acad. Sci. USA* **82:**3916–3920; 1985.

[3] Gibson, G. E.; Sheu, K. F.; Blass, J. P.; Baker, A.; Carlson, K. C.; Harding, B.; Perrino, P. Reduced activities of thiamine-dependent enzymes in the brains and peripheral tissues of patients with Alzheimer's disease. *Arch. Neurol.* **45:**836–840; 1988.

[4] Perry, G.; Castellani, R. J.; Smith, M. A.; Harris, P. L.; Kubat, Z.; Ghanbari, K.; Jones, P. K.; Cordone, G.; Tabaton, M.; Wolozin, B.; Ghanbari, H. Oxidative damage in the olfactory system in Alzheimer's disease. *Acta Neuropathol. (Berlin)* **106:**552–556; 2003.

[5] Ghanbari, H. A.; Ghanbari, K.; Harris, P. L.; Jones, P. K.; Kubat, Z.; Castellani, R. J.; Wolozin, B. L.; Smith, M. A.; Perry, G. Oxidative damage in cultured human olfactory neurons from Alzheimer's disease patients. *Aging Cell* **3:**41–44; 2004.

[6] Migliore, L.; Fontana, I.; Trippi, F.; Colognato, R.; Coppede, F.; Tognoni, G.; Nucciarone, B.; Siciliano, G. Oxidative DNA damage in peripheral leukocytes of mild cognitive impairment and AD patients. *Neurobiol. Aging* **26:**567–573; 2005.

[7] Smith, M. A.; Sayre, L. M.; Vitek, M. P.; Monnier, V. M.; Perry, G. Early AGEing and Alzheimer's. *Nature* **374:**316; 1995.

[8] Sayre, L. M.; Zelasko, D. A.; Harris, P. L.; Perry, G.; Salomon, R. G.; Smith, M. A. 4-Hydroxynonenal-derived advanced lipid peroxidation end products are increased in Alzheimer's disease. *J. Neurochem.* **68:**2092–2097; 1997.

[9] Nunomura, A.; Perry, G.; Pappolla, M. A.; Wade, R.; Hirai, K.; Chiba, S.; Smith, M. A. RNA oxidation is a prominent feature of vulnerable neurons in Alzheimer's disease. *J. Neurosci.* **19:**1959–1964; 1999.

[10] Nunomura, A.; Perry, G.; Aliev, G.; Hirai, K.; Takeda, A.; Balraj, E. K.; Jones, P. K.; Ghanbari, H.; Wataya, T.; Shimohama, S.; Chiba, S.; Atwood, C. S.; Petersen, R. B.; Smith, M. A. Oxidative damage is the earliest event in Alzheimer disease. *J. Neuropathol. Exp. Neurol.* **60:**759–767; 2001.

[11] Sayre, L. M.; Smith, M. A.; Perry, G. Chemistry and biochemistry of oxidative stress in neurodegenerative disease. *Curr. Med. Chem.* **8:**721–738; 2001.

[12] Nunomura, A.; Castellani, R. J.; Zhu, X.; Moreira, P. I.; Perry, G.; Smith, M. A. Involvement of oxidative stress in Alzheimer disease. *J. Neuropathol. Exp. Neurol.* **65:**631–641; 2006.

[13] Butterfield, D. A.; Poon, H. F.; St Clair, D.; Keller, J. N.; Pierce, W. M.; Klein, J. B.; Markesbery, W. R. Redox proteomics identification of oxidatively modified hippocampal proteins in mild cognitive impairment: insights into the development of Alzheimer's disease. *Neurobiol. Dis.* **22:**223–232; 2006.

[14] Ding, Q.; Markesbery, W. R.; Chen, Q.; Li, F.; Keller, J. N. Ribosome dysfunction is an early event in Alzheimer's disease. *J. Neurosci.* **25:**9171–9175; 2005.

[15] Keller, J. N.; Schmitt, F. A.; Scheff, S. W.; Ding, Q.; Chen, Q.; Butterfield, D. A.; Markesbery, W. R. Evidence of increased oxidative damage in subjects with mild cognitive impairment. *Neurology* **64:**1152–1156; 2005.

[16] Markesbery, W. R.; Kryscio, R. J.; Lovell, M. A.; Morrow, J. D. Lipid peroxidation is an early event in the brain in amnestic mild cognitive impairment. *Ann. Neurol.* **58:**730–735; 2005.

[17] Wang, J.; Markesbery, W. R.; Lovell, M. A. Increased oxidative damage in nuclear and mitochondrial DNA in mild cognitive impairment. *J. Neurochem.* **96:**825–832; 2006.

[18] Ischiropoulos, H.; Beckman, J. S. Oxidative stress and nitration in neurodegeneration: cause, effect, or association? *J. Clin. Invest.* **111:**163–169; 2003.

[19] Andersen, J. K. Oxidative stress in neurodegeneration: cause or consequence? *Nat. Med.* **10:**S18–S25 (Suppl.); 2004.

[20] Barnham, K. J.; Masters, C. L.; Bush, A. I. Neurodegenerative diseases and oxidative stress. *Nat. Rev. Drug Discovery* **3:**205–214; 2004.

[21] Ames, B. N.; Shigenaga, M. K.; Hagen, T. M. Oxidants, antioxidants, and the degenerative diseases of aging. *Proc. Natl. Acad. Sci. USA* **90:**7915–7922; 1993.

[22] Melov, S. Modeling mitochondrial function in aging neurons. *Trends Neurosci.* **27:**601–606; 2004.

[23] Lu, T.; Pan, Y.; Kao, S. Y.; Li, C.; Kohane, I.; Chan, J.; Yankner, B. A. Gene regulation and DNA damage in the ageing human brain. *Nature* **429:**883–891; 2004.

[24] Beal, M. F. Mitochondria take center stage in aging and neurodegeneration. *Ann. Neurol.* **58:**495–505; 2005.

[25] Wallace, D. C. Mitochondrial diseases in man and mouse. *Science* **283:**1482–1488; 1999.

[26] Smith, M. A.; Taneda, S.; Richey, P. L.; Miyata, S.; Yan, S. D.; Stern, D.; Sayre, L. M.; Monnier, V. M.; Perry, G. Advanced Maillard reaction end products are associated with Alzheimer disease pathology. *Proc. Natl. Acad. Sci. USA* **91:**5710–5714; 1994.

[27] Smith, M. A.; Harris, P. L.; Sayre, L. M.; Perry, G. Iron accumulation in Alzheimer disease is a source of redox-generated free radicals. *Proc. Natl. Acad. Sci. USA* **94:**9866–9868; 1997.

[28] Ferri, K. F.; Kroemer, G. Organelle-specific initiation of cell death pathways. *Nat. Cell Biol.* **3:**E255–E263; 2001.

[29] Pereira, C.; Ferreiro, E.; Cardoso, S. M.; de Oliveira, C. R. Cell degeneration induced by amyloid-beta peptides: implications for Alzheimer's disease. *J. Mol. Neurosci.* **23:**97–104; 2004.

[30] Zhu, X.; Wang, Y.; Ogawa, O.; Lee, H. G.; Raina, A. K.; Siedlak, S. L.; Harris, P. L.; Fujioka, H.; Shimohama, S.; Tabaton, M.; Atwood, C. S.; Petersen, R. B.; Perry, G.; Smith, M. A. Neuroprotective properties of Bcl-w in Alzheimer disease. *J. Neurochem.* **89:**1233–1240; 2004.

[31] Raina, A. K.; Hochman, A.; Zhu, X.; Rottkamp, C. A.; Nunomura, A.; Siedlak, S. L.; Boux, H.; Castellani, R. J.; Perry, G.; Smith, M. A. Abortive apoptosis in Alzheimer's disease. *Acta Neuropathol. (Berlin)* **101:**305–310; 2001.

[32] Perry, G.; Nunomura, A.; Lucassen, P.; Lassmann, H.; Smith, M. A. Apoptosis and Alzheimer's disease. *Science* **282:**1268–1269; 1998.

[33] Zhu, X.; Raina, A. K.; Perry, G.; Smith, M. A. Apoptosis in Alzheimer disease: a mathematical improbability. *Curr. Alzheimer Res.* **3:**393–396; 2006.

[34] Hirai, K.; Aliev, G.; Nunomura, A.; Fujioka, H.; Russell, R. L.; Atwood, C. S.; Johnson, A. B.; Kress, Y.; Vinters, H. V.; Tabaton, M.; Shimohama, S.; Cash, A. D.; Siedlak, S. L.; Harris, P. L.; Jones, P. K.; Petersen, R. B.; Perry, G.; Smith, M. A. Mitochondrial abnormalities in Alzheimer's disease. *J. Neurosci.* **21:**3017–3023; 2001.

[35] Moreira, P. I.; Siedlak, S. L.; Wang, X.; Santos, M. S.; Oliveira, C. R.; Tabaton, M.; Nunomura, A.; Szweda, L. I.; Aliev, G.; Smith, M. A.; Zhu, X.; Perry, G. Autophagocytosis of mitochondria is prominent in Alzheimer disease. *J. Neuropathol. Exp. Neurol.* **66:**525–532; 2007.

[36] Cash, A. D.; Aliev, G.; Siedlak, S. L.; Nunomura, A.; Fujioka, H.; Zhu, X.; Raina, A. K.; Vinters, H. V.; Tabaton, M.; Johnson, A. B.; Paula-Barbosa, M.; Avila, J.; Jones, P. K.; Castellani, R. J.; Smith, M. A.; Perry, G. Microtubule reduction in Alzheimer's disease and aging is independent of tau filament formation. *Am. J. Pathol.* **162:**1623–1627; 2003.

[37] Pappolla, M. A.; Chyan, Y. J.; Omar, R. A.; Hsiao, K.; Perry, G.; Smith, M. A.; Bozner, P. Evidence of oxidative stress and in vivo neurotoxicity of beta-amyloid in a transgenic mouse model of Alzheimer's disease: a chronic oxidative paradigm for testing antioxidant therapies in vivo. *Am. J. Pathol.* **152:**871–877; 1998.

[38] Smith, M. A.; Hirai, K.; Hsiao, K.; Pappolla, M. A.; Harris, P. L.; Siedlak, S. L.; Tabaton, M.; Perry, G. Amyloid-beta deposition in Alzheimer transgenic mice is associated with oxidative stress. *J. Neurochem.* **70:**2212–2215; 1998.

[39] Aliev, G.; Smith, M. A.; de la Torre, J. C.; Perry, G. Mitochondria as a primary target for vascular hypoperfusion and oxidative stress in Alzheimer's disease. *Mitochondrion* **4:**649–663; 2004.

[40] Aliyev, A.; Chen, S. G.; Seyidova, D.; Smith, M. A.; Perry, G.; de la Torre, J.; Aliev, G. Mitochondria DNA deletions in atherosclerotic hypoperfused brain microvessels as a primary target for the development of Alzheimer's disease. *J. Neurol. Sci.* **229–230:**285–292; 2005.

[41] Moreira, P. I.; Santos, M. S.; Oliveira, C. R. Alzheimer's disease: a lesson from mitochondrial dysfunction. *Antioxid. Redox Signal.* **9:**1621–1630; 2007.

[42] Moreira, P. I.; Cardoso, S. M.; Santos, M. S.; Oliveira, C. R. The key role of mitochondria in Alzheimer's disease. *J. Alzheimers Dis.* **9:**101–110; 2006.

[43] Zhu, X.; Su, B.; Wang, X.; Smith, M. A.; Perry, G. Causes of oxidative stress in Alzheimer disease. *Cell. Mol. Life Sci.* **64:**2202–2210; 2007.

[44] Connor, J. R.; Milward, E. A.; Moalem, S.; Sampietro, M.; Boyer, P.; Percy, M. E.; Vergani, C.; Scott, R. J.; Chorney, M. Is hemochromatosis a risk factor for Alzheimer's disease? *J. Alzheimers Dis.* **3:**471–477; 2001.

[45] Lovell, M. A.; Robertson, J. D.; Teesdale, W. J.; Campbell, J. L.; Markesbery, W. R. Copper, iron and zinc in Alzheimer's disease senile plaques. *J. Neurol. Sci.* **158:**47–52; 1998.

[46] Sayre, L. M.; Perry, G.; Harris, P. L.; Liu, Y.; Schubert, K. A.; Smith, M. A. In situ oxidative catalysis by neurofibrillary tangles and senile plaques in Alzheimer's disease: a central role for bound transition metals. *J. Neurochem.* **74:**270–279; 2000.

[47] Smith, M. A.; Perry, G.; Richey, P. L.; Sayre, L. M.; Anderson, V. E.; Beal, M. F.; Kowall, N. Oxidative damage in Alzheimer's. *Nature* **382:**120–121; 1996.

[48] Atwood, C. S.; Perry, G.; Zeng, H.; Kato, Y.; Jones, W. D.; Ling, K. Q.; Huang, X.; Moir, R. D.; Wang, D.; Sayre, L. M.; Smith, M. A.; Chen, S. G.; Bush, A. I. Copper mediates dityrosine cross-linking of Alzheimer's amyloid-beta. *Biochemistry (Moscow)* **43:**560–568; 2004.

[49] Rogers, J. T.; Randall, J. D.; Cahill, C. M.; Eder, P. S.; Huang, X.; Gunshin, H.; Leiter, L.; McPhee, J.; Sarang, S. S.; Utsuki, T.; Greig, N. H.; Lahiri, D. K.; Tanzi, R. E.; Bush, A. I.; Giordano, T.; Gullans, S. R. An iron-responsive element type II in the 5′-untranslated region of the Alzheimer's amyloid precursor protein transcript. *J. Biol. Chem.* **277:**45518–45528; 2002.

[50] Yan, S. D.; Yan, S. F.; Chen, X.; Fu, J.; Chen, M.; Kuppusamy, P.; Smith, M. A.; Perry, G.; Godman, G. C.; Nawroth, P., et al. Non-enzymatically glycated tau in Alzheimer's disease induces neuronal oxidant stress resulting in cytokine gene expression and release of amyloid beta-peptide. *Nat. Med.* **1:**693–699; 1995.

[51] Tamagno, E.; Parola, M.; Bardini, P.; Piccini, A.; Borghi, R.; Guglielmotto, M.; Santoro, G.; Davit, A.; Danni, O.; Smith, M. A.; Perry, G.; Tabaton, M. Beta-site APP cleaving enzyme up-regulation induced by 4-hydroxynonenal is mediated by stress-activated protein kinases pathways. *J. Neurochem.* **92:**628–636; 2005.

[52] Huang, X.; Moir, R. D.; Tanzi, R. E.; Bush, A. I.; Rogers, J. T. Redox-active metals, oxidative stress, and Alzheimer's disease pathology. *Ann. N. Y. Acad. Sci.* **1012:**153–163; 2004.

[53] Rottkamp, C. A.; Raina, A. K.; Zhu, X.; Gaier, E.; Bush, A. I.; Atwood, C. S.; Chevion, M.; Perry, G.; Smith, M. A. Redox-active iron mediates amyloid-beta toxicity. *Free Radic. Biol. Med.* **30:**447–450; 2001.

[54] Smith, M. A.; Kutty, R. K.; Richey, P. L.; Yan, S. D.; Stern, D.; Chader, G. J.; Wiggert, B.; Petersen, R. B.; Perry, G. Heme oxygenase-1 is associated with the neurofibrillary pathology of Alzheimer's disease. *Am. J. Pathol.* **145:**42–47; 1994.

[55] Premkumar, D. R.; Smith, M. A.; Richey, P. L.; Petersen, R. B.; Castellani, R.; Kutty, R. K.; Wiggert, B.; Perry, G.; Kalaria, R. N. Induction of heme oxygenase-1 mRNA and protein in neocortex and cerebral vessels in Alzheimer's disease. *J. Neurochem.* **65:**1399–1402; 1995.

[56] Keyse, S. M.; Tyrrell, R. M. Heme oxygenase is the major 32-kDa stress protein induced in human skin fibroblasts by UVA radiation, hydrogen peroxide, and sodium arsenite. *Proc. Natl. Acad. Sci. USA* **86:**99–103; 1989.

[57] Honda, K.; Smith, M. A.; Zhu, X.; Baus, D.; Merrick, W. C.; Tartakoff, A. M.; Hattier, T.; Harris, P. L.; Siedlak, S. L.; Fujioka, H.; Liu, Q.; Moreira, P. I.; Miller, F. P.; Nunomura, A.; Shimohama, S.; Perry, G. Ribosomal RNA in Alzheimer disease is oxidized by bound redox-active iron. *J. Biol. Chem.* **280:**20978–20986; 2005.

[58] Nunomura, A.; Chiba, S.; Lippa, C. F.; Cras, P.; Kalaria, R. N.; Takeda, A.; Honda, K.; Smith, M. A.; Perry, G. Neuronal RNA oxidation is a prominent feature of familial Alzheimer's disease. *Neurobiol. Dis.* **17:**108–113; 2004.

[59] Hayashi, T.; Shishido, N.; Nakayama, K.; Nunomura, A.; Smith, M. A.; Perry, G.; Nakamura, M. Lipid peroxidation and 4-hydroxy-2-nonenal formation by copper ion bound to amyloid-β peptide. *Free Radic. Biol. Med.* **43:**1552–1559; 2007.

[60] Nakamura, M.; Shishido, N.; Nunomura, A.; Smith, M. A.; Perry, G.; Hayashi, Y.; Nakayama, K.; Hayashi, T. Three histidine residues of amyloid-beta peptide control the redox activity of copper and iron. *Biochemistry (Moscow)* **46:**12737–12743; 2007.

[61] Huang, X.; Atwood, C. S.; Hartshorn, M. A.; Multhaup, G.; Goldstein, L. E.; Scarpa, R. C.; Cuajungco, M. P.; Gray, D. N.; Lim, J.; Moir, R. D.; Tanzi, R. E.; Bush, A. I. The A beta peptide of Alzheimer's disease directly produces hydrogen peroxide through metal ion reduction. *Biochemistry (Moscow)* **38:**7609–7616; 1999.

[62] Finefrock, A. E.; Bush, A. I.; Doraiswamy, P. M. Current status of metals as therapeutic targets in Alzheimer's disease. *J. Am. Geriatr. Soc.* **51:**1143–1148; 2003.

[63] Bayer, T. A.; Schafer, S.; Simons, A.; Kemmling, A.; Kamer, T.; Tepest, R.; Eckert, A.; Schussel, K.; Eikenberg, O.; Sturchler-Pierrat, C.; Abramowski, D.; Staufenbiel, M.; Multhaup, G. Dietary Cu stabilizes brain superoxide dismutase 1 activity and reduces amyloid Abeta production in APP23 transgenic mice. *Proc. Natl. Acad. Sci. USA* **100:**14187–14192; 2003.

[64] Sparks, D. L.; Schreurs, B. G. Trace amounts of copper in water induce beta-amyloid plaques and learning deficits in a rabbit model of Alzheimer's disease. *Proc. Natl. Acad. Sci. USA* **100:**11065–11069; 2003.

[65] Pajonk, F. G.; Kessler, H.; Supprian, T.; Hamzei, P.; Bach, D.; Schweickhardt, J.; Herrmann, W.; Obeid, R.; Simons, A.; Falkai, P.; Multhaup, G.; Bayer, T. A. Cognitive decline correlates with low plasma concentrations of copper in patients with mild to moderate Alzheimer's disease. *J. Alzheimers Dis.* **8:**23–27; 2005.

[66] Adlard, P. A.; Bush, A. I. Metals and Alzheimer's disease. *J. Alzheimers Dis.* **10:**145–163; 2006.

[67] Sohal, R. S.; Weindruch, R. Oxidative stress, caloric restriction, and aging. *Science* **273:**59–63; 1996.

[68] Markesbery, W. R.; Lovell, M. A. DNA oxidation in Alzheimer's disease. *Antioxid. Redox Signal.* **8:**2039–2045; 2006.

[69] Collins, A. R. Oxidative DNA damage, antioxidants, and cancer. *Bioessays* **21:**238–246; 1999.

[70] Steenken, S. Purine bases, nucleosides, and nucleotides: aqueous solution redox chemistry and transformation reactions of their radical cations and e- and OH adducts. *Chem. Rev.* **89:**503–520; 1989.

[71] Cooke, M. S.; Evans, M. D.; Dizdaroglu, M.; Lunec, J. Oxidative DNA damage: mechanisms, mutation, and disease. *FASEB J.* **17:**1195–1214; 2003.

[72] O'Connor, T. R.; Boiteux, S.; Laval, J. Ring-opened 7-methylguanine residues in DNA are a block to in vitro DNA synthesis. *Nucleic Acids Res.* **16:**5879–5894; 1988.

[73] Malins, D. C.; Hellstrom, K. E.; Anderson, K. M.; Johnson, P. M.; Vinson, M. A. Antioxidant-induced changes in oxidized DNA. *Proc. Natl. Acad. Sci. USA* **99:**5937–5941; 2002.

[74] Breimer, L. H. Molecular mechanisms of oxygen radical carcinogenesis and mutagenesis: the role of DNA base damage. *Mol. Carcinog.* **3:**188–197; 1990.

[75] Breen, A. P.; Murphy, J. A. Reactions of oxyl radicals with DNA. *Free Radic. Biol. Med.* **18:**1033–1077; 1995.

[76] Halliwell, B.; Dizdaroglu, M. The measurement of oxidative damage to DNA by HPLC and GC/MS techniques. *Free Radic. Res. Commun.* **16:**75–87; 1992.

[77] Herbert, K. E.; Evans, M. D.; Finnegan, M. T.; Farooq, S.; Mistry, N.; Podmore, I. D.; Farmer, P.; Lunec, J. A novel HPLC procedure for the analysis of 8-oxoguanine in DNA. *Free Radic. Biol. Med.* **20:**467–472; 1996.

[78] Dizdaroglu, M.; Jaruga, P.; Birincioglu, M.; Rodriguez, H. Free radical-induced damage to DNA: mechanisms and measurement. *Free Radic. Biol. Med.* **32:**1102–1115; 2002.

[79] Weissman, L.; de Souza-Pinto, N. C.; Stevnsner, T.; Bohr, V. A. DNA repair, mitochondria, and neurodegeneration. *Neuroscience* **145:**1318–1329; 2007.

[80] Nilsen, H.; Otterlei, M.; Haug, T.; Solum, K.; Nagelhus, T. A.; Skorpen, F.; Krokan, H. E. Nuclear and mitochondrial uracil-DNA glycosylases are generated by alternative splicing and transcription from different positions in the UNG gene. *Nucleic Acids Res.* **25:**750–755; 1997.

[81] Ohtsubo, T.; Nishioka, K.; Imaiso, Y.; Iwai, S.; Shimokawa, H.; Oda, H.; Fujiwara, T.; Nakabeppu, Y. Identification of human MutY homolog (hMYH) as a repair enzyme for 2-hydroxyadenine in DNA and detection of multiple forms of hMYH located in nuclei and mitochondria. *Nucleic Acids Res.* **28:**1355–1364; 2000.

[82] Klungland, A.; Rosewell, I.; Hollenbach, S.; Larsen, E.; Daly, G.; Epe, B.; Seeberg, E.; Lindahl, T.; Barnes, D. E. Accumulation of premutagenic DNA lesions in mice defective in removal of oxidative base damage. *Proc. Natl. Acad. Sci. USA* **96:**13300–13305; 1999.

[83] de Souza-Pinto, N. C.; Eide, L.; Hogue, B. A.; Thybo, T.; Stevnsner, T.; Seeberg, E.; Klungland, A.; Bohr, V. A. Repair of 8-oxodeoxyguanosine lesions in mitochondrial DNA depends on the oxoguanine DNA glycosylase (OGG1) gene and 8-oxoguanine accumulates in the mitochondrial DNA of OGG1-defective mice. *Cancer Res.* **61:**5378–5381; 2001.

[84] Le Page, F.; Klungland, A.; Barnes, D. E.; Sarasin, A.; Boiteux, S. Transcription coupled repair of 8-oxoguanine in murine cells: the ogg1 protein is required for repair in nontranscribed sequences but not in transcribed sequences. *Proc. Natl. Acad. Sci. USA* **97:**8397–8402; 2000.

[85] Nishioka, K.; Ohtsubo, T.; Oda, H.; Fujiwara, T.; Kang, D.; Sugimachi, K.; Nakabeppu, Y. Expression and differential intracellular localization of two major forms of human 8-oxoguanine DNA glycosylase encoded by alternatively spliced OGG1 mRNAs. *Mol. Biol. Cell* **10:**1637–1652; 1999.

[86] Morland, I.; Rolseth, V.; Luna, L.; Rognes, T.; Bjoras, M.; Seeberg, E. Human DNA glycosylases of the bacterial Fpg/MutM superfamily: an

alternative pathway for the repair of 8-oxoguanine and other oxidation products in DNA. *Nucleic Acids Res.* **30:**4926–4936; 2002.

[87] Hazra, T. K.; Izumi, T.; Boldogh, I.; Imhoff, B.; Kow, Y. W.; Jaruga, P.; Dizdaroglu, M.; Mitra, S. Identification and characterization of a human DNA glycosylase for repair of modified bases in oxidatively damaged DNA. *Proc. Natl. Acad. Sci. USA* **99:**3523–3528; 2002.

[88] Hu, J.; de Souza-Pinto, N. C.; Haraguchi, K.; Hogue, B. A.; Jaruga, P.; Greenberg, M. M.; Dizdaroglu, M.; Bohr, V. A. Repair of formamido-pyrimidines in DNA involves different glycosylases: role of the OGG1, NTH1, and NEIL1 enzymes. *J. Biol. Chem.* **280:**40544–40551; 2005.

[89] Rivkees, S. A.; Kelley, M. R. Expression of a multifunctional DNA repair enzyme gene, apurinic/apyrimidinic endonuclease (APE; Ref-1) in the suprachiasmatic, supraoptic and paraventricular nuclei. *Brain Res.* **666:**137–142; 1994.

[90] Wilson, T. M.; Rivkees, S. A.; Deutsch, W. A.; Kelley, M. R. Differential expression of the apurinic/apyrimidinic endonuclease (APE/ref-1) multi-functional DNA base excision repair gene during fetal development and in adult rat brain and testis. *Mutat. Res.* **362:**237–248; 1996.

[91] Fung, H.; Kow, Y. W.; Van Houten, B.; Taatjes, D. J.; Hatahet, Z.; Janssen, Y. M.; Vacek, P.; Faux, S. P.; Mossman, B. T. Asbestos increases mammalian AP-endonuclease gene expression, protein levels, and enzyme activity in mesothelial cells. *Cancer Res.* **58:**189–194; 1998.

[92] Tsuchimoto, D.; Sakai, Y.; Sakumi, K.; Nishioka, K.; Sasaki, M.; Fujiwara, T.; Nakabeppu, Y. Human APE2 protein is mostly localized in the nuclei and to some extent in the mitochondria, while nuclear APE2 is partly associated with proliferating cell nuclear antigen. *Nucleic Acids Res.* **29:**2349–2360; 2001.

[93] Kaguni, L. S. DNA polymerase gamma, the mitochondrial replicase. *Annu. Rev. Biochem.* **73:**293–320; 2004.

[94] Lakshmipathy, U.; Campbell, C. The human DNA ligase III gene encodes nuclear and mitochondrial proteins. *Mol. Cell. Biol.* **19:**3869–3876; 1999.

[95] Lakshmipathy, U.; Campbell, C. Mitochondrial DNA ligase III function is independent of Xrcc1. *Nucleic Acids Res.* **28:**3880–3886; 2000.

[96] Atamna, H.; Cheung, I.; Ames, B. N. A method for detecting abasic sites in living cells: age-dependent changes in base excision repair. *Proc. Natl. Acad. Sci. USA* **97:**686–691; 2000.

[97] Chen, D.; Cao, G.; Hastings, T.; Feng, Y.; Pei, W.; O'Horo, C.; Chen, J. Age-dependent decline of DNA repair activity for oxidative lesions in rat brain mitochondria. *J. Neurochem.* **81:**1273–1284; 2002.

[98] Imam, S. Z.; Karahalil, B.; Hogue, B. A.; Souza-Pinto, N. C.; Bohr, V. A. Mitochondrial and nuclear DNA-repair capacity of various brain regions in mouse is altered in an age-dependent manner. *Neurobiol. Aging* **27:**1129–1136; 2006.

[99] Wilson III, D. M.; Bohr, V. A. The mechanics of base excision repair, and its relationship to aging and disease. *DNA Repair* **6:**544–559; 2007.

[100] Shen, G. P.; Galick, H.; Inoue, M.; Wallace, S. S. Decline of nuclear and mitochondrial oxidative base excision repair activity in late passage human diploid fibroblasts. *DNA Repair* **2:**673–693; 2003.

[101] Intano, G. W.; McMahan, C. A.; McCarrey, J. R.; Walter, R. B.; McKenna, A. E.; Matsumoto, Y.; MacInnes, M. A.; Chen, D. J.; Walter, C. A. Base excision repair is limited by different proteins in male germ cell nuclear extracts prepared from young and old mice. *Mol. Cell. Biol.* **22:**2410–2418; 2002.

[102] Intano, G. W.; Cho, E. J.; McMahan, C. A.; Walter, C. A. Age-related base excision repair activity in mouse brain and liver nuclear extracts. *J. Gerontol. A Biol. Sci. Med. Sci.* **58:**205–211; 2003.

[103] Krishnan, S.; Chi, E. Y.; Wood, S. J.; Kendrick, B. S.; Li, C.; Garzon-Rodriguez, W.; Wypych, J.; Randolph, T. W.; Narhi, L. O.; Biere, A. L.; Citron, M.; Carpenter, J. F. Oxidative dimer formation is the critical rate-limiting step for Parkinson's disease alpha-synuclein fibrillogenesis. *Biochemistry (Moscow)* **42:**829–837; 2003.

[104] Cabelof, D. C.; Raffoul, J. J.; Ge, Y.; Van Remmen, H.; Matherly, L. H.; Heydari, A. R. Age-related loss of the DNA repair response following exposure to oxidative stress. *J. Gerontol. A Biol. Sci. Med. Sci.* **61:**427–434; 2006.

[105] Szczesny, B.; Hazra, T. K.; Papaconstantinou, J.; Mitra, S.; Boldogh, I. Age-dependent deficiency in import of mitochondrial DNA glycosylases

required for repair of oxidatively damaged bases. *Proc. Natl. Acad. Sci. USA* **100:**10670–10675; 2003.

[106] Szczesny, B.; Bhakat, K. K.; Mitra, S.; Boldogh, I. Age-dependent modulation of DNA repair enzymes by covalent modification and subcellular distribution. *Mech. Ageing Dev.* **125:**755–765; 2004.

[107] Sai, K.; Takagi, A.; Umemura, T.; Hasegawa, R.; Kurokawa, Y. Changes of 8-hydroxydeoxyguanosine levels in rat organ DNA during the aging process. *J. Environ. Pathol. Toxicol. Oncol.* **11:**139–143; 1992.

[108] Hirano, T.; Yamaguchi, R.; Asami, S.; Iwamoto, N.; Kasai, H. 8-Hydroxyguanine levels in nuclear DNA and its repair activity in rat organs associated with age. *J. Gerontol. A Biol. Sci. Med. Sci.* **51:**B303–B307; 1996.

[109] Hamilton, M. L.; Van Remmen, H.; Drake, J. A.; Yang, H.; Guo, Z. M.; Kewitt, K.; Walter, C. A.; Richardson, A. Does oxidative damage to DNA increase with age? *Proc. Natl. Acad. Sci. USA* **98:**10469–10474; 2001.

[110] Cardozo-Pelaez, F.; Song, S.; Parthasarathy, A.; Hazzi, C.; Naidu, K.; Sanchez-Ramos, J. Oxidative DNA damage in the aging mouse brain. *Mov. Disord.* **14:**972–980; 1999.

[111] Giovannelli, L.; Decorosi, F.; Dolara, P.; Pulvirenti, L. Vulnerability to DNA damage in the aging rat substantia nigra: a study with the comet assay. *Brain Res.* **969:**244–247; 2003.

[112] Mecocci, P.; Polidori, M. C.; Ingegni, T.; Cherubini, A.; Chionne, F.; Cecchetti, R.; Senin, U. Oxidative damage to DNA in lymphocytes from AD patients. *Neurology* **51:**1014–1017; 1998.

[113] Morocz, M.; Kalman, J.; Juhasz, A.; Sinko, I.; McGlynn, A. P.; Downes, C. S.; Janka, Z.; Rasko, I. Elevated levels of oxidative DNA damage in lymphocytes from patients with Alzheimer's disease. *Neurobiol. Aging* **23:**47–53; 2002.

[114] Kadioglu, E.; Sardas, S.; Aslan, S.; Isik, E.; Esat Karakaya, A. Detection of oxidative DNA damage in lymphocytes of patients with Alzheimer's disease. *Biomarkers* **9:**203–209; 2004.

[115] Lovell, M. A.; Gabbita, S. P.; Markesbery, W. R. Increased DNA oxidation and decreased levels of repair products in Alzheimer's disease ventricular CSF. *J. Neurochem.* **72:**771–776; 1999.

[116] Mecocci, P.; MacGarvey, U.; Kaufman, A. E.; Koontz, D.; Shoffner, J. M.; Wallace, D. C.; Beal, M. F. Oxidative damage to mitochondrial DNA shows marked age-dependent increases in human brain. *Ann. Neurol.* **34:**609–616; 1993.

[117] Mecocci, P.; MacGarvey, U.; Beal, M. F. Oxidative damage to mitochondrial DNA is increased in Alzheimer's disease. *Ann. Neurol.* **36:**747–751; 1994.

[118] Wang, J.; Xiong, S.; Xie, C.; Markesbery, W. R.; Lovell, M. A. Increased oxidative damage in nuclear and mitochondrial DNA in Alzheimer's disease. *J. Neurochem.* **93:**953–962; 2005.

[119] Gabbita, S. P.; Lovell, M. A.; Markesbery, W. R. Increased nuclear DNA oxidation in the brain in Alzheimer's disease. *J. Neurochem.* **71:**2034–2040; 1998.

[120] Mullaart, E.; Boerrigter, M. E.; Ravid, R.; Swaab, D. F.; Vijg, J. Increased levels of DNA breaks in cerebral cortex of Alzheimer's disease patients. *Neurobiol. Aging* **11:**169–173; 1990.

[121] Stadelmann, C.; Bruck, W.; Bancher, C.; Jellinger, K.; Lassmann, H. Alzheimer disease: DNA fragmentation indicates increased neuronal vulnerability, but not apoptosis. *J. Neuropathol. Exp. Neurol.* **57:**456–464; 1998.

[122] Adamec, E.; Vonsattel, J. P.; Nixon, R. A. DNA strand breaks in Alzheimer's disease. *Brain Res.* **849:**67–77; 1999.

[123] Te Koppele, J. M.; Lucassen, P. J.; Sakkee, A. N.; Van Asten, J. G.; Ravid, R.; Swaab, D. F.; Van Bezooijen, C. F. 8OHdG levels in brain do not indicate oxidative DNA damage in Alzheimer's disease. *Neurobiol. Aging* **17:**819–826; 1996.

[124] Seidl, R.; Greber, S.; Schuller, E.; Bernert, G.; Cairns, N.; Lubec, G. Evidence against increased oxidative DNA-damage in Down syndrome. *Neurosci. Lett.* **235:**137–140; 1997.

[125] Lyras, L.; Cairns, N. J.; Jenner, A.; Jenner, P.; Halliwell, B. An assessment of oxidative damage to proteins, lipids, and DNA in brain from patients with Alzheimer's disease. *J. Neurochem.* **68:**2061–2069; 1997.

[126] de la Monte, S. M.; Luong, T.; Neely, T. R.; Robinson, D.; Wands, J. R. Mitochondrial DNA damage as a mechanism of cell loss in Alzheimer's disease. *Lab. Invest.* **80:**1323–1335; 2000.

[127] Coskun, P. E.; Beal, M. F.; Wallace, D. C. Alzheimer's brains harbor somatic mtDNA control-region mutations that suppress mitochondrial transcription and replication. *Proc. Natl. Acad. Sci. USA* **101:**10726–10731; 2004.

[128] Robbins, J. H.; Brumback, R. A.; Polinsky, R. J.; Wirtschafter, J. D.; Tarone, R. E.; Scudiero, D. A.; Otsuka, F. Hypersensitivity to DNA-damaging agents in abiotrophies: a new explanation for degeneration of neurons, photoreceptors, and muscle in Alzheimer, Parkinson and Huntington diseases, retinitis pigmentosa, and Duchenne muscular dystrophy. *Basic Life Sci.* **35:**315–344; 1985.

[129] Parshad, R. P.; Sanford, K. K.; Price, F. M.; Melnick, L. K.; Nee, L. E.; Schapiro, M. B.; Tarone, R. E.; Robbins, J. H. Fluorescent light-induced chromatid breaks distinguish Alzheimer disease cells from normal cells in tissue culture. *Proc. Natl. Acad. Sci. USA* **93:**5146–5150; 1996.

[130] Jacobsen, E.; Beach, T.; Shen, Y.; Li, R.; Chang, Y. Deficiency of the Mre11 DNA repair complex in Alzheimer's disease brains. *Brain Res. Mol. Brain Res.* **128:**1–7; 2004.

[131] Kinsella, T. J.; Dobson, P. P.; Fornace Jr., A. J.; Barrett, S. F.; Ganges, M. B.; Robbins, J. H. Alzheimer's disease fibroblasts have normal repair of *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine-induced DNA damage determined by the alkaline elution technique. *Biochem. Biophys. Res. Commun.* **149:**355–361; 1987.

[132] Edwards, J. A.; Wang, L. G.; Setlow, R. B.; Kaminskas, E. O^6-methylguanine-DNA methyltransferase in lymphocytes of the elderly with and without Alzheimer's disease. *Mutat. Res.* **219:**267–272; 1989.

[133] Costa, F. F. Non-coding RNAs: new players in eukaryotic biology. *Gene* **357:**83–94; 2005.

[134] Cao, X.; Yeo, G.; Muotri, A. R.; Kuwabara, T.; Gage, F. H. Noncoding RNAs in the mammalian central nervous system. *Annu. Rev. Neurosci.* **29:**77–103; 2006.

[135] Mehler, M. F.; Mattick, J. S. Non-coding RNAs in the nervous system. *J. Physiol.* **575:**333–341; 2006.

[136] Taft, R. J.; Pheasant, M.; Mattick, J. S. The relationship between non-protein-coding DNA and eukaryotic complexity. *Bioessays* **29:**288–299; 2007.

[137] Perkins, D. O.; Jeffries, C.; Sullivan, P. Expanding the 'central dogma': the regulatory role of nonprotein coding genes and implications for the genetic liability to schizophrenia. *Mol. Psychiatry* **10:**69–78; 2005.

[138] Bregeon, D.; Sarasin, A. Hypothetical role of RNA damage avoidance in preventing human disease. *Mutat. Res.* **577:**293–302; 2005.

[139] Li, Z.; Wu, J.; Deleo, C. J. RNA damage and surveillance under oxidative stress. *IUBMB Life* **58:**581–588; 2006.

[140] Hofer, T.; Seo, A. Y.; Prudencio, M.; Leeuwenburgh, C. A method to determine RNA and DNA oxidation simultaneously by HPLC-ECD: greater RNA than DNA oxidation in rat liver after doxorubicin administration. *Biol. Chem.* **387:**103–111; 2006.

[141] Shen, Z.; Wu, W.; Hazen, S. L. Activated leukocytes oxidatively damage DNA, RNA, and the nucleotide pool through halide-dependent formation of hydroxyl radical. *Biochemistry* **39:**5474–5482; 2000.

[142] Wamer, W. G.; Wei, R. R. In vitro photooxidation of nucleic acids by ultraviolet A radiation. *Photochem. Photobiol.* **65:**560–563; 1997.

[143] Hofer, T.; Badouard, C.; Bajak, E.; Ravanat, J. L.; Mattsson, A.; Cotgreave, I. A. Hydrogen peroxide causes greater oxidation in cellular RNA than in DNA. *Biol. Chem.* **386:**333–337; 2005.

[144] Barciszewski, J.; Barciszewska, M. Z.; Siboska, G.; Rattan, S. I.; Clark, B. F. Some unusual nucleic acid bases are products of hydroxyl radical oxidation of DNA and RNA. *Mol. Biol. Rep.* **26:**231–238; 1999.

[145] Evans, M. D.; Dizdaroglu, M.; Cooke, M. S. Oxidative DNA damage and disease: induction, repair and significance. *Mutat. Res.* **567:**1–61; 2004.

[146] Ishibashi, T.; Hayakawa, H.; Ito, R.; Miyazawa, M.; Yamagata, Y.; Sekiguchi, M. Mammalian enzymes for preventing transcriptional errors caused by oxidative damage. *Nucleic Acids Res.* **33:**3779–3784; 2005.

[147] Cooke, M. S.; Olinski, R.; Evans, M. D. Does measurement of oxidative damage to DNA have clinical significance? *Clin. Chim. Acta* **365:**30–49; 2006.

[148] Yanagawa, H.; Ogawa, Y.; Ueno, M. Redox ribonucleosides: isolation and characterization of 5-hydroxyuridine, 8-hydroxyguanosine, and 8-hydroxyadenosine from Torula yeast RNA. *J. Biol. Chem.* **267:**13320–13326; 1992.

[149] Taddei, F.; Hayakawa, H.; Bouton, M.; Cirinesi, A.; Matic, I.; Sekiguchi, M.; Radman, M. Counteraction by MutT protein of transcriptional errors caused by oxidative damage. *Science* **278:**128–130; 1997.

[150] Shan, X.; Tashiro, H.; Lin, C. L. The identification and characterization of oxidized RNAs in Alzheimer's disease. *J. Neurosci.* **23:**4913–4921; 2003.

[151] Tanaka, M.; Chock, P. B.; Stadtman, E. R. Oxidized messenger RNA induces translation errors. *Proc. Natl. Acad. Sci. USA* **104:**66–71; 2007.

[152] Gong, X.; Tao, R.; Li, Z. Quantification of RNA damage by reverse transcription polymerase chain reactions. *Anal. Biochem.* **357:**58–67; 2006.

[153] Ding, Q.; Markesbery, W. R.; Cecarini, V.; Keller, J. N. Decreased RNA, and increased RNA oxidation, in ribosomes from early Alzheimer's disease. *Neurochem. Res.* **31:**705–710; 2006.

[154] Shan, X.; Chang, Y.; Lin, C. L. Messenger RNA oxidation is an early event preceding cell death and causes reduced protein expression. *FASEB J.* **21:**2753–2764; 2007.

[155] Bellacosa, A.; Moss, E. G. RNA repair: damage control. *Curr. Biol.* **13:**R482–R484; 2003.

[156] Deutscher, M. P. Degradation of RNA in bacteria: comparison of mRNA and stable RNA. *Nucleic Acids Res.* **34:**659–666; 2006.

[157] Sheth, U.; Parker, R. Decapping and decay of messenger RNA occur in cytoplasmic processing bodies. *Science* **300:**805–808; 2003.

[158] Aas, P. A.; Otterlei, M.; Falnes, P. O.; Vagbo, C. B.; Skorpen, F.; Akbari, M.; Sundheim, O.; Bjoras, M.; Slupphaug, G.; Seeberg, E.; Krokan, H. E. Human and bacterial oxidative demethylases repair alkylation damage in both RNA and DNA. *Nature* **421:**859–863; 2003.

[159] Krokan, H. E.; Kavli, B.; Slupphaug, G. Novel aspects of macro-molecular repair and relationship to human disease. *J. Mol. Med.* **82:**280–297; 2004.

[160] Nakabeppu, Y.; Kajitani, K.; Sakamoto, K.; Yamaguchi, H.; Tsuchimoto, D. MTH1, an oxidized purine nucleoside triphosphatase, prevents the cytotoxicity and neurotoxicity of oxidized purine nucleotides. *DNA Repair* **5:**761–772; 2006.

[161] Kajitani, K.; Yamaguchi, H.; Dan, Y.; Furuichi, M.; Kang, D.; Nakabeppu, Y. MTH1, an oxidized purine nucleoside triphosphatase, suppresses the accumulation of oxidative damage of nucleic acids in the hippocampal microglia during kainate-induced excitotoxicity. *J. Neurosci.* **26:**1688–1698; 2006.

[162] Furuta, A.; Iida, T.; Nakabeppu, Y.; Iwaki, T. Expression of hMTH1 in the hippocampi of control and Alzheimer's disease. *Neuroreport* **12:**2895–2899; 2001.

[163] Hayakawa, H.; Hofer, A.; Thelander, L.; Kitajima, S.; Cai, Y.; Oshiro, S.; Yakushiji, H.; Nakabeppu, Y.; Kuwano, M.; Sekiguchi, M. Metabolic fate of oxidized guanine ribonucleotides in mammalian cells. *Biochemistry* **38:**3610–3614; 1999.

[164] Hayakawa, H.; Kuwano, M.; Sekiguchi, M. Specific binding of 8-oxoguanine-containing RNA to polynucleotide phosphorylase protein. *Biochemistry* **40:**9977–9982; 2001.

[165] Hayakawa, H.; Sekiguchi, M. Human polynucleotide phosphorylase protein in response to oxidative stress. *Biochemistry* **45:**6749–6755; 2006.

[166] Hayakawa, H.; Uchiumi, T.; Fukuda, T.; Ashizuka, M.; Kohno, K.; Kuwano, M.; Sekiguchi, M. Binding capacity of human YB-1 protein for RNA containing 8-oxoguanine. *Biochemistry* **41:**12739–12744; 2002.

[167] Yang, W. H.; Bloch, D. B. Probing the mRNA processing body using protein macroarrays and "autoantigenomics". *RNA* **13:**704–712; 2007.

[168] van Leeuwen, F. W.; de Kleijn, D. P.; van den Hurk, H. H.; Neubauer, A.; Sonnemans, M. A.; Sluijs, J. A.; Koycu, S.; Ramdjielal, R. D.; Salehi, A.; Martens, G. J.; Grosveld, F. G.; Peter, J.; Burbach, H.; Hol, E. M. Frameshift mutants of beta amyloid precursor protein and ubiquitin-B in Alzheimer's and Down patients. *Science* **279:**242–247; 1998.

[169] Lee, J. W.; Beebe, K.; Nangle, L. A.; Jang, J.; Longo-Guess, C. M.; Cook, S. A.; Davisson, M. T.; Sundberg, J. P.; Schimmel, P.; Ackerman, S. L. Editing-defective tRNA synthetase causes protein misfolding and neurodegeneration. *Nature* **443:**50–55; 2006.

[170] Rhee, Y.; Valentine, M. R.; Termini, J. Oxidative base damage in RNA detected by reverse transcriptase. *Nucleic Acids Res.* **23:**3275–3282; 1995.

[171] Shan, X.; Lin, C. L. Quantification of oxidized RNAs in Alzheimer's disease. *Neurobiol. Aging* **27:**657–662; 2006.

[172] Moreira, P. I.; Honda, K.; Zhu, X.; Nunomura, A.; Casadesus, G.; Smith, M. A.; Perry, G. Brain and brawn: parallels in oxidative strength. *Neurology* **66:**S97–S101; 2006.

[173] Aliev, G.; Seyidova, D.; Neal, M. L.; Shi, J.; Lamb, B. T.; Siedlak, S. L.; Vinters, H. V.; Head, E.; Perry, G.; Lamanna, J. C.; Friedland, R. P.; Cotman, C. W. Atherosclerotic lesions and mitochondria DNA deletions in brain microvessels as a central target for the development of human AD and AD-like pathology in aged transgenic mice. *Ann. N. Y. Acad. Sci.* **977:**45–64; 2002.

[174] Aliev, G.; Smith, M. A.; Seyidov, D.; Neal, M. L.; Lamb, B. T.; Nunomura, A.; Gasimov, E. K.; Vinters, H. V.; Perry, G.; LaManna, J. C.; Friedland, R. P. The role of oxidative stress in the pathophysiology of cerebrovascular lesions in Alzheimer's disease. *Brain Pathol.* **12:**21–35; 2002.

[175] Nunomura, A.; Perry, G.; Pappolla, M. A.; Friedland, R. P.; Hirai, K.; Chiba, S.; Smith, M. A. Neuronal oxidative stress precedes amyloid-beta deposition in Down syndrome. *J. Neuropathol. Exp. Neurol.* **59:**1011–1017; 2000.

[176] Sayre, L. M.; Perry, G.; Smith, M. A. In situ methods for detection and localization of markers of oxidative stress: application in neurodegenerative disorders. *Methods Enzymol.* **309:**133–152; 1999.

[177] Fishel, M. L.; Kelley, M. R. The DNA base excision repair protein Ape1/Ref-1 as a therapeutic and chemopreventive target. *Mol. Aspects Med.* **28:**375–395; 2007.

[178] Bohr, V. A.; Ottersen, O. P.; Tonjum, T. Genome instability and DNA repair in brain, ageing and neurological disease. *Neuroscience* **145:**1183–1186; 2007.