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.

Keywords: Alzheimer disease; DNA oxidation; DNA repair; Oxidative stress; RNA damage; RNA repair; Free radicals

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Abbreviations: 8OHG, 8-hydroxyguanosine; 8-oxoG, 8-oxoguanine; AD, Alzheimer disease; AβPP, amyloid-β protein precursor; BER, base excision repair; CSF, cerebrospinal fluid; ER, endoplasmic reticulum; HO-1, heme oxygenase-1; MCI, mild cognitive impairment; mtDNA, mitochondrial DNA; MTH1, MutT homolog 1; ncRNA, noncoding RNA; nDNA, nuclear DNA; NFT, neurofibrillary tangles; NUDT5, Nudix type 5; NEIL, Nei-like homolog; OGG, oxoguanine-DNA glycosylase; PNP, polynucleotide phosphorylase; ROS, reactive oxygen species; YB-1, Y-box-binding protein-1.

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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 (H2O2), hydroxyl ('OH), and superoxide (O2·−), 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 O2·− that can be detoxified by the mitochondrial manganese superoxide dismutase to give H2O2 that, in turn, can be converted to H2O by glutathione peroxidase. However, O2·− in the presence of nitric oxide (NO·), formed during the conversion of arginine to citrulline by nitric oxide synthase, can generate peroxynitrite (ONOO−) and lead to protein modification [26]. Furthermore, H2O2 in the presence of reduced transition metals can be converted to toxic '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 nuclei 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 critical to metal dynamics within the neuronal cytoplasm. It is also known to 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,
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-hydroxyguanine 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 (OGG1) 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. OGG1−/− 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 OGG1−/− 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 H2O2; 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 decrease 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 H2O2, 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 machinery 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 tRNA, extremely abundant in neurons, contained 8OHG in AD brain. Remarkably, tRNA showed higher binding capacity to redox-active iron than tRNA, and consequently the oxidation of tRNA 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

ROS increase and the repair mechanisms decline, leading to the oxidative lesion of several biomolecules including nuclear and mtDNA and RNA. The accumulation of glycoxidation products indicates a 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 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


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