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RNA oxidation: A contributing factor or an epiphenomenon in the process of neurodegeneration

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Abstract

In the past decade, RNA oxidation has caught the attention of many researchers, working to uncover its role in the pathogenesis of neurodegenerative diseases. It has been well documented that RNA oxidation is involved in a wide variety of neurological diseases and is an early event in the process of neurodegeneration. The analysis of oxidized RNA species revealed that at least messenger RNA (mRNA) and ribosomal RNA (rRNA) are damaged in several neurodegenerative diseases, including Alzheimer's disease and amyotrophic lateral sclerosis (ALS). The magnitude of the RNA oxidation, at least in mRNA, is significantly high at the early stage of the disease. Oxidative damage to mRNA is not random but selective and many oxidized mRNAs are related to the pathogenesis of the disease. Several studies have suggested that oxidative modification of RNA affects the translational process and consequently produces less protein and/or defective protein. Furthermore, several proteins have been identified to be involved in handling of damaged RNA. Although a growing body of studies suggests that oxidative damage to RNA may be associated with neuron deterioration, further investigation and solid evidence are needed. In addition, further uncovering of the consequences and cellular handling of the oxidatively damaged RNA should be important focuses in this area and may provide significant insights into the pathogenesis of neurodegenerative diseases.

Keywords: *RNA oxidation, oxidative damage, oxidative stress, neurodegeneration, Alzheimer's disease, ALS*

RNA oxidation is involved in a wide variety of neurological diseases

Oxidative damage is involved in many neurological disorders, such as Alzheimer's disease (AD), Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS), spinal cord injury, epilepsy, etc. [1–3]. It results from modification of vital cellular components, including proteins, lipids, carbohydrates and nucleic acids, by free radicals. Reactive oxygen species can attack and hydroxylate guanine to produce 8-oxo-7, 8-dihydro-2′-deoxyguanosine (8-OHdG) in DNA and 8-oxo-7, 8-dihydroguanosine (8-OHG) in RNA. 8-OHdG and 8-OHG are two of the best characterized and the most abundant oxidized bases [4,5]. Nunomura et al. [6] used an *in situ* immunohistochemical approach to identify oxidized nucleosides in postmortem brains of patients with AD. They found that oxidative damage to nucleic acids occurs predominantly in cytoplasmic RNA rather than in nuclear DNA and is restricted to vulnerable neurons in AD. Similar RNA oxidation in neuronal cytoplasm was also observed in other neurological disorders, including Parkinson's disease [7], Down syndrome [8], dementia with Lewy bodies [9], prion diseases [10,11], subacute sclerosing panencephalitis (caused by

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persistent brain infection of a mutated measles virus) [12], xeroderma pigmentosum group A (caused by a genetic defect of nucleotide excision repair mechanisms) [13] and ALS [14]. The oxidative damage to RNA was demonstrated in both sporadic and familial forms of these diseases. Consistent with the studies in post-mortem tissues, a significant increase in the concentration of 8-OHG has also been reported in cerebrospinal fluid (CSF) from patients with AD and PD [15,16]. Therefore, RNA oxidation is a common feature in many neurological diseases.

RNA oxidation is an early event far preceding neurodegeneration

Studies on the relationship between oxidative damage and histopathological changes in AD, i.e. amyloid β (Aβ) plaques, neurofibrillary tangles as well as duration of dementia suggested that increased RNA oxidation may occur prior to histopathological changes in AD [17]. Similar results were also reported in post-mortem brains of patients with Down syndrome [8]. Furthermore, increased RNA oxidation was observed in a pre-symptomatic case with a familial AD mutation [18] and in subjects with mild cognitive impairment (MCI), who possibly represent the prodromal stage of AD [19]. Consistently, investigations on the relationship between the level of the oxidized RNA nucleoside, 8-OHG, in the CSF of AD patients and the duration as well as severity of dementia also suggested that RNA oxidation may occur in the early stage of AD [15]. Similar results were also reported in a study on the CSF of PD patients [16].

Interpretation of human post-mortem tissue pathological changes needs to be confirmed by other approaches, since post-mortem tissue represents the very end stage of the neurological disease and reflects the cells that remain but not necessarily those that are at risk. Our group has investigated RNA oxidation in transgenic mice expressing familial ALS-linked mutant copper-zinc superoxide dismutase (SOD1) [14]. The transgenic mice expressing human mutant $SOD1^{G93A}$ develop similar clinical and pathological phenotypes as ALS patients. These mice begin to lose motor neurons and associated motor function at ~ 3 months of age and eventually die at ~ 4–5 months of age. There is no motor neuron loss or obvious pathological change at 2 months of age. In these mice, increased RNA oxidation, primarily in the motor neurons and oligodendrocytes of the spinal cord, occurs as early as 1 month of age, progressively increasing with age until it peaks at 2–2.5 months of age and then diminishes when the motor neurons begin to degenerate. Similarly, increased RNA oxidation also occurs in the pre-symptomatic stage of mice expressing other ALS-linked mutant SOD1, including $SOD1^{G37R}$, $SOD1^{G85R}$, $SOD1^{G127X}$ and $SOD1^{H46R/H48Q}$, suggesting that RNA oxidation may be a common early event preceding motor neuron degeneration in ALS [14].

Furthermore, we used primary rat cortical cultures to investigate the relationship between RNA oxidation and neuron degeneration induced by various insults, including hydrogen peroxide, glutamate and amyloid β peptide [20]. The results showed that RNA oxidation occurs at an early stage, primarily in a distinct group of neurons that died later. Taken together, the above studies (post-mortem tissues, disease mouse models and cultured primary neurons) support that RNA oxidation is an early event far preceding neuron degeneration.

What type of RNA is affected and how much is oxidized?

Our group has developed a novel procedure to isolate, quantify and identify oxidized RNA [21]. We separated oxidized RNAs from non-oxidized RNAs by immunoprecipitation with specific antibodies recognizing 8-OHG in the oxidized RNA and quantitatively analysed both RNA fractions. We found that up to 50% of the messenger RNAs (mRNA) isolated from AD frontal cortices were oxidized, while less than 2% of the mRNAs were oxidized in age-matched normal controls [22]. Lower amounts of the oxidized mRNAs ($\sim$10%) were found in AD hippocampus, the most affected area, when compared with frontal cortex. Moreover, the magnitude of the mRNA oxidation was lower in the patients diagnosed as advanced stage compared with the patients diagnosed as mild or moderate stage. This is expected because more neurons are degenerated in more affected areas and more advanced patients and RNA oxidation is restricted to vulnerable neurons and is an early event. Further, we also determined the magnitude of mRNA oxidation in ALS post-mortem tissues [14]. About 6–10% of the mRNAs were oxidized in ALS-affected areas, i.e. the motor cortex and spinal cord, which was observed in both sporadic and familial forms of ALS. Notably, the post-mortem interval did not affect the amount of oxidized mRNAs. Increased mRNA oxidation did not result from the agonal state of disease because it was not observed in the unaffected areas of the patients.

Importantly, in transgenic mice expressing mutant $SOD1^{G93A}$, $\sim$30% of total spinal cord mRNAs were oxidized at pre-symptomatic stage (2-months-old) [14]. Considering that RNA oxidation primarily occurs in motor neurons and oligodendrocytes, which account for a small portion of the total spinal cord cell population, it is conceivable that significant amounts of mRNAs are oxidized in these cells. Lower amounts of total spinal cord mRNAs were oxidized ($\sim$10% or

less) in the end stage, which is consistent with the study in ALS post-mortem tissues. From the results of the SOD1^{G93A} mice study, we speculate that significantly increased mRNA oxidation may occur in the motor neurons and oligodendrocytes of ALS-affected areas at the prodromal stage. This may contribute to neuronal deterioration and in combination with other factors (toxicities) eventually leading to motor neuron degeneration and the disease symptoms. At the end stage of the disease, significant numbers of motor neurons are degenerated and the detected oxidized mRNAs are probably from the survived or dying motor neurons and the surrounding glial cells.

The next important question is which mRNA species are oxidatively damaged. We cloned and identified oxidized mRNAs isolated from AD frontal cortices [21]. Two interesting phenomena were observed. First, mRNA oxidation is not random but highly selective. Thus, some mRNA species are more susceptible to oxidative damage. The mechanisms underlying the selective mRNA oxidation remain unknown. Secondly, many identified oxidized mRNA species are related to AD—either the transcripts have been characterized in AD or their protein functions have been implicated in the pathogenesis of AD, which include p21ras, mitogen-activated protein kinase (MAPK) kinase 1, carbonyl reductase, SOD1, apolipoprotein D, glutamate dehydrogenase, etc.

These phenomena were also observed in the oxidized mRNAs prepared from spinal cords of 2- month-old (pre-symptomatic stage) SOD1^{G93A} mice [14]. In this study, we applied DNA microarrays to identify oxidized mRNA species. A total of 3409 mRNA species were identified. The analysis of these oxidized mRNA species revealed that mRNAs encoded proteins involved in several biological processes, including mitochondrial electron transport, protein biosynthesis, myelination, protein folding and degradation, cytoskeleton, tricarboxylic acid cycle and glycolysis, were over-represented in the pool of highly oxidized mRNAs. Selective mRNA oxidation was not due to the abundance of mRNA species. For instance, β-actin and MAP-2 mRNAs are highly abundant mRNA species, but only very small amounts of β-actin and MAP-2 mRNAs were oxidized. Importantly, many oxidized mRNAs are related to the pathogenesis of ALS, such as SOD1, dynactin 1, vesicle-associated membrane protein 1, neurofilament sub-units and metallothioneins. These studies suggest that mRNA oxidation may be an important initiation factor causing motor neuron deterioration.

Non-coding RNAs, such as ribosomal RNA (rRNA), transfer RNA (tRNA) and microRNA (miRNA), function directly as structural, catalytic or regulatory molecules rather than serving as templates for protein synthesis [23]. Honda et al. [24] reported that rRNA in AD-affected areas is oxidized by bound redox-active iron. Further, Ding et al. [19] also reported increased 8-OHG in total RNA pool, especially rRNA, in AD-affected areas. However, the magnitude of the rRNA oxidation in AD was not addressed in these studies and remains to be determined. Furthermore, whether other types of cytoplasmic RNAs, such as tRNA and miRNA, are oxidatively damaged in diseased brains remains to be explored.

What is the biological consequence of oxidized RNA?

More than 20 different types of oxidatively altered purine and pyrimidine bases have been detected in nucleic acids [25,26]. Guanine is the most reactive nucleic acid base [27]. The biological consequence of RNA containing oxidatively altered bases remains largely unexplored. Our group has investigated how oxidative modification of mRNA affects the downstream translational process by expressing oxidized mRNAs in cell lines. We observed that oxidative modification of mRNA causes reduced protein expression and associated function [21]. Polyribosome analysis indicated that oxidized bases on transcript may cause ribosome stalling on the transcript or slow the translation process, leading to a decrease of protein expression. In addition, a recent study [28] demonstrates that oxidized mRNA induces translation errors, producing short polypeptides because of premature termination or translation error-induced degradation. It is still unclear whether oxidized bases on mRNA can alter pairing capacity with tRNA and consequently produce mutated proteins. The detailed mechanism of how oxidized base affects the translational process is currently being investigated.

We have examined protein expression levels for the oxidized mRNA species in transgenic mice expressing mutant SOD1^{G93A} [14]. The results showed that protein levels of cytochrome c oxidase VIb, NADH-ubiquinol oxidoreductase sub-unit 39 kDa and myelin basic protein, whose mRNAs were highly oxidized, were significantly decreased in the motor neurons or oligodendrocytes of 60 day-old SOD1^{G93A} lumber spinal cords. On the other hand, the neuronal glutamate transporter EAAT3, whose mRNA was not oxidized, was not decreased. Importantly, the reduced protein expression levels were partially restored when the oxidative damage of their mRNAs was attenuated by treating the mice with vitamin E. These results support that oxidative modification of mRNA alters protein expression.

Ding et al. [19,29] reported a significant impairment in ribosome function in the affected areas of AD and MCI subjects; this impairment is associated with a decreased rate and capacity for protein

synthesis, decreased rRNA and tRNA levels and increased RNA oxidation. Further, impaired protein synthesis and decreased RNA levels were also observed in cultured neurons treated with oxidative stressor (hydrogen peroxide) [30]. Taken together, these studies indicate that oxidative modification of RNA affects the translational process and consequently produces less protein and/or defective protein, which may have detrimental effects on cellular function.

However, cells have developed surveillance mechanisms to prevent accumulation of damaged RNA, which are important for maintaining cellular health and preventing disease. To date, there are three surveillance mechanisms that have been identified. First, oxidation of nucleotides can occur in the cellular nucleotide pool and these oxidized nucleotides can be incorporated into DNA and RNA. MutT protein in *E. coli* and its mammalian homologues, MutT homologue (MTH1) and Nudix type 5 (NUDT5), have the ability to prevent incorporation of oxidized nucleotides into DNA and RNA by hydrolysing the oxidized nucleoside diphosphates and/or triphosphates to the monophosphates [31,32]. Secondly, recognizing and then removing oxidized RNA is one way to prevent accumulation of damaged RNA. Human Y box-binding protein (YB-1) has the ability to bind specifically to 8OHG-containing RNA, which may discriminate the oxidized RNA from normal RNA, thereby sequestrating the damaged RNA from the translation and directing the oxidized RNA to degradation [33,34]. Thirdly, damaged RNA can be repaired. Alkylation damage in RNA can be repaired by the *E. coli* enzyme AlkB and its human homologue hABH3 by hydroxylation of methyl group on damaged RNA bases, thereby directly reversing alkylation damage [35]. Although no repair enzyme for oxidized mRNA has yet been found, there is a possibility that such an enzyme is present in cells, as mRNA oxidation occurs no less frequently than methylation. Further investigation is needed to better understand how the cell handles RNA oxidation.

Does oxidative damage to RNAs contribute to neurodegeneration?

In the past decade, we have learned that RNA oxidation is involved in a wide variety of neurological diseases and appears to be an early event preceding neuron degeneration. The magnitude of the RNA oxidation, at least in mRNA, appears to be high at the early stage of the disease. In addition, oxidative modification of RNA can affect the translational process. However, there is no direct evidence supporting that oxidative damage to RNA can cause or contribute to neurodegeneration. There are at least two suggestions indicating that RNA oxidation may be a contributing factor and not simply an epiphenomenon. First, studies on various anti-cancer agents have shown that RNA damage can lead to cell-cycle arrest and cell death, much as DNA damage does [36]. This suggests that apoptosis can be induced when the damage to RNA is substantial. Secondly, in our study with $SOD1^{G93A}$ mice, we observed that oxidative mRNA damage occurs at an early age of mice, but significant oxidative damages to protein, lipid and DNA do not occur until the active disease progression stage. Importantly, vitamin E treatment can reduce RNA oxidation and partially protect motor neurons from degeneration in mice at the early symptomatic stage [14], which suggests that mRNA oxidation may be an important initiation factor causing motor neuron deterioration.

In conclusion, we are still in an early stage of understanding the role of RNA oxidation in the process of neurodegeneration. Although a growing body of studies suggests that oxidative damage to RNA may be associated with neuron deterioration, further investigation and solid evidence are needed. Furthermore, uncovering of the consequences and cellular handling of the oxidatively damaged RNA should be important focuses in this area and may provide significant insights into the pathogenesis of the disease. It is possible that RNA oxidation is an important factor in neuronal deterioration and blocking RNA oxidation at the prodromal stage may prevent/slow the disease progression.

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