Review Article

# RNA modifications by oxidation: A novel disease mechanism?

Henrik E. Poulsen [a,b,*], Elisabeth Specht [a], Kasper Broedbaek [a,b], Trine Henriksen [a,b], Christina Ellervik [c,d], Thomas Mandrup-Poulsen [e,f], Morten Tonnesen [g], Peter E. Nielsen [h], Henrik U. Andersen [i], Allan Weimann [a,b]

[a] Laboratory of Clinical Pharmacology Q7642, Rigshospitalet, DK-2100 Copenhagen, Denmark
[b] Department of Clinical Pharmacology, Bispebjerg Hospital, DK-2400 Copenhagen, Denmark
[c] Department of Clinical Biochemistry, Naestved Sygehus, DK-4700 Naestved, Denmark
[d] Department of Diagnostic Sciences, Faculty of Health Science, University of Copenhagen, DK-2200 Copenhagen N, Denmark
[e] Department of Biomedical Sciences, Faculty of Health Science, University of Copenhagen, DK-2200 Copenhagen N, Denmark
[f] Department of Medicine and Surgery, Karolinska Institutet, S-171 77 Stockholm, Sweden
[g] Hagedorn Research Institute, DK-2820 Gentofte, Denmark
[h] Department of Cellular and Molecular Medicine, Faculty of Health Science, University of Copenhagen, DK-2200 Copenhagen N, Denmark
[i] Steno Diabetes Center Ltd., DK-2820 Gentofte, Denmark

## ARTICLE INFO

## ABSTRACT

The past decade has provided exciting insights into a novel class of central (small) RNA molecules intimately involved in gene regulation. Only a small percentage of our DNA is translated into proteins by mRNA, yet 80% or more of the DNA is transcribed into RNA, and this RNA has been found to encompass various classes of novel regulatory RNAs, including, e.g., microRNAs. It is well known that DNA is constantly oxidized and repaired by complex genome maintenance mechanisms. Analogously, RNA also undergoes significant oxidation, and there are now convincing data suggesting that oxidation, and the consequent loss of integrity of RNA, is a mechanism for disease development. Oxidized RNA is found in a large variety of diseases, and interest has been especially devoted to degenerative brain diseases such as Alzheimer disease, in which up to 50–70% of specific mRNA molecules are reported oxidized, whereas other RNA molecules show virtually no oxidation. The iron-storage disease hemochromatosis exhibits the most prominent general increase in RNA oxidation ever observed. Oxidation of RNA primarily leads to strand breaks and to oxidative base modifications. Oxidized mRNA is recognized by the ribosomes, but the oxidation results in ribosomal stalling and dysfunction, followed by decreased levels of functional protein as well as the production of truncated proteins that do not undergo proper folding and may result in protein aggregation within the cell. Ribosomal dysfunction may also signal apoptosis by p53-independent pathways. There are very few reports on interventions that reduce RNA oxidation, one interesting observation being a reduction in RNA oxidation by ingestion of raw olive oil. High urinary excretion of 8-oxo-guanosine, a biomarker for RNA oxidation, is highly predictive of death in newly diagnosed type 2 diabetics; this demonstrates the clinical relevance of RNA oxidation. Taken collectively the available data suggest that RNA oxidation is a contributing factor in several diseases such as diabetes, hemochromatosis, heart failure, and β-cell destruction. The mechanism involves free iron and hydrogen peroxide from mitochondrial dysfunction that together lead to RNA oxidation that in turn gives rise to truncated proteins that may cause aggregation. Thus RNA oxidation may well be an important novel contributing mechanism for several diseases.

© 2012 Elsevier Inc. All rights reserved.

## Contents

* Corresponding author at: Laboratory of Clinical Pharmacology Q7642, Rigshospitalet, DK-2100 Copenhagen, Denmark.
  E-mail address: hepo@rh.dk (H.E. Poulsen).

## Introduction

DNA has historically been the main focus of nucleic acid studies, since the discovery of "the book of life" embedded in this molecule [1]. In 2001 there were great expectations from the Human Genome Project detailing the information of the complete sequence map [2,3]. The paradigm seemed straightforward, as the coding DNA genes determine protein sequence and function, and changes in the DNA sequence (mutations) may alter protein function. However, only a small fraction of our genome actually codes for proteins. Indeed our approximately 21,000 genes account for only about 1.5% of the total 3 billion bases in our genome, yet 80% of the DNA is transcribed to RNA [4]. Furthermore, the homology between the mouse and the human genome is surprisingly large, not only concerning protein-coding genes but also within the large part of non-protein-encoding DNA [5,6]. Such a conservation during the 75 million years that has elapsed since the lineage of mice and humans separated indicates that the non-protein-encoding DNA (>90% of the genome) does indeed harbor essential information needed in addition to the protein sequence contained in protein-coding DNA regions. During the past few decades entirely new classes of central regulatory RNA molecules have been discovered [7]. Thus apart from tRNA (accounting for about 2% of total RNA) and the stable ribosomal RNAs, a large number of small RNA molecules have been described, e.g., gene-regulatory microRNAs (including interfering RNA), and more recently larger RNA molecules termed large intervening noncoding RNAs, also participating in the regulation of genes, have been characterized. The number of RNA classes is growing as is the number of described RNAs in each class, and many databases now exist with detailed information on these noncoding RNAs, including their known functions.

For decades it has been well known that both endogenous and exogenous agents can damage cellular DNA, proteins, and lipids, and among the endogenous agents reactive oxygen species (ROS) have attracted particular interest. The DNA modifications by oxidation are particularly well examined [8,9], and 8-hydroxylation of guanine constitutes the prototype oxidation lesion that can be measured in the DNA as well as as an excretion product (8-oxo-deoxyguanosine (8-oxodG) nucleoside) in urine [10,11].

Cells have protective mechanisms against oxidative damage, e.g., through the use of antioxidant-scavenging enzymes such as superoxide dismutases, catalases, and glutathione peroxidases [12]. However, oxidative stress occurs when the ROS level overwhelms defensive mechanisms, causing a cellular imbalance between pro- and antioxidant factors [13,14]. The resulting cell damage seems to be mediated directly through DNA/RNA damage and destabilization of membranes surrounding lysosomes and mitochondria that swell and rupture, but also indirectly by potentiating cellular stress signaling, e.g., via the NF-κB pathway and activation of proapoptotic processes.

We reported some years ago that in humans RNA damage is more abundant than DNA damage [11]. Recently we further reported an increased RNA oxidation in the iron-overload disease hemochromatosis [15], and in yet another study of twins we found that the oxidation of RNA is minimally determined by genetic factors [16], and we have just published that 8-oxo-guanosine (8-oxoGuo) is predictive of death in type 2 diabetics [17].

RNA comprises an incredibly diverse group of nucleic acids with many and varying functions important for cellular function and protein synthesis. It would be surprising if nature had not evolved mechanisms to maintain integrity for such a complex and central regulatory system. The discovery of an RNA repair mechanism [18] provides support for the hypothesis that evolution has given priority to mechanisms that maintain RNA integrity. However, it is not clear to what quantitative extent RNA repair operates.

We find that the potential for RNA oxidation as a disease mechanism is compelling, and therefore this review focuses on the present knowledge about oxidative modifications in RNA, the mechanism of RNA oxidation, and the possible importance for development of certain diseases and their complications.

## The oxidative chemical modifications of RNA and their determination

DNA and RNA differ only by the 2′-hydroxyl group found in RNA and by the 5-methyl group found in thymine in DNA (uracil in RNA), and it is therefore to be expected that the base modifications (in particular concerning guanine) found in oxidized DNA and RNA would be very similar. In DNA the number of products found after in vitro oxidation is very high and was described several decades ago, in particular by the pioneers Miral Dizdaroglu [9] and Jean Cadet [8]. Oxidation of DNA can affect the sugar as well as the base moiety, and oxidation can also break the glycoside bond or the sugar phosphate backbone leading to abasic sites and strand breaks. Still, the majority of the investigations in vivo are restricted to measurement of the 8-hydroxylation product of guanine, the 8-oxodG lesion. In RNA a similar focus has been on the oxidation of guanine. This presumably relates to the fact that these lesions can be measured by liquid chromatography with electrochemical detection and immunological methods, both relatively affordable and practical methodologies.

In yeast RNA 5-hydroxycytidine, 5-hydroxyuridine, 8-hydroxyguanosine, and 8-hydroxyadenosine have been described [19]. In human urine [11,20,21], cerebrospinal fluid [22–24], and tissue, immunohistochemical analysis has so far focused only on oxidized guanosine (Table 1).

Recent studies have demonstrated that the flash-quench method and direct oxidation by a metal oxo complex can be used to study the nature of guanine oxidation. For instance it was shown that runs of multiple guanines in an RNA molecule are relatively more susceptible to oxidation. Thus the method opens up the way for a more detailed understanding of the more susceptible sites and RNA conformational changes [58]. As detailed later, the localization of oxidation within RNA is not a random process and there are large differences in the oxidation of different RNAs in the same cell.

## Detection methods for oxidized RNA

The major principles for analysis of oxidized RNA are based on immunological or chromatographic procedures [25,51], primer extension and reverse transcriptase [59], the use of an aldehyde-reactive probe [60,61], reverse transcription to cDNA, DIG labeling of sUTPs, and Southern blot analysis [51]. Antibody specificity is repeatedly questioned, so the results obtained with these methods need confirmation by more specific methods.

**Table 1**
Diseases and conditions with RNA oxidation changes.

| Disease/condition | Observed in | Finding | Method | Ref. |
|---|---|---|---|---|
| Alcohol | Rat pituitary gland | Increased RNA oxidation | Immunohistochemistry | [25] |
| Olive oil | Human urine | Decreased RNA oxidation | HPLC–MS/MS | [26] |
| Doxorubicin | Rat liver, brain, heart, muscle | Increased RNA oxidation in liver, no increase in DNA oxidation | HPLC–ECD | [21] |
| Oltipraz | Human urine | Oltipraz does not influence RNA oxidation | HPLC–MS/MS | [27] |
| Irbesartan | Human urine | No influence on RNA oxidation | | [28] |
| Endogenous melatonin production | Human urine | Low melatonin production marginally associated with high RNA oxidation | | [29] |
| 2-Nitropropane | Rat liver | Increased 8-oxoGuo | HPLC–ECD | [30] |
| Low birth weight | Human urine | Low birth weight at normal term gives no change in RNA oxidation later in life | HPLC–MS/MS | [31] |
| Prion disease; Gerstmann–Straussler–Scheinker syndrome | Pyramidal neurons in hippocampus | Increased RNA oxidation | Immunohistochemistry | [32] |
| Emphysema | Human alveolar lung cells | More RNA oxidation than DNA oxidation | Immunohistochemistry | [33] |
| Type 1 diabetes with ketoacidosis | Pyramidal neurons in hippocampus | Increase compared to controls | Immunohistochemistry | [34] |
| Type 2 diabetes | Human urine | High excretion vs low predicts death and death from diabetic complications | UPLC–MS/MS | [17] |
| Xeroderma pigmentosum; Cockayne syndrome | Human brain, globus pallidus | Increased in xeroderma pigmentosum but not in Cockayne syndrome | Immunohistochemistry | [35] |
| Dentatorubral-pallidoluysian atrophy | Brain, lenticulate nucleus | Increased RNA oxidation | Immunohistochemistry | [36] |
| Subacute sclerosing panencephalitis | Human cerebral cortex | Increased 8-oxoGua in neuronal and glial cells | Immunohistochemistry | [37] |
| Normal humans | Urine | Higher RNA (8-oxoGuo) than DNA (8-oxodG) oxidation | HPLC/immunoaffinity/UV, UPLC with UV or MS/MS | [11,20,38] |
| Normal humans | Urine | No circadian rhythm in 8-oxoGuo | HPLC with MS/MS | [39] |
| Twin humans | Urine | 8-OxoGuo not influenced by genetics, identical in monozygotic versus dizygotic twins | UPLC–MS/MS | [16] |
| Normal humans; cancer patients | Urine, reference values sex, age, smoking vs nonsmoking | Males excrete more 8-oxoGuo than females; no effect of smoking; correlation with age; 8-oxoGuo excretion increased in cancer patients | HPLC–MS/MS | [40] |
| Normal humans; caloric restriction/exercise | WBCs, urine | Training and exercise reduced RNA oxidation in WBCs, no change in urinary excretion of 8-oxoGuo | HPLC–MS/MS | [41] |
| Low-grade inflammation, elderly humans | Urine | No relation to high IL-6, and CRP within normal range; strong relation to iron status | UPLC–MS/MS | [42] |
| Styrene exposure | Human urine, WBCs | Increased urinary excretion of 8-oxoGuo, unchanged urinary excretion of 8-oxodG, unchanged levels in WBCs | HPLC–MS/MS | [43] |
| Cirrhosis with hepatic encephalopathy | Human cerebral cortex | Increased 8-oxoGuo in cirrhosis patients vs controls | Northwestern blot analysis | [44] |
| Hemochromatosis | Human urine | Increased 8-oxoGuo excretion, same 8-oxodG excretion as in controls; 8-oxoGuo excretion reduced to control values after treatment | UPLC–MS/MS | [15] |
| Atherosclerosis | Human and rabbit carotid artery, aorta | Increased 8-oxoGuo, loss of RNA integrity | Immunohistochemistry, qualitative RT-PCR | [45,46] |
| Amyotrophic lateral sclerosis | Motor neurons and oligodendrocytes | Increased in early stages of the disease | Southern blot, HPLC–ECD | [47] |
| Parkinson disease | Substantia nigra | Cytoplasmic RNA oxidation increased in midbrain, no nuclear DNA oxidation | Immunohistochemistry | [48] |
| Parkinson disease | Cerebrospinal fluid | Increased in patients vs controls | HPLC/immunoaffinity/UV | [22] |
| Alzheimer human | Cerebrospinal fluid | Increased in patients vs controls; decreased with increasing duration of disease | HPLC–ECD | [23] |
| Alzheimer human | Cerebrospinal fluid | High 8-oxoGuo in CSF, correlated with homocysteine concentration | HPLC–ECD | [49] |
| Alzheimer human | Brain | mRNA oxidized in frontal cortex, some RNAs more susceptible than others | Northwestern blotting | [50] |
| Alzheimer human | Brain | 50–70% of mRNA in frontal cortex is oxidized | Reverse transcription semiquantitative RT–PCR | [51] |
| Alzheimer human | Brain | mRNA oxidation is an early event that causes ribosome stalling and reduced protein expression | Immunoaffinity, fluorescence, Western blot, Northern blot | [52] |
| Alzheimer human; Down syndrome | Cerebral cortex | Oxidized mRNA increased | Immunohistochemistry | [53,54] |
| Alzheimer human | Cerebral cortex and neocortex | Oxidized RNA increased | Immunohistochemistry | [55] |
| Alzheimer human | Brain | 8-OxoGua correlates with intraneuronal amyloid-β in hippocampus | Immunohistochemistry | [56] |
| Alzheimer human | Brain | Multiple cortical areas show increased 8-oxoGuo, decreased tRNA and rRNA, and impaired protein synthesis | Immunohistochemistry, reverse transcription PCR, Western blotting | [57] |

WBCs, white blood cells.

Chromatographic methods with UV [38], electrochemistry [21], or mass spectrometry [11,20,39,40] or UPLC separation with multiple ions detected by tandem mass spectrometry are highly specific [20].

It is emphasized that urinary collection and measurement represent an average of the rate of oxidation of all RNAs in the organism and consequently are best suited to situations in which all or a large part of the cells of the body are affected [62]. At present, the chromatographic methods require rather large tissues samples (50–100 mg), which makes it difficult to examine organ localizations in detail.

The chromatographic methods for measurement of 8-oxodG in tissue have undergone an international multilaboratory validation [63,64], and the results can presumably be extended to the RNA counterpart 8-oxoGuo, which can measured by the same principles [11,20]. For analysis of 8-oxodG in urine a similar multilaboratory effort is ongoing [65,66], but has not been extended to the RNA counterpart 8-oxoGuo.

Plasma levels of 8-oxoGuo have not been reported, but clearly 8-oxoGuo must be transported from the cells via the bloodstream for urinary excretion. The plasma levels cannot be used as a biomarker as they reflect the balance between cellular production and urinary excretion and are difficult to interpret because the plasma levels are mainly dependent on kidney function as indicated by conventional physiological considerations.

## Consequences of RNA oxidation

The past decade has clearly revealed that RNA is oxidized analogous to DNA, and it may even be more prone to oxidation, presumably because of its intracellular location closer to the ROS-generating mitochondria and because it contains larger single-stranded regions and is less protected by protecting proteins. The general assumption has been that oxidation of RNA would simply lead to degradation and replacement by new synthesis. Consequently, it has been considered to be just "The Messenger in the Middle" [7] and has not attracted much attention in a damage and repair context.

Oxidation does indeed result in RNA modifications, but it should be recognized that the most abundant result of oxidation is probably direct strand scission, suggested to constitute 40–90% of the reactions. One mechanism involves initial formation of a nucleobase radical (and/or the respective peroxyl radical), which subsequently abstracts a hydrogen atom from the ribose ring ultimately resulting in strand scission [67].

Furthermore, cell culture studies in which RNA oxidation and neuron degeneration were induced by various insults, including hydrogen peroxide, glutamate, and β-amyloid peptide, showed that RNA oxidation occurred primarily in a distinct group of neurons that later died [52].

It is also important to note that RNA oxidation is not a random phenomenon; some RNA species are more prone to oxidation than others. In Alzheimer disease 50–70% of mRNA molecules are oxidized [51], causing severe ribosome dysfunction [57,68,69].

In a study of patients with amyotrophic lateral sclerosis (ALS) combined with studies on transgenic mice expressing mutant superoxide dismutase (SOD) 1, it was demonstrated that some mRNA species are particularly susceptible to oxidative damage. The highly abundant mRNAs for β-actin and microtubule-associated protein 2 showed very low mRNA oxidation levels, whereas 60 other mRNAs were highly oxidized and 298 were clearly oxidized, and all were related to functional disturbances in ALS, such as in neurodegeneration, the electron transport chain, citric acid cycle, protein folding, and a long list of other relevant functions [47]. In these mouse studies vitamin E reversed RNA oxidation and delayed disease onset but did not influence mortality [47]. This is suggestive of RNA oxidation as an important mechanism in this disease.

In the brain increased levels of 8-oxoGuo lead to a reduction in rRNA and tRNA levels and to decreased protein synthesis [57,68,69],

indicating ribosome dysfunction. In Escherichia coli a polynucleotide phosphorylase binds with higher affinity to 8-oxoGuo-containing RNA than to intact RNA [70], and also proteins that preferentially bind to oxidized RNA in humans have been described [71], indicating that RNA degradation could be a mechanism by which the cell controls the levels of oxidized RNA by increased degradation. It cannot be ruled out that the very high levels of oxidized mRNA described [51] could be the result of a defect and/or saturation of degradation pathways, or increased half-life and/or increased formation of the modified RNA, or a combination thereof. In view of the spurious oxidation during extraction and analysis of DNA, e.g., by the ESCODD program, the observations need confirmation by different and improved methods.

In a study in which RNA subjected to oxidation was translated in a rabbit reticulate lysate or transfected into human HEK cells a resulting reduction in protein synthesis and production of short polypeptides correlated with increased 8-oxoGuo content, whereas no major degradation (scission) of the RNA was detected [72]. These experiments indicate that RNA oxidation leads to suppression of protein synthesis and that oxidized RNA is translated to form dysfunctional full-length proteins as well as truncated proteins. It is not clear whether part of the truncated protein products originate from oxidatively cleaved mRNA.

Taken collectively these data indicate that oxidation of coding RNAs leads to ribosomal dysfunction with formation of nonfunctional or truncated proteins and to reduced levels of functional proteins. Finally, mutated proteins may be formed from mistranslation of oxidized RNA, which in turn can lead to incorrect folding of the protein [56], resulting in formation of aggregates that are the hallmarks of degenerative brain diseases [73,74].

## RNA repair and prevention of oxidative damage

The DNA repair mechanisms constitute a complex system in which defects in maintaining genome integrity are associated with aging and disease development [75]. Damaged RNA was for long thought to be degraded and not repaired because at least single-stranded RNA does not have the template that in DNA serves as the backup for correction of errors. However, it has recently been suggested that cells have at least one specific mechanism to repair RNA damage [18]. The apurinic endonuclease also possesses activity toward RNA [76] and plays a role in the rRNA quality control process [77]. Several other proteins have similar properties [71,78–80].

Oxidation of guanine can also take place in the nucleotide pool, and oxidized guanine nucleotides could be incorporated into DNA as well as into RNA if there were no preventive mechanisms. Such mechanisms have been described in detail and include mammalian MutT homologue MTH1 and Nudix type 5 protein, which function to sanitize the nucleotide pool for oxidized nucleotides, although this does not seem to be a function that is totally tight [81]. Currently, the sensitivity of available methods does not allow direct measurement of the oxidized pool size. An indirect experiment argues that oxidation of the guanine nucleotide pool is a major contribution to the urinary excretion [82]. However, in that experiment 120 min was used as the time point at which to examine the presence of 8-oxodG in the cell medium, a time point at which excision and repair of the lesion in DNA have not yet taken place [83]. Assuming that a mammalian cell contains about 30 pg RNA [82,84,85] and that total GPT is about 0.4 fmol/cell [86] there is about 75-fold more guanine in RNA than in the dGTP pool; thus it would require a considerable oxidation of the GTP pool to contribute to the urinary excretion. We therefore argue that oxidation of the ribonucleotide pool does not contribute to the urinary excretion of 8-oxoGuo.

Although there are reports on repair mechanisms for oxidized RNA, the major mechanism by which cells have to deal with modified RNA seems to be by elimination and degradation. In DNA the first step in the repair process is scission. Some of the proteins involved in scission also have affinity toward RNA, but most likely contribute to

elimination or degradation rather than to repair. We believe that repair of RNA most probably is a minor mechanism by which cells deal with oxidative RNA modifications, and the major mechanisms to prevent deleterious effects of RNA oxidation are degradation and elimination, as well as prevention of incorporation of oxidized nucleotides from the nucleotide pool.

## RNA oxidation in various conditions and diseases

This field is very young and the published data mainly originate from the past decade. In the original publications the methodologies used are mainly immunohistochemistry on postmortem brain tissue and HPLC–ECD for cerebrospinal fluid analysis (Table 1). The results are quite consistent with increased cytosolic levels of 8-oxoGuo in frontal, cortical, and hippocampal regions of the brain from Alzheimer patients and negative findings in brains from age-matched controls without a history of dementia or significant neuropathological changes [51]. Furthermore, it has been demonstrated that these changes occur in the early stages at which the clinical symptoms are very discrete [47,52].

In the above-cited review papers on RNA oxidation there are many references to a link between misfolding of proteins and the ribosomal dysfunction seen with RNA damage from oxidation. A cytosolic ribonuclease with RNA-cleaving properties is blocked by several drugs that interfere with RNA synthesis [87] and represents an example that RNA damage can lead to cell cycle arrest and apoptosis by a p53-independent pathway. It is not known for certain—but clearly possible—that RNA damage induced by oxidation elicits a similar response.

It can be argued that RNA oxidation is an epiphenomenon rather than a contributing pathogenetic mechanism [88]. As indicated earlier in this review RNA oxidation leads to disturbed ribosomal function, stalling of the ribosomes, reduced protein synthesis, and production of truncated proteins and furthermore there is some evidence for translational errors and "mutated" protein production [72]. Even though these data taken together suggest that RNA damage induced by oxidation is a contributing mechanistic factor rather than just an epiphenomenon, this principle needs to be investigated further, in particular regarding to what extent it contributes to the development of neurological and other disease.

Although the major focus of the research on RNA oxidation has been on Alzheimer disease, it has also been reported for other neurological diseases such as Parkinson disease, for chromosomal errors such as Down syndrome, and in ALS, hemochromatosis, hepatic cirrhosis, emphysema, rare syndromes, and diabetes (for details see Table 1) and also in connection with alcohol-, drug-, and carcinogen (such as 2-nitropropane)-induced cell damage. The RNA oxidation does not seem to be significantly influenced by genetics, i.e., no genetic trait in healthy people has been found to correlate with the degree of RNA oxidation [16]; this leaves ample room for therapeutic interventions.

In Alzheimer disease increased RNA oxidation is evidenced by increased intracellular levels and also by increased 8-oxoGuo concentrations in the cerebrospinal fluid (see Table 1).

In vivo studies on the human brain or other organs are few. However, it is possible to measure the rate of RNA oxidation by collecting and measuring urine over a defined period of time [20,62]. Such a measure could be clinically important in the cases in which all or the majority of the cells in larger tissues suffer from increased oxidative stress, but will most probably be insensitive to oxidative stress localized to small organs or few cells. Thus in degenerative brain diseases there is localized oxidative stress that will not be detectable in urine because of the huge contribution from the entire organism. In diseases in which all body cells are affected, e.g., xeroderma pigmentosum, hemochromatosis, and diabetes, global increased oxidative stress to RNA can be measured by urine collection.

A suggested mechanism for the initiation of RNA oxidation and its consequences is presented in Fig. 1 and explained in the legend.

In summary, there is evidence in favor of increased RNA oxidation levels in many different diseases and very few negative studies so far. The present data indicate that RNA oxidation is a prominent feature in, but not restricted to, degenerative brain diseases; thus increased oxidative stress to RNA may be the rule rather than the exception in a variety of diseases. However, it must be acknowledged that there are indications that the methodologies used are not sufficiently specific and may overestimate the levels reported.

Recently we reported that in newly diagnosed type 2 diabetic patients, followed for almost 20 years, urinary excretion of 8-oxoGuo is predictive of death; those in the quintile with the highest 8-oxoGuo excretion showed a 50% mortality 5–8 years earlier that those in the lowest quintile [17]. To our knowledge this is first direct demonstration that RNA oxidation is of clinical relevance in type 2 diabetes and thereby of its importance in the pathogenesis of diabetic complications.

## The role of iron in RNA oxidation

Redox-active metals together with reactive oxygen species have long been suggested to have a pivotal role in oxidative stress in vivo [89–91]. It has been demonstrated that iron interacts with rRNA and alters protein synthesis [92], and it has long been known that there is increased redox-active iron in the brains of Alzheimer patients [93].
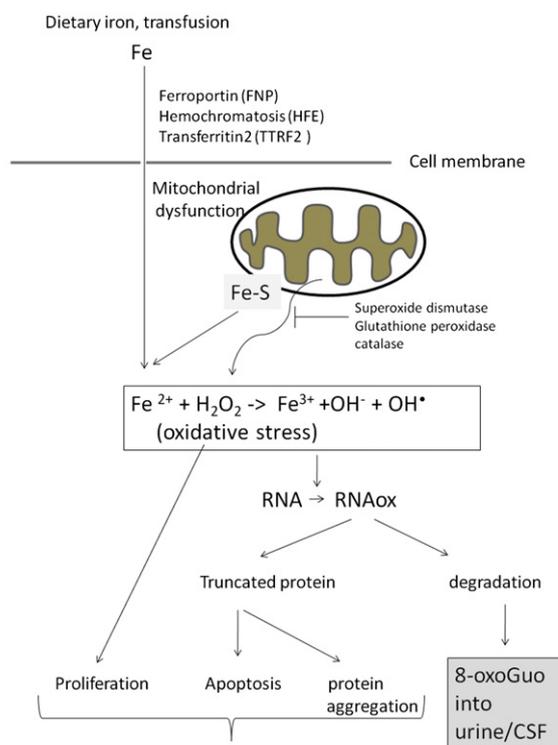


**Fig. 1.** Suggestion for a mechanism by which insulin resistance/mitochondrial dysfunction leads to oxidative stress by the Fenton reaction that is fueled by available or liberated iron and hydrogen peroxide from the mitochondria. The oxidation of RNA leads to the translation of truncated proteins that can induce apoptosis and protein aggregation and ultimately to hemochromatosis and diabetes and its complications, heart failure, and Alzheimer disease. References for the individual processes are given in the text. Degradation of oxidized RNA leads to 8-oxo-guanosine (8-oxoGuo) in the cerebrospinal fluid (CSF) and excretion in the urine and is suggested as a biomarker that can provide risk estimate and treatment effect in these diseases.

The cellular iron level is carefully controlled, as iron overload leads to free radical damage by the Fenton reaction. This occurs when iron ($Fe^{2+}$) reacts with hydrogen peroxide ($H_2O_2$) to generate hydroxyl radicals ($HO^{\bullet}$) (and $OH^-$) and highly reactive intermediates, causing oxidative stress in the cell [94].

The organism carefully controls iron homeostasis. Iron deficiency is a considerable problem in many parts of the world, often associated with malnutrition. However, iron overload may be derived from multiple blood transfusions, high iron intake, or mutations in genes such as HFE (coding for High Iron Fe), HAMP (coding for hepcidin), TRF2 (coding for transferrin receptor 2), and SCL40A1 (coding for ferroportin) [95,96]. In hemochromatosis the disease risks are well known, but in other diseases the role of iron is less examined, particularly in the situation in which food is abundant and overweight/metabolic syndrome is rising and a synergistic effect could be operating.

Excess iron is broadly stored in organs and tissues including the pancreatic β cells [97,98], the liver, and the skin [99]. Without treatment, the iron deposits damage these organs and tissues. Because iron amplifies oxidative damage, tissues with high mitochondrial activity are prime targets of iron-driven cellular damage. Brain cells, hepatocytes, cardiomyocytes, chondrocytes, pituitary cells, and pancreatic β cells fall in this category. Hence, cirrhosis and hepatoma, heart failure, arthritis, hypogonadotropic hypogonadism, and insulinopenic diabetes correlate with iron-overload conditions such as hereditary hemochromatosis (HH) and transfusional iron overload [100–103].

Cross talk between impaired β-cell function and iron dysmetabolism may contribute to the pathogenesis in HH. The etiology of the development of diabetes in HH is complex. Generally, two mechanisms may contribute to the progression to diabetes in HH patients: liver iron overload, leading to insulin resistance, and pancreatic β-cell iron accumulation, resulting in cell damage and diminished insulin secretion [104]. The latter was suggested by findings of oxidative stress, β-cell apoptosis, and decreased insulin secretory capacity in mouse models of hemochromatosis [105]. Thus, β-cells may be especially sensitive to oxidative stress due to reduced expression of antioxidant-scavenging enzymes such as SOD, catalase, and glutathione peroxidase. Furthermore, insulin secretion is significantly increased by correcting iron overload with phlebotomy in patients with hereditary or secondary hemochromatosis [106,107]. Lowering elevated serum ferritin levels with desferroxamine in type 2 diabetic patients or animal diabetes models correlated with improvements in glycemia, insulin sensitivity and secretion, and triglyceride levels [108–111].

Mitochondrial dysfunction/insulin resistance occurs in the metabolic syndrome, diabetes, heart failure, hereditary hemochromatosis, gout, and Alzheimer disease [106,107,112–117]. Many mitochondrial enzymes harbor Fe–S clusters. These ancient and evolutionarily conserved inorganic cofactors are important for controlling a variety of oxidative processes, including the respiratory chain [118]. It is of interest that Fe–S clusters are under redox control, and in oxidative stress concomitant with mitochondrial dysfunction the clusters can release the iron, which then becomes freely available [118–122]. Iron released from Fe–S clusters can be an additive mechanism for increased oxidative stress and mitochondrial dysfunction that should be further investigated, also from a quantitative point of view.

Epidemiological studies have shown a positive association between high body iron stores, as measured by circulating ferritin level or transferrin saturation, and risk of both type 1 [123] and type 2 diabetes [124–128] and in other insulin-resistant states such as the metabolic syndrome [124,126,129], gestational diabetes [130], and polycystic ovarian syndrome [131]. Furthermore, hemochromatosis genotype C282Y/C282Y is associated with late-onset type 1 diabetes [132] and any diabetes type [133] in Northern Europeans.

It is likely that chronic iron-catalyzed oxidative stress in the liver, muscle, and adipose tissue causes an inflammatory response and insulin resistance in these tissues.

Also, in the general population, high iron load measured as transferrin saturation levels shows a "dose–response" relationship, with overall survival having a hazards ratio up to 2.2 (95% CI 1.4–3.3) [66], and there is a significant correlation between RNA oxidation and iron concentration/ferritin levels [42]. Taken together these data suggest that iron could have an important role in determining RNA oxidation, which may be a contributing mechanism in cellular functional decline and premature aging or death. The chemical mechanism is most probably a Fenton reaction with cytosolic iron and hydrogen peroxide generated by the mitochondria, as depicted in Fig. 1. It has been advocated that there are considerable risks from iron and copper toxicity with aging, particularly because the elimination processes for these transition metals cannot cope with the overload and accumulation that happens with increasing age [90]. In addition to the increased risk of death cited above there is epidemiological evidence for links between copper and iron levels and the development of age-related degenerative diseases such as Alzheimer disease and diabetes, and there are also suggestions of links between Alzheimer disease and diabetes. The epidemiological evidence is compelling, reviewed by Brewer [90], and oxidative stress as a common mechanism is also compelling as reviewed by Grunblatt et al. [114] and Takeda et al. [134]. Furthermore, the possible pathophysiological links have been reviewed by Akter et al. [112], who also suggest that Alzheimer disease might constitute type 3 diabetes and that the treatment modalities could be the same, particularly concerning the antidiabetic drugs. Interestingly, amyloid activates the inflammasome via macrophage signaling [135], leading to the processing of interleukin-1, a proinflammatory cytokine that causes pancreatic β-cell apoptosis in part by upregulating the divalent metal transporter 1 in the β cell, leading to excessive iron import, ROS formation, and death (M. Tonnesen et al., unpublished data).

Although the epidemiological evidence is convincing, it indicates only association and not causation, and additional studies are needed for rigorous testing of causal links between diabetes, Alzheimer disease, transition metals, and oxidative stress. We propose a mechanism for the relationship between iron, oxidative stress, and RNA oxidation as depicted in Fig. 1. The proposed mechanism needs further studies to confirm proof of principle, and clinical studies to determine if it is quantitatively important, but every individual step can be tested experimentally or in clinical studies.

Diabetes type 2 is particularly interesting to examine for this mechanism because of the high glucose and the mitochondrial dysfunction. Also heart failure has been associated with insulin resistance [116], and in hemochromatosis high RNA oxidation has been demonstrated [15], so these diseases are the prime candidates in which the mechanism could operate.

## Conclusion

In conclusion, oxidation of RNA occurs in vivo in humans. RNA oxidation occurs very early in degenerative brain diseases and concomitant with ribosomal dysfunction by ribosomal stalling and production of truncated proteins. There are indications that the ribosomal dysfunction also results in protein misfolding and protein aggregation and that oxidative stress per se in a dose-dependent manner can signal proliferation and apoptosis. The evidence that oxidized RNA miscodes protein synthesis is indirect and needs more detailed investigation. However, RNA oxidation is a possible and intriguing mechanism for many diseases, in particular degenerative brain diseases, diabetes, and hemochromatosis, and this deserves further investigation. We propose that the degradation product from RNA oxidation, 8-oxo-guanosine, excreted into urine and present in cerebrospinal fluid, can be exploited as a biomarker that may provide information about the risk of complications and the treatment effects in the diseases mentioned.

## Acknowledgments

## References

[1] Crick, F. H. On protein synthesis. *Symp. Soc. Exp. Biol.* **12:**138–163; 1958.

[2] Lander, E. S.; Linton, L. M.; Birren, B., et al. Initial sequencing and analysis of the human genome. *Nature* **409:**860–921; 2001.

[3] Venter, J. C.; Adams, M. D.; Myers, E. W., et al. The sequence of the human genome. *Science* **291:**1304–1351; 2001.

[4] Birney, E.; Stamatoyannopoulos, J. A.; Dutta, A., et al. Identification and analysis of functional elements in 1% of the human genome by the ENCODE pilot project. *Nature* **447:**799–816; 2007.

[5] Pennacchio, L. A. Insights from human/mouse genome comparisons. *Mamm. Genome* **14:**429–436; 2003.

[6] Waterston, R. H.; Lindblad-Toh, K.; Birney, E., et al. Initial sequencing and comparative analysis of the mouse genome. *Nature* **420:**520–562; 2002.

[7] Nelson, P. T.; Keller, J. N. RNA in brain disease: no longer just "the messenger in the middle. *J. Neuropathol. Exp. Neurol.* **66:**461–468; 2007.

[8] Cadet, J.; Douki, T.; Gasparutto, D.; Ravanat, J. L. Oxidative damage to DNA: formation, measurement and biochemical features. *Mutat. Res.* **531:**5–23; 2003.

[9] 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.

[10] Poulsen, H. E.; Loft, S. Interpretation of oxidative DNA modification: relation between tissue levels, excretion of urinary repair products and single cell gel electrophoresis (comet assay). In: Aruoma, O.I., Halliwell, B. (Eds.), DNA & Free Radicals: Techniques, Mechanisms and Applications. OICA Int, London,pp. 261–270; 1998.

[11] Weimann, A.; Belling, D.; Poulsen, H. E. Quantification of 8-oxo-guanine and guanine as the nucleobase, nucleoside and deoxynucleoside forms in human urine by high-performance liquid chromatography–electrospray tandem mass spectrometry. *Nucleic Acids Res.* **30:**E7; 2002.

[12] Genestra, M. Oxyl radicals, redox-sensitive signalling cascades and antioxidants. *Cell. Signal.* **19:**1807–1819; 2007.

[13] Sies, H. Introductory remarks. In: Sies, H. (Ed.), Oxidative Stress. Academic Press, London,pp. 1–8; 1985.

[14] Sies, H. Oxidative stress: oxidants and antioxidants. *Exp. Physiol.* **82:**291–295; 1997.

[15] Broedbaek, K.; Poulsen, H. E.; Weimann, A.; Kom, G. D.; Schwedhelm, E.; Nielsen, P.; Boger, R. H. Urinary excretion of biomarkers of oxidatively damaged DNA and RNA in hereditary hemochromatosis. *Free Radic. Med.* **47:**1230–1233; 2009.

[16] Broedbaek, K.; Ribel-Madsen, R.; Henriksen, T.; Weimann, A.; Petersen, M.; Andersen, J. T.; Afzal, S.; Hjelvang, B.; Roberts, L. J.; Vaag, A.; Poulsen, P.; Poulsen, H. E. Genetic and environmental influences on oxidative damage assessed in elderly Danish twins. *Free Radic. Biol. Med.* **50:**1488–1491; 2011.

[17] Broedbaek, K.; Siersma, V.; Henriksen, T.; Weimann, A.; Petersen, M.; Andersen, J. T.; Jimenez-Solem, E.; Stovgaard, E. S.; Hansen, L. J.; Henriksen, J. E.; Bonnema, S. J.; de Fine, O. N.; Poulsen, H. E. Urinary markers of nucleic acid oxidation and long-term mortality of newly diagnosed type 2 diabetic patients. *Diabetes Care* **34:**2594–2596; 2011.

[18] 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.

[19] 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.

[20] Henriksen, T.; Hillestrom, P. R.; Poulsen, H. E.; Weimann, A. Automated method for the direct analysis of 8-oxo-guanosine and 8-oxo-2′-deoxyguanosine in human urine using ultraperformance liquid chromatography and tandem mass spectrometry. *Free Radic. Biol. Med.* **47:**629–635; 2009.

[21] 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.

[22] Abe, T.; Isobe, C.; Murata, T.; Sato, C.; Tohgi, H. Alteration of 8-hydroxyguanosine concentrations in the cerebrospinal fluid and serum from patients with Parkinson's disease. *Neurosci. Lett.* **336:**105–108; 2003.

[23] Abe, T.; Tohgi, H.; Isobe, C.; Murata, T.; Sato, C. Remarkable increase in the concentration of 8-hydroxyguanosine in cerebrospinal fluid from patients with Alzheimer's disease. *J. Neurosci. Res.* **70:**447–450; 2002.

[24] Bogdanov, M. B.; Beal, M. F.; McCabe, D. R.; Griffin, R. M.; Matson, W. R. A carbon column-based liquid chromatography electrochemical approach to routine 8-hydroxy-2′-deoxyguanosine measurements in urine and other biologic matrices: a one-year evaluation of methods. *Free Radic. Biol. Med.* **27:**647–666; 1999.

[25] Ren, J.; Banan, A.; Keshavarzian, A.; Zhu, Q.; Lapaglia, N.; McNulty, J.; Emanuele, N. V.; Emanuele, M. A. Exposure to ethanol induces oxidative damage in the pituitary gland. *Alcohol* **35:**91–101; 2005.

[26] Machowetz, A.; Poulsen, H. E.; Gruendel, S.; Weimann, A.; Fito, M.; Marrugat, J.; de la, T. R.; Salonen, J. T.; Nyyssonen, K.; Mursu, J.; Nascetti, S.; Gaddi, A.; Kiesewetter, H.; Baumler, H.; Selmi, H.; Kaikkonen, J.; Zunft, H. J.; Covas, M. I.; Koebnick, C. Effect of olive oils on biomarkers of oxidative DNA stress in Northern and Southern Europeans. *FASEB J.* **21:**45–52; 2007.

[27] Glintborg, B.; Weimann, A.; Kensler, T. W.; Poulsen, H. E. Oltipraz chemoprevention trial in Qidong, People's Republic of China: unaltered oxidative biomarkers. *Free Radic. Biol. Med.* **41:**1010–1014; 2006.

[28] Broedbaek, K.; Henriksen, T.; Weimann, A.; Petersen, M.; Andersen, J. T.; Afzal, S.; Jimenez-Solem, E.; Persson, F.; Parving, H. H.; Rossing, P.; Poulsen, H. E. Long-term effects of irbesartan treatment and smoking on nucleic acid oxidation in patients with type 2 diabetes and microalbuminuria: an Irbesartan in Patients with Type 2 Diabetes and Microalbuminuria (IRMA 2) substudy. *Diabetes Care* **34:**1192–1198; 2011.

[29] Davanipour, Z.; Poulsen, H. E.; Weimann, A.; Sobel, E. Endogenous melatonin and oxidatively damaged guanine in DNA. *BMC Endocr. Disord.* **9:**22; 2009.

[30] Fiala, E. S.; Conaway, C. C.; Mathis, J. E. Oxidative DNA and RNA damage in the livers of Sprague–Dawley rats treated with the hepatocarcinogen 2-nitropropane. *Cancer Res.* **49:**5518–5522; 1989.

[31] Hillestrom, P. R.; Weimann, A.; Jensen, C. B.; Storgaard, H.; Vaag, A. A.; Poulsen, H. E. Consequences of low birthweight on urinary excretion of DNA markers of oxidative stress in young men. *Scand. J. Clin. Lab. Invest.* **66:**363–370; 2006.

[32] Petersen, R. B.; Siedlak, S. L.; Lee, H. G.; Kim, Y. S.; Nunomura, A.; Tagliavini, F.; Ghetti, B.; Cras, P.; Moreira, P. I.; Castellani, R. J.; Guentchev, M.; Budka, H.; Ironside, J. W.; Gambetti, P.; Smith, M. A.; Perry, G. Redox metals and oxidative abnormalities in human prion diseases. *Acta Neuropathol.* **110:**232–238; 2005.

[33] Deslee, G.; Woods, J. C.; Moore, C.; Conradi, S. H.; Gierada, D. S.; Atkinson, J. J.; Battaile, J. T.; Liu, L.; Patterson, G. A.; ir-Kirk, T. L.; Holtzman, M. J.; Pierce, R. A. Oxidative damage to nucleic acids in severe emphysema. *Chest* **135:**965–974; 2009.

[34] Hoffman, W. H.; Siedlak, S.; Wang, Y.; Castellani, R. J.; Smith, M. A. Oxidative damage is present in the fatal brain edema of diabetic ketoacidosis. *Brain Res.* **1369:**194–202; 2011.

[35] Hayashi, M.; Araki, S.; Kohyama, J.; Shioda, K.; Fukatsu, R. Oxidative nucleotide damage and superoxide dismutase expression in the brains of xeroderma pigmentosum group A and Cockayne syndrome. *Brain Dev.* **27:**34–38; 2005.

[36] Miyata, R.; Hayashi, M.; Tanuma, N.; Shioda, K.; Fukatsu, R.; Mizutani, S. Oxidative stress in neurodegeneration in dentatorubral–pallidoluysian atrophy. *J. Neurol. Sci.* **264:**133–139; 2008.

[37] Hayashi, M.; Arai, N.; Satoh, J.; Suzuki, H.; Katayama, K.; Tamagawa, K.; Morimatsu, Y. Neurodegenerative mechanisms in subacute sclerosing panencephalitis. *J. Child Neurol.* **17:**725–730; 2002.

[38] Park, E. M.; Shigenaga, M. K.; Degan, P.; Korn, T. S.; Kitzler, J. W.; Wehr, C. M.; Kolachana, P.; Ames, B. N. Assay of excised oxidative DNA lesions: isolation of 8-oxoguanine and its nucleoside derivatives from biological fluids with a monoclonal antibody column. *Proc. Natl. Acad. Sci. U. S. A.* **89:**3375–3379; 1992.

[39] Andreoli, R.; Manini, P.; De, P. G.; Alinovi, R.; Goldoni, M.; Niessen, W. M.; Mutti, A. Quantitative determination of urinary 8-oxo-7,8-dihydro-2′-deoxyguanosine, 8-oxo-7,8-dihydroguanine, 8-oxo-7,8-dihydroguanosine, and their non-oxidized forms: daily concentration profile in healthy volunteers. *Biomarkers* **15:**221–231; 2010.

[40] Andreoli, R.; Mutti, A.; Goldoni, M.; Manini, P.; Apostoli, P.; De, P. G. Reference ranges of urinary biomarkers of oxidized guanine in (2′-deoxy)ribonucleotides and nucleic acids. *Free Radic. Biol. Med.* **50:**254–261; 2011.

[41] Hofer, T.; Fontana, L.; Anton, S. D.; Weiss, E. P.; Villareal, D.; Malayappan, B.; Leeuwenburgh, C. Long-term effects of caloric restriction or exercise on DNA and RNA oxidation levels in white blood cells and urine in humans. *Rejuvenation Res.* **11:**793–799; 2008.

[42] Broedbaek, K.; Siersma, V.; Andersen, J. T.; Petersen, M.; Afzal, S.; Hjelvang, B.; Weimann, A.; Semba, R. D.; Ferrucci, L.; Poulsen, H. E. The association between low-grade inflammation, iron status and nucleic acid oxidation in the elderly. *Free Radic. Res.* **45:**409–416; 2011.

[43] Manini, P.; De, P. G.; Andreoli, R.; Marczynski, B.; Hanova, M.; Mozzoni, P.; Naccarati, A.; Vodickova, L.; Hlavac, P.; Mutti, A.; Vodicka, P. Biomarkers of nucleic acid oxidation, polymorphism in, and expression of, hOGG1 gene in styrene-exposed workers. *Toxicol. Lett.* **190:**41–47; 2009.

[44] Gorg, B.; Qvartskhava, N.; Bidmon, H. J.; Palomero-Gallagher, N.; Kircheis, G.; Zilles, K.; Haussinger, D. Oxidative stress markers in the brain of patients with cirrhosis and hepatic encephalopathy. *Hepatology* **52:**256–265; 2010.

[45] Martinet, W.; De Meyer, G. R.; Herman, A. G.; Kockx, M. M. Reactive oxygen species induce RNA damage in human atherosclerosis. *Eur. J. Clin. Invest.* **34:**323–327; 2004.

[46] Martinet, W.; De Meyer, G. R.; Herman, A. G.; Kockx, M. M. RNA damage in human atherosclerosis: pathophysiological significance and implications for gene expression studies. *RNA Biol.* **2:**4–7; 2005.

[47] Chang, Y.; Kong, Q.; Shan, X.; Tian, G.; Ilieva, H.; Cleveland, D. W.; Rothstein, J. D.; Borchelt, D. R.; Wong, P. C.; Lin, C. L. Messenger RNA oxidation occurs early in disease pathogenesis and promotes motor neuron degeneration in ALS. *PLoS One* **3:**e2849; 2008.

[48] Zhang, J.; Perry, G.; Smith, M. A.; Robertson, D.; Olson, S. J.; Graham, D. G.; Montine, T. J. Parkinson's disease is associated with oxidative damage to cytoplasmic DNA and RNA in substantia nigra neurons. *Am. J. Pathol.* **154:**1423–1429; 1999.

[49] Isobe, C.; Abe, T.; Terayama, Y. Homocysteine may contribute to pathogenesis of RNA damage in brains with Alzheimer's disease. *Neurodegener Dis* **6:**252–257; 2009.

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

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

[52] 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.

[53] Nunomura, A.; Perry, G.; Hirai, K.; Aliev, G.; Takeda, A.; Chiba, S.; Smith, M. A. Neuronal RNA oxidation in Alzheimer's disease and Down's syndrome. *Ann. N. Y. Acad. Sci.* **893:**362–364; 1999.

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

[55] 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.

[56] Nunomura, A.; Tamaoki, T.; Tanaka, K.; Motohashi, N.; Nakamura, M.; Hayashi, T.; Yamaguchi, H.; Shimohama, S.; Lee, H. G.; Zhu, X.; Smith, M. A.; Perry, G. Intraneuronal amyloid β accumulation and oxidative damage to nucleic acids in Alzheimer disease. *Neurobiol. Dis.* **37:**731–737; 2010.

[57] 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.

[58] Holcomb, D. R.; Ropp, P. A.; Theil, E. C.; Thorp, H. H. Nature of guanine oxidation in RNA via the flash-quench technique versus direct oxidation by a metal oxo complex. *Inorg. Chem.* **49:**786–795; 2010.

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

[60] Cooke, M. S. A commentary on "Urea, the most abundant component in urine, cross-reacts with a commercial 8-OH-dG ELISA kit and contributes to overestimation of urinary 8-OH-dG." What is ELISA detecting? *Free Radic. Biol. Med.* **47:**30–31; 2009.

[61] Tanaka, M.; Song, H.; Kupfer, P. A.; Leumann, C. J.; Sonntag, W. E. An assay for RNA oxidation induced abasic sites using the aldehyde reactive probe. *Free Radic. Res.* **45:**237–247; 2011.

[62] Poulsen, H. E. Oxidative DNA modifications. *Exp. Toxicol. Pathol.* **57** (Suppl. 1)**:** 161–169; 2005.

[63] Collins, A. R.; Brown, J.; Bogdanov, M.; Cadet, J.; Cooke, M.; Douki, T.; Dunster, C.; Eakins, J.; Epe, B.; Evans, M.; Farmer, P.; Gedik, C. M.; Halliwell, B.; Herbert, K.; Hofer, T.; Hutchinson, R.; Jenner, A.; Jones, G. D. D.; Kasai, H.; Kelly, F.; Lloret, A.; Loft, S.; Lunec, J.; McEwan, M.; Moller, L.; Olinski, R.; Podmore, I.; Poulsen, H.; Ravanat, J. L.; Rees, J. F.; Reetz, F.; Shertzer, H.; Spiegelhalder, B.; Turesky, R.; Tyrrell, R.; Vina, J.; Vinicombe, D.; Weimann, A.; de Wergifosse, B.; Wood, S. G. Comparison of different methods of measuring 8-oxoguanine as a marker of oxidative DNA damage. *Free Radic. Res.* **32:**333–341; 2000.

[64] Collins, A. R.; Cadet, J.; Moller, L.; Poulsen, H. E.; Vina, J. Are we sure we know how to measure 8-oxo-7,8-dihydroguanine in DNA from human cells? *Arch. Biochem. Biophys.* **423:**57–65; 2004.

[65] Cooke, M. S.; Barregard, L.; Mistry, V.; Potdar, N.; Rozalski, R.; Gackowski, D.; Siomek, A.; Foksinski, M.; Svoboda, P.; Kasai, H.; Konje, J. C.; Sallsten, G.; Evans, M. D.; Olinski, R. Interlaboratory comparison of methodologies for the measurement of urinary 8-oxo-7,8-dihydro-2′-deoxyguanosine. *Biomarkers* **14:** 103–110; 2009.

[66] Ellervik, C.; Tybjaerg-Hansen, A.; Nordestgaard, B. G. Total mortality by transferrin saturation levels: two general population studies and a metaanalysis. *Clin. Chem.* **57:**459–466; 2011.

[67] Jacobs, A. C.; Resendiz, M. J.; Greenberg, M. M. Direct strand scission from a nucleobase radical in RNA. *J. Am. Chem. Soc.* **132:**3668–3669; 2010.

[68] Ding, Q.; Dimayuga, E.; Keller, J. N. Oxidative damage, protein synthesis, and protein degradation in Alzheimer's disease. *Curr. Alzheimer Res.* **4:**73–79; 2007.

[69] Ding, Q.; Dimayuga, E.; Keller, J. N. Oxidative stress alters neuronal RNA- and protein-synthesis: implications for neural viability. *Free Radic. Res.* **41:** 903–910; 2007.

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

[71] Hayakawa, H.; Fujikane, A.; Ito, R.; Matsumoto, M.; Nakayama, K. I.; Sekiguchi, M. Human proteins that specifically bind to 8-oxoguanine-containing RNA and their responses to oxidative stress. *Biochem. Biophys. Res. Commun.* **403:**220–224; 2010.

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

[73] 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.

[74] 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.

[75] Hoeijmakers, J. H. DNA damage, aging, and cancer. *N. Engl. J. Med.* **361:** 1475–1485; 2009.

[76] Barnes, T.; Kim, W. C.; Mantha, A. K.; Kim, S. E.; Izumi, T.; Mitra, S.; Lee, C. H. Identification of apurinic/apyrimidinic endonuclease 1 (APE1) as the endoribonuclease that cleaves c-myc mRNA. *Nucleic Acids Res.* **37:**3946–3958; 2009.

[77] Vascotto, C.; Fantini, D.; Romanello, M.; Cesaratto, L.; Deganuto, M.; Leonardi, A.; Radicella, J. P.; Kelley, M. R.; D'Ambrosio, C.; Scaloni, A.; Quadrifoglio, F.; Tell, G. APE1/Ref-1 interacts with NPM1 within nucleoli and plays a role in the rRNA quality control process. *Mol. Cell. Biol.* **29:**1834–1854; 2009.

[78] 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.

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

[80] 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.

[81] Henderson, P. T.; Evans, M. D.; Cooke, M. S. Salvage of oxidized guanine derivatives in the (2′-deoxy)ribonucleotide pool as source of mutations in DNA. *Mutat. Res.* **703:**11–17; 2010.

[82] Haghdoost, S.; Sjolander, L.; Czene, S.; Harms-Ringdahl, M. The nucleotide pool is a significant target for oxidative stress. *Free Radic. Biol. Med.* **41:**620–626; 2006.

[83] 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.

[84] Bestwick, R. K.; Moffett, G. L.; Mathews, C. K. Selective expansion of mitochondrial nucleoside triphosphate pools in antimetabolite-treated HeLa cells. *J. Biol. Chem.* **257:**9300–9304; 1982.

[85] Collins, A.; Oates, D. J. Hydroxyurea: effects on deoxyribonucleotide pool sizes correlated with effects on DNA repair in mammalian cells. *Eur. J. Biochem.* **169:** 299–305; 1987.

[86] Korte, d. D.; Haverkort, W. A.; de, B. M.; van Gennip, A. H.; Roos, D. Imbalance in the nucleotide pools of myeloid leukemia cells and HL-60 cells: correlation with cell-cycle phase, proliferation, differentiation, and transformation. *Cancer Res.* **47:**1841–1847; 1987.

[87] Iordanov, M. S.; Ryabinina, O. P.; Wong, J.; Dinh, T. H.; Newton, D. L.; Rybak, S. M.; Magun, B. E. Molecular determinants of apoptosis induced by the cytotoxic ribonuclease onconase: evidence for cytotoxic mechanisms different from inhibition of protein synthesis. *Cancer Res.* **60:**1983–1994; 2000.

[88] Kong, Q.; Shan, X.; Chang, Y.; Tashiro, H.; Lin, C. L. RNA oxidation: a contributing factor or an epiphenomenon in the process of neurodegeneration. *Free Radic. Res.* **42:**773–777; 2008.

[89] Brewer, G. J. The risks of copper toxicity contributing to cognitive decline in the aging population and to Alzheimer's disease. *J. Am. Coll. Nutr.* **28:**238–242; 2009.

[90] Brewer, G. J. Risks of copper and iron toxicity during aging in humans. *Chem. Res. Toxicol.* **23:**319–326; 2010.

[91] Jomova, K.; Valko, M. Advances in metal-induced oxidative stress and human disease. *Toxicology* **283:**65–87; 2011.

[92] 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.

[93] 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. U. S. A.* **94:**9866–9868; 1997.

[94] Lenzen, S. Oxidative stress: the vulnerable β-cell. *Biochem. Soc. Trans.* **36:** 343–347; 2008.

[95] Pietrangelo, A. Hereditary hemochromatosis—a new look at an old disease. *N. Engl. J. Med.* **350:**2383–2397; 2004.

[96] Theil, E. C. Iron homeostasis and nutritional iron deficiency. *J. Nutr.* **141:** 724S–728S; 2011.

[97] Lu, J. P.; Hayashi, K. Selective iron deposition in pancreatic islet B cells of transfusional iron-overloaded autopsy tissue. *Pathol. Int.* **44:**194–199; 1994.

[98] Lu, J. P.; Hayashi, K.; Okada, S.; Awai, M. Transferrin receptors and selective iron deposition in pancreatic B cells of iron-overloaded rats. *Acta Pathol. Jpn.* **41:** 647–652; 1991.

[99] Fowler, C. Hereditary hemochromatosis: pathophysiology, diagnosis, and management. *Crit. Care Nurs. Clin. North Am.* **20:**191–201 vi; 2008.

[100] Aldouri, M. A.; Wonke, B.; Hoffbrand, A. V.; Flynn, D. M.; Ward, S. E.; Agnew, J. E.; Hilson, A. J. High incidence of cardiomyopathy in β-thalassaemia patients receiving regular transfusion and iron chelation: reversal by intensified chelation. *Acta Haematol.* **84:**113–117; 1990.

[101] Eaton, J. W.; Qian, M. Molecular bases of cellular iron toxicity. *Free Radic. Biol. Med.* **32:**833–840; 2002.

[102] Gabutti, V.; Borgna-Pignatti, C. Clinical manifestations and therapy of transfusional haemosiderosis. *Baillieres Clin. Haematol.* **7:**919–940; 1994.

[103] Niederau, C.; Fischer, R.; Sonnenberg, A.; Stremmel, W.; Trampisch, H. J.; Strohmeyer, G. Survival and causes of death in cirrhotic and in noncirrhotic patients with primary hemochromatosis. *N. Engl. J. Med.* **313:**1256–1262; 1985.

[104] Pietrangelo, A. Hereditary hemochromatosis. *Annu. Rev. Nutr.* **26:**251–270; 2006.

[105] Cooksey, R. C.; Jouihan, H. A.; Ajioka, R. S.; Hazel, M. W.; Jones, D. L.; Kushner, J. P.; McClain, D. A. Oxidative stress, β-cell apoptosis, and decreased insulin secretory capacity in mouse models of hemochromatosis. *Endocrinology* **145:**5305–5312; 2004.

[106] Abraham, D.; Rogers, J.; Gault, P.; McClain, D. A. Increased insulin secretory capacity but decreased insulin sensitivity after correction of iron overload by phlebotomy in hereditary haemochromatosis. *Diabetologia* **49:** 2546–2551; 2006.

[107] McClain, D. A.; Abraham, D.; Rogers, J.; Brady, R.; Gault, P.; Ajioka, R.; Kushner, J. P. High prevalence of abnormal glucose homeostasis secondary to decreased insulin secretion in individuals with hereditary haemochromatosis. *Diabetologia* **49:**1661–1669; 2006.

[108] Brittenham, G. M.; Griffith, P. M.; Nienhuis, A. W.; McLaren, C. E.; Young, N. S.; Tucker, E. E.; Allen, C. J.; Farrell, D. E.; Harris, J. W. Efficacy of deferoxamine in preventing complications of iron overload in patients with thalassemia major. *N. Engl. J. Med.* **331:**567–573; 1994.

[109] Cooksey, R. C.; Jones, D.; Gabrielsen, S.; Huang, J.; Simcox, J. A.; Luo, B.; Soesanto, Y.; Rienhoff, H.; Abel, E. D.; McClain, D. A. Dietary iron restriction or iron chelation

protects from diabetes and loss of β-cell function in the obese (ob/ob lep−/−) mouse. *Am. J. Physiol. Endocrinol. Metab.* **298:**E1236–E1243; 2010.

[110] Cutler, P. Deferoxamine therapy in high-ferritin diabetes. *Diabetes* **38:** 1207–1210; 1989.

[111] Minamiyama, Y.; Takemura, S.; Kodai, S.; Shinkawa, H.; Tsukioka, T.; Ichikawa, H.; Naito, Y.; Yoshikawa, T.; Okada, S. Iron restriction improves type 2 diabetes mellitus in Otsuka Long-Evans Tokushima fatty rats. *Am. J. Physiol. Endocrinol. Metab.* **298:**E1140–E1149; 2010.

[112] Akter, K.; Lanza, E. A.; Martin, S. A.; Myronyuk, N.; Rua, M.; Raffa, R. B. Diabetes mellitus and Alzheimer's disease: shared pathology and treatment? *Br. J. Clin. Pharmacol.* **71:**365–376; 2011.

[113] Choi, H. K.; Ford, E. S.; Li, C.; Curhan, G. Prevalence of the metabolic syndrome in patients with gout: the Third National Health and Nutrition Examination Survey. *Arthritis Rheum.* **57:**109–115; 2007.

[114] Grunblatt, E.; Bartl, J.; Riederer, P. The link between iron, metabolic syndrome, and Alzheimer's disease. *J. Neural Transm.* **118:**371–379; 2011.

[115] Henriksen, E. J.; Diamond-Stanic, M. K.; Marchionne, E. M. Oxidative stress and the etiology of insulin resistance and type 2 diabetes. *Free Radic. Biol. Med.* **51:** 993–999; 2010.

[116] Li, C.; Ford, E. S.; McGuire, L. C.; Mokdad, A. H. Association of metabolic syndrome and insulin resistance with congestive heart failure: findings from the Third National Health and Nutrition Examination Survey. *J. Epidemiol. Community Health* **61:**67–73; 2007.

[117] Lowell, B. B.; Shulman, G. I. Mitochondrial dysfunction and type 2 diabetes. *Science* **307:**384–387; 2005.

[118] Xu, X. M.; Moller, S. G. Iron–sulfur clusters: biogenesis, molecular mechanisms, and their functional significance. *Antioxid. Redox Signal.* **15:**271–307; 2011.

[119] Kohanski, M. A.; Dwyer, D. J.; Collins, J. J. How antibiotics kill bacteria: from targets to networks. *Nat. Rev. Microbiol.* **8:**423–435; 2010.

[120] Kohanski, M. A.; Dwyer, D. J.; Hayete, B.; Lawrence, C. A.; Collins, J. J. A common mechanism of cellular death induced by bactericidal antibiotics. *Cell* **130:**797–810; 2007.

[121] Outten, F. W.; Theil, E. C. Iron-based redox switches in biology. *Antioxid. Redox Signal.* **11:**1029–1046; 2009.

[122] Rouault, T. A.; Tong, W. H. Iron–sulfur cluster biogenesis and human disease. *Trends Genet.* **24:**398–407; 2008.

[123] Ellervik, C.; Mandrup-Poulsen, T.; Andersen, H. U.; Tybjerg-Hansen, A.; Frandsen, M.; Birgens, H.; Nordestgaard, B. G. Elevated transferrin saturation and risk of diabetes. *Diabetes Care* **34:**2256–2258; 2011.

[124] Fernandez-Real, J. M.; Lopez-Bermejo, A.; Ricart, W. Cross-talk between iron metabolism and diabetes. *Diabetes* **51:**2348–2354; 2002.

[125] Hernandez, C.; Lecube, A.; Carrera, A.; Simo, R. Soluble transferrin receptors and ferritin in type 2 diabetic patients. *Diabetes Med.* **22:**97–101; 2005.

[126] Rajpathak, S. N.; Crandall, J. P.; Wylie-Rosett, J.; Kabat, G. C.; Rohan, T. E.; Hu, F. B. The role of iron in type 2 diabetes in humans. *Biochim. Biophys. Acta* **1790:** 671–681; 2009.

[127] Reddy, M. B.; Clark, L. Iron, oxidative stress, and disease risk. *Nutr. Rev.* **62:** 120–124; 2004.

[128] Wilson, J. G.; Lindquist, J. H.; Grambow, S. C.; Crook, E. D.; Maher, J. F. Potential role of increased iron stores in diabetes. *Am. J. Med. Sci.* **325:**332–339; 2003.

[129] Sheu, W. H.; Chen, Y. T.; Lee, W. J.; Wang, C. W.; Lin, L. Y. A relationship between serum ferritin and the insulin resistance syndrome is present in non-diabetic women but not in non-diabetic men. *Clin. Endocrinol. (Oxford)* **58:**380–385; 2003.

[130] Lao, T. T.; Chan, P. L.; Tam, K. F. Gestational diabetes mellitus in the last trimester—a feature of maternal iron excess? *Diabetes Med.* **18:**218–223; 2001.

[131] Luque-Ramirez, M.; Alvarez-Blasco, F.; Botella-Carretero, J. I.; Sanchon, R.; San Millan, J. L.; Escobar-Morreale, H. F. Increased body iron stores of obese women with polycystic ovary syndrome are a consequence of insulin resistance and hyperinsulinism and are not a result of reduced menstrual losses. *Diabetes Care* **30:**2309–2313; 2007.

[132] Ellervik, C.; Mandrup-Poulsen, T.; Nordestgaard, B. G.; Larsen, L. E.; Appleyard, M.; Frandsen, M.; Petersen, P.; Schlichting, P.; Saermark, T.; Tybjaerg-Hansen, A.; Birgens, H. Prevalence of hereditary haemochromatosis in late-onset type 1 diabetes mellitus: a retrospective study. *Lancet* **358:** 1405–1409; 2001.

[133] Ellervik, C.; Birgens, H.; Tybjaerg-Hansen, A.; Nordestgaard, B. G. Hemochromatosis genotypes and risk of 31 disease endpoints: meta-analyses including 66,000 cases and 226,000 controls. *Hepatology* **46:** 1071–1080; 2007.

[134] Takeda, S.; Sato, N.; Rakugi, H.; Morishita, R. Molecular mechanisms linking diabetes mellitus and Alzheimer disease: β-amyloid peptide, insulin signaling, and neuronal function. *Mol. Biosyst.* **7:**1822–1827; 2011.

[135] Masters, S. L.; Dunne, A.; Subramanian, S. L.; Hull, R. L.; Tannahill, G. M.; Sharp, F. A.; Becker, C.; Franchi, L.; Yoshihara, E.; Chen, Z.; Mullooly, N.; Mielke, L. A.; Harris, J.; Coll, R. C.; Mills, K. H.; Mok, K. H.; Newsholme, P.; Nunez, G.; Yodoi, J.; Kahn, S. E.; Lavelle, E. C.; O'Neill, L. A. Activation of the NLRP3 inflammasome by islet amyloid polypeptide provides a mechanism for enhanced IL-1β in type 2 diabetes. *Nat. Immunol.* **11:**897–904; 2010.