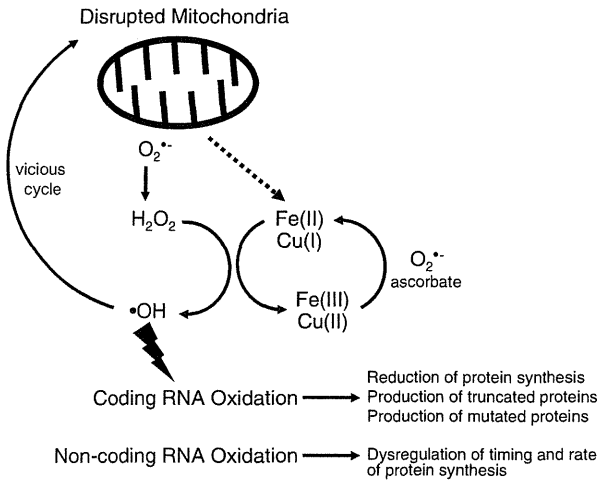

Fig. 2 Hypothetical schema of upstream mediators and downstream consequences of cellular RNA oxidation. Disrupted mitochondria (Hirai et al. 2001) likely play a central role in producing ROS as well as supplying redox-active metals (iron and copper) in the cytosol to catalyze •OH production through the Fenton/Harber–Weiss reactions. Oxidation of coding RNAs leads to reduced level of functional proteins, and to formation of non-functional, truncated proteins or mutated proteins. Non-coding RNAs could also be oxidized and their complex regulatory functions in protein synthesis should be impeded, while this interesting aspect still remains largely unexplored

(Schapira et al. 1990; Gu et al. 2002) and metal ion dysregulation (Sofic et al. 1988; Berg et al. 2002) are also found in the substantia nigra of PD, making this mechanism a common theme in neurodegenerative cascades.

Conclusion

Involvement of RNA oxidation in the process of aging and neurodegeneration has been demonstrated in human brain tissue and experimental models. Indeed, remarkable RNA oxidation has been observed in vulnerable neuronal population of AD, PD, DLB, ALS, DRPLA (a CAG-repeat disease), and prion diseases (CJD and GSS). Particular emphasis should be placed on the early-stage involvement of RNA oxidation in the process of neurodegeneration, which suggests a primary role of RNA oxidation in the pathomechanisms. Indeed, oxidized RNA is associated with a disturbance in protein synthesis in vitro and in vivo. There are only a small number of studies suggesting the existence of coping mechanisms for RNA damage at present. Understanding of the consequences and cellular handling mechanisms of the oxidative RNA damage may provide clues to the underlying mechanisms of aging as well as pathophysiology of chronic degenerative disorders and lead to better anti-aging and therapeutic strategies.

References

Aas PA, Otterlei M, Falnes PO et al (2003) Human and bacterial oxidative demethylases repair alkylation damage in both RNA and DNA. Nature 421:859–863

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

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

Aluise CD, Robinson RA, Beckett TL et al (2010) Preclinical Alzheimer disease; brain oxidative stress, Aβ peptide and proteomics. Neurobiol Dis 39:221–228

Aluise CD, Robinson RA, Cai J, Pierce WM, Markesbery WR, Butterfield DA (2011) Redox proteomics analysis of brains from subjects with amnestic mild cognitive impairment compared to brains from subjects with preclinical Alzheimer's disease; insights into memory loss in MCI. J Alzheimers Dis 23:257–269

Ames BN, Gold LS (1991) Endogenous mutagens and the causes of aging and cancer. Mutat Res 250:3–16

Andersen JK (2004) Oxidative stress in neurodegeneration: cause or consequence? Nat Med 10(Suppl):S18–S25

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

Barber SC, Mead RJ, Shaw PJ (2006) Oxidative stress in ALS: a mechanism of neurodegeneration and a therapeutic target. Biochim Biophys Acta 1762:1051–1067

Barciszewski J, Barciszewska MZ, Siboska G, Rattan SI, Clark BF (1999) Some unusual nucleic acid bases are products of hydroxyl radical oxidation of DNA and RNA. Mol Biol Rep 26:231–238

Barnham KJ, Masters CL, Bush AI (2004) Neurodegenerative diseases and oxidative stress. Nat Rev Drug Discov 3:205–214

Bellacosa A, Moss EG (2003) RNA repair: damage control. Curr Biol 13:R482–R484

Berg D, Roggendorf W, Schroder U et al (2002) Echogenicity of the substantia nigra: association with increased iron content and marker for susceptibility to nigrostriatal injury. Arch Neurol 59:999–1005

Bradley MA, Markesbery WR, Lovell MA (2010) Increased levels of 4-hydroxynonenal and acrolein in the brain in preclinical Alzheimer disease. Free Radic Biol Med 48:1570–1576

Brégeon D, Sarasin A (2005) Hypothetical role of RNA damage avoidance in preventing human disease. Mutat Res 577:293–302

Broedbaek K, Poulsen HE, Weimann A et al (2009) Urinary excretion of biomarkers of oxidatively damaged DNA and RNA in hereditary hemochromatosis. Free Radic Biol Med 47:1230–1233

Broedbaek K, Siersma V, Henriksen T et al (2011) Urinary markers of nucleic acid oxidation and long-term mortality of newly diagnosed type 2 diabetic patients. Diabetes Care 34:2594–2596

Butterfield DA, Reed TT, Perluigi M et al (2007) Elevated levels of 3-nitrotyrosine in brain from subjects with amnestic mild cognitive impairment: implications for the role of nitration in the progression of Alzheimer's disease. Brain Res 1148:243–248

Calabrese V, Cornelius C, Dinkova-Kostova AT, Calabrese EJ, Mattson MP (2010) Cellular stress responses, the hormesis paradigm, and vitagenes: novel targets for therapeutic intervention in neurodegenerative disorders. Antioxid Redox Signal 13:1763–1811

Calabrese V, Cornelius C, Dinkova-Kostova AT et al (2012) Cellular stress responses, hormetic phytochemicals and vitagenes in aging and longevity. Biochim Biophys Acta 1822:753–783

Cao X, Yeo G, Muotri AR, Kuwabara T, Gage FH (2006) Noncoding RNAs in the mammalian central nervous system. Annu Rev Neurosci 29:77–103

Casadesus G, Smith MA, Basu S et al (2007) Increased isoprostane and prostaglandin are prominent in neurons in Alzheimer disease. Mol Neurodegener 2:2

Castellani RJ, Harris PL, Sayre LM et al (2001) Active glycation in neurofibrillary pathology of Alzheimer disease: N(epsilon)-(carboxymethyl) lysine and hexitol-lysine. Free Radic Biol Med 31:175–180

Chang Y, Kong Q, Shan X et al (2008) Messenger RNA oxidation occurs early in disease pathogenesis and promotes motor neuron degeneration in ALS. PLoS ONE 3:e2849

Che Y, Wang JF, Shao L, Young T (2010) Oxidative damage to RNA but not DNA in the hippocampus of patients with major mental illness. J Psychiatry Neurosci 35:296–302

Cooke MS, Olinski R, Evans MD (2006) Does measurement of oxidative damage to DNA have clinical significance? Clin Chim Acta 365:30–49

Costa FF (2005) Non-coding RNAs: new players in eukaryotic biology. Gene 357:83–94

Coyle JT, Puttfarcken P (1993) Oxidative stress, glutamate, and neurodegenerative disorders. Science 262:689–695

Cui L, Hofer T, Rani A, Leeuwenburgh C, Foster TC (2009) Comparison of lifelong and late life exercise on oxidative stress in the cerebellum. Neurobiol Aging 30:903–909

Deutscher MP (2006) Degradation of RNA in bacteria: comparison of mRNA and stable RNA. Nucleic Acids Res 34:659–666

Ding Q, Dimayuga E, Markesbery WR, Keller JN (2004) Proteasome inhibition increases DNA and RNA oxidation in astrocyte and neuron cultures. J Neurochem 91:1211–1218

Ding Q, Markesbery WR, Chen Q, Li F, Keller JN (2005) Ribosome dysfunction is an early event in Alzheimer's disease. J Neurosci 25:9171–9175

Ding Q, Markesbery WR, Cecarini V, Keller JN (2006) Decreased RNA, and increased RNA oxidation, in ribosomes from early Alzheimer's disease. Neurochem Res 31:705–710

Ding Q, Cecarini V, Keller JN (2007) Interplay between protein synthesis and degradation in the CNS: physiological and pathological implications. Trends Neurosci 30:31–36

Evans MD, Dizdaroglu M, Cooke MS (2004) Oxidative DNA damage and disease: induction, repair and significance. Mutat Res 567:1–61

Fiala ES, Conaway CC, Mathis JE (1989) Oxidative DNA and RNA damage in the livers of Sprague-Dawley rats treated with the hepatocarcinogen 2-nitropropane. Cancer Res 49:5518–5522

Foksinski M, Rozalski R, Guz J et al (2004) Urinary excretion of DNA repair products correlates with metabolic rates as well as with maximum life spans of different mammalian species. Free Radic Biol Med 37:1449–1454

Furuta A, Iida T, Nakabeppu Y, Iwaki T (2001) Expression of hMTH1 in the hippocampi of control and Alzheimer's disease. NeuroReport 12:2895–2899

Gemma C, Vila J, Bachstetter A, Bickford PC (2007) Oxidative stress and the aging brain: from theory to prevention, Chapter 15. In: Riddle DR (ed) Brain aging: models, methods, and mechanisms. CRC Press, Boca Raton, FL

Gong X, Tao R, Li Z (2006) Quantification of RNA damage by reverse transcription polymerase chain reactions. Anal Biochem 357:58–67

Görg B, Qvartskhava N, Keitel V et al (2008) Ammonia induces RNA oxidation in cultured astrocytes and brain in vivo. Hepatology 48:567–579

Görg B, Qvartskhava N, Bidmon HJ et al (2010) Oxidative stress markers in the brain of patients with cirrhosis and hepatic encephalopathy. Hepatology 52:256–265

Gu G, Reyes PE, Golden GT et al (2002) Mitochondrial DNA deletions/rearrangements in Parkinson disease and related neurodegenerative disorders. J Neuropathol Exp Neurol 61:634–639

Guentchev M, Siedlak SL, Jarius C et al (2002) Oxidative damage to nucleic acids in human prion disease. Neurobiol Dis 9:275–281

Guidi I, Galimberti D, Lonati S et al (2006) Oxidative imbalance in patients with mild cognitive impairment and Alzheimer's disease. Neurobiol Aging 27:262–269

Gurney ME, Pu H, Chiu AY et al (1994) Motor neuron degeneration in mice that express a human Cu, Zn superoxide dismutase mutation. Science 264:1772–1775

Halliwell B (1992) Reactive oxygen species and the central nervous system. J Neurochem 59:1609–1623

Hayakawa H, Sekiguchi M (2006) Human polynucleotide phosphorylase protein in response to oxidative stress. Biochemistry (Mosc) 45:6749–6755

Hayakawa H, Hofer A, Thelander L et al (1999) Metabolic fate of oxidized guanine ribonucleotides in mammalian cells. Biochemistry (Mosc) 38:3610–3614

Hayakawa H, Kuwano M, Sekiguchi M (2001) Specific binding of 8-oxoguanine-containing RNA to polynucleotide phosphorylase protein. Biochemistry (Mosc) 40:9977–9982

Hayakawa H, Uchiumi T, Fukuda T et al (2002) Binding capacity of human YB-1 protein for RNA containing 8-oxoguanine. Biochemistry (Mosc) 41:12739–12744

Hayakawa H, Fujikane A, Ito R, Matsumoto M, Nakayama KI, Sekiguchi M (2010) Human proteins that specifically bind to 8-oxoguanine-containing RNA and their responses to oxidative stress. Biochem Biophys Res Commun 403:220–224

Hayashi M, Arai N, Satoh J et al (2002) Neurodegenerative mechanisms in subacute sclerosing panencephalitis. J Child Neurol 17:725–730

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

Herrup K (2010) Reimagining Alzheimer's disease—an age-based hypothesis. J Neurosci 30:16755–16762

Hirai K, Aliev G, Nunomura A et al (2001) Mitochondrial abnormalities in Alzheimer's disease. J Neurosci 21:3017–3023

Hirtz D, Thurman DJ, Gwinn-Hardy K, Mohamed M, Chaudhuri AR, Zalutsky R (2007) How common are the "common" neurologic disorders? Neurology 68:326–337

Hofer T, Badouard C, Bajak E, Ravanat JL, Mattsson A, Cotgreave IA (2005) Hydrogen peroxide causes greater oxidation in cellular RNA than in DNA. Biol Chem 386:333–337

Hofer T, Seo AY, Prudencio M, Leeuwenburgh C (2006) 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

Hofer T, Fontana L, Anton SD et al (2008a) 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

Hofer T, Marzetti E, Xu J et al (2008b) Increased iron content and RNA oxidative damage in skeletal muscle with aging and disuse atrophy. Exp Gerontol 43:563–570

Hoffman WH, Siedlak SL, Wang Y, Castellani RJ, Smith MA (2011) Oxidative damage is present in the fatal brain edema of diabetic ketoacidosis. Brain Res 1369:194–202

Holcomb DR, Ropp PA, Theil EC, Thorp HH (2010) Nature of guanine oxidation in RNA via the flash-quench technique versus direct oxidation by a metal oxo complex. Inorg Chem 49:786–795

Honda K, Smith MA, Zhu X et al (2005) Ribosomal RNA in Alzheimer disease is oxidized by bound redox-active iron. J Biol Chem 280:20978–20986

Honig LS, Kukull W, Mayeux R (2005) Atherosclerosis and AD: analysis of data from the US National Alzheimer's Coordinating Center. Neurology 64:494–500

Huang WL, King VR, Curran OE et al (2007) A combination of intravenous and dietary docosahexaenoic acid significantly improves outcome after spinal cord injury. Brain 130:3004–3019

Ischiropoulos H, Beckman JS (2003) Oxidative stress and nitration in neurodegeneration: cause, effect, or association? J Clin Invest 111:163–169

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

Ito R, Hayakawa H, Sekiguchi M, Ishibashi T (2005) Multiple enzyme activities of *Escherichia coli* MutT protein for sanitization of DNA and RNA precursor pools. Biochemistry (Mosc) 44:6670–6674

Jacobs AC, Resendiz MJ, Greenberg MM (2010) Direct strand scission from a nucleobase radical in RNA. J Am Chem Soc 132:3668–3669

Javitch JA, D'Amato RJ, Strittmatter SM, Snyder SH (1985) Parkinsonism-inducing neurotoxin, N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine: uptake of the metabolite N-methyl-4-phenylpyridine by dopamine neurons explains selective toxicity. Proc Natl Acad Sci USA 82:2173–2177

Jenner P (2003) Oxidative stress in Parkinson's disease. Ann Neurol 53(Suppl 3):S26–S36

Joenje H (1989) Genetic toxicology of oxygen. Mutat Res 219:193–208

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

Kasai H, Crain PF, Kuchino Y, Nishimura S, Ootsuyama A, Tanooka H (1986) Formation of 8-hydroxyguanine moiety in cellular DNA by agents producing oxygen radicals and evidence for its repair. Carcinogenesis 7:1849–1851

Kedersha N, Stoecklin G, Ayodele M et al (2005) Stress granules and processing bodies are dynamically linked sites of mRNP remodeling. J Cell Biol 169:871–884

Keller JN, Schmitt FA, Scheff SW et al (2005) Evidence of increased oxidative damage in subjects with mild cognitive impairment. Neurology 64:1152–1156

Kikuchi A, Takeda A, Onodera H et al (2002) Systemic increase of oxidative nucleic acid damage in Parkinson's disease and multiple system atrophy. Neurobiol Dis 9:244–248

King VR, Huang WL, Dyall SC, Curran OE, Priestley JV, Michael-Titus AT (2006) Omega-3 fatty acids improve recovery, whereas omega-6 fatty acids worsen outcome, after spinal cord injury in the adult rat. J Neurosci 26:4672–4680

Krokan HE, Kavli B, Slupphaug G (2004) Novel aspects of macromolecular repair and relationship to human disease. J Mol Med 82:280–297

Lee JW, Beebe K, Nangle LA et al (2006) Editing-defective tRNA synthetase causes protein misfolding and neurodegeneration. Nature 443:50–55

Li Z, Wu J, Deleo CJ (2006) RNA damage and surveillance under oxidative stress. IUBMB Life 58:581–588

Lin MT, Beal MF (2006) Mitochondrial dysfunction and oxidative stress in neurodegenerative diseases. Nature 443:787–795

Liu J, Head E, Gharib AM et al (2002) Memory loss in old rats is associated with brain mitochondrial decay and RNA/DNA oxidation: partial reversal by feeding acetyl-L-carnitine and/or R-alpha -lipoic acid. Proc Natl Acad Sci USA 99:2356–2361

Liu Q, Xie F, Rolston R et al (2007) Prevention and treatment of Alzheimer disease and aging: antioxidants. Mini Rev Med Chem 7:171–180

Logroscino G, Beghi E, Zoccolella S et al (2005) Incidence of amyotrophic lateral sclerosis in southern Italy: a population based study. J Neurol Neurosurg Psychiatry 76:1094–1098

Lovell MA, Markesbery WR (2008) Oxidatively modified RNA in mild cognitive impairment. Neurobiol Dis 29:169–175

Lovell MA, Xiong S, Lyubartseva G, Markesbery WR (2009) Organoselenium (Sel-Plex diet) decreases amyloid burden and RNA and DNA oxidative damage in APP/PS1 mice. Free Radic Biol Med 46:1527–1533

Lovell MA, Soman S, Bradley MA (2011) Oxidatively modified nucleic acids in preclinical Alzheimer's disease (PCAD) brain. Mech Ageing Dev 132:443–448

Mangialasche F, Solomon A, Winblad B, Mecocci P, Kivipelto M (2010) Alzheimer's disease: clinical trials and drug development. Lancet Neurol 9:702–716

Manini P, De Palma G, Andreoli R et al (2009) Biomarkers of nucleic acid oxidation, polymorphism in, and expression of, hOGG1 gene in styrene-exposed workers. Toxicol Lett 190:41–47

Manini P, De Palma G, Andreoli R et al (2010) Occupational exposure to low levels of benzene: biomarkers of exposure and nucleic acid oxidation and their modulation by polymorphic xenobiotic metabolizing enzymes. Toxicol Lett 193:229–235

Martinet W, de Meyer GR, Herman AG, Kockx MM (2004) Reactive oxygen species induce RNA damage in human atherosclerosis. Eur J Clin Invest 34:323–327

Mattson MP, Chan SL, Duan W (2002) Modification of brain aging and neurodegenerative disorders by genes, diet, and behavior. Physiol Rev 82:637–672

Mehler MF, Mattick JS (2006) Non-coding RNAs in the nervous system. J Physiol 575:333–341

Mehler MF, Mattick JS (2007) Noncoding RNAs and RNA editing in brain development, functional diversification, and neurological disease. Physiol Rev 87:799–823

Migliore L, Fontana I, Trippi F et al (2005) Oxidative DNA damage in peripheral leukocytes of mild cognitive impairment and AD patients. Neurobiol Aging 26:567–573

Miyata R, Hayashi M, Tanuma N, Shioda K, Fukatsu R, Mizutani S (2008) Oxidative stress in neurodegeneration in dentatorubral-pallidoluysian atrophy. J Neurol Sci 264:133–139

Moreira PI, Honda K, Zhu X et al (2006a) Brain and brawn: parallels in oxidative strength. Neurology 66(Suppl 1):S97–S101

Moreira PI, Zhu X, Nunomura A, Smith MA, Perry G (2006b) Therapeutic options in Alzheimer's disease. Expert Rev Neurother 6:897–910

Moreira PI, Nunomura A, Nakamura M et al (2008) Nucleic acid oxidation in Alzheimer disease. Free Radic Biol Med 44:1493–1505

Mundt JM, Hah SS, Sumbad RA, Schramm V, Henderson PT (2008) Incorporation of extracellular 8-oxodG into DNA and RNA requires purine nucleoside phosphorylase in MCF-7 cells. Nucleic Acids Res 36:228–236

Nakabeppu Y, Tsuchimoto D, Ichinoe A et al (2004) Biological significance of the defense mechanisms against oxidative damage in nucleic acids caused by reactive oxygen species: from mitochondria to nuclei. Ann N Y Acad Sci 1011:101–111

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

Nelson PT, Wang WX, Rajeev BW (2008) MicroRNAs (miRNAs) in neurodegenerative diseases. Brain Pathol 18:130–138

Nunomura A, Perry G, Pappolla MA et al (1999) RNA oxidation is a prominent feature of vulnerable neurons in Alzheimer's disease. J Neurosci 19:1959–1964

Nunomura A, Perry G, Pappolla MA et al (2000) Neuronal oxidative stress precedes amyloid-β deposition in Down syndrome. J Neuropathol Exp Neurol 59:1011–1017

Nunomura A, Perry G, Aliev G et al (2001) Oxidative damage is the earliest event in Alzheimer disease. J Neuropathol Exp Neurol 60:759–767

Nunomura A, Chiba S, Kosaka K et al (2002) Neuronal RNA oxidation is a prominent feature of dementia with Lewy bodies. NeuroReport 13:2035–2039

Nunomura A, Chiba S, Lippa CF et al (2004) Neuronal RNA oxidation is a prominent feature of familial Alzheimer's disease. Neurobiol Dis 17:108–113

Nunomura A, Castellani RJ, Zhu X, Moreira PI, Perry G, Smith MA (2006) Involvement of oxidative stress in Alzheimer disease. J Neuropathol Exp Neurol 65:631–641

Nunomura A, Moreira PI, Lee HG et al (2007) Neuronal death and survival under oxidative stress in Alzheimer and Parkinson diseases. CNS Neurol Disord: Drug Targets 6:411–423

Nunomura A, Tamaoki T, Tanaka K et al (2010) Intraneuronal amyloid β accumulation and oxidative damage to nucleic acids in Alzheimer disease. Neurobiol Dis 37:731–737

Nunomura A, Tamaoki T, Motohashi N et al (2012) The earliest stage of cognitive impairment in transition from normal aging to Alzheimer disease is marked by prominent RNA oxidation in vulnerable neurons. J Neuropathol Exp Neurol 71:233–241

Park EM, Shigenaga MK, Degan P et al (1992) 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 USA 89:3375–3379

Perkins DO, Jeffries C, Sullivan P (2005) Expanding the 'central dogma': the regulatory role of nonprotein coding genes and implications for the genetic liability to schizophrenia. Mol Psychiatry 10:69–78

Perry G, Nunomura A, Cash AD et al (2002a) Reactive oxygen: its sources and significance in Alzheimer disease. J Neural Transm Suppl 62:69–75

Perry G, Nunomura A, Hirai K et al (2002b) Is oxidative damage the fundamental pathogenic mechanism of Alzheimer's and other neurodegenerative diseases? Free Radic Biol Med 33:1475–1479

Petersen RB, Siedlak SL, Lee HG et al (2005) Redox metals and oxidative abnormalities in human prion diseases. Acta Neuropathol 110:232–238

Praticò D, Clark CM, Liun F, Rokach J, Lee VY, Trojanowski JQ (2002) Increase of brain oxidative stress in mild cognitive impairment: a possible predictor of Alzheimer disease. Arch Neurol 59:972–976

Rahkonen T, Eloniemi-Sulkava U, Rissanen S, Vatanen A, Viramo P, Sulkava R (2003) Dementia with Lewy bodies according to the consensus criteria in a general population aged 75 years or older. J Neurol Neurosurg Psychiatry 74:720–724

Rhee Y, Valentine MR, Termini J (1995) Oxidative base damage in RNA detected by reverse transcriptase. Nucleic Acids Res 23:3275–3282

Rinaldi P, Polidori MC, Metastasio A et al (2003) Plasma antioxidants are similarly depleted in mild cognitive impairment and in Alzheimer's disease. Neurobiol Aging 24:915–919

Rosen DR, Siddique T, Patterson D et al (1993) Mutations in Cu/Zn superoxide dismutase gene are associated with familial amyotrophic lateral sclerosis. Nature 362:59–62

Row BW, Liu R, Xu W, Kheirandish L, Gozal D (2003) Intermittent hypoxia is associated with oxidative stress and spatial learning deficits in the rat. Am J Respir Crit Care Med 167:1548–1553

Satterlee JS, Barbee S, Jin P et al (2007) Noncoding RNAs in the Brain. J Neurosci 27:11856–11859

Sayre LM, Zelasko DA, Harris PL, Perry G, Salomon RG, Smith MA (1997) 4-Hydroxynonenal-derived advanced lipid peroxidation end products are increased in Alzheimer's disease. J Neurochem 68:2092–2097

Sayre LM, Perry G, Smith MA (1999) In situ methods for detection and localization of markers of oxidative stress: application in neurodegenerative disorders. Methods Enzymol 309:133–152

Sayre LM, Smith MA, Perry G (2001) Chemistry and biochemistry of oxidative stress in neurodegenerative disease. Curr Med Chem 8:721–738

Schapira AH, Cooper JM, Dexter D, Clark JB, Jenner P, Marsden CD (1990) Mitochondrial complex I deficiency in Parkinson's disease. J Neurochem 54:823–827

Schneider JE Jr, Phillips JR, Pye Q, Maidt ML, Price S, Floyd RA (1993) Methylene blue and rose bengal photoinactivation of RNA bacteriophages: comparative studies of 8-oxoguanine formation in isolated RNA. Arch Biochem Biophys 301:91–97

Schubert J, Wilmer JW (1991) Does hydrogen peroxide exist "free" in biological systems? Free Radic Biol Med 11:545–555

Seo AY, Hofer T, Sung B, Judge S, Chung HY, Leeuwenburgh C (2006) Hepatic oxidative stress during aging: effects of 8% long-term calorie restriction and lifelong exercise. Antioxid Redox Signal 8:529–538

Seo AY, Xu J, Servais S et al (2008) Mitochondrial iron accumulation with age and functional consequences. Aging Cell 7:706–716

Shan X, Lin CL (2006) Quantification of oxidized RNAs in Alzheimer's disease. Neurobiol Aging 27:657–662

Shan X, Tashiro H, Lin CL (2003) The identification and characterization of oxidized RNAs in Alzheimer's disease. J Neurosci 23:4913–4921

Shan X, Chang Y, Lin CL (2007) Messenger RNA oxidation is an early event preceding cell death and causes reduced protein expression. FASEB J 21:2753–2764

Shao C, Xiong S, Li GM et al (2008) Altered 8-oxoguanine glycosylase in mild cognitive impairment and late-stage Alzheimer's disease brain. Free Radic Biol Med 45:813–819

Shen Z, Wu W, Hazen SL (2000) Activated leukocytes oxidatively damage DNA, RNA, and the nucleotide pool through halide-dependent formation of hydroxyl radical. Biochemistry (Mosc) 39:5474–5482

Sheth U, Parker R (2003) Decapping and decay of messenger RNA occur in cytoplasmic processing bodies. Science 300:805–808

Shi Q, Gibson GE (2011) Up-regulation of the mitochondrial malate dehydrogenase by oxidative stress is mediated by miR-743a. J Neurochem 118:440–448

Shi F, Gan W, Nie B et al (2012) Greater nucleic acids oxidation in the temporal lobe than the frontal lobe in SAMP8. NeuroReport 23:508–512

Shimura-Miura H, Hattori N, Kang D, Miyako K, Nakabeppu Y, Mizuno Y (1999) Increased 8-oxo-dGTPase in the mitochondria of substantia nigral neurons in Parkinson's disease. Ann Neurol 46:920–924

Smith MA, Perry G, Richey PL et al (1996) Oxidative damage in Alzheimer's. Nature 382:120–121

Smith MA, Nunomura A, Zhu X, Takeda A, Perry G (2000) Metabolic, metallic, and mitotic sources of oxidative stress in Alzheimer disease. Antioxid Redox Signal 2:413–420

Sofic E, Riederer P, Heinsen H et al (1988) Increased iron (III) and total iron content in post mortem substantia nigra of parkinsonian brain. J Neural Transm 74:199–205

Song XN, Zhang LQ, Liu DG et al (2011) Oxidative damage to RNA and expression patterns of MTH1 in the hippocampi of senescence-accelerated SAMP8 mice and Alzheimer's disease patients. Neurochem Res 36:1558–1565

Sperling RA, Aisen PS, Beckett LA et al (2011) Toward defining the preclinical stages of Alzheimer's disease; recommendations from the National Institute on Aging-Alzheimer's Association

workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimers Dement 7:280–292

Szymanski M, Barciszewska MZ, Erdmann VA, Barciszewski J (2005) A new frontier for molecular medicine: noncoding RNAs. Biochim Biophys Acta 1756:65–75

Taddei F, Hayakawa H, Bouton M et al (1997) Counteraction by MutT protein of transcriptional errors caused by oxidative damage. Science 278:128–130

Taft RJ, Pheasant M, Mattick JS (2007) The relationship between non-protein-coding DNA and eukaryotic complexity. BioEssays 29:288–299

Takahashi MA, Asada K (1983) Superoxide anion permeability of phospholipid membranes and chloroplast thylakoids. Arch Biochem Biophys 226:558–566

Tanaka M, Chock PB, Stadtman ER (2007) Oxidized messenger RNA induces translation errors. Proc Natl Acad Sci USA 104:66–71

Tanaka M, Han S, Küpfer PA, Leumann CJ, Sonntag WE (2011a) Quantification of oxidized levels of specific RNA species using an aldehyde reactive probe. Anal Biochem 417:142–148

Tanaka M, Han S, Küpfer PA, Leumann CJ, Sonntag WE (2011b) An assay for RNA oxidation induced abasic sites using the Aldehyde Reactive Probe. Free Radic Res 45:237–247

Tateyama M, Takeda A, Onodera Y et al (2003) Oxidative stress and predominant Aβ42(43) deposition in myopathies with rimmed vacuoles. Acta Neuropathol 105:581–585

Taylor JP, Hardy J, Fischbeck KH (2002) Toxic proteins in neurodegenerative disease. Science 296:1991–1995

van Leeuwen FW, de Kleijn DP, van den Hurk HH et al (1998) Frameshift mutants of β amyloid precursor protein and ubiquitin-B in Alzheimer's and Down patients. Science 279:242–247

Vascotto C, Fantini D, Romanello M et al (2009) APE1/Ref-1 interacts with NPM1 within nucleoli and plays a role in the rRNA quality control process. Mol Cell Biol 29:1834–1854

Wamer WG, Wei RR (1997) In vitro photooxidation of nucleic acids by ultraviolet A radiation. Photochem Photobiol 65:560–563

Wang Q, Yu S, Simonyi A, Sun GY, Sun AY (2005) Kainic acid-mediated excitotoxicity as a model for neurodegeneration. Mol Neurobiol 31:3–16

Wang J, Markesbery WR, Lovell MA (2006) Increased oxidative damage in nuclear and mitochondrial DNA in mild cognitive impairment. J Neurochem 96:825–832

Weidner AM, Bradley MA, Beckett TL et al (2011) RNA oxidation adducts 8-OHG and 8-OHA change with Aβ42 levels in late-stage Alzheimer's disease. PLoS ONE 6(9):e24930

Weimann A, Belling D, Poulsen HE (2002) Quantification of 8-oxo-guanine and guanine as the nucleobase, nucleoside and deoxy-nucleoside forms in human urine by high-performance liquid chromatography-electrospray tandem mass spectrometry. Nucleic Acids Res 30:e7

Weissman L, Jo DG, Sørensen MM et al (2007) Defective DNA base excision repair in brain from individuals with Alzheimer's disease and amnestic mild cognitive impairment. Nucleic Acids Res 35:5545–5555

Wu J, Li Z (2008) Human polynucleotide phosphorylase reduces oxidative RNA damage and protects HeLa cell against oxidative stress. Biochem Biophys Res Commun 372:288–292

Yamaguchi H, Kajitani K, Dan Y et al (2006) MTH1, an oxidized purine nucleoside triphosphatase, protects the dopamine neurons from oxidative damage in nucleic acids caused by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. Cell Death Differ 13:551–563

Yanagawa H, Ogawa Y, Ueno M (1992) Redox ribonucleosides. Isolation and characterization of 5-hydroxyuridine, 8-hydroxyguanosine, and 8-hydroxyadenosine from Torula yeast RNA. J Biol Chem 267:13320–13326

Yang WH, Bloch DB (2007) Probing the mRNA processing body using protein macroarrays and "autoantigenomics". RNA 13:704–712

Yin B, Whyatt RM, Perera FP, Randall MC, Cooper TB, Santella RM (1995) Determination of 8-hydroxydeoxyguanosine by an immunoaffinity chromatography-monoclonal antibody-based ELISA. Free Radic Biol Med 18:1023–1032

Zhang J, Perry G, Smith MA et al (1999) Parkinson's disease is associated with oxidative damage to cytoplasmic DNA and RNA in substantia nigra neurons. Am J Pathol 154:1423–1429

Free Radical Research, April 2012; 46(4): 565–576

informa
healthcare

REVIEW ARTICLE

Nuclear and mitochondrial DNA oxidation in Alzheimer's disease

RENATO X. SANTOS[1,2], SÓNIA C. CORREIA[1,2], XIONGWEI ZHU[3], HYOUNG-GON LEE[3], ROBERT B. PETERSEN[3], AKIHIKO NUNOMURA[4], MARK A. SMITH[3], GEORGE PERRY[5] & PAULA I. MOREIRA[1,6]

[1]*Center for Neuroscience and Cell Biology, University of Coimbra, Coimbra, Portugal,* [2]*Department of Life Sciences, Faculty of Sciences and Technology, University of Coimbra, Coimbra, Portugal,* [3]*Department of Pathology, Case Western Reserve University, Cleveland, Ohio, USA,* [4]*Department of Neuropsychiatry, Interdisciplinary Graduate School of Medicine and Engineering, University of Yamanashi, Chuo, Yamanashi, Japan,* [5]*UTSA Neurosciences Institute and Department of Biology, University of Texas at San Antonio, San Antonio, Texas, USA, and* [6]*Faculty of Medicine, Institute of Physiology, University of Coimbra, Coimbra, Portugal*

(Received date: 17 November 2011; Accepted date: 6 December 2011)

Abstract

The study of Alzheimer's disease neuropathology has been intimately associated with the field of oxidative stress for nearly 20 years. Indeed, increased markers of oxidative stress have been associated with this neurodegenerative condition, resulting from oxidation of lipids, proteins and nucleic acids. Increased nuclear and mitochondrial DNA oxidation are observed in Alzheimer's disease, stemming from increased reactive oxygen species attack to DNA bases and from the impairment of DNA repair mechanisms. Moreover, mitochondrial DNA is found to be more extensively oxidized than nuclear DNA. This review is intended to summarizes the most important cellular reactive oxygen species producers and how mitochondrial dysfunction, redox-active metals dyshomeostasis and NADPH oxidases contribute to increased oxidative stress in Alzheimer's disease. A summary of the antioxidant system malfunction will also be provided. Moreover, we will highlight the mechanisms of DNA oxidation and repair. Importantly, we will discuss evidence relating the DNA repair machinery and accumulated DNA oxidation with Alzheimer's disease.

Keywords: *BER, 8-hydroxyguanine, hydroxyl radical, mitochondria, nucleus*

Introduction

Alzheimer's disease (AD) is a highly prevalent and, to date, incurable neurodegenerative disorder. The prevalence of AD in 2009 was estimated at 35.6 million cases worldwide, a number predicted to be more than double by 2050 [1]. The formation of neurofibrillary tangles (hyperphosphorylated tau protein) and senile plaques (aberrant aggregates of amyloid-β (Aβ) protein) are distinctive hallmarks of the disease process [1,2]. Two subsets of patients are considered according to the ethiogenesis of AD: those with a mid-life onset form of disease, usually referred to as early onset and due to its usual association with other affected family members, familial form of AD (fAD); while the vast majority of cases are represented by the late onset sporadic forms of AD (sAD). For fAD, some mutations and duplications of genes associated with Aβ production have been identified [3,4]. Concerning sAD, only risk factors that affect the onset and course of disease have been identified. Some of these risk factors support the mitochondrial cascade hypotheses (for further detail, see [5,6]).

Despite the controversy surrounding the causes of AD, a growing body of evidence reveals oxidative stress as an early event during the cascade of degenerative events. Indeed, oxidation of several biomolecules such as lipids, proteins and DNA has been shown to occur in mild cognitive impairment (MCI),

[1]Correspondence: Paula I. Moreira, Institute of Physiology- Faculty of Medicine and Center for Neuroscience and Cell Biology, University of Coimbra, 3000-354 Coimbra, Portugal. E-mail: venta@ci.uc.pt/pismoreira@gmail.com George Perry, College of Sciences, University of Texas at San Antonio, San Antonio, Texas, USA. E-mail: george.perry@utsa.edu

ISSN 1071-5762 print/ISSN 1029-2470 online
DOI: 10.3109/10715762.2011.648188

a stage from which most individuals progress to AD [7–15] (Nunomura et al. unpublished). Oxidative stress underlies, at least in part, the pathological relation that supports diabetes as a risk factor for the development of AD [16,17]. The generation of reactive oxygen species (ROS) primarily stems from mitochondrial metabolism, and while these radicals can have damaging effects, they also act as important cellular secondary messengers. The effect of ROS as oxidative-modifying agents seems to underlie the aging process [18]. The exacerbation of ROS production in post-mitotic cells, such as neurons, which have limited self-renewal, results in dramatic alterations to their function and viability [19]. The alteration of the oxidative environment is also often a consequence of the reduction in the antioxidant capacity of the cell [13,20].

The hydroxyl radical (•OH) is highly reactive, meaning it is a ROS that has an uncoupled electron that can directly damage DNA. •OH can react with purines, pyrimidines and even with the deoxyribose backbone [21,22]. The most extensively studied alteration on DNA due to oxidative damage is the formation of 8-hydroxyguanine (8OHG) and its deoxynucleoside equivalent (8OHdG) [23,24]. 8OHG assumed such preponderance as a marker of oxidative DNA damage because guanine is the base with the lowest oxidation potential and thus most susceptible to damage [25]. Oxidative damage to DNA induces mutagenic properties and can alter the binding of transcription factors resulting in epigenetic regulation [26]. Therefore, both nuclear and mitochondrial DNA (nDNA and mtDNA, respectively) oxidation is a key event in the neurodegenerative process.

The purpose of this review is to summarize aspects that result in the alteration of oxidative status in AD. We will also provide an outline about nDNA and mtDNA oxidation and DNA repair mechanisms. Finally, we will discuss evidence showing accumulated nDNA and mtDNA oxidation in AD.

Oxidative disequilibrium in Alzheimer's disease

ROS production

A considerable amount of literature implicates a number of cellular constituents in the formation of ROS, particularly in AD neuropathology. Among the most relevant producers of ROS, it is mandatory to consider the contribution of mitochondria (1), redox-active metals (2) and NADPH oxidases (3).

Mitochondria. Mitochondria are mid-placed in the convergence of several cellular processes. Aerobic oxidative phosphorylation occurs by the generation of an electron stream throughout the mitochondrial respiratory complexes I–IV. Along with electron flow, the efflux of protons from the matrix to intermembrane space generates energetic potential that drives the phosphorylation of ADP to ATP by the FoF1-ATP synthase or mitochondrial complex V [27,28]. Mitochondria account for more than 90% of cellular energetic production [29]. Neurons are highly reliant on mitochondrial energetic generation due to limited glycolytic capacity, making them highly dependent on aerobic oxidative phosphorylation; furthermore, neuronal function is extremely energy demanding [30]. The final acceptor of electrons is molecular oxygen that is reduced to water. However, electron leak can occur, especially by complexes I and III, participating in a single-electron reduction of oxygen, thereby, generating superoxide anion ($O_2^{\bullet-}$) that is considered the primary ROS [31,32]. Whereas complex I produces $O_2^{\bullet-}$ only within the matrix, complex III can produce $O_2^{\bullet-}$ to both sides of the mitochondrial inner membrane. $O_2^{\bullet-}$ in the intermembrane space theoretically accesses cytosol more easily, either assuming its function as signalling molecule or harmful agent [32–34]. $O_2^{\bullet-}$ can be readily dismutated by superoxide dismutases (SOD) present in the mitochondria (manganese SOD, MnSOD) and in the cytosol and intermembrane space (copper/zinc SOD, Cu/ZnSOD) forming hydrogen peroxide (H_2O_2) [35]. Subsequently, glutathione peroxidase (GPx) or peroxiredoxin III (Prx III) convert H_2O_2 to water [36]. Damage to cellular structures occurs when the balance between ROS production and scavenging is affected, as it occurs in AD. Imbalance in endogenous enzymatic and non-enzymatic antioxidants in the course of AD will be further discussed below. Also mitochondrial matrix enzymes such as glycerol-3-phosphate dehydrogenase, and those belonging to the tricarboxylic acid (TCA) cycle like α-keto dehydrogenase (KDH) and pyruvate dehydrogenase (PDH) produce $O_2^{\bullet-}$ in the matrix; however, the contribution of these enzymes to total mitochondrial ROS production remains unclear [24,37]. Furthermore, also monoamine oxidase in the mitochondrial outer membrane produces free radicals generating considerable amounts of H_2O_2 [24]. Under conditions of elevated oxidative stress, the opening of the mitochondrial permeability transition pore is promoted allowing apoptotic factors such as cytochrome c and the apoptosis-inducing factor to be released to the cytosol [38]. Thus, ultimately, chronic elevations in ROS levels may cause cell death, which culminates in neurodegeneration.

Several mitochondrial defects potentially causative of an increase in ROS production, a situation that occurs in AD, have been abundantly described in the literature. Multiple studies suggest alterations in the activity of mitochondrial enzymes involved in TCA cycle and electron transport chain (ETC) [39,40]. Indeed, mitochondrial bioenergetic deficits are an early event that precedes AD pathology in

animal models of AD [41,42]. Accordingly, it has been previously shown in the brains of AD patients a decrease in cytochrome oxidase (COX) activity, an increase in free radical generation and a reduction in energy metabolism prior to senile plaque formation [43–49]. During the disease progression, Aβ is translocated towards mitochondria [50,51] enabling its interaction with critical redox centres of the subunit I of COX [52,53] and Aβ-binding alcohol dehydrogenase (ABAD) [54,55]. Both interaction of Aβ with the subunit I of COX and ABAD potentiate mitochondrial dysfunction and further increase ROS production. Likewise, a molecular interaction between amyloid-β protein precursor (AβPP), heat shock proteins and Bcl-2, decreases protection against insults, which is likely to lead to a diverse array of mitochondrial disturbances including apoptosis [56]. Some mtDNA mutations have been associated with increased incidence of AD [57,58], in addition to brains having increased and unique mtDNA mutations compared to control cases, this being further enhanced in an age-dependent fashion and preferentially in mtDNA regulatory elements [58]. Other types of mitochondrial defects have been described in AD, including abnormal mitochondrial dynamics, biogenesis and transport through axons [59–63], possibly leading to the impairment of efficient mitochondrial turnover.

Accumulating evidence places mitochondria in a strategic position in determining cellular fate as primary energy producers, ROS sources and cell executioners. In AD, mitochondrial energetic function is impaired, exacerbating ROS production and, consequently, cell damage and death.

Redox-active metals. Brain is relatively enriched in transition metals with redox activity such as copper, iron and manganese. Such biometals assume important functions in synapses and are part of active centres of metalloproteins with enzymatic activity like aconitase, COX (iron) and cytosolic SOD (copper and zinc). The interplay between redox-active metals and AD assumes at least two dimensions: (1) metals intervene in Aβ aggregation and (2) redox cycling/ROS production [64].

It has been reported that Aβ deposition and AβPP processing and synthesis are promoted in the presence of iron [15,65]. Furthermore, iron homeostasis dysregulation in the brain is suggested to be an early neuropathological event, being involved in increased oxidative stress and inflammation [15]. Indeed, recent data demonstrates that iron-mediated enhancement of oxidative stress occurs in pre-clinical AD and MCI [66]. This is in agreement with previous data reporting increased redox-active iron in cerebrospinal fluid (CSF) from AD subjects [67]. Notably, it is known that iron accumulates in the brain as a function of age [68], likely assuming a role not only in the triggering of disease process but also in the escalation of neuropathological processes. This is in line with evidence showing that besides iron, zinc and copper also participate in the initiation of Aβ-mediated seeding process and Aβ oligomerization [69]. Similarly, more recently, Liu and collaborators [70] demonstrated that iron delays the ordered fibrilization of Aβ, enhancing its toxicity, but only if the metal is present throughout the aggregation process. Two compelling studies analyzed the role of the different transition metals in the process of Aβ aggregation and concluded that while zinc promotes the formation of oligomers and protofibrils, copper and iron inhibit fibril formation [71,72].

The redox state of the cell is primarily dependent on an iron (and copper) redox couple and is maintained within strict physiological limits [73]. As a transition metal, iron is involved in the formation of •OH via the Fenton reaction [74]. The Fenton reaction consists of a molecule of H_2O_2, originated from $O_2^{\bullet-}$ dismutation, reacting with Fe^{2+} to generate Fe^{3+} and hydroxide anion (OH^-) with •OH. Fe^{3+} can be again reduced by $O_2^{\bullet-}$, generating a redox cycle by which $O_2^{\bullet-}$ facilitates the Fenton reaction by making Fe^{2+} available [73]. Copper can also participate in the Fenton reaction to generate ROS [75,76]. •OH can also be produced by direct reaction of $O_2^{\bullet-}$ with H_2O_2, a reaction known as the Haber–Weiss reaction [73]. The •OH is a highly reactive radical with a half-life of approximately 10^{-9} s, therefore reacting and damaging structures close to its source [22]. Mitochondria consubstantiate a microenvironment highly enriched in iron since many mitochondrial enzymes possess heme groups and iron-sulfur cluster in their active centres making them favourable locations of •OH production [23]. Hence, mitochondria are prone to oxidative damage and particularly susceptible to •OH-mediated oxidation, which has a major role in DNA oxidation.

NADPH oxidases. Nicotinamide adenine dinucleotide phosphate (NADPH) oxidase is a multimeric enzyme that belongs to the NOX gene family. NADPH oxidase enzymatic complex is composed of cytochrome b558 (a heterodimer comprising a 22-KDa alpha-subunit—p22phox—and a glycosylated approximately 91-KDa beta-subunit—gp91phox), several cytosolic proteins (p47phox, p67phox, p40phox) and the Rac G-protein. NADPH oxidase is activated and translocated to form the enzymatic complex with the membrane bound cytochrome b558 [77]. NADPH oxidase is known to have seven different isoforms from which NOX1, NOX2 and NOX4 are expressed in neurons, astrocytes and microglia. Recently, it has been suggested that several NOX isoforms can be expressed within the same cell [78].

NOX2 is the isoform expressed in the phagocytes in the immune system and microglia, the resident

macrophages in the CNS. The physiological function of this enzyme in the immune system is to produce $O_2^{\bullet-}$ in response to microorganism invasion as a form of 'oxidative defense' [79]. During pathological events that occur in AD, such as neuroinflammation and in reaction to Aβ peptide, NADPH oxidase contributes to the generation of abnormal and deleterious levels of ROS [80,81]. Aβ is known to directly activate the NADPH oxidase complex in macrophages, rat microglia and, importantly, in cortical neurons resulting in increased production of $O_2^{\bullet-}$ and H_2O_2 [82–84]. Likewise, microglial expression of NADPH oxidase subunit p22phox is enhanced in AD brain [85]. Interestingly, NADPH oxidase alterations have been placed as one of the earliest events of AD, being up-regulated in superior and middle temporal gyri from MCI patients both by expression and activity, but not at pre-clinical or late stages of the disease [86]. A recent study expanded this analysis and also detected increased expression of several NADPH oxidase complex subunits as well as increased activity in frontal and temporal cortical regions of MCI, early and mild-to-moderate AD patients [87]. Cerebrovascular dysfunction is believed to be one of the earliest events in the progression of AD [88]. Indeed, Aβ-induced NADPH oxidase-mediated generation of ROS is described to induce cerebrovascular dysfunction, contributing to the malfunctioning of the neurovascular unit and behavioral dysfunction in rodents [89,90].

Overall, these data demonstrate a critical role for NADPH oxidase to the total dysregulation of oxidative status during the progression of AD.

Endogenous antioxidant imbalance

Besides exacerbated ROS production, also the impairment of endogenous antioxidant defences, both enzymatic and non-enzymatic, contributes to 'unbalance the scale' of the oxidative status equilibrium in AD. Regarding enzymatic antioxidant defences, Cu/ZnSOD and/or MnSOD dismutate $O_2^{\bullet-}$, forming H_2O_2 that can be readily converted to water by the action of GPx or catalase [91]. An animal strain generated by crossing an AD transgenic mice with mice partially deficient in MnSOD demonstrated a ROS-dependent increase in Aβ levels [92], tau phosphorylation [93] and significant worsening of the behavioral outcome [94]. Overexpression of MnSOD in both Tg19959 [95] and Tg2576 [96] AD animal models alleviated protein oxidative damage, microglia activation and Aβ levels, rescuing behavioral deficits.

Along with MnSOD, also Cu/ZnSOD and catalase are significantly reduced in the frontal and temporal cortex of AD patients [97]. Moreover, Cu/ZnSOD activity decreased peripherally in AD patients [98]. In agreement, others have documented that impaired Cu/ZnSOD activity contributes to oxidative damage in Thy1-APP751(SL) transgenic mice [99]. It was hypothesized that catalase activity inhibition is due to the interaction of Aβ peptide with this antioxidant enzyme, a hypothesis supported by data showing significant intracellular co-localization of Aβ with catalase [100]. Aβ-mediated catalase inhibition also induces increased ROS production, this effect being reversed by small molecule inhibitors of Aβ-catalase interactions [100].

A group of antioxidants known as plasma membrane redox system (PMRS), which include some membrane associated quinone reductases, lipophilic antioxidants (coenzyme Q and α-tocopherol) and the cytosolic electron donor NADPH, is expressed in neural cells and up-regulated in response to mitochondrial impairment to preserve energy metabolism and protect the cells against oxidative stress [101–105]. PMRS has been recently demonstrated to be impaired in the hippocampus and cortex of the triple transgenic mouse model of AD (3xTgAD) [106].

The non-enzymatic antioxidant defence system is also compromised in AD brains. A study from our laboratory performed in the 3xTgAD mice showed low levels of glutathione (GSH) and vitamin E and high levels of lipid peroxidation occurring before the appearance of Aβ plaques and neurofibrillary tangles [107]. The decrease in peripheral vitamin E levels was suggested to be related with the progression from MCI, considered the prodromal phase of AD, to AD and even with the deterioration of cognitive performance [13]. Interestingly, the maintenance of vitamin E physiological levels seems to be involved in the clearance of Aβ from the brain [108]. It has also been reported that GSH levels are changed in specific regions of CNS of AD patients [109]. A decrease in GSH levels and GSH/GSSG ratio in erythrocytes of AD and MCI patients has been reported [110]. Similarly, Rinaldi and collaborators [111] reported that the peripheral levels and activities of antioxidants are similarly lower in MCI and AD patients relative to control subjects. Accordingly, Cardoso and Oliveira [112] reported that GSH cycle impairment mediates Aβ-induced cell toxicity.

Altogether, these data show the critical position that endogenous antioxidants, occupy in the fine regulation of oxidative status. A complex impairment of the enzymatic and non-enzymatic antioxidant system is involved in AD neuropathology.

DNA oxidation and repair mechanisms

Nuclear DNA (nDNA)

As already discussed, the DNA is particularly susceptible to oxidation by $^{\bullet}OH$, which is originated mainly by the Fenton reaction. In general, upon chronic oxidative-mediated DNA damage several persistent

alterations may occur such as nucleotide base modifications, single- and double-strand DNA breaks, sister chromatid exchanges and DNA crosslinks [23,113]. Because •OH half-life is very short, Fenton reactions must occur in the close vicinity of nDNA, triggering oxidative injury of nDNA. Lysosomes are cellular compartments highly enriched in iron in the ferrous form (Fe^{2+}) favouring Fenton reactions [114–117]. It has been suggested that lysosomal rupture strongly enhances oxidative injury to nDNA mainly due to intralysosomal iron translocation to the nucleus [118,119]. Lysosomal rupture and subsequent release of the luminal content, which includes transition metals, potentiate nDNA oxidative damage that may have implications in AD, since the loss of lysosomal membrane integrity in AD late stages has been described [120,121]. Aside from the direct effects of the Fenton chemistry on DNA oxidative injury, also trans-4-hydroxy-2-nonenal (HNE, a major product of lipid peroxidation) undergoes oxidation, forming an epoxide that readily reacts with DNA bases, forming HNE-DNA adducts [122]. Formation of modified DNA bases could result in alterations in DNA replication or incorrect base pairing, producing mutations. All four bases (purines- adenine, guanine; pyrimidines- cytosine, thymine) and respective deoxynucleosides are susceptible to oxidative damage. Nevertheless, guanine has the lowest oxidation potential, being the most readily oxidized base [23]. 8OHG and its deoxynucleoside equivalent, 8OHdG, along with 2,6-diamino-4-hydroxy-5-formamidopyrimidine (FapydG) are the most abundantly formed and studied oxidized forms of DNA bases [123,124]. While mutagenesis is stimulated by the accumulation of 8OHdG by pairing with adenine as well as cytosine [125], the FapydG lesions inhibit DNA synthesis [126,127]. 8OHdG can be further oxidized and yield more mutagenic lesions, including guanidinohydantoin and spiroiminodihydantoin [128,129]. Overall, more than 20 oxidized base adducts can be formed from ROS attack on DNA [130,131].

Cellular defences against insults to nDNA consist of four major repair mechanisms: nucleotide excision repair (NER), base excision repair (BER), mismatch repair (MMR) and double-strand break repair (DSBR). NER excises bulky helix-distorting DNA lesions and BER repairs damage to a single nucleotide base, whereas MMR corrects mismatches of the normal bases and DSBR intervenes when both strands of the DNA backbone break (the breakage of only one strand is handled by the BER pathway) [122]. BER is the primary pathway for the repair of 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 DNA phosphodiester backbone, and this site will be, in a second step, cleaved by an AP lyase or AP endonuclease [132–135]. nDNA glycosylases are classified in monofunctional (hydroxylmethyluracil DNA glycosylase, UDG) or bifunctional (8OHG DNA glycosylase – OGG1, NTH1 and Nei-like homologs – NEILs), which have an intrinsic 3′ AP lyase activity in addition to the glycosylase activity. Nevertheless, in human cells, oxidative pyrimidine lesions are generally removed by NTH1 or NEILs whereas oxidative purine lesions are removed by OGG1 [136–138]. OGG1 is the primary enzyme for the repair of 8OHG in both nDNA and mtDNA [139,140]. The AP endonuclease that incises the DNA backbone immediately 5′ to the AP site is APE1 [141,142]. In the case of OGG1, a byfunctional DNA glycosylase, the incision to the DNA backbone is made immediately 3′ to the AP site and then APE1 proceeds (for further detail, see [122]). After that, the repair mechanism continues through one of two possible sub-pathways, the short- or long-patch BER (SP-BER or LP-BER, respectively). The SP-BER involves the incorporation of a single nucleotide into the gap by DNA polymerase. The LP-BER involves incorporation of several nucleotides, typically 2–7, followed by cleavage of the resulting 5′ flap (for further detail, see [122]). Finally, the nick left behind by DNA polymerases needs to be sealed, a process performed by ligases, ligase I in the case of LP-BER and ligase III in the case of SP-BER.

Over time, DNA oxidation can induce mutagenesis, this process being tightly regulated by the intricate machinery that regulates DNA repair. It is evident that a failure of this system can lead to deleterious events that characterize or underlie several pathological conditions such as AD.

Mitochondrial DNA (mtDNA)

mtDNA integrity is mandatory for normal function of ETC since it encodes several subunits of mitochondrial respiratory chain complexes as well as other mitochondrial proteins [143]. In contrast to nDNA, mtDNA is a circular double-stranded molecule packaged into nucleoid-like structure that is not well protected from insults due to: (1) nDNA is tightly wound around histones that are chief proteins involved in the compaction of chromatin; (2) compared to mtDNA, nDNA is farther from the major source of ROS production. Furthermore, the fact that human mtDNA has none or only a few non-coding bases [144], strongly enhances the odds of oxidation-induced mutagenesis in some coding regions. Due to these characteristics, mtDNA are more prone to oxidation and mutagenesis. Indeed, oxidative injury to mtDNA is 10–20 fold higher compared to nDNA [145], and mtDNA damage is more extensive and persists longer than nDNA damage in human cells [146]. Basically, all the products formed from nDNA oxidation can

also be formed from mtDNA oxidation, thus 8OHdG also represents the most frequent oxidized base in mtDNA.

The main differences between nDNA and mtDNA arise from the repair mechanisms, although the basics of the BER pathway are essentially the same. The BER proteins of the nuclear mechanism are fundamentally the same as those of the mitochondrial mechanism; indeed, all the components of the pathway are nuclear encoded [133]. Briefly, UDG1 and UDG2 are isoforms encoded by the same UDG gene that are generated by alternative splicing and transcription, the first being a mitochondrial and the latter a nuclear glycosylase [147]. Also, OGG1 has two isoforms, α-OGG1 that is localized in both nucleus and mitochondria, and β-OGG1 that is localized solely to mitochondria [148]. NEILs [136,137,149], APE1 [150–152], and ligase III [153,154] are localized both in the nucleus and in mitochondria. Concerning DNA polymerases, polymerase β is present in the nucleus while polymerase γ is present in mitochondria [155,156].

Overall, nucleus and mitochondria have similar BER machinery, presenting the same enzymes or, at least, mitochondrial versions of the nuclear enzymes. However, as discussed above, mitochondria are more susceptible to DNA oxidation.

Oxidative damage to nDNA and mtDNA in Alzheimer's disease

Because aging is the main risk factor for the majority of AD cases, two questions remain: Is DNA oxidation a key factor in late-onset sAD? If so, then is it expected that oxidative damage to DNA accumulates during aging? Henceforward, several facts will be discussed that shed some light on these questions.

A growing body of evidence demonstrates that as a cell or an organism gets older, lower is its ability to efficiently repair DNA oxidative injury. Intano and collaborators [157,158] demonstrated that nuclear extracts from brain, liver and germ-cells from aged mice had a decreased BER machinery activity. Furthermore, extracts from aged brains show an 85% decline in the repair activity of a synthetic DNA substrate containing a single mismatch [158]. Decreased content of DNA polymerase β has also been reported in brains of aged mice [158]. Likewise, also mitochondrial BER machinery has been reported to be impaired, particularly OGG1, UDG, APE and DNA polymerase γ [159,160]. The decline in the efficiency of BER machinery in mitochondria was observed in five distinct cerebral regions, caudate nucleus, frontal cortex, hippocampus, cerebellum and brain stem [159]. As a function of age, BER enzymes are negatively modulated by covalent modification, which is putatively induced by decreased acetylation [161] or by iron and copper dyshomeostasis [162]. Rather than just a matter of decreased activity of BER with age, there is also an impairment of: (1) the machinery induction (at the transcriptional, translational and enzyme activation levels) upon exposure to a DNA oxidizing agent [163], (2) the import of repair enzymes to mitochondria [164] and nucleus [161]. Interestingly, Du and collaborators [165] showed that oxidative damage to p62 promoter and subsequent decrease in p62 expression was positively correlated with an age-dependent increase in oxidative stress both in aged mice and human brains, as well as in 3xTg-AD mice and human AD brains. A deficiency/absence of p62 led to the loss of aggresomes and neuronal cell death [166] and resulted in an AD-like phenotype in mice [167].

These observations in aged animals are paralleled by findings reporting disrupted BER in AD. Reduced expression and activity levels of UDG and DNA polymerase β in the inferior parietal lobule of post-mortem sAD and MCI patients have been documented [168]. Interestingly, DNA repair activity was inversely correlated with the severity of disease as assessed by Braak stage [168]. Recently, it was shown in lymphocytes from AD patients both an increase in DNA oxidation and a decrease in OGG1 expression [169]. Others have suggested that BER decline is an early event in AD progression either by the observation of a decrease in neuronal OGG1 levels in some AD brain regions [170], or by the increase in the levels of 8OHG in the brains of pre-clinical AD subjects [14]. Curiously, transgenic AβPP and 3xTg-AD mice with 10 months of age did not present a significant decrease in 8OHdG, uracil incision activity and DNA repair synthesis by DNA polymerase β [171]. However, an age-dependent decline in mouse synaptic mitochondrial BER proteins and axonal transport was observed, although no differences in the repair capacity were found between wild-type and AD mice in any age group [172].

A higher content of oxidized bases was detected in lymphocytes and leukocytes of AD patients compared with controls [12,173–175], and an increase in the levels of 8OHdG were observed in DNA from ventricular CSF of AD patients [176]. Moreover, increased oxidative damage in leukocytes [12] and brain tissue [11] of MCI subjects suggest that DNA oxidation may constitute an early event in the progression of AD. As discussed above, mtDNA is much more susceptible to oxidation than nDNA. In this regard, it is noteworthy that an increased oxidation in both mtDNA and nDNA in frontal, parietal, and especially, in temporal lobes was observed in AD cases although mtDNA oxidation was approximately 10-fold higher than nDNA oxidation [177]. Some mtDNA mutations have been associated with increased incidence of AD [57,58]. AD brains have increased and unique mtDNA mutations compared

to control cases, these mutations being further enhanced in an age-dependent fashion and, preferentially, in mtDNA regulatory elements [58]. Importantly, mutations in regulatory elements of mtDNA could account for some of the mitochondrial defects in oxidative phosphorylation observed in AD, namely a reduction in the level of ND6 complex I transcript [58]. The impairment in oxidative phosphorylation results in an exacerbation of ROS generation, promoting an augment of the number of mtDNA mutations in a vicious positive feedback cycle [178,179].

It is not completely clear whether the failure of BER machinery occurs during AD pathology or if it is an age-dependent etiological event underlying the disease triggering events. Nevertheless, the failure of BER certainly plays a critical role, along with elevated oxidative stress levels, in the accumulation of DNA oxidative damage in AD.

Conclusion

It is widely recognized that increased oxidative stress is present since the early stages of AD. This alteration in oxidative status results from an increased production of ROS and a failure of the antioxidant system (Figure 1). A number of cellular components contribute to the exacerbation of ROS production in AD, such as mitochondria, redox-active metals and NADPH oxidases (Figure 1). Increased ROS levels are tightly associated with increased damage to several biomolecules such as proteins, lipids and nucleic acids. •OH is particularly associated with DNA oxidation, however, products of lipid peroxidation can also lead to the formation HNE-DNA adducts, which suggest that oxidative stress can damage DNA directly and indirectly. Noteworthy, in AD there is accumulated oxidative damage to nDNA and mtDNA, which likely results from both oxidative attack to DNA bases and impaired DNA repair mechanisms (Figure 1). Interestingly, such events are observed early during AD development suggesting that targeting DNA repair machinery could be of therapeutic interest to delay the progression or even prevent the onset of the disease. Nevertheless, it is mandatory to clarify the precise role of DNA oxidation in the complex multitude of pathophysiological events involved in AD onset and progression.

Acknowledgements

The authors dedicate this article to the memory of Mark A. Smith and Lawrence M. Sayre.

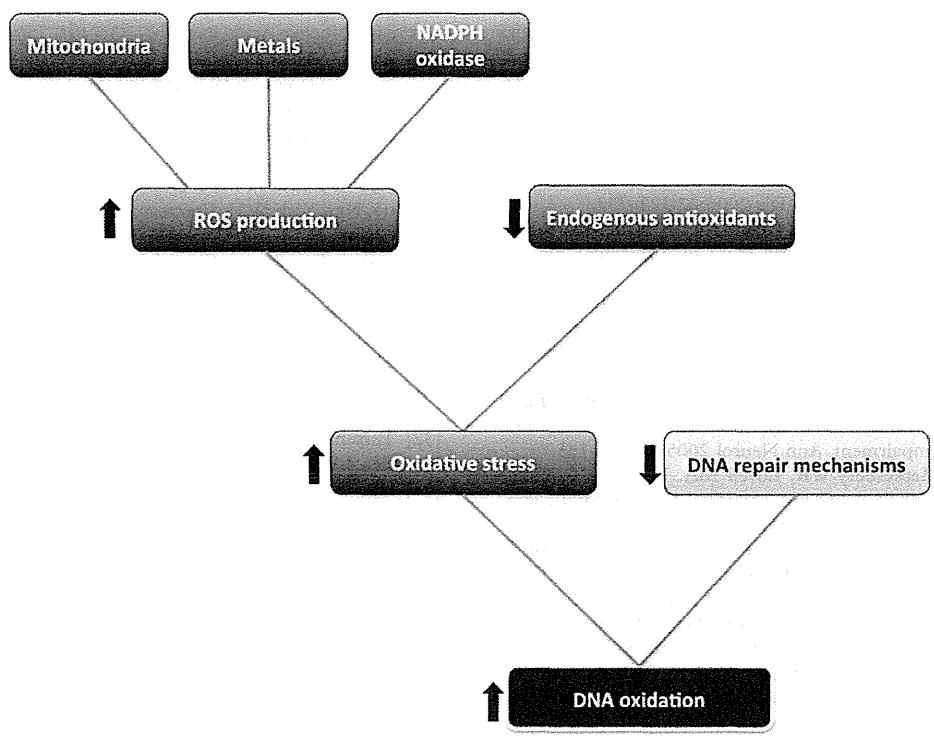

Figure 1. Oxidative stress, DNA oxidation, and repair in AD. Oxidative stress results from the imbalance between ROS production and scavenging. In AD, the exacerbation of ROS production by several sources, such as mitochondria, redox-active metals and NADPH oxidases, is poorly controlled due to impaired antioxidant defences, which leads to an increase in the oxidative damage of several biomolecules including DNA. Under physiological conditions the DNA repair mechanisms are able to neutralize this damage; however, in AD a failure of these mechanisms occurs leading to increased oxidation of DNA.

Declaration of interest

None of the authors have any other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript.

Work in the Portuguese authors' laboratories is supported by Fundação para a Ciência e a Tecnologia and Fundo Europeu de Desenvolvimento Regional (PTDC/SAU-NEU/103325/2008 and PTDC/SAU-NMC/110990/2009).

RXS and SCC are recipients of PhD fellowships from the Fundação para a Ciência e a Tecnologia (SFRH/BD/43972/2008 and SFRH/BD/40702/2007, respectively).

References

[1] Ittner LM, Gotz J. Amyloid-beta and tau–a toxic pas de deux in Alzheimer's disease. Nat Rev Neurosci 2011;12:65–72.

[2] Querfurth HW, LaFerla FM. Alzheimer's disease. N Engl J Med 2010;362:329–344.

[3] Santos RX, Moreira PI. mTOR is a potential therapeutic target for Alzhiemer's disease. In: Sun M-K, editor. Cognitive Sciences. Hauppauge, NY: Nova Science Publishers, Inc.; 2012. in press.

[4] Eisenstein M. Genetics: finding risk factors. Nature 2011; 475:S20–S22.

[5] Swerdlow RH, Burns JM, Khan SM. The Alzheimer's disease mitochondrial cascade hypothesis. J Alzheimers Dis 2010;20(Suppl 2):S265–279.

[6] Santos RX, Correia SC, Wang X, Perry G, Smith MA, Moreira PI, Zhu X. Alzheimer's disease: diverse aspects of mitochondrial malfunctioning. Int J Clin Exp Pathol 2010;3:570–581.

[7] Butterfield DA, Poon HF, St Clair D, Keller JN, Pierce WM, Klein JB, Markesbery WR. Redox proteomics identification of oxidatively modified hippocampal proteins in mild cognitive impairment: insights into the development of Alzheimer's disease. Neurobiol Dis 2006;22:223–232.

[8] Ding Q, Markesbery WR, Chen Q, Li F, Keller JN. Ribosome dysfunction is an early event in Alzheimer's disease. J Neurosci 2005;25:9171–9175.

[9] Keller JN, Schmitt FA, Scheff SW, Ding Q, Chen Q, Butterfield DA, Markesbery WR. Evidence of increased oxidative damage in subjects with mild cognitive impairment. Neurology 2005;64:1152–1156.

[10] Markesbery WR, Kryscio RJ, Lovell MA, Morrow JD. Lipid peroxidation is an early event in the brain in amnestic mild cognitive impairment. Ann Neurol 2005;58:730–735.

[11] Wang J, Markesbery WR, Lovell MA. Increased oxidative damage in nuclear and mitochondrial DNA in mild cognitive impairment. J Neurochem 2006;96:825–832.

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

[13] Baldeiras I, Santana I, Proenca MT, Garrucho MH, Pascoal R, Rodrigues A, Duro D, Oliveira CR. Oxidative damage and progression to Alzheimer's disease in patients with mild cognitive impairment. J Alzheimers Dis 2010;21:1165–1177.

[14] Lovell MA, Soman S, Bradley MA. Oxidatively modified nucleic acids in preclinical Alzheimer's disease (PCAD) brain. Mech Ageing Dev 2011;132:443–448.

[15] Gallagher JJ, Finnegan ME, Grehan B, Dobson J, Collingwood JF, Lynch MA. Modest amyloid deposition is associated with iron dysregulation, microglial activation, and oxidative stress. J Alzheimers Dis 2011;doi: 10.3233/JAD-2011-110614.

[16] Sims-Robinson C, Kim B, Rosko A, Feldman EL. How does diabetes accelerate Alzheimer disease pathology? Nat Rev Neurol 2010;6:551–559.

[17] Moreira PI, Duarte AI, Santos MS, Rego AC, Oliveira CR. An integrative view of the role of oxidative stress, mitochondria and insulin in Alzheimer's disease. J Alzheimers Dis 2009;16:741–761.

[18] Simm A, Brömme H-J. Reactive oxygen species (ROS) and aging: do we need them — can we measure them — should we block them? Signal Transd 2005;5:115–125.

[19] Terman A, Kurz T, Navratil M, Arriaga EA, Brunk UT. Mitochondrial turnover and aging of long-lived postmitotic cells: the mitochondrial-lysosomal axis theory of aging. Antioxid Redox Signal 2010;12:503–535.

[20] Sayre LM, Perry G, Smith MA. Oxidative stress and neurotoxicity. Chem Res Toxicol 2008;21:172–188.

[21] Halliwell B, Cross CE. Oxygen-derived species: their relation to human disease and environmental stress. Environ Health Perspect 1994;102(Suppl 10):5–12.

[22] Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol 2007;39:44–84.

[23] Moreira PI, Nunomura A, Nakamura M, Takeda A, Shenk JC, Aliev G, Smith MA, Perry G. Nucleic acid oxidation in Alzheimer disease. Free Radic Biol Med 2008;44: 1493–1505.

[24] Mao P, Reddy PH. Aging and amyloid beta-induced oxidative DNA damage and mitochondrial dysfunction in Alzheimer's disease: implications for early intervention and therapeutics. Biochim Biophys Acta 2011;1812:1359–1370.

[25] Lovell MA, Markesbery WR. Oxidative DNA damage in mild cognitive impairment and late-stage Alzheimer's disease. Nucleic Acids Res 2007;35:7497–7504.

[26] Zawia NH, Lahiri DK, Cardozo-Pelaez F. Epigenetics, oxidative stress, and Alzheimer disease. Free Radic Biol Med 2009;46:1241–1249.

[27] Scheffler IE. Mitochondria. Hoboken, NJ: John Wiley & Sons, Inc.; 2008.

[28] Nelson DL, Cox MM. Lehninger Principles of Biochemistry. New York: W.H. Freeman; 2004.

[29] Chance B, Sies H, Boveris A. Hydroperoxide metabolism in mammalian organs. Physiol Rev 1979;59:527–605.

[30] Moreira PI, Zhu X, Wang X, Lee HG, Nunomura A, Petersen RB, Perry G, Smith MA. Mitochondria: a therapeutic target in neurodegeneration. Biochim Biophys Acta 2010;1802: 212–220.

[31] Murphy MP. How mitochondria produce reactive oxygen species. Biochem J 2009;417:1–13.

[32] Turrens JF. Mitochondrial formation of reactive oxygen species. J Physiol 2003;552:335–344.

[33] Han D, Antunes F, Canali R, Rettori D, Cadenas E. Voltage-dependent anion channels control the release of the superoxide anion from mitochondria to cytosol. J Biol Chem 2003;278:5557–5563.

[34] Muller FL, Liu Y, Van Remmen H. Complex III releases superoxide to both sides of the inner mitochondrial membrane. J Biol Chem 2004;279:49064–49073.

[35] Wallace DC. A mitochondrial paradigm of metabolic and degenerative diseases, aging, and cancer: a dawn for evolutionary medicine. Annu Rev Genet 2005;39:359–407.

[36] Green K, Brand MD, Murphy MP. Prevention of mitochondrial oxidative damage as a therapeutic strategy in diabetes. Diabetes 2004;53(Suppl 1):S110–S118.

[37] Hamanaka RB, Chandel NS. Mitochondrial reactive oxygen species regulate cellular signaling and dictate biological outcomes. Trends Biochem Sci 2010;35:505–513.

[38] Ribe EM, Serrano-Saiz E, Akpan N, Troy CM. Mechanisms of neuronal death in disease: defining the models and the players. Biochem J 2008;415:165–182.
[39] Moreira PI, Siedlak SL, Wang X, Santos MS, Oliveira CR, Tabaton M, et al. Autophagocytosis of mitochondria is prominent in Alzheimer disease. J Neuropathol Exp Neurol 2007;66:525–532.
[40] Fattoretti P, Balietti M, Casoli T, Giorgetti B, Di Stefano G, Bertoni-Freddari C, Lattanzio F, Sensi SL. Decreased numeric density of succinic dehydrogenase-positive mitochondria in CA1 pyramidal neurons of 3xTg-AD mice. Rejuvenation Res 2010;13:144–147.
[41] Hauptmann S, Scherping I, Drose S, Brandt U, Schulz KL, Jendrach M, et al. Mitochondrial dysfunction: an early event in Alzheimer pathology accumulates with age in AD transgenic mice. Neurobiol Aging 2009;30:1574–1586.
[42] Yao J, Irwin RW, Zhao L, Nilsen J, Hamilton RT, Brinton RD. Mitochondrial bioenergetic deficit precedes Alzheimer's pathology in female mouse model of Alzheimer's disease. Proc Nat Acad Sci USA 2009;106:14670–14675.
[43] Lin MT, Beal MF. Mitochondrial dysfunction and oxidative stress in neurodegenerative diseases. Nature 2006;443: 787–795.
[44] Nunomura A, Perry G, Aliev G, Hirai K, Takeda A, Balraj EK, et al. Oxidative damage is the earliest event in Alzheimer disease. J Neuropathol Exp Neurol 2001;60:759–767.
[45] Reddy PH, Beal MF. Are mitochondria critical in the pathogenesis of Alzheimer's disease? Brain Res Brain Res Rev 2005;49:618–632.
[46] Moreira PI, Carvalho C, Zhu X, Smith MA, Perry G. Mitochondrial dysfunction is a trigger of Alzheimer's disease pathophysiology. Biochim Biophys Acta 2010;1802:2–10.
[47] Maurer I, Zierz S, Moller HJ. A selective defect of cytochrome c oxidase is present in brain of Alzheimer disease patients. Neurobiol Aging 2000;21:455–462.
[48] Russell RL, Siedlak SL, Raina AK, Bautista JM, Smith MA, Perry G. Increased neuronal glucose-6-phosphate dehydrogenase and sulfhydryl levels indicate reductive compensation to oxidative stress in Alzheimer disease. Arch Biochem Biophys 1999;370:236–239.
[49] Parker WD Jr. Cytochrome oxidase deficiency in Alzheimer's disease. Ann NY Acad Sci 1991;640:59–64.
[50] Takuma K, Fang F, Zhang W, Yan S, Fukuzaki E, Du H, et al. RAGE-mediated signaling contributes to intraneuronal transport of amyloid-beta and neuronal dysfunction. Proc Natl Acad Sci USA 2009;106:20021–20026.
[51] Humpel C, Marksteiner J. Cerebrovascular damage as a cause for Alzheimer's disease. Curr Neurovasc Res 2005;2: 341–347.
[52] Atamna H, Frey WH 2nd. A role for heme in Alzheimer's disease: heme binds amyloid beta and has altered metabolism. Proc Natl Acad Sci USA 2004;101:11153–11158.
[53] Atamna H. Heme binding to Amyloid-beta peptide: mechanistic role in Alzheimer's disease. J Alzheimers Dis 2006;10: 255–266.
[54] Takuma K, Yao J, Huang J, Xu H, Chen X, Luddy J, et al. ABAD enhances Abeta-induced cell stress via mitochondrial dysfunction. FASEB J 2005;19:597–598.
[55] Lustbader JW, Cirilli M, Lin C, Xu HW, Takuma K, Wang N, et al. ABAD directly links Abeta to mitochondrial toxicity in Alzheimer's disease. Science 2004;304:448–452.
[56] Yang TT, Hsu CT, Kuo YM. Amyloid precursor protein, heat-shock proteins, and Bcl-2 form a complex in mitochondria and modulate mitochondria function and apoptosis in N2a cells. Mech Ageing Dev 2009;130:592–601.
[57] Wallace DC, Stugard C, Murdock D, Schurr T, Brown MD. Ancient mtDNA sequences in the human nuclear genome: a potential source of errors in identifying pathogenic mutations. Proc Natl Acad Sci USA 1997;94:14900–14905.
[58] Coskun PE, Beal MF, Wallace DC. Alzheimer's brains harbor somatic mtDNA control-region mutations that suppress mitochondrial transcription and replication. Proc Natl Acad Sci USA 2004;101:10726–10731.
[59] Wang X, Su B, Siedlak SL, Moreira PI, Fujioka H, Wang Y, Casadesus G, Zhu X. Amyloid-beta overproduction causes abnormal mitochondrial dynamics via differential modulation of mitochondrial fission/fusion proteins. Proc Natl Acad Sci USA 2008;105:19318–19323.
[60] Wang X, Su B, Lee HG, Li X, Perry G, Smith MA, Zhu X. Impaired balance of mitochondrial fission and fusion in Alzheimer's disease. J Neurosci 2009;29:9090–9103.
[61] Manczak M, Calkins MJ, Reddy PH. Impaired mitochondrial dynamics and abnormal interaction of amyloid beta with mitochondrial protein Drp1 in neurons from patients with Alzheimer's disease: implications for neuronal damage. Hum Mol Genet 2011;20:2495–2509.
[62] Calkins MJ, Manczak M, Mao P, Shirendeb U, Reddy PH. Impaired mitochondrial biogenesis, defective axonal transport of mitochondria, abnormal mitochondrial dynamics and synaptic degeneration in a mouse model of Alzheimer's disease. Hum Mol Genet 2011;20:4515–4529.
[63] Sheng B, Wang X, Su B, Lee HG, Casadesus G, Perry G, Zhu X. Impaired mitochondrial biogenesis contributes to mitochondrial dysfunction in Alzheimer's disease. J Neurochem 2011; doi: 10.1111/j.1471–4159.2011.07581.x.
[64] Budimir A. Metal ions, Alzheimer's disease and chelation therapy. Acta Pharm 2011;61:1–14.
[65] Rogers JT, Randall JD, Cahill CM, Eder PS, Huang X, Gunshin H, et al. An iron-responsive element type II in the 5′-untranslated region of the Alzheimer's amyloid precursor protein transcript. J Biol Chem 2002;277:45518–45528.
[66] Smith MA, Zhu X, Tabaton M, Liu G, McKeel DW Jr, Cohen ML, et al. Increased iron and free radical generation in preclinical Alzheimer disease and mild cognitive impairment. J Alzheimers Dis 2010;19:363–372.
[67] Lavados M, Guillon M, Mujica MC, Rojo LE, Fuentes P, Maccioni RB. Mild cognitive impairment and Alzheimer patients display different levels of redox-active CSF iron. J Alzheimers Dis 2008;13:225–232.
[68] Moreira PI, Santos MS, Oliveira CR, Shenk JC, Nunomura A, Smith MA, Zhu X, Perry G. Alzheimer disease and the role of free radicals in the pathogenesis of the disease. CNS Neurol Disord Drug Targets 2008;7:3–10.
[69] Huang X, Moir RD, Tanzi RE, Bush AI, Rogers JT. Redox-active metals, oxidative stress, and Alzheimer's disease pathology. Ann N Y Acad Sci 2004;1012:153–163.
[70] Liu B, Moloney A, Meehan S, Morris K, Thomas SE, Serpell LC, et al. Iron promotes the toxicity of amyloid beta peptide by impeding its ordered aggregation. J Biol Chem 2011;286:4248–4256.
[71] Bolognin S, Messori L, Drago D, Gabbiani C, Cendron L, Zatta P. Aluminum, copper, iron and zinc differentially alter amyloid-Abeta(1-42) aggregation and toxicity. Int J Biochem Cell Biol 2011;43:877–885.
[72] Chen WT, Liao YH, Yu HM, Cheng IH, Chen YR. Distinct effects of Zn2 +, Cu2 +, Fe3 +, and Al3 + on amyloid-beta stability, oligomerization, and aggregation: amyloid-beta destabilization promotes annular protofibril formation. J Biol Chem 2011;286:9646–9656.
[73] Jomova K, Valko M. Advances in metal-induced oxidative stress and human disease. Toxicology 2011;283:65–87.
[74] Sayre LM, Perry G, Harris PL, Liu Y, Schubert KA, Smith MA. In situ oxidative catalysis by neurofibrillary tangles and senile plaques in Alzheimer's disease: a central role for bound transition metals. J Neurochem 2000;74:270–279.
[75] Huang X, Atwood CS, Hartshorn MA, Multhaup G, Goldstein LE, Scarpa RC, et al. The A beta peptide of Alzheimer's disease directly produces hydrogen peroxide

through metal ion reduction. Biochemistry (Mosc) 1999;38: 7609–7616.
[76] Finefrock AE, Bush AI, Doraiswamy PM. Current status of metals as therapeutic targets in Alzheimer's disease. J Am Geriatr Soc 2003;51:1143–1148.
[77] Block ML. NADPH oxidase as a therapeutic target in Alzheimer's disease. BMC Neurosci 2008;9(Suppl 2):S8.
[78] Simonyi A, He Y, Sheng W, Sun AY, Wood WG, Weisman GA, Sun GY. Targeting NADPH oxidase and phospholipases A2 in Alzheimer's disease. Mol Neurobiol 2010;41:73–86.
[79] Babior BM. NADPH oxidase. Curr Opin Immunol 2004;16:42–47.
[80] Zhu X, Su B, Wang X, Smith MA, Perry G. Causes of oxidative stress in Alzheimer disease. Cell Mol Life Sci 2007;64: 2202–2210.
[81] Sorce S, Krause KH. NOX enzymes in the central nervous system: from signaling to disease. Antioxid Redox Signal 2009;11:2481–2504.
[82] Klegeris A, McGeer PL. Beta-amyloid protein enhances macrophage production of oxygen free radicals and glutamate. J Neurosci Res 1997;49:229–235.
[83] Shelat PB, Chalimoniuk M, Wang JH, Strosznajder JB, Lee JC, Sun AY, Simonyi A, Sun GY. Amyloid beta peptide and NMDA induce ROS from NADPH oxidase and AA release from cytosolic phospholipase A2 in cortical neurons. J Neurochem 2008;106:45–55.
[84] Van Muiswinkel FL, Veerhuis R, Eikelenboom P. Amyloid beta protein primes cultured rat microglial cells for an enhanced phorbol 12-myristate 13-acetate-induced respiratory burst activity. J Neurochem 1996;66:2468–2476.
[85] Akiyama H, Barger S, Barnum S, Bradt B, Bauer J, Cole GM, et al. Inflammation and Alzheimer's disease. Neurobiol Aging 2000;21:383–421.
[86] Bruce-Keller AJ, Gupta S, Parrino TE, Knight AG, Ebenezer PJ, Weidner AM, et al. NOX activity is increased in mild cognitive impairment. Antioxid Redox Signal 2010;12: 1371–1382.
[87] Ansari MA, Scheff SW. NADPH-oxidase activation and cognition in Alzheimer disease progression. Free Radic Biol Med 2011;51:171–178.
[88] Iadecola C. Neurovascular regulation in the normal brain and in Alzheimer's disease. Nat Rev Neurosci 2004;5: 347–360.
[89] Park L, Zhou P, Pitstick R, Capone C, Anrather J, Norris EH, et al. Nox2-derived radicals contribute to neurovascular and behavioral dysfunction in mice overexpressing the amyloid precursor protein. Proc Natl Acad Sci USA 2008;105: 1347–1352.
[90] Park L, Anrather J, Zhou P, Frys K, Pitstick R, Younkin S, Carlson GA, Iadecola C. NADPH-oxidase-derived reactive oxygen species mediate the cerebrovascular dysfunction induced by the amyloid beta peptide. J Neurosci 2005;25: 1769–1777.
[91] Dumont M, Lin MT, Beal MF. Mitochondria and antioxidant targeted therapeutic strategies for Alzheimer's disease. J Alzheimers Dis 2010;20(Suppl 2):S633–S643.
[92] Li F, Calingasan NY, Yu F, Mauck WM, Toidze M, Almeida CG, et al. Increased plaque burden in brains of APP mutant MnSOD heterozygous knockout mice. J Neurochem 2004; 89:1308–1312.
[93] Melov S, Adlard PA, Morten K, Johnson F, Golden TR, Hinerfeld D, et al. Mitochondrial oxidative stress causes hyperphosphorylation of tau. PLoS ONE 2007;2:e536.
[94] Esposito L, Raber J, Kekonius L, Yan F, Yu GQ, Bien-Ly N, et al. Reduction in mitochondrial superoxide dismutase modulates Alzheimer's disease-like pathology and accelerates the onset of behavioral changes in human amyloid precursor protein transgenic mice. J Neurosci 2006;26: 5167–5179.
[95] Dumont M, Wille E, Stack C, Calingasan NY, Beal MF, Lin MT. Reduction of oxidative stress, amyloid deposition, and memory deficit by manganese superoxide dismutase overexpression in a transgenic mouse model of Alzheimer's disease. FASEB J 2009;23:2459–2466.
[96] Massaad CA, Washington TM, Pautler RG, Klann E. Overexpression of SOD-2 reduces hippocampal superoxide and prevents memory deficits in a mouse model of Alzheimer's disease. Proc Natl Acad Sci USA 2009;106:13576–13581.
[97] Marcus DL, Thomas C, Rodriguez C, Simberkoff K, Tsai JS, Strafaci JA, Freedman ML. Increased peroxidation and reduced antioxidant enzyme activity in Alzheimer's disease. Exp Neurol 1998;150:40–44.
[98] Straface E, Matarrese P, Gambardella L, Vona R, Sgadari A, Silveri MC, Malorni W. Oxidative imbalance and cathepsin D changes as peripheral blood biomarkers of Alzheimer disease: a pilot study. FEBS Lett 2005;579:2759–2766.
[99] Schuessel K, Schafer S, Bayer TA, Czech C, Pradier L, Muller-Spahn F, Muller WE, Eckert A. Impaired Cu/Zn-SOD activity contributes to increased oxidative damage in APP transgenic mice. Neurobiol Dis 2005;18:89–99.
[100] Habib LK, Lee MT, Yang J. Inhibitors of catalase-amyloid interactions protect cells from beta-amyloid-induced oxidative stress and toxicity. J Biol Chem 2010;285: 38933–38943.
[101] Hyun DH, Hernandez JO, Mattson MP, de Cabo R. The plasma membrane redox system in aging. Ageing Res Rev 2006;5:209–220.
[102] Hyun DH, Hunt ND, Emerson SS, Hernandez JO, Mattson MP, de Cabo R. Up-regulation of plasma membrane-associated redox activities in neuronal cells lacking functional mitochondria. J Neurochem 2007;100:1364–1374.
[103] Navas P, Villalba JM, de Cabo R. The importance of plasma membrane coenzyme Q in aging and stress responses. Mitochondrion 2007;7(Suppl):S34–40.
[104] Rodriguez-Aguilera JC, Lopez-Lluch G, Santos-Ocana C, Villalba JM, Gomez-Diaz C, Navas P. Plasma membrane redox system protects cells against oxidative stress. Redox Rep 2000;5:148–150.
[105] Villalba JM, Navas P. Plasma membrane redox system in the control of stress-induced apoptosis. Antioxid Redox Signal 2000;2:213–230.
[106] Hyun DH, Mughal MR, Yang H, Lee JH, Ko EJ, Hunt ND, de Cabo R, Mattson MP. The plasma membrane redox system is impaired by amyloid beta-peptide and in the hippocampus and cerebral cortex of 3xTgAD mice. Exp Neurol 2010;225:423–429.
[107] Resende R, Moreira PI, Proenca T, Deshpande A, Busciglio J, Pereira C, Oliveira CR. Brain oxidative stress in a triple-transgenic mouse model of Alzheimer disease. Free Radic Biol Med 2008;44:2051–2057.
[108] Nishida Y, Ito S, Ohtsuki S, Yamamoto N, Takahashi T, Iwata N, et al. Depletion of vitamin E increases amyloid beta accumulation by decreasing its clearances from brain and blood in a mouse model of Alzheimer disease. J Biol Chem 2009; 284:33400–33408.
[109] Gu M, Owen AD, Toffa SE, Cooper JM, Dexter DT, Jenner P, Marsden CD, Schapira AH. Mitochondrial function, GSH and iron in neurodegeneration and Lewy body diseases. J Neurol Sci 1998;158:24–29.
[110] Bermejo P, Martin-Aragon S, Benedi J, Susin C, Felici E, Gil P, Ribera JM, Villar AM. Peripheral levels of glutathione and protein oxidation as markers in the development of Alzheimer's disease from mild cognitive impairment. Free Radic Res 2008;42:162–170.
[111] Rinaldi P, Polidori MC, Metastasio A, Mariani E, Mattioli P, Cherubini A, et al. Plasma antioxidants are similarly depleted in mild cognitive impairment and in Alzheimer's disease. Neurobiol Aging 2003;24:915–919.

[112] Cardoso SM, Oliveira CR. Glutathione cycle impairment mediates A beta-induced cell toxicity. Free Radic Res 2003;37:241–250.
[113] Svilar D, Goellner EM, Almeida KH, Sobol RW. Base excision repair and lesion-dependent subpathways for repair of oxidative DNA damage. Antioxid Redox Signal 2011;14: 2491–2507.
[114] Pisoni RL, Acker TL, Lisowski KM, Lemons RM, Thoene JG. A cysteine-specific lysosomal transport system provides a major route for the delivery of thiol to human fibroblast lysosomes: possible role in supporting lysosomal proteolysis. J Cell Biol 1990;110:327–335.
[115] Brun A, Brunk U. Histochemical indications for lysosomal localization of heavy metals in normal rat brain and liver. J Histochem Cytochem 1970;18:820–827.
[116] Petrat F, de Groot H, Rauen U. Subcellular distribution of chelatable iron: a laser scanning microscopic study in isolated hepatocytes and liver endothelial cells. Biochem J 2001;356:61–69.
[117] Schafer FQ, Buettner GR. Acidic pH amplifies iron-mediated lipid peroxidation in cells. Free Radic Biol Med 2000;28:1175–1181.
[118] Tenopoulou M, Doulias PT, Barbouti A, Brunk U, Galaris D. Role of compartmentalized redox-active iron in hydrogen peroxide-induced DNA damage and apoptosis. Biochem J 2005;387:703–710.
[119] Kurz T, Leake A, Von Zglinicki T, Brunk UT. Relocalized redox-active lysosomal iron is an important mediator of oxidative-stress-induced DNA damage. Biochem J 2004;378: 1039–1045.
[120] Ditaranto K, Tekirian TL, Yang AJ. Lysosomal membrane damage in soluble Abeta-mediated cell death in Alzheimer's disease. Neurobiol Dis 2001;8:19–31.
[121] Yang AJ, Chandswangbhuvana D, Margol L, Glabe CG. Loss of endosomal/lysosomal membrane impermeability is an early event in amyloid Abeta1-42 pathogenesis. J Neurosci Res 1998;52:691–698.
[122] Jeppesen DK, Bohr VA, Stevnsner T. DNA repair deficiency in neurodegeneration. Prog Neurobiol 2011;94: 166–200.
[123] Dizdaroglu M, Kirkali G, Jaruga P. Formamidopyrimidines in DNA: mechanisms of formation, repair, and biological effects. Free Radic Biol Med 2008;45:1610–1621.
[124] Kasai H, Nishimura S. Hydroxylation of deoxyguanosine at the C-8 position by ascorbic acid and other reducing agents. Nucleic Acids Res 1984;12:2137–2145.
[125] Maki H. Origins of spontaneous mutations: specificity and directionality of base-substitution, frameshift, and sequence-substitution mutageneses. Annu Rev Genet 2002;36: 279–303.
[126] O'Connor TR, Boiteux S, Laval J. Ring-opened 7-methylguanine residues in DNA are a block to in vitro DNA synthesis. Nucleic Acids Res 1988;16:5879–5894.
[127] Malins DC, Hellstrom KE, Anderson KM, Johnson PM, Vinson MA. Antioxidant-induced changes in oxidized DNA. Proc Natl Acad Sci USA 2002;99:5937–5941.
[128] Neeley WL, Essigmann JM. Mechanisms of formation, genotoxicity, and mutation of guanine oxidation products. Chem Res Toxicol 2006;19:491–505.
[129] Hailer MK, Slade PG, Martin BD, Rosenquist TA, Sugden KD. Recognition of the oxidized lesions spiroiminodihydantoin and guanidinohydantoin in DNA by the mammalian base excision repair glycosylases NEIL1 and NEIL2. DNA Repair (Amst) 2005;4:41–50.
[130] Cooke MS, Evans MD, Dizdaroglu M, Lunec J. Oxidative DNA damage: mechanisms, mutation, and disease. FASEB J 2003;17:1195–1214.
[131] Steenken S. Purine bases, nucleosides, and nucleotides: aqueous solution redox chemistry and transformation reactions of their radical cations and e- and OH adducts. Chem Rev 1989;89:503–520.
[132] Dizdaroglu M. Base-excision repair of oxidative DNA damage by DNA glycosylases. Mutat Res 2005;591:45–59.
[133] Weissman L, de Souza-Pinto NC, Stevnsner T, Bohr VA. DNA repair, mitochondria, and neurodegeneration. Neuroscience 2007;145:1318–1329.
[134] Huffman JL, Sundheim O, Tainer JA. DNA base damage recognition and removal: new twists and grooves. Mutat Res 2005;577:55–76.
[135] Stivers JT, Jiang YL. A mechanistic perspective on the chemistry of DNA repair glycosylases. Chem Rev 2003;103: 2729–2759.
[136] Morland I, Rolseth V, Luna L, Rognes T, Bjoras M, Seeberg E. Human DNA glycosylases of the bacterial Fpg/MutM superfamily: an alternative pathway for the repair of 8-oxoguanine and other oxidation products in DNA. Nucleic Acids Res 2002;30:4926–4936.
[137] Hazra TK, Izumi T, Boldogh I, Imhoff B, Kow YW, Jaruga P, Dizdaroglu M, Mitra S. Identification and characterization of a human DNA glycosylase for repair of modified bases in oxidatively damaged DNA. Proc Natl Acad Sci USA 2002;99:3523–3528.
[138] Ide H, Kotera M. Human DNA glycosylases involved in the repair of oxidatively damaged DNA. Biol Pharm Bull 2004;27:480–485.
[139] Klungland A, Rosewell I, Hollenbach S, Larsen E, Daly G, Epe B, et al. Accumulation of premutagenic DNA lesions in mice defective in removal of oxidative base damage. Proc Natl Acad Sci USA 1999;96:13300–13305.
[140] de Souza-Pinto NC, Eide L, Hogue BA, Thybo T, Stevnsner T, Seeberg E, Klungland A, Bohr VA. Repair of 8-oxodeoxyguanosine lesions in mitochondrial dna depends on the oxoguanine dna glycosylase (OGG1) gene and 8-oxoguanine accumulates in the mitochondrial dna of OGG1-defective mice. Cancer Res 2001;61:5378–5381.
[141] Demple B, Sung JS. Molecular and biological roles of Ape1 protein in mammalian base excision repair. DNA Repair (Amst) 2005;4:1442–1449.
[142] Wilson DM 3rd, Barsky D. The major human abasic endonuclease: formation, consequences and repair of abasic lesions in DNA. Mutat Res 2001;485:283–307.
[143] Bohr VA. Repair of oxidative DNA damage in nuclear and mitochondrial DNA, and some changes with aging in mammalian cells. Free Radic Biol Med 2002;32:804–812.
[144] Anderson S, Bankier AT, Barrell BG, de Bruijn MH, Coulson AR, Drouin J, et al. Sequence and organization of the human mitochondrial genome. Nature 1981;290:457–465.
[145] Stowe DF, Camara AK. Mitochondrial reactive oxygen species production in excitable cells: modulators of mitochondrial and cell function. Antioxid Redox Signal 2009;11: 1373–1414.
[146] Yakes FM, Van Houten B. Mitochondrial DNA damage is more extensive and persists longer than nuclear DNA damage in human cells following oxidative stress. Proc Natl Acad Sci USA 1997;94:514–519.
[147] Nilsen H, Otterlei M, Haug T, Solum K, Nagelhus TA, Skorpen F, Krokan HE. Nuclear and mitochondrial uracil-DNA glycosylases are generated by alternative splicing and transcription from different positions in the UNG gene. Nucleic Acids Res 1997;25:750–755.
[148] Nishioka K, Ohtsubo T, Oda H, Fujiwara T, Kang D, Sugimachi K, Nakabeppu Y. Expression and differential intracellular localization of two major forms of human 8-oxoguanine DNA glycosylase encoded by alternatively spliced OGG1 mRNAs. Mol Biol Cell 1999;10:1637–1652.
[149] Hu J, de Souza-Pinto NC, Haraguchi K, Hogue BA, Jaruga P, Greenberg MM, Dizdaroglu M, Bohr VA. Repair of formamidopyrimidines in DNA involves different glycosylases:

role of the OGG1, NTH1, and NEIL1 enzymes. J Biol Chem 2005;280:40544–40551.
[150] Fung H, Kow YW, Van Houten B, Taatjes DJ, Hatahet Z, Janssen YM, et al. Asbestos increases mammalian AP-endonuclease gene expression, protein levels, and enzyme activity in mesothelial cells. Cancer Res 1998;58:189–194.
[151] Rivkees SA, Kelley MR. Expression of a multifunctional DNA repair enzyme gene, apurinic/apyrimidinic endonuclease (APE; Ref-1) in the suprachiasmatic, supraoptic and paraventricular nuclei. Brain Res 1994;666:137–142.
[152] Wilson TM, Rivkees SA, Deutsch WA, Kelley MR. Differential expression of the apurinic/apyrimidinic endonuclease (APE/ref-1) multifunctional DNA base excision repair gene during fetal development and in adult rat brain and testis. Mutat Res 1996;362:237–248.
[153] Lakshmipathy U, Campbell C. Mitochondrial DNA ligase III function is independent of Xrcc1. Nucleic Acids Res 2000;28:3880–3886.
[154] Lakshmipathy U, Campbell C. The human DNA ligase III gene encodes nuclear and mitochondrial proteins. Mol Cell Biol 1999;19:3869–3876.
[155] Hansen AB, Griner NB, Anderson JP, Kujoth GC, Prolla TA, Loeb LA, Glick E. Mitochondrial DNA integrity is not dependent on DNA polymerase-beta activity. DNA Repair (Amst) 2006;5:71–79.
[156] Kaguni LS. DNA polymerase gamma, the mitochondrial replicase. Annu Rev Biochem 2004;73:293–320.
[157] Intano GW, McMahan CA, McCarrey JR, Walter RB, McKenna AE, Matsumoto Y, et al. Base excision repair is limited by different proteins in male germ cell nuclear extracts prepared from young and old mice. Mol Cell Biol 2002;22:2410–2418.
[158] Intano GW, Cho EJ, McMahan CA, Walter CA. Age-related base excision repair activity in mouse brain and liver nuclear extracts. J Gerontol A Biol Sci Med Sci 2003;58:205–211.
[159] Imam SZ, Karahalil B, Hogue BA, Souza-Pinto NC, Bohr VA. Mitochondrial and nuclear DNA-repair capacity of various brain regions in mouse is altered in an age-dependent manner. Neurobiol Aging 2006;27:1129–1136.
[160] Chen D, Cao G, Hastings T, Feng Y, Pei W, O'Horo C, Chen J. Age-dependent decline of DNA repair activity for oxidative lesions in rat brain mitochondria. J Neurochem 2002;81:1273–1284.
[161] Szczesny B, Bhakat KK, Mitra S, Boldogh I. Age-dependent modulation of DNA repair enzymes by covalent modification and subcellular distribution. Mech Ageing Dev 2004;125:755–765.
[162] Hegde ML, Hegde PM, Holthauzen LM, Hazra TK, Rao KS, Mitra S. Specific Inhibition of NEIL-initiated repair of oxidized base damage in human genome by copper and iron: potential etiological linkage to neurodegenerative diseases. J Biol Chem 2010;285:28812–28825.
[163] Cabelof DC, Raffoul JJ, Ge Y, Van Remmen H, Matherly LH, Heydari AR. Age-related loss of the DNA repair response following exposure to oxidative stress. J Gerontol A Biol Sci Med Sci 2006;61:427–434.
[164] Szczesny B, Hazra TK, Papaconstantinou J, Mitra S, Boldogh I. Age-dependent deficiency in import of mitochondrial DNA glycosylases required for repair of oxidatively damaged bases. Proc Natl Acad Sci USA 2003;100:10670–10675.

This paper was first published online on Early Online on 6 October 2011.

[165] Du Y, Wooten MC, Gearing M, Wooten MW. Age-associated oxidative damage to the p62 promoter: implications for Alzheimer disease. Free Radic Biol Med 2009;46:492–501.
[166] Nakaso K, Yoshimoto Y, Nakano T, Takeshima T, Fukuhara Y, Yasui K, et al. Transcriptional activation of p62/A170/ZIP during the formation of the aggregates: possible mechanisms and the role in Lewy body formation in Parkinson's disease. Brain Res 2004;1012:42–51.
[167] Ramesh Babu J, Lamar Seibenhener M, Peng J, Strom AL, Kemppainen R, Cox N, et al. Genetic inactivation of p62 leads to accumulation of hyperphosphorylated tau and neurodegeneration. J Neurochem 2008;106:107–120.
[168] Weissman L, Jo DG, Sorensen MM, de Souza-Pinto NC, Markesbery WR, Mattson MP, Bohr VA. Defective DNA base excision repair in brain from individuals with Alzheimer's disease and amnestic mild cognitive impairment. Nucleic Acids Res 2007;35:5545–5555.
[169] Dezor M, Dorszewska J, Florczak J, Kempisty B, Jaroszewska-Kolecka J, Rozycka A, et al. Expression of 8-oxoguanine DNA glycosylase 1 (OGG1) and the level of p53 and TNF-alphalpha proteins in peripheral lymphocytes of patients with Alzheimer's disease. Folia Neuropathol 2011;49:123–131.
[170] Shao C, Xiong S, Li GM, Gu L, Mao G, Markesbery WR, Lovell MA. Altered 8-oxoguanine glycosylase in mild cognitive impairment and late-stage Alzheimer's disease brain. Free Radic Biol Med 2008;45:813–819.
[171] Weissman L, de Souza-Pinto NC, Mattson MP, Bohr VA. DNA base excision repair activities in mouse models of Alzheimer's disease. Neurobiol Aging 2009;30:2080–2081.
[172] Gredilla R, Weissman L, Yang JL, Bohr VA, Stevnsner T. Mitochondrial base excision repair in mouse synaptosomes during normal aging and in a model of Alzheimer's disease. Neurobiol Aging 2010; doi:10.1016/j.neurobiolaging.2010.1006.1019.
[173] Kadioglu E, Sardas S, Aslan S, Isik E, Esat Karakaya A. Detection of oxidative DNA damage in lymphocytes of patients with Alzheimer's disease. Biomarkers 2004;9:203–209.
[174] Mecocci P, Polidori MC, Ingegni T, Cherubini A, Chionne F, Cecchetti R, Senin U. Oxidative damage to DNA in lymphocytes from AD patients. Neurology 1998;51:1014–1017.
[175] Morocz M, Kalman J, Juhasz A, Sinko I, McGlynn AP, Downes CS, Janka Z, Rasko I. Elevated levels of oxidative DNA damage in lymphocytes from patients with Alzheimer's disease. Neurobiol Aging 2002;23:47–53.
[176] Lovell MA, Gabbita SP, Markesbery WR. Increased DNA oxidation and decreased levels of repair products in Alzheimer's disease ventricular CSF. J Neurochem 1999;72:771–776.
[177] Wang J, Xiong S, Xie C, Markesbery WR, Lovell MA. Increased oxidative damage in nuclear and mitochondrial DNA in Alzheimer's disease. J Neurochem 2005;93:953–962.
[178] Fukui H, Moraes CT. The mitochondrial impairment, oxidative stress and neurodegeneration connection: reality or just an attractive hypothesis? Trends Neurosci 2008;31:251–256.
[179] Petrozzi L, Ricci G, Giglioli NJ, Siciliano G, Mancuso M. Mitochondria and neurodegeneration. Biosci Rep 2007;27:87–104.

ARS-2012-5039-ver9-Santos_1P
Type: review-article

ANTIOXIDANTS & REDOX SIGNALING
Volume 00, Number 00, 2012

DOI: 10.1089/ars.2012.5039

FORUM REVIEW ARTICLE

Mitochondrial DNA Oxidative Damage and Repair in Aging and Alzheimer's Disease

Renato X. Santos,[1,2] Sónia C. Correia,[1,2] Xiongwei Zhu,[3] Mark A. Smith,[3] Paula I. Moreira,[1,4] Rudy J. Castellani,[5] Akihiko Nunomura,[6] and George Perry[7]

Abstract

Significance: Mitochondria are fundamental to the life and proper functioning of cells. These organelles play a key role in energy production, in maintaining homeostatic levels of second messengers (*e.g.*, reactive oxygen species and calcium), and in the coordination of apoptotic cell death. The role of mitochondria in aging and in pathophysiological processes is constantly being unraveled, and their involvement in neurodegenerative processes, such as Alzheimer's disease (AD), is very well known. ***Recent Advances:*** A considerable amount of evidence points to oxidative damage to mitochondrial DNA (mtDNA) as a determinant event that occurs during aging, which may cause or potentiate mitochondrial dysfunction which favors neurodegenerative events. Concomitantly to reactive oxygen species production, an inefficient mitochondrial base excision repair (BER) machinery has also been pointed to favor the accumulation of oxidized bases in mtDNA during aging and AD progression. ***Critical Issues:*** The accumulation of oxidized mtDNA bases during aging increases the risk of sporadic AD, an event that is much less relevant in the familial forms of the disease. This aspect is critical for the interpretation of data arising from tissue of AD patients and animal models of AD, as the major part of animal models rely on mutations in genes associated with familial forms of the disease. ***Future Directions:*** Further investigation is important to unveil the role of mtDNA and BER in aging brain and AD in order to design more effective preventive and therapeutic strategies. *Antioxid. Redox Signal.* 00, 000–000.

Introduction

THE SUSCEPTIBILITY of mitochondrial DNA (mtDNA) to damage is much higher than that of nuclear DNA (nDNA), resulting in higher mutation rates in mtDNA (132). mtDNA has multiple copies, and each mitochondrion contains 2 to 10 molecules of DNA, which are organized as nucleoids (51). The existence of several copies of mtDNA means that mutated and wild-type mtDNA can co-exist, a condition known as heteroplasmy (188). The ratio between wild-type and mutant mtDNA may define a threshold where a biochemical abnormality may determine a pathological phenotype. Indeed, it is estimated that in many patients with clinical manifestations of mitochondrial disorders, the proportion of mutant mtDNA exceeds 50% (138). The mitochondrial genome contains 37 genes, 13 of which encode for subunits of electron transport chain (ETC) complexes, 22 for transfer RNAs, and 2 for ribosomal RNAs (115, 59). Therefore, mtDNA integrity is mandatory for normal function of ETC, as it encodes several subunits of mitochondrial respiratory chain complexes as well as other mitochondrial proteins (19). If a proper ETC function is not ensured, reactive oxygen species (ROS) production is largely increased, as observed in mitochondrial diseases, or in experimental and animal models of oxidative phosphorylation (OXPHOS) deficiencies (125, 196). Increased generation of ROS and oxidative damage occur

[1]Center for Neuroscience and Cell Biology, University of Coimbra, Coimbra, Portugal.
[2]Department of Life Sciences, Faculty of Sciences and Technology, University of Coimbra, Coimbra, Portugal.
[3]Department of Pathology, Case Western Reserve University, Cleveland, Ohio.
[4]Laboratory of Physiology, Faculty of Medicine, University of Coimbra, Coimbra, Portugal.
[5]Department of Pathology, University of Maryland, Baltimore, Maryland.
[6]Department of Neuropsychiatry, University of Yamanashi, Yamanashi, Japan.
[7]UTSA Neurosciences Institute and Department of Biology, University of Texas at San Antonio, San Antonio, Texas.

during aging as well as several age-related degenerative diseases, including Alzheimer's disease (AD) (20, 10, 165). Furthermore, it has been suggested that age-associated deficiencies in the repair of oxidative DNA damage correlate with cognitive decline and neurodegenerative diseases that are prominent in the aged population (199, 177).

This review addresses several aspects of mitochondrial (dys) function and the involvement of mitochondria in aging, and AD is also discussed. Special attention is given to mtDNA and its repair mechanisms.

Mitochondria: Cell Keepers or Executioners?

The survival of eukaryotic cells greatly relies on mitochondrial function. The classical appraisal of mitochondrial function is based on energy-producing capacity. Nevertheless, the importance of mitochondria to the cells is far more complex and includes a number of functions that span from energy production, calcium (Ca^{2+}) homeostasis, and production of second messengers, to the control of apoptotic cell death (Fig. 1). In addition, the canonical view of mitochondria as bean-shaped organelles has been revoked and redefined to a more dynamic perspective, fusing, dividing, and moving within cells (50). Mitochondria are able to change from a network-like appearance, forming long tubules, to a more individualized state, appearing similar to small round vesicles. The stimuli that alter this equilibrium toward highly branched or completely fragmented morphology are linked to the cell compartmentalization, developmental stage, stress stimulus, and the functional state of the mitochondria, among others (14). Disturbing either mitochondrial fission or fusion may affect mitochondrial membrane stability with possible negative consequences for ETC functionality (38, 116, 40).

Mitochondrial bioenergetic production depends on the formation of a "protonmotive force," which is generated through the extrusion of protons to the intermembrane space driven by the electron flow throughout ETC, from complexes with lower to complexes with higher oxidation potentials. Protons are driven back to the matrix through the ATP synthase during ATP production (52). Although the electron flow through ETC complexes is a very efficient process, a small amount of superoxide anions ($O_2^{\bullet -}$) is produced, due to electron leak mostly from complexes I and III (163, 56, 33, 10). At low/moderate levels, ROS act as second messengers within cells; however, exacerbated ROS production is deleterious for the cell, contributing to a variety of pathological processes (192, 1). Redox imbalance will be further discussed in a subsequent section of the article.

Mitochondria are also intracellular buffers of cytoplasmic Ca^{2+}, thus playing a key role in normal neurotransmission, short- and long-term plasticity, excitotoxicity, and regulation of gene transcription, processes that are highly dependent on Ca^{2+} levels (35, 152, 208, 153, 210, 169, 154, 203). Ca^{2+} is internalized into mitochondria *via* the Ca^{2+}uniporter, a protein that is still to be fully identified and biochemically characterized. Nevertheless, a candidate protein, which was named MCU (from "mitochondrial Ca^{2+} uniporter"), proved to be essential for high-capacity Ca^{2+} transport into mitochondria in a number of *in vitro* and *in vivo* experimental models (13, 48). On the other hand, Ca^{2+} release is mediated by Na^+/Ca^{2+} or H^+/Ca^{2+} exchangers (203). It was shown that mitochondria are involved in cells' Ca^{2+} buffering impairment, a situation which occurs in the aging brain and AD (26, 29). The impairment of Ca^{2+} homeostasis is intimately associated with mitochondrial permeability transition (MPT). MPT is potentiated by oxidative stress, high phosphate concentrations, and adenine nucleotide depletion and is characterized by the opening of a high conductance pore known as mitochondrial permeability transition pore (MPTP) that enables the release of ions and solutes from the matrix to the

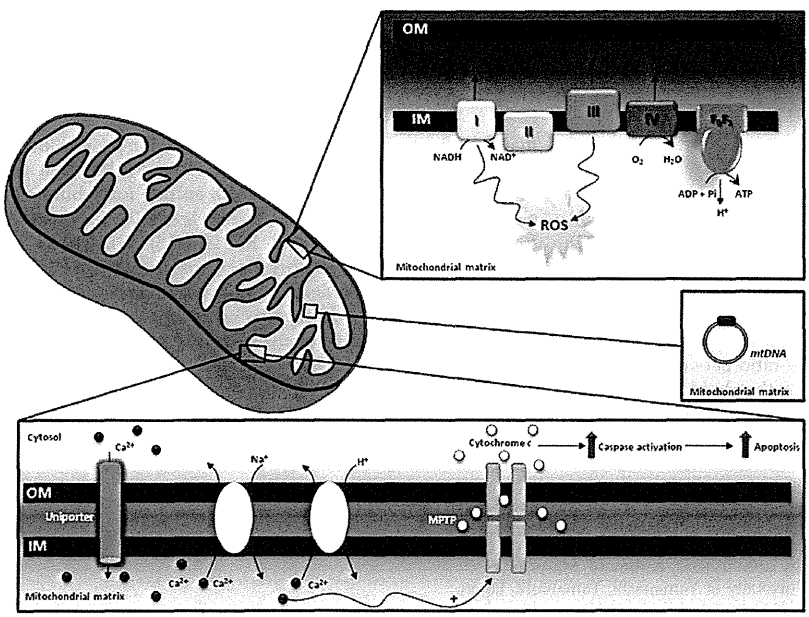

FIG. 1. Physiological functions of mitochondria. Mitochondria are centrally positioned in diverse aspects of cellular physiology such as homeostasis of second messengers (*e.g.*, reactive oxygen species (ROS), calcium (Ca^{2+})), apoptosis and energy production. mitochondrial permeability transition pore, mitochondrial permeability transition pore; CI, CII, CIII, and CIV, complexes I, II, III, and IV of the respiratory chain; FoF1, ATP synthase.

cytosol (209, 44). The MPTP eventually culminates in cell death due to the release of proapoptotic factors such as cytochrome c and apoptosis-inducing factor (80, 189, 150).

Ros Imbalance in Aging and AD

Endogenous production and scavenging of ros

The balance between ROS production and scavenging enables cells to achieve a physiological equilibrium where the levels of free radicals might play a role in cell transduction (178). ROS interfere with the macromolecules of cells; however, under physiological conditions, the cells' quality control systems are able to overcome this damage, avoiding the development of a pathological state (159). During aging, the quality control systems become defective, resulting in an accumulation of damaged components, which, accompanied by a redox disequilibrium, may elicit a pathological condition (156).

In cells, there are multiple sources of ROS, including mitochondria, nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (NOX), xanthine oxidase, and nitric oxide synthase (NOS) (147). Mitochondria are recognized as the hub of ROS production during normal aerobic activity. The electrons leak from the ETC directly to molecular oxygen, producing short-lived free radicals such as $O_2^{\bullet -}$ (133, 191). While complex I releases $O_2^{\bullet -}$ only to the matrix, complex III releases $O_2^{\bullet -}$ to both the matrix and intermembrane space (23). $O_2^{\bullet -}$ can be converted into nonradical derivates such as hydrogen peroxide (H_2O_2) either by a spontaneous dismutation reaction or catalyzed by the manganese superoxide dismutase that resides in the mitochondrial matrix (73). H_2O_2 can be converted into hydroxyl radicals ($^{\bullet}OH$) through the Fenton reaction. In the Fenton reaction, a molecule of H_2O_2 reacts with ferrous iron (Fe^{2+}) to generate ferric iron (Fe^{3+}), hydroxide anion (OH^-), and $^{\bullet}OH$. Fe^{3+} can be reduced by $O_2^{\bullet -}$, generating a redox cycle in which the $O_2^{\bullet -}$ facilitates the Fenton reaction by making Fe^{2+} available (92). Similar to iron, copper also participates in the Fenton reaction, which exacerbates ROS production (60, 85, 157). $^{\bullet}OH$ can also be produced by a direct reaction of $O_2^{\bullet -}$ with H_2O_2, a reaction known as the Haber–Weiss reaction (92). Mitochondria consubstantiate a microenvironment that is highly enriched in iron, as many mitochondrial enzymes possess heme groups and iron-sulfur clusters in their active centers, making them favorable locations of $\bullet OH$ production (128). Hence, mitochondria are prone to oxidative damage and particularly susceptible to $\bullet OH$-mediated oxidation, which plays a major role in DNA oxidation. Apart from the ETC, several other sites in the mitochondria have also been reported to generate $O_2^{\bullet -}$, including pyruvate dehydrogenase, α-ketoglutarate dehydrogenase (171), glycerol-3-phosphate dehydrogenase, and fatty acid β-oxidation (23). Recently, important advances toward understanding mitochondrial ROS generation have been made. Transient quantal $O_2^{\bullet -}$ flashes were observed in excitable cells such as neurons, which are associated with and required for the opening of MPTP, which represents a new facet of mitochondrial ROS (57, 198). To counteract an exaggerated production of ROS, mitochondria possess a very efficient antioxidant system, including glutathione peroxidase, catalase, and peroxiredoxin III, which are responsible for converting H_2O_2 to water (71).

As mentioned earlier, another source of cellular ROS is the NOX family proteins that are enzymatic complexes which catalyze the electron transfer from NADPH to molecular oxygen and generate $O_2^{\bullet -}$ and its downstream reactive species (16, 65). NADPH oxidase is composed of cytochrome b558 (an heterodimer comprising a 22-KDa alpha-subunit-p22phox- and a glycosylated approximately 91-KDa beta-subunit-gp91phox), several cytosolic proteins (p47phox, p67phox, and p40phox), and the Rac G-protein. According to the new terminology, the NOX family refers to the catalytic subunit of NADPH oxidase, and these include NOX2 and its six homologs (NOX1, NOX3, NOX4, NOX5, DUOX1, and DUOX2)(65). It is known that NOX1, NOX2, and NOX4 are expressed in neurons, astrocytes, and microglia. Under normal circumstances, NOX is latent. However, on stimulation, NOX is translocated to the membrane and forms an heterodimeric enzymatic complex with cytochrome b558 that catalyzes the reduction of molecular oxygen to $O_2^{\bullet -}$ (16).

In this way, compromised mitochondrial functioning, NOX overactivation, or the failure of free radical-scavenging systems could constitute critical events underlying oxidative damage in brain aging and AD.

Oxidative stress in the aging brain

Aging is an inevitable biological process that is characterized by a progressive decline in physiological function, including cognition, and by the increased susceptibility to disease, representing a major risk factor for the development of AD (36, 76). Oxidative stress and mitochondrial malfunction are two interdependent mechanisms that play a central role in brain aging (36). The brain is particularly vulnerable to oxidative damage as a consequence of its high levels of polyunsaturated fatty acids, high oxygen consumption, high content in transition metals, and poor antioxidant defenses (139). Compelling evidence reports that the aging brain is associated with the accumulation of markers of proteins, lipids, and DNA oxidative damage (37, 61, 66, 78, 166, 28). It was previously shown that the aged brain is characterized by increased levels of protein carbonyls, 3-nitrotyrosine, thiobarbituric acid reactive substances (TBARS), and diminished content of cardiolipin and protein thiols (41, 67, 160).

Along with oxidative stress, mitochondrial dysfunction also contributes to the aging brain. The most important functional deficits documented in aged brain are the loss of the mitochondrial membrane potential and OXPHOS capacity, decreased respiration and ATP synthesis, and increased susceptibility to MPTP opening (9, 22, 41, 58, 143).

Oxidative stress in AD

AD is the most prevalent age-related neurodegenerative disorder that affects approximately 35 million people worldwide (149). Clinically, AD is characterized by the progressive loss of cognitive function and behavioral disturbances (149). These traits are accompanied by two distinctive pathological features, the massive deposition of aggregated amyloid-β (Aβ) peptide in the extracellular space as senile plaques, and the presence of intracellular neurofibrillary tangles, mainly composed of hyperphosphorylated tau protein (34, 131).

The pathogenic road map leading to AD pathology is still not entirely understood; however, multiple pieces of evidence support the key involvement of oxidative stress and mitochondrial malfunction in the onset and progression of the disease (127, 155, 180). Oxidative stress is manifested by the