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Neurobiology of Aging 27 (2006) 596–605

NEUROBIOLOGY OF AGING

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DNA end joining activity is reduced in Alzheimer's disease

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Received 17 August 2004; received in revised form 22 February 2005; accepted 9 March 2005
Available online 23 May 2005

Abstract

Evidence indicates that oxidative stress-induced damage to DNA, protein, and other cellular components contributes to the progression of Alzheimer's disease (AD). Several studies indicate that postmitotic neurons have a reduced capacity for some types of DNA repair, which is further compromised by aging. Thus in AD, the cellular response to increased oxidative DNA damage may be inadequate to protect the genome. Mammalian cells use several mechanisms to repair DNA damage generated during normal oxidative metabolism or by genotoxic insults. The predominant mechanism to repair double strand breaks is non-homologous end joining (NHEJ) which utilizes the DNA-dependent protein kinase (DNA-PK) complex. A cell-free DNA end joining assay was employed to determine if NHEJ was reduced in nuclear cortical extracts from brains of AD versus normal subjects. This report demonstrates that end joining activity and protein levels of DNA-PK catalytic subunit are significantly lower in AD brains compared to normal controls. The amount of end joining activity correlates with the expression of DNA-PK and is dependent on DNA-PK catalytic activity. This indicates that repair of DNA double-strand breaks by the DNA-PK-dependent NHEJ pathway may be deficient in AD.

Keywords: Alzheimer's disease; DNA damage; DNA repair; DNA-dependent protein kinase; DNA end joining; Non-homologous end joining; Cerebral cortex

1. Introduction

Alzheimer's disease is a neurodegenerative disease identified by progressive memory loss and cognitive impairment. The distinguishing hallmarks of the disease are neuronal cell loss in the hippocampus and cerebral cortex, extracellular neuritic plaques formed by insoluble deposits of β-amyloid peptide, and intracellular neurofibrillary tangles formed by aggregation of hyperphosphorylated tau protein into paired helical filaments. Although multiple risk factors, including advanced age, are associated with AD, a primary cause has not been determined. Evidence indicates that oxidative stress contributes to the progression of AD as well as other neurodegenerative disorders (for review see [3,36,37,46]). Recent studies stress the role of the redox-active metals Cu and Fe in generating reactive oxygen species (for review see [3,18,20]). Normal aging results in an elevation of Cu and Fe in the brain and further dysregulation of metal homeostasis is noted in AD. Through the Fenton reaction, H_2O_2 in the presence of Fe is converted to the highly reactive hydroxyl radical, which can induce much of the oxidative damage observed in AD. Thus, the association of AD with advanced age may be due to a general increase in free radical production in the brain exacerbated by deposition of β-amyloid peptide in AD.

Increased oxidative damage detected in AD brains includes lipid peroxidation, DNA base hydroxylation, protein carbonylation and nitration, and glycation and glycoxidation of lysine residues [3,36,37,46]. In addition hydroxyl radicals cause DNA single and double-strand breaks, which are detected in AD brains although the origin and time course of the damage is not known [1,27,33,38,53–55]. Several studies indicate that postmitotic neurons have a reduced capacity for some types of DNA repair, which is further compromised by aging [32,41,42]. Thus, the cellular response to increased oxidative DNA damage during the course of Alzheimer's disease may be inadequate to protect the genome, contributing to neuronal loss.

Double strand breaks (DSBs) are a particularly dangerous form of DNA damage. Unrepaired DSBs can lead to

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doi:10.1016/j.neurobiolaging.2005.03.009

induction of cell cycle arrest or apoptosis. The consequences of errors in DSB repair can be chromosome translocation, genomic instability, and a predisposition to cancer (for review see [24,58]). Mammalian cells use predominantly non-homologous end joining (NHEJ) to repair DSBs, which can yield accurate restoration of the sequence or loss or addition of nucleotides before religation. The alternative mechanism of homologous recombination is used to a lesser extent in higher eukaryotes and is mainly proficient during late S and G2 phases of the cell cycle when a sister chromatid is available to serve as a template and thus may be less important in terminally differentiated neurons.

Genetic and biochemical studies identified five major components of the NHEJ machinery. These include the large catalytic subunit of DNA-dependent protein kinase (DNA-PKcs; 469 kDa), Ku70 (70 kDa), Ku 80 (or Ku86; 83 kDa), DNA ligase IV (96 kDa), and XRCC4 (38 kDa) (for review see [28,29,57,58]). DNA-PKcs is a member of the phosphatidylinositol-3 kinase-related serine/threonine protein kinase family that includes the cell cycle checkpoint kinases ATM and ATR. Its substrates include itself, Ku70 and Ku80, and XRCC4. Ku70 and Ku80 heterodimerize to form an open ring-like structure that can accommodate double-stranded DNA. Ku70/80 binding to DSBs recruits DNA-PKcs to DNA ends resulting in kinase activation and also binding of the DNA ligase IV/XRCC4 multimer. Additional proteins, such as Artemis and the complex of Mre11/Rad50/NBS1, have been demonstrated to interact with DNA-PKcs or Ku and may play a role in processing DNA ends by contributing nuclease activity [28].

Previously, we demonstrated that Ku binding to DNA and protein levels of DNA-PKcs are reduced, but not significantly different, in extracts of postmortem AD midfrontal cortex compared to age-matched controls [12]. Ku DNA binding and DNA-PK protein expression in the AD cases, however, correlates with synaptophysin levels, a measure of synaptic loss, which is a major correlate of cognitive deficits in AD. The results suggest that the non-homologous end joining machinery may be less proficient in AD. In this report a cell-free DNA end joining assay was employed to determine if NHEJ was reduced in nuclear cortical extracts from brains of AD versus normal subjects. In human cells, the assay is dependent on DNA-PKcs, Ku, and DNA ligase IV/XRCC4 [4]. The results demonstrate that the amount of end joining activity correlates with the expression of DNA-PKcs and is significantly lower in AD brains compared to normal controls. This indicates that repair of DNA double-strand breaks by the DNA-PK-dependent NHEJ pathway may be deficient in AD.

2. Methods

2.1. Subjects

Frozen human postmortem brain tissue was obtained from the Alzheimer's Disease Research Consortium (ADRC) at the University of California, San Diego. Tissue from midfrontal cortex of 46 neuropathologically confirmed AD cases and 13 aged-matched, non-demented normal cases were analyzed. Of these, 39 AD and 7 normal cases were used in an earlier study [12] and are identified by the case numbers used previously. The seven added AD cases were Braak stage III, whereas the earlier panel consisted of Braak IV (3 samples), V (13 samples), and VI (18 samples) cases. Five additional non-AD cases with short postmortem intervals excluded from the controls were evaluated previously [12] and in the current study to ensure that activity levels were not significantly affected by the postmortem delay.

The following tests of mental status were available for most of the cases: Mini-Mental State Examination (MMSE; 53 cases), Blessed Information-Memory-Concentration Test (51 cases), and Dementia Rating Scale (DRS; 42 cases). The neuropathology report from the ADRC provided Braak staging and counts of plaques/neuritic plaques and neurofibrillary tangles (counted in 100× and 500× fields, respectively, to maximize densities) in the midfrontal cortex for each case.

2.2. Preparation of nuclear extracts

Extracts from midfrontal cortex were prepared as described previously [12,52]. Briefly, midfrontal cortex tissue (0.1–0.2 g) was homogenized in four volumes of homogenization buffer A (10 mM HEPES, pH 7.9, 0.5 mM dithiothreitol, 10 mM KCl, 1.5 mM $MgCl_2$, and protease inhibitor cocktail) using 10 strokes with a Dounce homogenizer (B-type pestle) on ice. The final concentration of protease inhibitors in the buffers was 0.2 mM phenylmethylsulfonyl fluoride (PMSF), 5 mM benzamidine, 0.05 mg/ml leupeptin, 0.025 mg/ml pepstatin A, and 0.05 mg/ml aprotinin. Homogenates were centrifuged at 5000 × *g* for 15 min at 4 °C. The supernatants were considered the cytosolic fraction. Pellets were resuspended in an equal volume of high salt buffer C (20 mM HEPES, pH 7.9, 25% glycerol, 1.5 mM $MgCl_2$, 1 M NaCl, 0.2 mM EDTA, 0.5 mM dithiothreitol, and protease inhibitor cocktail), extracted for 30 min at 4 °C with continuous gentle mixing, and centrifuged at 70,000 × *g* for 30 min at 4 °C. This supernatant was the nuclear protein extract. The nuclear fractions were dialyzed for 6 h at 4 °C against 20 mM HEPES, pH 7.9, 20% glycerol, 100 mM KCl, 0.2 mM EDTA, 0.5 mM dithiothreitol, and 0.2 mM PMSF, centrifuged at 16,000 × *g* for 30 min at 4 °C, aliquoted and frozen at −70 °C. Protein concentrations were determined by the BCA protein assay (Pierce, Rockford, IL) using bovine gamma-globulin as the standard protein. In addition, extracts were prepared from the human endothelial cell line HeLa and the mouse hippocampal cell line HT22.

2.3. DNA end joining assay

The end joining assay was based on procedures described by Baumann and West [4]. The substrate was pGEX-2T plasmid DNA (Amersham Pharmacia Biotech, Piscataway, NJ)