Free Radical Biology and Medicine 71 (2014) 270–280

Contents lists available at ScienceDirect

Free Radical Biology and Medicine

journal homepage: www.elsevier.com/locate/freeradbiomed

Original Contribution

Redox proteomics analysis of HNE-modified proteins in Down syndrome brain: clues for understanding the development of Alzheimer disease

Fabio Di Domenico [a], Gilda Pupo [a], Antonella Tramutola [a], Alessandra Giorgi [a], Maria Eugenia Schininà [a], Raffaella Coccia [a], Elizabeth Head [b], D. Allan Butterfield [b,c], Marzia Perluigi [a,*]

[a] Department of Biochemical Sciences, Sapienza University of Rome, 00185 Rome, Italy
[b] Sanders-Brown Center on Aging, University of Kentucky, Lexington, KY 40506-0055, USA
[c] Department of Chemistry and Center of Membrane Sciences, University of Kentucky, Lexington, KY 40506-0055, USA

ARTICLE INFO

Article history:
Received 20 January 2014
Received in revised form
4 March 2014
Accepted 18 March 2014
Available online 25 March 2014

Keywords:
Protein oxidation
Down syndrome
Alzheimer disease
Redox proteomics
Lipid peroxidation
HNE
Trisomy 21
Free radicals

ABSTRACT

Down syndrome (DS) is the most common genetic cause of intellectual disability, due to partial or complete triplication of chromosome 21. DS subjects are characterized by a number of abnormalities including premature aging and development of Alzheimer disease (AD) neuropathology after approximately 40 years of age. Several studies show that oxidative stress plays a crucial role in the development of neurodegeneration in the DS population. Increased lipid peroxidation is one of the main events causing redox imbalance within cells through the formation of toxic aldehydes that easily react with DNA, lipids, and proteins. In this study we used a redox proteomics approach to identify specific targets of 4-hydroxynonenal modifications in the frontal cortex from DS cases with and without AD pathology. We suggest that a group of identified proteins followed a specific pattern of oxidation in DS vs young controls, probably indicating characteristic features of the DS phenotype; a second group of identified proteins showed increased oxidation in DS/AD vs DS, thus possibly playing a role in the development of AD. The third group of comparison, DS/AD vs old controls, identified proteins that may be considered specific markers of AD pathology. All the identified proteins are involved in important biological functions including intracellular quality control systems, cytoskeleton network, energy metabolism, and antioxidant response. Our results demonstrate that oxidative damage is an early event in DS, as well as dysfunctions of protein-degradation systems and cellular protective pathways, suggesting that DS subjects are more vulnerable to oxidative damage accumulation that might contribute to AD development. Further, considering that the majority of proteins have been already demonstrated to be oxidized in AD brain, our results strongly support similarities with AD in DS.

Down syndrome (DS) is one of the most frequent chromosomal abnormalities, resulting from the triplication of the part of chromosome 21 [1–5] that causes intellectual disability. In addition to cognitive deficits, individuals with DS show signs of premature aging, immune disorders, and other clinical pathologies [6]. By 35–40 years of age, a marked accumulation of senile plaques (SPs) and neurofibrillary tangles, the common neuropathological features of Alzheimer disease (AD), can be observed in DS brain [7,8]. Interestingly, there are reports of diffuse SPs in 8- to 12-year-old persons with DS [9,10], in whom the onset of dementia appears later, after 50 years of age, suggesting a prodromal period in which clinical signs are undetectable or minimal [11]. By studying autopsy samples from individuals with DS of various ages provides critical information regarding AD pathogenesis. Several studies showed that oxidative stress (OS) plays an important role in DS pathogenesis and in the development of AD pathology [12–15]. A recent study from our group reported increased OS conditions in brain of young DS people as indexed by increased carbonylation of specific proteins in the frontal cortex of DS subjects compared with non-DS cases [15]. Oxidative damage targets primarily proteasome and autophagy systems and may contribute to the disturbance of the proteostasis network in DS, thus potentially contributing to the development of AD [16]. Increased OS in DS most likely occurs as a consequence of overexpression of a subset of genes encoded by chromosome 21; among these, the most relevant

* Corresponding author. Fax: +39 064440062.
E-mail address: Marzia.perluigi@uniroma1.it (M. Perluigi).

http://dx.doi.org/10.1016/j.freeradbiomed.2014.03.027
0891-5849/

as potent OS inducers are APP (amyloid precursor protein) and SOD1 (superoxide dismutase 1). APP is the protein from which Aβ(1–42) peptide, the major protein in SPs in both DS and AD, is produced and Aβ(1–42) has been demonstrated to cause OS [17,18]. SOD1 has an important role in antioxidant defense, catalyzing the dismutation of $O_2^{\bullet -}$ to molecular oxygen and H_2O_2, the latter being decomposed in water by glutathione peroxide (GPX) and catalase (CAT). In DS the triplication of chromosome 21 leads to an increased production of H_2O_2, which is not followed by a similar increase in CAT and GPX, thus leading to an accumulation of H_2O_2 [19–21]. This compound, through the reaction with the reduced form of redox-active transition metal ions, leads to the formation of the hydroxyl radicals that are able to react and damage biological macromolecules, such as DNA, lipids, and proteins. In addition, superoxide can also react with nitric oxide, leading to the formation of peroxynitrite and other reactive nitrogen species.

In conditions of oxidative/nitrosative stress, proteins are highly vulnerable and may be the target of a number of modifications that may affect their functions. Further, if the oxidized proteins are not properly repaired or removed, they may accumulate within cells and become toxic. Different types of oxidative modifications, including, among others, carbonylation, formation of mixed disulfides, nitration, and formation of adducts with lipid peroxidation products, can be detected as indexes of tissue-specific damage.

Lipid peroxidation is one of the main events causing redox imbalance and subsequent buildup of oxidative stress within the cell. Lipid peroxidation is able to directly damage membranes, but reactive oxygen species (ROS) can also interact with polyunsaturated fatty acids, leading to the formation of copious amounts of reactive electrophilic aldehydes that are able to covalently bind proteins by forming adducts with specific amino acids [22]. According to a series of factors, such as acyl chain length and degree of unsaturation, the lipid hydroperoxide that is formed by reaction of a carbon radical with oxygen can decompose to produce reactive products such as malondialdehyde, 4-hydroxy-2-*trans*-nonenal (HNE), and acrolein. HNE is one of the most abundant and toxic aldehydes generated through ROS-mediated peroxidation of lipids and it is a highly reactive electrophile [23]. HNE can accumulate in cells and cause cell toxicity, membrane damage, disruption of Ca^{2+} homeostasis, and cell death and, with the other toxic aldehydes, is elevated in several neurodegenerative disease [24]. This compound can covalently modify protein residues of cysteine, lysine, and histidine by Michael addition, altering protein structure and causing loss of function and activity [25,26].

In this study, we investigated the role of lipid peroxidation in DS to shed light on the molecular mechanisms that may trigger the development of AD in DS subjects. Redox proteomics approaches [15] were used to analyze the frontal cortex of DS brain with and without AD pathology compared with age-matched controls to identify HNE-modified brain proteins.

Materials and methods

Subjects

DS, DS with AD pathology (DS/AD), and age-matched young (CTRY) and old (CTRO) control cases (six for each group) were obtained from the University of California at Irvine Alzheimer's Disease Research Center Brain Tissue Repository. Table 1 shows the characteristics and demographic data of all included subjects in the study. All DS cases were under the age of 40, whereas all cases with both DS and AD were over the age of 40. Thus, for this study, controls were split into two groups, either less than or equal to 40 years or older than 40 years of age at death. The postmortem interval (PMI) was different across groups, 9.96 ± 2.88 h for young controls, 12.5 ± 1.51 h for DS, 5.4 ± 2.8 h for DS/AD, and 8.9 ± 6.2 h for old controls. Subgroups used in this study were selected to maintain homogeneous age and gender inside the groups and were part of the entire cohort used in a previous experiment to investigate insoluble Aβ and total oxidation as a function of age in DS [15].

Sample preparation

Brain tissues (frontal cortex, around 20 mg per sample) from DS, DS/AD, and controls (six per group) were homogenized in Media 1 lysis buffer (pH 7.4) containing 320 mM sucrose; 1% of 990 mM Tris–HCl (pH 8.8); 0.098 mM $MgCl_2$; 0.076 mM EDTA; the proteinase inhibitors leupeptin (0.5 mg/ml), pepstatin (0.7 μg/ml), aprotinin (0.5 mg/ml), and phenylmethanesulfonyl fluoride (40 μg/ml); and phosphatase inhibitor cocktail (Sigma–Aldrich, St. Louis, MO, USA). Homogenates were centrifuged at 14,000 *g* for 10 min to remove debris. Protein concentration in the supernatant was determined by the Bradford assay (Pierce, Rockford, IL, USA).

Measurement of total protein-bound HNE

For the analysis of HNE-bound protein levels, 10 μl of frontal cortex homogenate was incubated with 10 μl of Laemmli buffer containing 0.125 M Tris base, pH 6.8, 4% (v/v) sodium dodecyl sulfate (SDS), and 20% (v/v) glycerol. The resulting samples (250 ng per well) were loaded onto a nitrocellulose membrane with a slot-blot apparatus under vacuum pressure. The membrane was blocked for 2 h with a solution of 3% (w/v) bovine serum albumin in phosphate-buffered saline containing 0.01% (w/v) sodium azide and 0.2% (v/v) Tween 20 and incubated with a rabbit polyclonal anti-4-hydroxynonenal antibody (1:3000; HNE-13M, Alpha Diagnostics, San Antonio, TX, USA) for 2 h at room temperature. This antibody specifically recognizes HNE-modified proteins. Membranes were washed and incubated with anti-rabbit IgG alkaline phosphatase secondary antibody (1:5000; Sigma–Aldrich) for 1 h at room temperature. Blots were dried and scanned by GS-800 densitometer (Bio-Rad) and analyzed by Quantity One (4.6.9 version; Bio-Rad, Hercules, CA, USA).

Two-dimensional (2D) electrophoresis

Brain sample proteins (200 μg) were precipitated in a 15% final concentration of trichloroacetic acid for 10 min in ice. Subsequently each sample was centrifuged at 10,000 *g* for 5 min and precipitates were washed in ice-cold ethanol:ethyl acetate 1:1 solution three times. The final pellet was dissolved in 200 μl rehydration buffer (8 M urea, 20 mM dithiothreitol (DTT), 2.0% (w/v) Chaps, 0.2% Bio-Lyte, 2 M thiourea, and bromophenol blue). First-dimension electrophoresis (isoelectric focusing) was performed with ReadyStrip IPG strips (11 cm, pH 3–10; Bio-Rad) at 300 V for 2 h linearly, 500 V for 2 h linearly, 1000 V for 2 h linearly, 8000 V for 8 h linearly, and 8000 V for 10 h rapidly. All the above processes were carried out at room temperature. After the first-dimension run the strips were equilibrated two times, first for 10 min in 50 mM Tris–HCl (pH 6.8) containing 6 M urea, 1% (w/v) SDS, 30% (v/v) glycerol, and 0.5% DTT and again for another 10 min in the same buffer containing 4.5% iodoacetamide in place of DTT. The second dimension was performed using 12% precast Criterion gels (Bio-Rad). The gels were incubated in fixing solution (7% acetic acid, 10% methanol) for 45 min and then stained for 1 h in Bio-Safe Coomassie gel stain (Bio-Rad) and destained overnight in deionized water. The Coomassie gels were scanned using a GS 800 densitometer (Bio-Rad).