The identification of protein biomarkers for oxidative stress in Down syndrome

Expert Rev. Proteomics 8(4), 427–429 (2011)

Marzia Perluigi

Department of Biochemical Sciences, Faculty of Pharmacy and Medicine, Sapienza University of Rome, 00185 Rome, Italy

D Allan Butterfield

Author for correspondence
Department of Chemistry, Center of Membrane Sciences and Sanders-Brown Center on Aging, University of Kentucky, Lexington, KY 40506, USA
Tel.: +1 859 257 3184
Fax: +1 859 257 5876
dabcns@uky.edu

"The identification of oxidative stress biomarkers is becoming an intense and challenging field of research, which aims to gain insight into the role of oxidative stress in the multifaceted phenotypes of Down syndrome."

Down syndrome (DS) is a chromosomal abnormality due to partial or complete triplication of chromosome 21 (HSA21), and is the most common genetic cause of intellectual disability. DS may be considered a multifactorial disease, where an abnormal expression of trisomic genes arises not only from genetic, but also environmental factors [1]. Thus, trisomy leads to a deregulated scenario that also affects disomic genes and that ultimately results in largely different phenotypes [2]. In fact, DS patients present a high variability of symptoms, including premature aging, mental retardation and Alzheimer's-like dementia. Thus, above 40 years of age these persons develop a form of dementia with several clinical and neuropathologic characteristics of Alzheimer's disease (AD), although with an earlier age of onset [3].

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Owing to the prolonged life expectancy and improved quality of life of DS subjects, the comprehension of neurodegenerative phenomena that affect DS has received much attention from researchers. In particular, growing interest has been given to better understanding the role of oxidative stress (OS) in the appearance and exacerbation of severe clinical symptoms.

Is OS the bridge between DS & Alzheimer's disease?

Accumulating evidence has demonstrated a major role of OS in DS clinical outcomes [4,5]. One of the most corroborated hypotheses that has been proposed to explain the enhanced OS in DS is the increased intracellular activity of cytosolic copper/zinc superoxide dismutase (SOD-1), the gene for which is located in HSA21. This increased activity of SOD-1 leads to the over-production of H_2O_2, which at high, toxic levels may be responsible not only for neuronal damage/loss observed in DS, but may also be involved in the impairment of other cellular functions. H_2O_2 produced by SOD-1 is further detoxified by glutathione peroxidase or catalase. If the increased release of H_2O_2 is not followed by a parallel increase of glutathione peroxidase and catalase, which detoxify H_2O_2, this imbalance could result in OS conditions, leading to damage to all classes of biological macromolecules. Based on this view, the variability observed in antioxidant enzyme levels within the DS population may explain the different susceptibility to oxidative insult that may correlate to the degree of intellectual disability, premature aging and dementia seen in DS [6].

Keywords: Alzheimer's disease • Down syndrome • oxidative stress • protein oxidation • redox proteomics

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 10.1586/EPR.11.36 ISSN 1478-9450

In addition, the overexpression of the *APP* gene, which is also located on HSA21, could conceivably explain the overproduction of Aβ(1–42) peptide, the major protein in senile plaques in DS subjects, which is one of the important hallmarks of AD pathology. Accumulation of Aβ(1–42) peptide has been found in the brain of DS children, and deposits increased with age [7]. Many studies demonstrated that Aβ(1–42) is able to induce OS [8–10].

Post-mortem and *in vitro* studies have shown that OS plays a role in the pathogenesis of many of the clinical features of DS. Elevated OS has been demonstrated in the brains of DS patients, as indexed by increased levels of thiobarbituric acid reactive substances (TBARs), total protein carbonyls and advanced glycation end products in the cortex from DS fetal brain compared with controls [11], and a marked accumulation of 8-hydroxy-2-deoxyguanosine (8OHdG), oxidized proteins and nitrotyrosine in the cytoplasm of cerebral neurons in DS [12]. Elevated levels of isoprostane 8,12-iso-iPF2α (iPF2α), a specific marker of lipid peroxidation, have been demonstrated in living adults with DS [13]. Recently, evaluation of some biomarkers of oxidative/nitrosative stress in the urine from adults compared with adolescent DS subjects demonstrated that advanced glycation end products, dityrosine, H_2O_2 and nitrite/nitrate could be considered as OS biomarkers in DS in contrast to other markers, such as 8-OHG, 15-F(2t)-IsoP and TBARS, which gave contrasting results [14]. However, additional studies on large populations are needed to confirm the reproducibility of these results.

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Studies on an animal model of the disease [15] demonstrated an increased level of reactive oxygen species and mitochondrial dysfunction in primary cultured astrocytes and neurons from DS transgenic mice, suggesting that the 'gene-dosage' hypothesis is sufficient to explain at least the major part of OS phenomena observed in this animal model of DS. Using the redox proteomics approach, the authors also identified the putative target proteins that were modified by lipid peroxidation-derived products [15]. ATP synthase mitochondrial F1 complex β-subunit, enolase and triosephosphate isomerase 1 were identified as proteins modified by 3-hydroperoxy-9Z,11E-octadecadienoic acid (13-HPODE). Neurofilament light polypeptide, internexin neuronal intermediate filament, neuron-specific enolase, peroxiredoxin 6, phosphoglycerate kinase 1 and triosephosphate isomerase were shown to be 4-hydroxy-trans-2-nonenal-modified proteins. Thus, dysfunction of these proteins as a consequence of oxidative damage may affect ATP production, the neuronal cytoskeleton system and antioxidant network function. Some of these proteins have already been found to be modified by hydroxynonenal, a reactive product of lipid peroxidation, in AD and mild cognitive impairment brains [16–18], suggesting that these redox proteomics-identifed brain proteins might play a central role in the cognitive dysfunction and neurodegenerative processes occurring in DS. In addition, these findings may not only underlie some putative pathways that lead to an increased risk of DS patients developing AD, but also underscore how OS may link DS to AD.

To gain insight into this matter, we have recently studied the amniotic fluid from women carrying DS fetuses in comparison with those of pregnant women with healthy fetuses. We found increased levels of OS, as indexed by increased protein oxidation, lipid peroxidation, reduction of glutathione and thioredoxin levels and induction of the heat-shock response [19]. Other investigators have also reported increased levels of isoprostanes in second trimester amniotic fluid samples from DS fetuses [20].

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By using a redox proteomics approach, we identified selective proteins that showed increased oxidation in the amniotic fluid of mothers carrying DS fetuses compared with amniotic fluid from mothers with healthy fetuses. The identified proteins are involved in iron homeostasis (ceruloplasmin and transferrin), lipid metabolism (zinc-α2-glycoprotein, retinol-binding protein 4 and apolipoprotein A1) and inflammation (complement C9, α-1B-glycoprotein and collagen α-1V chain). We suggest that the increased oxidation of specific proteins could correlate with some characteristic features of DS, including early aging, cognitive impairment and also increased risk for cancer and immunodeficiency. Our study further demonstrates that OS occurs early in the pathogenesis of DS and might play a crucial role in the severity of DS phenotypes. Thus, it would be desirable to establish a set of OS biomarkers that may be evaluated postnatally throughout life to follow the development of clinical manifestations.

OS occurs early in the pathogenesis of DS

The emerging consideration that arises from this review is that oxidative damage is not only a common feature of neurodegenerative disorders but, more interestingly, might play a crucial role in the pathogenesis of the disease. Indeed, OS occurs very early in the pathogenesis of DS, many years before the appearance of 'defective' phenotypes, not only related to cognition.

The currently available findings point to the fact that DS and AD share some common mechanisms closely linked both by genetic and biochemical similarities that translate into protein dysfunctions. These abnormalities seem to converge into the 'OS hypothesis', which has been proposed and demonstrated for many neurodegenerative diseases. OS is a crucial factor because it affects multiple pathways related to cell growth/death, gene expression and protein function, among many others. Many studies have linked OS to neurodegeneration, but in the case of DS and AD, this correlation has many additional overlapping features due to the fact that some of the genes responsible for the familial form of AD are expressed

on HSA21. Although this involvement has not been revealed in every detail, investigations of DS and AD brains and non-neuronal tissues have demonstrated many signs of reactive oxygen species attack, including lipid peroxidation, protein and DNA oxidation and mitochondrial abnormalities. Due to these genetic similarities, DS may be also regarded as 'prodromal' AD and provides an *in vivo* model to study the pathogenesis and progression of AD. However, these patients have several other clinical symptoms that complicate the identification of the molecular pathways of neurodegeneration. A promising and unexplored insight may be offered by the analysis of DS brain or brain from DS-relevant transgenic mice. Such studies are ongoing in our laboratories.

The identification of OS biomarkers is becoming an intense and challenging field of research, which aims to gain insight into the role of OS in the multifaceted phenotypes of DS. Our OS and redox proteomics study, together with results from other researchers, provide the groundwork for developing new pharmacological and/or nutritional interventions, based on antioxidant compounds, in DS patients from the earliest life stages for the prevention, or at least slowing, of the multiple complications observed in DS.

Financial & competing interests disclosure

This work was supported, in part, by a NIH grant to D Allan Butterfield (AG-05119). The authors have no 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 apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

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