REVIEW

Unraveling the complexity of neurodegeneration in brains of subjects with Down syndrome: Insights from proteomics

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Down syndrome (DS) is one of the most common genetic causes of intellectual disability characterized by multiple pathological phenotypes, among which neurodegeneration is a key feature. The neuropathology of DS is complex and likely results from impaired mitochondrial function, increased oxidative stress, and altered proteostasis. After the age of 40 years, many (most) DS individuals develop a type of dementia that closely resembles that of Alzheimer's disease with deposition of senile plaques and neurofibrillary tangles. A number of studies demonstrated that increased oxidative damage, accumulation of damaged/misfolded protein aggregates, and dysfunction of intracellular degradative systems are critical events in the neurodegenerative processes. This review summarizes the current knowledge that demonstrates a "chronic" condition of oxidative stress in DS pointing to the putative molecular pathways that could contribute to accelerate cognition and memory decline. Proteomics and redox proteomics studies are powerful tools to unravel the complexity of DS phenotypes, by allowing to identifying protein expression changes and oxidative PTMs that are proved to be detrimental for protein function. It is reasonable to suggest that changes in the cellular redox status in DS neurons, early from the fetal period, could provide a fertile environment upon which increased aging favors neurodegeneration. Thus, after a critical age, DS neuropathology can be considered a human model of early Alzheimer's disease and could contribute to understanding the overlapping mechanisms that lead from normal aging to development of dementia.

Keywords:
Brain / Down syndrome / Neurodegeneration / Oxidative stress / Proteasome

1 Introduction

Down syndrome (DS) is the most common chromosomal abnormality with an estimated 70–80% prenatal lethality and an incidence of 1:700 births. This syndrome re-

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Abbreviations: **Aβ**, amyloid beta; **AD**, Alzheimer's disease; **AF**, amniotic fluid; **APP**, amyloid precursor protein; **Cα**, collagen alpha; **CAT**, catalase; **CBR**, carbonyl reductase; **CSTB**, cystatin B; **DS**, Down syndrome; **DSCR**, Down syndrome critical region; **ES**, embryonic stem; **ETS**, E26 transformation specific; **GPX**, glutathione peroxidase; **4-HNE**, 4-hydroxy-*t a* -2-nonenal; **13-HPODE**, 3-hydroperoxy-9Z,11E-octadecadienoic acid; **MMP2**, matrix metalloproteinase 2; **OS**, oxidative stress; **SOD**, superoxide dismutase; **SP**, senile plaques; **UCH-L1**, ubiquitin-carboxy-hydroxyl lyase 1

S100B, an astroglial-derived Ca^{2+}-binding protein acting as a neurotrophic factor on neurons and glial cells, is also encoded on Chr21. S100B is involved in the regulation of energy metabolism in brain cells by stimulating the enzymatic activity of fructose-1,6-bisphosphate aldolase and phosphoglucomuatse [37]. It modulates the proliferation and differentiation of neurons and glia, and it interacts with many immunological functions of the brain. S100B exerts a protective effect as long as its intracellular concentration is at physiological levels. However, once secreted, its local concentration dictates its beneficial or detrimental effects. At nanomolar concentrations neuroprotective effects prevail, while at micromolar concentrations neurodegenerative or apoptosis-inducing ef-

proteolytic machinery and is achieved by ubiquitin C-terminal hydrolases, ubiquitin isopeptidases, or deubiquitinating enzymes.

Interestingly, a defective protein ubiquitination could result in reduced intracellular protein degradation, also in the presence of adequate proteasome activity. Several changes in intracellular protein ubiquitination with age have been reported, showing that aged cells have less free ubiquitin and more ubiquitin-protein conjugates than young cells [60]. The fact that changes in protein ubiquitination do not always indicate changes in protein degradation rates may reflect the participation of ubiquitin in intracellular processes other than protein degradation [61]. It is likely that conjugation with ubiquitin competes with other types of PTMs among which phosphorylation, acetylation, as in the case of p53, are fundamental for its activation [62].

In order to prevent protein aggregation, oxidized proteins have to be efficiently degraded. Therefore, specific systems are required to both recognize and degrade damaged/misfolded proteins. The proteasomal system is the major proteolytic system responsible for the removal of oxidized proteins. Since one of the main functions of the proteasome is the removal of oxidatively damaged proteins, proteasomal activity is regulated by OS. After protein exposure to oxidants, increased susceptibility to proteolytic attack by various proteases is well documented [63]. Thus, oxidation processes correlate with intracellular proteolysis [64]. However, oxidized proteins accumulate within cells if oxidative damage is faster than the rate of proteolysis. It is generally accepted that intracellular protein turnover declines during aging, while oxidatively modified and damaged proteins accumulate [65–70]. This accumulation of oxidatively modified and ubiquitinated proteins and the general decline in protein turnover have raised the possibility that proteasome function is impaired with age. The aggregates thus formed are called plaques, aggresomes, age pigments, or Lewy bodies, depending on their composition and location. Changes in proteasomal function have also been observed in senescent cells, whereas proteasome inhibition in young cells induces premature senescence [71].

DS fetal brains have a selective upregulation of the proteasome zeta chain and isopeptidase T [72]. Very recently, our group demonstrated using redox proteomics that ubiquitin-carboxy-hydroxyl lyase 1 (UCH-L1) is a target of oxidative damage in DS brains, with a reduction of its enzymatic activity [73]. Indeed, UCH-L1 is responsible for recycling of ubiquitin through hydrolysis of peptide-ubiquitin bonds and processing of ubiquitin precursors, but it also has ubiquitin ligase activity [40]. We suggest that aberrant ubiquitin hydrolase and/or ligase activity for the identified oxidative modifications of UCH-L1 might lead to dysfunction of the neuronal ubiquitination/deubiquitination machinery, causing synaptic deterioration and neuronal degeneration in

brain studies [78, 85]. Of the other remaining proteins, six of them were matrix and structural proteins (annexin 4; plastin-3 T-isoform; keratin complex 2; Vil2, microtubule-associated protein RP/EB 2; and calponin 3); three were heat shock/stress proteins (HSP84–1, HASP70, and HSP86–1); three were degradation proteins or translational regulators (UCH-L1, eukaryotic translation elongation factor, and ubiquitin thioesterase); two were nuclear transcriptional factors (heterogeneous nuclear ribonucleoprotein H1 and heterogeneous nuclear ribonucleoprotein); and two were enzymes for energy and macromolecular metabolism (vacuolar ATPase subunit a isoform 1 (ATP6v1a1) and vacuolar ATPase subunit b isoform 2 (ATP6v1b2)). To better understand the expression patterns of these altered proteins throughout neuronal differentiation, the corresponding spot intensities in the 2DE gel in TT2F and TT2F/hChr21 cells at day 0 (D0), day 3 (D3), day 6 (D6), and day 10 (D10) of differentiation were also analyzed. Both protein subunits Atp6v1a1 and Atp6v1b2 of the vacuolar ATPase proton pump, which mediate acidification of intracellular organelles for energy production and convention, as well as autophagy, were overexpressed. Three proteins, ubiquitin thioesterase, Eef1D, and UCH-L1 involved in protein catabolism or translation regulation were underexpressed. HSPs, HSP84–1, HSP70, and HSP86–1, demonstrated a stage-specific suppression on D0, D3, and D6, respectively. However, HSP84–1 protein expression did not change

Overall, the biochemical data presented are consistent with other reports showing little change in the expression of proteins from synaptosomes and PSDs isolated from the cerebri of adult Ts65Dn mice [97–99]. However, shifts in the phosphorylation of a variety of synaptic proteins including pre- and postsynaptic scaffold proteins and receptors such as synapsin, piccolo, liprin, dynamin, PSD-95, or NMDA (*N*-methyl-D-aspartate) receptors were observed. The results of this study suggest that the trisomic condition serves primarily to change the functional state of synaptic proteins, but may not result in a fundamental reorganization of synapses. Thus, cognitive impairment in people with DS cannot be reduced to compositional changes at excitatory synapses, but is dependent on higher order deficits in neurons and astrocytes.

A study by Ishihara et al. [100] on primary cultured astrocytes and neurons from Ts1CJe mouse model of DS demon-

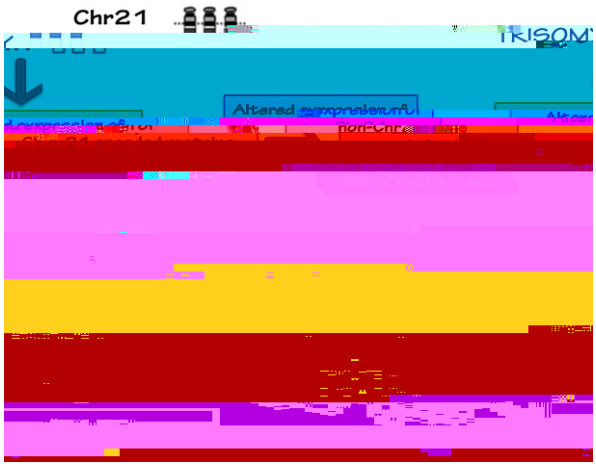

Figure 2. Putative scenario representing the contribution of

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