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Gelsolin Levels are increased in the brain as a Function of Age During Normal Development in Children That are further increased in Down Syndrome

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Abstract

Neuronal dysfunctions in several neurodegenerative diseases such as Down syndrome (DS) have been linked to oxidative stress. In this study, we observed that lipid peroxidation, a marker of oxidative stress, is significantly increased in the frontal cortex of brains of individuals with DS as compared to control subjects. We report here that gelsolin levels are increased in the frontal cortex of individuals with DS as compared to controls during early developmental ages (5–13 years). Interestingly, the levels of gelsolin in the frontal cortex were increased as a function of age in both DS and control subjects. Because cytoplasmic gelsolin has five free thiol groups (cysteine), and its levels are increased in response to oxidative stress, we propose that gelsolin may serve as an anti-oxidant protein.

Keywords

gelsolin; development; Down syndrome; oxidative stress

INTRODUCTION

Down syndrome (DS), a common genetic birth defect, is associated with mental retardation. Statistics shows that 1 in 800 children have DS, and the frequency becomes even higher with the age of the mother. Mothers past 35 years of age have increased chances of having a child with DS. Some of the features of DS are endocardial and immunological defects, hematological and endocrinal alterations, behavioral and cognitive deficits, and hypotonia.[1] An extra copy of chromosome 21, frequently referred to as trisomy 21, causes DS. The genes for superoxide dismutase 1 (SOD-1), beta-site Alzheimer's precursor protein (APP)-cleaving site 2 (BACE-2), and APP have been identified on chromosome 21.[2] Alzheimer's disease (AD) is caused by fibrillization of amyloid beta-protein (Aβ), a proteolytic product of APP. After 40 years of age, individuals with DS invariably develop AD.

The pathogenesis of a number of neurodegenerative diseases such as AD and DS has been associated with oxidative stress.[3] Oxidative stress occurs when the production of reactive oxygen species (ROS) exceeds the antioxidant capacity of a cell. According to the "gene dosage effect" hypothesis of DS[4], increased oxidative stress in DS is due to the presence of an extra copy of chromosome 21 and the consecutive overexpression of the genes located on it. APP and Cu^{2+}/Zn^{2+} SOD-1, localized on chromosome 21, are involved in the regulation of redox homeostasis[5], and in oxidative stress-mediated neuronal loss by apoptosis.

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ROS are highly unstable molecules that react to other biological molecules (proteins, DNA, lipids) and modify their functions or properties. Several studies have shown higher levels of markers of lipid peroxidation and protein oxidation in the brains of subjects diagnosed with both DS and AD.[3] We have recently reported that oxidative stress induces the expression of gelsolin in pheochromocytoma-12 (PC-12) cells.[6]

Gelsolin is present intracellularly in all cell types and in the plasma/cerebrospinal fluid (CSF) as secreted protein. Intracellularly, gelsolin is a major actin-binding protein that caps the actin filament's growing end, stimulates its nucleation, and severs the actin filaments.[7] Both plasma/CSF and intracellular gelsolin originate by the alternative splicing of a single gene.[8] Five Cys residues in human cytoplasmic gelsolin are free thiols, whereas three Cys residues in plasma/CSF gelsolin are free thiols, and the other two are disulfide-linked.[9] In addition to controlling the formation of cytoplasmic actin filaments, gelsolin plays important roles in apoptosis, amyloidosis, and cancer. Caspase-3 has been implicated as a key mediator of apoptosis, and gelsolin was identified as one of its substrates.[10] Gelsolin also prevents apoptosis by inhibiting apoptotic mitochondrial changes via closing voltage-dependent anion channels.[11] We and other groups have identified an anti-amyloidogenic role of gelsolin in AD.[12] We have reported that extracellular gelsolin, i.e., plasma gelsolin binds to amyloid beta-protein (Aβ)[13], inhibits its fibrillization, and solubilizes preformed Aβ fibrils.[14] Recently, we demonstrated the binding of cytoplasmic gelsolin to Aβ.[6] In addition, peripheral administration or transgene expression of gelsolin could reduce the amyloid load in the brains of a transgenic mouse model of AD.[15,16]

In this study, we investigated whether gelsolin levels are affected in the brains of individuals with DS. Here we report increased oxidative stress and increased gelsolin in the brains of individuals with DS as compared to age-matched control subjects. We also observed an age-dependent increase in gelsolin levels in the brains of individuals with DS and of normal control subjects.

Methods

Preparation of Brain Homogenate

Case demographics are presented in Table 1. The ages of the DS and control subjects were from 0.5 to 13 years. Frozen human frontal cortex tissues from five DS and five control cases were obtained from the Brain Bank of the New York State Institute for Basic Research in Developmental Disabilities. The tissues were homogenized in cold buffer containing 50 mM Tris-HCl (pH 7.4), 8.5% sucrose, 2 mM EDTA, and 10 mM β-mercaptoethanol plus protease inhibitor cocktail (Sigma-Aldrich). The homogenates were centrifuged at 4 °C, and the protein concentrations of the supernatants were assayed by Bradford's method.

Measurement of Lipid Peroxidation in Brain Homogenate

Malonyldialdehyde (MDA), an end product of lipid peroxidation, was measured by the thiobarbituric acid assay as described previously.[17] MDA content was calculated by using a molar extinction coefficient for MDA of 1.56×10^5.

Western Blotting

Fifteen micrograms of total protein from human brain homogenates from DS subjects and controls was separated by using a 10% SDS-polyacrylamide gel electrophoresis (PAGE) and then transferred to a nitrocellulose membrane (Bio-Rad). The membrane was blocked with phosphate-buffered saline (PBS) containing 5% fat-free dried milk for 2 h at room temperature, and further incubated overnight at 4 °C with a primary antibody against gelsolin or β-actin. The membrane was then washed with PBS-0.05% Tween 20 three times and incubated with

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horseradish peroxidase- conjugated secondary antibody for 2 h at room temperature. The membrane was washed again, and the immunoreactive protein was visualized using enhanced chemiluminescent reagent (Pierce).

Statistical Analysis

Statistical analysis was performed using GraphPad Prism version 4.0 for Windows (GraphPad Software, San Diego, California).

Results

Increased Lipid Peroxidation in the Frontal Cortex of DS brain

MDA is the end product of lipid peroxidation. Table 1 shows MDA contents in the frontal cortex from the brains of five DS subjects and five age-matched controls. The amount of MDA was significantly increased ($p < 0.05$) in the frontal cortex of subjects with DS as compared to controls.

Increased Gelsolin in the Frontal Cortex of Individuals with DS and During Normal Development

Figure 1 compares the levels of gelsolin and actin in brain homogenates from DS and control subjects of varying ages (0.5–13 years). Data from five age-matched pairs of DS and control subjects are shown. The Western blot (Figure 1A) showed increased gelsolin levels in DS subjects when compared to age-matched control subjects. The intensity of β-actin band was similar in different lanes for both DS and control groups, which confirmed that an equal amount of protein was loaded in each lane. There was no effect of postmortem interval (PMI) on gelsolin levels.

The density of different bands was analyzed by Image J software (NIH). The density of gelsolin bands as a function of age in DS and control groups is represented in Figure 1B. We observed that gelsolin levels were significantly higher in DS subjects as compared to controls (ANOVA, one-way analysis of variance, $p < 0.05$, and Bartlett's test for equal variances, $p < 0.0001$). Gelsolin expression was observed to increase with age in both DS ($r = 0.84$) and controls ($r = 0.94$), where r is the correlation coefficient.

Discussion

This study is the first to show increased gelsolin levels in the brains of DS and normal subjects as a function of age. We selected samples from 0.5 years to 13 years of age because development of the brain takes place during this period. Any assault in the developing phase may have deleterious effects on normal development of the brain. The brain is highly vulnerable to oxidative stress because of its limited antioxidant capacity, higher energy requirement, and higher amounts of lipids and iron.[18] The brain makes up about 2% of body mass but consumes 20% of metabolic oxygen. Due to the lack of glutathione-producing capacity by neurons, the brain has a limited capacity to detoxify ROS. Therefore, neurons are the first cells to be affected by increases in ROS and shortages of antioxidants and, as a result, are most susceptible to oxidative stress. Children are more vulnerable than adults to oxidative stress because of their naturally low glutathione levels from conception through infancy.[19] Subjects with DS have increased vulnerability to oxidative stress as a result of over expression of SOD 1 coded on chromosome 21. Oxidative stress has been associated with DS and with its major phenotypic features, such as early aging. Increased MDA contents in the frontal cortex of DS brain as compared to age-matched controls in our study suggests the presence of oxidative stress in subjects with DS as early as 0.5 years of age. This observation is supported by the finding of

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Perrone et al[20] who observed excessive oxidative stress in the amniotic fluid of pregnancies with DS.

Actin and its binding proteins play a major role in cellular signaling.[21] Actin responds to extracellular signaling by converting from soluble actin to polymerized actin, which is controlled by gelsolin. We have reported that both cytosolic and secretory forms of gelsolin bind to Aβ.[6, 14] We have also reported that PC 12 cells respond to oxidative stress by increasing the expression of gelsolin.[6] The present study suggests that gelsolin levels increase with age during development in the brains (Figure 1). It has been proposed that aging is caused by free radical–mediated damage.[22] Therefore, the increase of gelsolin in brain as a function of age may be related to free radical-mediated damage. In light of our previous report that gelsolin responds to oxidative stress by increasing its expression, an increase in gelsolin levels in the brains of individuals with DS as compared to age-matched controls subjects may be a response to increased oxidative stress. Taken together, the studies suggest multifunctions of gelsolin: (a) it binds to actin and regulates actin polymerization, (b) it binds to beta-amyloid and regulates its clearance, (c) its levels are reduced in cancer, (d) it prevents apoptosis by inhibiting apoptotic mitochondrial changes via closing voltage-dependent anion channels, and (v) it responds to oxidative stress by increasing its expression.

It is estimated that proteins can scavenge 50–75% of ROS generated within a cell.[23] The question arises: Can gelsolin function as an antioxidant protein? The answer may lie in its structure. Five Cys residues in human cytoplasmic gelsolin are free thiols[9], thus rendering it suitable to act as an antioxidant. Free thiol residues in proteins such as gelsolin are particularly susceptible to oxidation, and this can result in transient formation of intramolecular disulfide.[24] A recent report suggests that gelsolin is converted to disulfide bridges in response to menadione.[25] Its increased levels under oxidative stress conditions or upon induction of the oxidative stress suggests that gelsolin may function as an antioxidant protein.

In summary, our results show that gelsolin levels are increased in the brains of individuals with DS as compared to age-matched control subjects and that it also increases as a function of age in developing brains. We suggest that gelsolin may act as an antioxidant protein by virtue of the free thiol groups in its structure.

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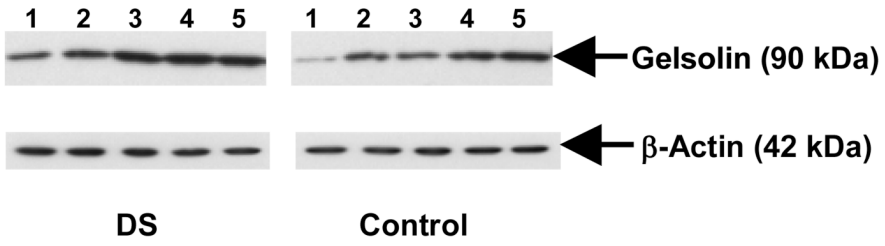

Figure 1A

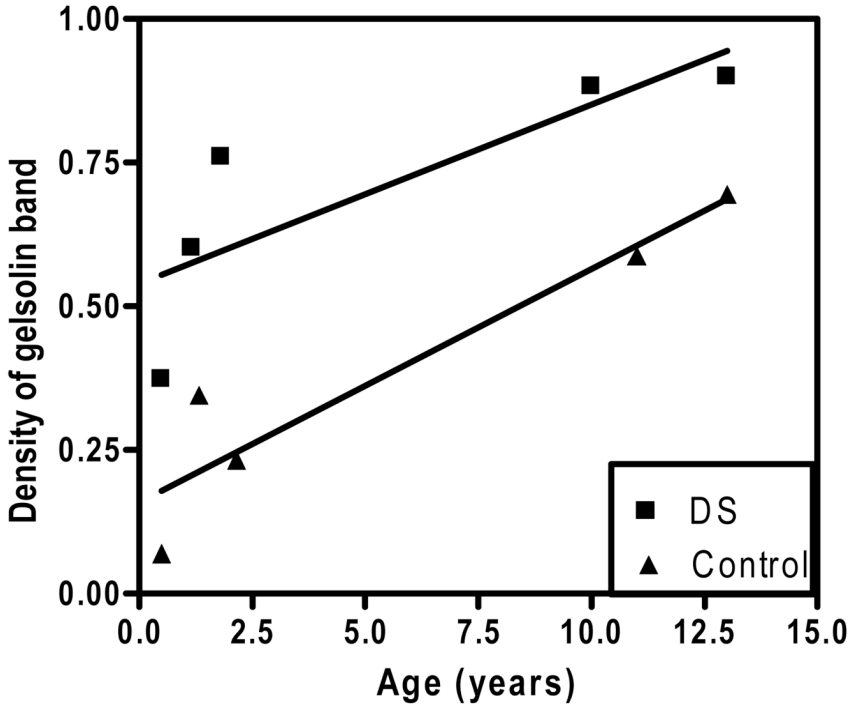

Figure 1B

Figure 1. Gelsolin levels in the frontal cortex of brains from DS subjects and age-matched controls

Brain proteins were separated on SDS-PAGE, and gelsolin and β-actin were detected by Western blotting as described in Methods (Figure 1A). Lanes 1–5 represent corresponding samples 1–5 from DS and control groups that are shown in Table 1. The densities of gelsolin bands in Figure 1A were analyzed by Image J software (NIH). The density of gelsolin as a function of age in DS and control subjects is shown in Figure 1B. Gelsolin levels were significantly increased as a function of age both in DS and controls, and also in DS as compared to age-matched controls. ANOVA, one-way analysis of variance, $p < 0.05$, and Bartlett's test for equal variances, $p < 0.0001$

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Table 1

Description of Five Pairs of Subjects with Down syndrome (DS) and Controls with Age, Gender and Postmortem Interval (PMI)

Pair	Group	Age (y)	Gender	PMI (h)
1	DS	0.5	F	28
	Control	0.5	F	1
2	DS	1.1	F	28
	Control	1.3	M	21
3	DS	1.8	M	17
	Control	2.2	F	21
4	DS	10.0	M	17
	Control	11.0	M	19
5	DS	13.0	M	25
	Control	13.0	M	5

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Table 2

Malonydialdehyde (MDA) Contents in the Frontal Cortex of Brains from DS and Control Subjects

	MDA contents (n mol / mg protein)
Control	17.87 ± 1.28
DS	23.59 ± 0.98*

* denotes $p < 0.02$ as compared to control group