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ACTIVATION OF p53 IN DOWN SYNDROME AND IN THE Ts65Dn MOUSE BRAIN IS ASSOCIATED WITH A PRO-APOPTOTIC PHENOTYPE

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Abstract

Down Syndrome (DS) is the most common genetic cause of intellectual disability resulting from trisomy of chromosome 21. The main feature of DS neuropathology includes early onset of Alzheimer's disease, with deposition of senile plaques and tangles. We hypothesized that apoptosis may be activated in the presence of AD neuropathology in DS, thus we measured proteins associated with upstream and downstream pathways of p53 in the frontal cortex from DS cases with and without AD pathology and from Ts65Dn mice, at different ages. We observed increased acetylation and phosphorylation of p53, coupled to reduced MDM2-mediated ubiquitination and lower levels of SIRT1. Activation of p53 was associated with a number of down-stream targets (bax, PARP1, caspase-3, heat shock proteins and PGC1α) that were modulated in both DS and DS/AD compared with age-matched controls. In particular, the most relevant changes (increased p-p53, acetyl-p53 and reduced formation of MDM2/p53 complex) were found to be modified only in the presence of AD pathology in DS. In addition, a similar pattern of alterations in the p53 pathway were found in Ts65Dn mice. These results suggest that p53 may integrate different signals, which can result in a pro-apoptotic-phenotype contributing to AD neuropathology in people with DS.

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Contributions.

M.P., D.A.B and F.DD conceived and designed the experiments. A.T., G.P., D.B. and A.A. performed the experiments. E.B., C.L and C.B. performed statistics and analyzed the data. M.P. and A.T. wrote the paper. E.H. and D.A.B. critically revised the manuscript. All the authors read and discussed the manuscript. Study supervision: M.P. and F.DD.

Competing interests.

The authors declare no competing financial interests.

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Keywords

apoptosis; bax; caspase; sirtuins; trisomy 21; Ts65Dn mouse model

INTRODUCTION

Down syndrome (DS) is the most frequent genetic disorder caused by the presence of all or part of a third copy of chromosome 21 (trisomy 21) affecting 1 in 1,000 infants. Characteristic features of DS are intellectual disability and the early appearance of Alzheimer's disease (AD) neuropathology by the age of 50 ys [1]. Among the molecular mechanisms proposed to be involved in the intellectual disability in DS individuals is the widespread hypo-cellularity observed in the brain. A reduction in neuronal number in the hippocampus, parahippocampal gyrus, cerebellum and neocortex of fetuses, children and adults with DS has been reported [2-4]. The brain cell loss may result from impaired neurogenesis during pre- and post-natal stages in fetal brain, also observed in the mouse model of DS [5-7] and defective proliferation of neural precursors. Thus, among putative mechanisms, it is likely that increased susceptibility of neurons to undergo apoptosis may be involved in the cell loss found in DS brain.

Within this scenario, a pivotal function in neuronal death is played by p53 as demonstrated by studies in several *in vitro* and *in vivo* models of neurodegeneration, showing increased p53 levels in affected neurons [8, 9]. The tumor suppressor protein p53 has been proposed “as the guardian of the genome” for its crucial role in regulating the transcription of a wide set of genes involved in cell cycle arrest, senescence, antioxidant system or apoptosis in response to various stress signals [10]. Although p53 promotes longevity by decreasing the risk of cancer through activation of apoptosis or cellular senescence, several findings suggest that the uncontrolled increase of its activity may have deleterious effects leading to “abnormal” aging phenotypes [11, 12].

Under normal, non-stressed physiological conditions, p53 protein is maintained at low levels within a cell by its negative regulator MDM2, an ubiquitin ligase responsible for p53 degradation [13]. Cellular stress affects the interaction between p53 and MDM2 leading to the accumulation of the former [14], and reactive oxygen (ROS) also modify p53 and its activity [15]. The regulatory events that affect the amount, stability and activity of p53 are in part derived from a variety of post-translational modifications, including phosphorylation, ubiquitination and acetylation [16].

The molecular basis underlying the choice between cell-cycle arrest and induction of apoptosis by p53 is not well understood. However, among the multiple posttranslational modifications of p53 that have been characterized, the acetylation of key lysine residues within the C-terminal region of p53 appears to be a determinant of activity [17]. Indeed, most studies suggest that acetylation stimulates p53 stabilization, sequence-specific binding activity, and activation of target genes [18, 19]. Further, following stress, p53 is also phosphorylated at multiple residues, thereby modifying its biochemical functions required for increased activity as a transcription factor. It has been reported that, other than being regulated by ROS, the activation of p53 leads to the generation of ROS [20, 21]. Studies

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from our groups and others support the idea that oxidative stress is a key “toxic” event, which may trigger neurodegenerative phenomena in DS brain accelerating the development of AD [22, 23]. We demonstrated that oxidative stress is an early event in DS phenotype [24] and we suggest that chronic, sublethal doses of ROS activates a plethora of signalling ultimately resulting in neuronal loss in the adult life.

Accumulating evidence suggests that defects in apoptosis may lead to neurodegenerative disorders. However, studies performed in different types of neurodegenerative diseases have led to some controversial results. It is unclear what role apoptotic processes have in DS. We hypothesize that p53 and its regulatory network in DS brain, is active prior to and after the development of AD pathology. Our findings suggest that activation of p53 may contribute to the development of AD neuropathology in DS brain.

MATERIALS AND METHODS

Subjects

Frozen frontal cortex samples from DS and control cases were obtained from the University of California-Irvine-ADRC Brain Tissue Repository, the NIH NeuroBioBank, and the University of Kentucky ADC. The human cases used in the present study are described in Table 1. DS cases were divided into two groups, with (DS) or without a neuropathology diagnosis of AD (DS/AD). All cases with both DS and AD were over the age of 40 years. Thus for the current study, controls were split into two groups, either less than or equal to 40 years (CTRY) or older than 40 years at death (CTRO). The post mortem interval (PMI) was different across groups ($p<0.05$).

Mouse colonies

Mice were generated by repeatedly backcrossing Ts65Dn trisomic females with (C57BL/6JxC3H/ HeJ) F1 hybrid males, the parental generation was purchased from Jackson Laboratories. These breeding pairs produce litters containing both trisomic (Ts65Dn) and disomic (2N) offspring. The pups were genotyped to establish trisomy using standard PCR, as described by [25]. In addition, the recessive retinal degeneration 1 mutation (Pdeb rd1), which is segregates in the colony and results in blindness in homozygotes, was detected in all the Tg animals used in the present study by using a standard PCR. Mice were housed in clear plexiglass cages (20×22×20 cm) under standard laboratory conditions with a temperature of 22±2°C and 70% humidity, a 12-h light/dark cycle and free access to food and water. Mice were sacrificed at 6 and at 12 months of age and the frontal cortex region was collected (Table 2). All the experiments were performed in strict compliance with the Italian National Laws (DL 116/92), the European Communities Council Directives (86/609/EEC). All efforts were made to minimize the number of animals used and their suffering.

Samples preparation for Western Blot

Brain tissue (Frontal Cortex) from human and mouse samples were homogenized by sonication in RIPA buffer (pH 7.4) containing 50 mMTris-HCl (pH 7.4), 150 mMNaCl, 1% NP-40, 0.25 % sodium deoxycholate,1mM EDTA, 0,1% SDS, 1mM PMSF, 1mM NaF and 1 mM Na3VO4. was centrifuged at 14,000 ×g for 30 min to remove cellular debris. The

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supernatant was extracted to determine the total protein concentration by the BCA method (Pierce, Rockford, IL).

Western Blot

For Western blot, 30 μg of proteins were separated by 12% SDS– PAGE using Criterion Gel TGX Stain free (Bio-Rad) and blotted onto a nitrocellulose membrane (Bio-Rad, Hercules, CA, USA). Before blotting, the gel image was used to obtain total protein load to normalize blot analysis. Membranes were blocked for 1h and 30 min with 3% bovine serum albumin in T-TBS at room temperature and then incubated with primary anti-body. The following antibodies were used: p53 (Human, 1:1000) and Acetyl-p53 (1:1000) from Millipore (Boston, MA, USA), from Abcam (Cambridge, MA, USA), Caspase-3 (1:500), p53 (Mouse, 1:1000), p-p53 (1:1000), Sirt1 and 2 (1:1000), Hsp70 (1:1000), Hsp27 (1:1000), PARP1 (1:1000), Bax (1:1000), PCG1alpha (1:1000), Mdm-2 (1:1000) from Santa Cruz Biotechnology (Santa Cruz, CA, USA). The membranes were incubated for 1 h at room temperature with secondary antibody horseradish peroxidase-conjugated anti-rabbit, anti-mouse or anti-goat IgG (1:5000; Sigma–Aldrich, St Louis, MO, USA). Membranes were developed with the Clarity Western ECL Substrate (Bio-Rad) acquired with ChemiDoc XP (Bio-Rad) and analyzed using Image Lab software (Bio-Rad).

Immunoprecipitation

Briefly, 100 μg of protein extracts were dissolved in RIA buffer (10mM Tris, pH 7.6; 140mM NaCl; 0.5% NP40 including protease inhibitors) and then incubated with 1 μg of primary antibody at 4°C overnight. Immunocomplexes were collected by using protein A/G beads (Santa Cruz, CA, USA) for 2 h at 4°C. Immunoprecipitated protein was recovered by resuspending the pellets in reducing SDS buffers and subjected to electrophoresis on 12% gels followed by western blot analysis.

Statistical analysis

Data are expressed as mean ± SD per group. All statistical analyses were performed using a nonparametric one-way ANOVA with post hoc Bonferroni t-tests. $p < 0.05$ (*) was considered significant. All statistical analysis was performed using GraphPad Prism 5.0 software.

Results

All the results obtained from human autopsy samples were analysed by considering the difference in PMI among groups. Correlation analysis did not show any association between all the reported measures and PMI (supplementary Table 3).

p53: protein level and post-translational modifications

p53 is activated in response to a wide variety of stresses that can damage the cell genome; once it is bound to sites of DNA damage, p53 promotes DNA repair and simultaneously stimulates the transcription of direct effectors of cell-cycle arrest [26]. Our results show that p53 expression was not altered across the four groups, indicating that trisomy does not affect p53 expression levels (Figure 1, A.3). However, the activity of p53 as a sequence-specific

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transcription factor is highly regulated by post-translational modifications (PTMs), protein–protein interactions and protein stabilization [27].

To investigate the activation of p53, which results from multiple PTMs, we focused our attention on two different PTMs, phosphorylation and acetylation. Both phosphorylation at Ser^{20} and acetylation of at Lys^{382} residues within the C-terminal region of p53, are a determinant of its activity [28]. The p-(Ser^{20})p53 /p53 ratio, which represents the majority of nuclear p53 protein and weakens the interaction of p53 with MDM2, was significantly increased in DS/AD vs. DS (~2 fold increase, *p<0.05) and DS/AD vs. CTRO (~ 150% increase, *p<0.05) (Figure 1, A.1). Similarly, the levels of acetyl-p53 at Lys382 (K382)/p53 were increased in DS/AD vs. DS (~100% increase, *p<0,05). This increase was not statistically significant, in DS/AD vs. CTRO (Figure 1, A.2).

Interestingly, a similar increase in p53 phosphorylation and acetylation was observed in brain of Ts65Dn mice at 12 months of age compared with Ts65Dn at 6 months of age (~ 30% increase in Ts65Dn 12M vs. 6M p<0.05; Figure 1, B.1 and B:2) however the alterations were also accompanied by increased p53 expression levels (~ 40% in Ts65Dn 12M vs. 6M *p<0.05) (Figure 1, B.3).

Upstream regulation of p53: SIRTs and MDM2

One major regulator of p53 activity is the sirtuin family [29]. By deacetylating p53, both SIRT1 and SIRT2 lead to the degradation of p53 suppressing apoptosis [30, 31]. We found that the levels of SIRT1 are significantly reduced in DS/AD samples vs. DS (*p<0.05) as well as in DS/AD vs. CTRO (~ 50% decrease, *p<0.05) (Figure 2, A.1). Decreased SIRT1 levels were also observed in 12 month-old Ts65Dn mice compared with 6 month-old Tg mice (~ 30% decrease, p<0.05) and also, between 12 month-old Ts65Dn mice and their age matched controls (~ 40% decrease, p<0.05) (Figure 2, B.1). Surprisingly, SIRT1 is expressed in higher amounts in 6 months Ts65Dn mice compared with euploid mice (Figure 2, B.1).

In contrast, SIRT2 did not show any significant decreases in both human and mouse samples, suggesting this protein may not be critically involved in DS or age associated AD neuropathology (Figure 2, A.2 and B.2).

MDM2, a p53-specific E3 ubiquitin ligase, is the principal cellular antagonist of p53, acting to limit the p53 growth-suppressive function in unstressed cells by constantly mono-ubiquitinating p53. The interaction between p53 and MDM2 is conformation-based and is tightly regulated at multiple levels. Disruption of the p53-MDM2 complex by multiple routes is the pivotal event for p53 activation, leading to p53 induction and its biological response [32]. In the present study, we immunoprecipitated p53 and evaluated the levels of p53-MDM2 complexes. As shown in Figure 3, A.1 we found reduced levels of MDM2/p53 complex in DS/AD vs. DS as well as in DS/AD vs. CTRO (about 30% decrease, *p<0.05), thus suggesting that p53 is activated and there is reduced antagonism by the interaction with MDM2 (Figure 3, A.1).

The same trend of reduced MDM2 bound to p53 was also observed in Ts65Dn mice as a function of age, as showed in Figure 3, B.1. A significant reduction of MDM2/p53 complex

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is observed in 12-month old Ts65Dn compared with 6-month old mice (~ 40% decrease, *p<0.05) but also compared with 12-month old euploid mice (Figure 3, B.1). These data suggest that in “old” Ts65Dn mice, p53 is likely less degraded and possibly transcriptionally active.

p53-down stream targets: apoptotic pathway (BAX, PARP1 and caspase 3), HSPSs and PGC1α

Western blot experiments show that the mitochondrial pro-apoptotic protein BAX was increased in DS/AD vs. CTRO and also in DS/AD vs DS (~ 100% and 80% increased respectively, p<0.05) (Figure 4, A.1). As showed in Figure 4 B.1, the same trend was also observed in Ts65Dn mice, once again showing a significant increase in 12-month old Ts65Dn mice vs. 6-month old Ts65Dn mice as well as vs. 12-month-old euploid mice (~ 100% increase, *p<0.05).

Another apoptotic effector activated in response to oxidative/metabolic stress is the DNA-binding enzymes PARP-1 and PARP-2, which are involved in base excision repair. We found that PARP1 protein levels are increased in DS/AD vs. CTRO and DS/AD vs. DS (~ 30% and 40% increase respectively, p<0.05) (Figure 4, A.2). Conversely, no significant increases were observed in older Ts65Dn mice (Figure 4, B.2).

The apoptotic executioner Caspase-3 was increased in DS/AD compared with DS and also with CTRO (~ 120% increase for both, p<0.05) (Figure 4, A.3), while no age changes were observed in Ts65Dn mice (Figure 4, B.3).

In order to address the presence of a pro-apoptotic phenotype in cerebral cortex of DS as a function of AD pathology, we measured the levels of specific heat shock proteins (HSPs) that are induced/repressed during different types of stress. As shown in Figure 5 Hsp70, that has an anti-apoptotic function, was not modulated in DS human samples (Figure 5, A.1), while an increase of approximately 50% (p<0.05) was observed in 12-month old Ts65Dn mice compared with 6-month-old Ts65Dn mice (Figure 5, B.1). However, this increase is likely due to an aging phenotype rather then specific of the trisomic phenotype.

Consistent with Hsp70, Hsp27 levels, with anti-apoptotic activity, were unchanged in DS human brain (Figure 5, A.2), while euploid mice a slight increase is observed as a function of age (Figure 5, B.2).

Last, we hypothesized that PGC1α, a downstream target of p53, that is a member of a family of transcriptional co-activators that plays a central role in the regulation of cellular energy metabolism, may be reduced in DS. Recent reports indicate that PGC1α could be a potential biomarker of AD, as reduced PGC1α mRNA and protein levels were detected in AD brains [33, 34]. We found that PGC1α protein levels were reduced only in DS vs. CTRY (~ 50% decrease, *p<0.05), with no further decrease in the presence of AD pathology (Figure 6, A. 1). The opposite effect was observed in Ts65Dn mice, PGC1α was not decreased in Ts65Dn mice but a slight increase was found in euploid mice with increasing age (~ 30% increase, p<0.05) (Figure 6, B.1).

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Discussion

People with DS consistently develop AD neuropathology, which includes deposition of senile plaques, neurofibrillary tangles, and neuronal loss following middle age [35]. Apoptosis has been proposed as a potential mechanism leading to the neuronal death observed in several neurodegenerative disorders, including AD [36, 37]. Increased immunoreactivity for activated caspase-3 is observed in neurons of AD and DS [38]; however, the underlying mechanism remains to be unraveled. In addition, p53 levels are up-regulated and aberrantly modified in the brains of patients with AD and Parkinsons' disease [39-42]. Elevated gene expression of apoptotic factors including the p53 protein was also detected in the brains of people with DS [43]. In the present study, we did not observe any significant changes in p53 protein levels in the frontal cortex of cases with DS, prior to and after development of AD pathology or in Ts65Dn mice. However, p53 undergoes PTMs that critically control its stability and function, including phosphorylation, acetylation, sumoylation, oxidation and others. We focused our attention on the acetylation of key lysine residues (Lys^{382}) within the C-terminal region of p53 that appear to be a determinant of activity [18, 45-47]. In addition, under different stress stimuli p53 is also phosphorylated at multiple residues. Specifically the phosphorylation of p53 at Ser20 residue is essential for the interaction between MDM2 and p53 [48]. Nevertheless, acetylation or phosphorylation alone are likely not sufficient for p53 activation but their coupled increase might result in the stimulation of an apoptotic pathway. Indeed, we found that, despite no change in the levels of p53, both acetylation and phosphorylation levels were significantly increased in DSAD vs. DS thus suggesting the either age-dependent or AD-dependent activation of p53.

Initial stabilization of p53 occurs through the disruption of the p53 and MDM2 interaction and the balance between acetylation, phosphorylation and ubiquitination appears to be critical to regulate p53 transcriptional activity of antiapoptotic genes. A reduced level of the p53/MDM2 complex was observed in DSAD vs. DS as well as vs. CTRO, confirming that p53 is transcriptionally active. The activation of p53 observed in this study may be in part regulated by the activity of SIRTs, a class of enzyme that deacetylate a variety of proteins, resulting in a robust, protective cellular response. By deacetylating p53, SIRT1 acts as a negative regulator of its activity thus inhibiting apoptosis. Reduced levels of SIRT1 were observed in DSAD compared with younger DS cases, which also supports higher levels of p53 acetylation. In addition, overexpression of SIRT1 is reported to reduce both Aβ production and plaques, whereas removing SIRT1 results in an increase of Aβ levels [49].

Moreover, SIRT1 can promote tau accumulation, by deacetylation [52]. In the presence of reduced SIRT1 levels and p53/MDM2 complex, the steady-state levels of p53 and its target genes could be not efficiently regulated [53]. Surprisingly, the constitutive levels of SIRT1 are increased in Ts65Dn mice compared with euploid mice. Although a decrease in SIRT1 in Ts65Dn with aging was observed, but is higher overall compared to euploid mice suggesting a constitutively higher expression independent of age. Taken together, our results suggest that p53 is transcriptionally active in the presence of AD pathology in DS, suggesting that molecular cascades associated with apoptosis are induced in the neurodegenerative process affecting DS individuals.

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To better understand if activation of p53 translates to increased apoptosis, we have evaluated p53-downstream targets. Among these, the levels of the pro-apoptotic factor Bax were increased in DSAD relative to DS and to CTRO. In agreement with our hypothesis, the up-regulation of Bax and the down-regulation of antiapoptotic Bcl-2 factors are observed in different cortical and cerebellar regions of DS foetuses [54, 55].

PARP-1 levels were also increased in the presence of AD pathology in DS brain and associated with the activation of caspase-3. Activation of PARP-1 is another early response event after DNA damage in neurons and mediates a variety of complex mechanisms involved in DNA repair or apoptotic signalling. Accordingly, a previous study by Salemi and colleagues [56] showed increased PARP1 expression in 72% of DS cases compared to controls. Increased immunoreactivity of activated caspase-3 [38] and TUNEL assay labelling [57] are observed in the brains of adults with DS. In addition, Sun *et al.* [58] showed that overexpression of RCAN1 in primary neurons activates caspase-9 and caspase-3, and subsequently induces neuronal apoptosis..

However, changes in PARP-1 and Caspase-3 levels were not observed in Ts65Dn mice, thus neuronal loss in these Tg mice may be caused only partially by programmed cell death. This may not be entirely surprising, as Ts65Dn mice do not develop AD neuropathology with age. Similarly, decreased expression levels of PGC1α were observed only in human autopsy samples while in Tg mice its levels showed an opposite trend. PGC1α, besides regulating mitochondrial biogenesis and function [59], it is involved in a wide range of cellular metabolic processes and thereby influences cell survival on multiple levels [60]. Interestingly, expression of PGC1α is reduced in AD patients and in the Tg2576 mouse model of AD [33, 34].

This scenario fits with the concept that one of the major contributors to the increased susceptibility of neurons to death is the elevation of oxidative stress, which occurs at early ages in DS brain [36, 61, 62]. Increased oxidative stress conditions in DS may be caused by the triplication of some of the genes encoded on chromosome 21, which directly or indirectly lead to increased free radical release [63-65]. In addition, both *in vitro* and *in vivo* studies suggest that in neurons, p53 plays a role in ROS-mediated apoptotic signalling (reviewed in [44]). However, the response of p53 to oxidative damage may depend on the extent of damage. Initially, at low levels of oxidative stress, p53 has primarily antioxidant functions and target genes of p53 are induced to counteract oxidative damage. This could explain the reason why we did not observe significant induction of apoptosis in young DS. However, when oxidative stress overwhelms antioxidant defences, p53 changes its function and serves as a prooxidant by stimulating pro-oxidative genes. This concept is reinforced by the finding that HSPs, a well-recognized protective and pro-survival response, are not induced in DS and DSAD in agreement with previous studies form our group showing that one of the member of HSP family, HO-1, is transcriptionally repressed by the trisomic gene Bach-1 [63].

p53 is activated by the orchestrated action of multiple PTMs, as observed in our study. It also interesting to note that PTMs of p53 may vary between neuronal cell type, depending on the source and severity of the “stress”, and may change during development and aging.

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However, with the accumulation of oxidative damage over the lifespan, and the consequent impairment of selected proteins [22, 66] as demonstrated by redox proteomics studies from our group, p53 transcriptional activity is increased and a pro-apoptotic phenotype prevails driving the accelerated development of AD neuropathology in DS.

In conclusion, the current study suggests that p53 is transcriptionally active in the brains of DS individuals and is associated with an increase in apoptotic markers in the presence of AD pathology. It is likely that Aβ-induced neurotoxicity and OS– mediated damage are chronic stimuli in neurons and may be responsible for increased susceptibility of neurons to death. We suggest that p53 is able to integrate different signals that lead to a pro-apoptotic phenotype that contributes to the development of AD neuropathology in DS.

Data obtained on Ts65Dn mice confirm the majority of the results obtained by human DS autopsy samples, however some discrepancies exist. We suggest that activation of apoptosis is associated with the development of AD in DS aging brain. Although Ts65Dn mice exhibit many of the neurodegenerative DS phenotypes, they do not display the neuropathological hallmarks of AD - plaques and tangles. Furthermore, differences between studies performed in DS individuals and in Ts65Dn mice, as well as in other trisomic models, could be due to the fact that many of the orthologous genes found in Hsa21 are not triplicated in these models. Additionally, some genes that are not overexpressed in DS are found in trisomy in these mice. Collectively, mouse models have certainly contributed to unravel putative molecular mechanisms responsible of learning and memory deficits in DS and to identify neuronal targets to be tested in clinical trial. Novel Tg mouse models would provide further understanding of the complexity of DS phenotypes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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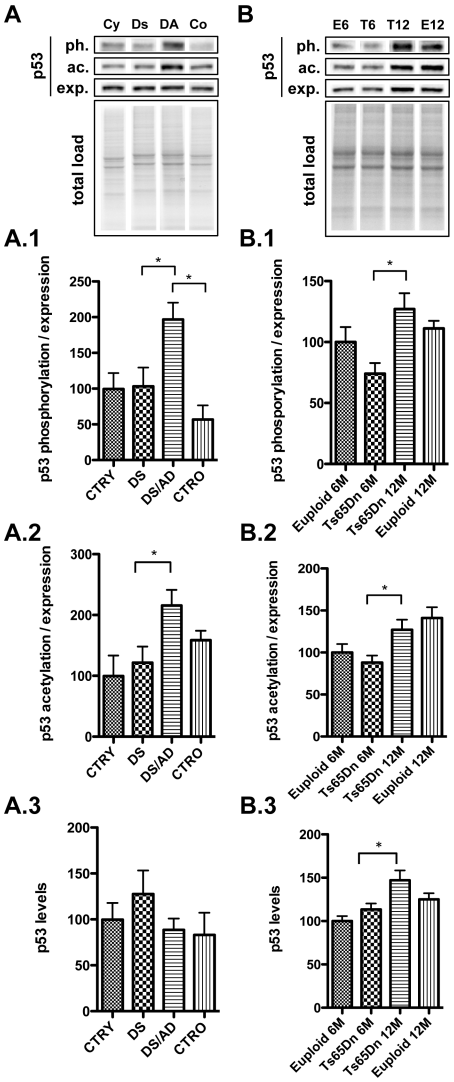

Figure 1. p53 protein levels and post-translational modifications

p53 phosphorylation at Ser-20 (1)(ph.), acetylation at Lys382 (K382) (2)(ac.) and total expression (exp.) levels (3)were measured by Western Blot in the frontal cortex of controls and DS cases (Panel A) and in the frontal cortex of Ts65Dn mice at 6 and 12 months of age (T6, T12) compared to age-matched euploid animals (E6, E12 -Panel B). Densitometric values shown in the bargraph are the mean of 8 samples per group normalized to total protein load and are given as percentage of control (E6 mice), set as 100%. On the top a representative blot image with protein bands is shown (*$p < 0.05$).

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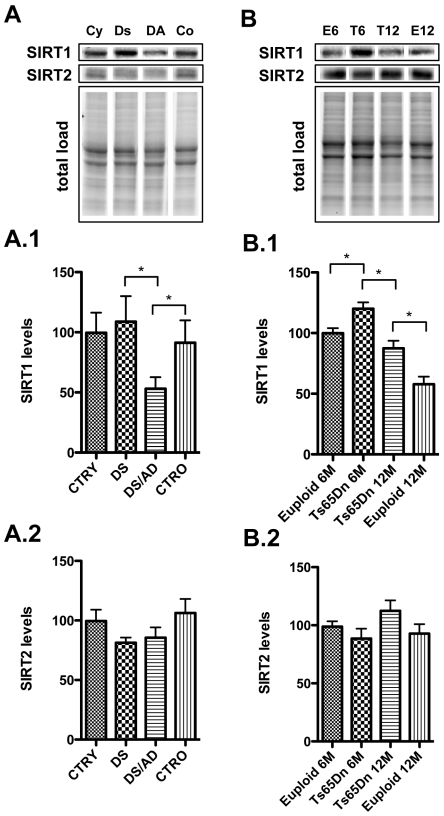

Figure 2. Upstream regulation of p53

Sirtuins: SIRT1 (1) and SIRT2 (2) levels were measured by Western Blot in the frontal cortex of controls and DS cases (Panel A) and in the frontal cortex of euploid and Ts65Dn mice at 6 and 12 months of age (Panel B). Densitometric values shown in the bargraph are the mean of 8 samples per group normalized per total protein load and are given as percentage of control, set as 100%. On the top a representative blot image with protein bands is shown (*$p < 0.05$).

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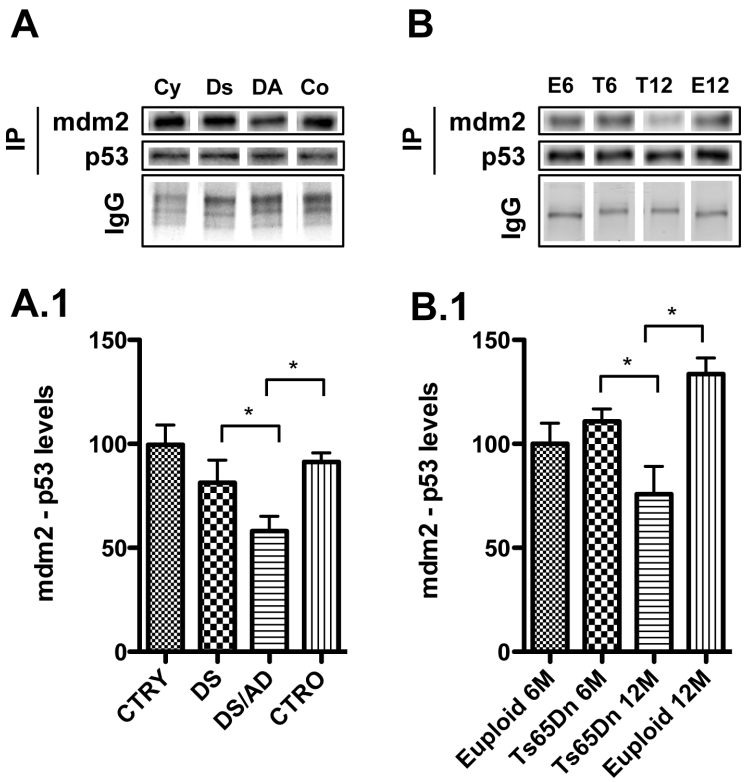

Figure 3. p53-MDM2 complex

Levels of p53-MDM2 complex were measured by immunoprecipitation/Western Blot in the frontal cortex of controls and DS cases (panel A) and in the frontal cortex of euploid and Ts65Dn mice at 6 and 12 months of age (Panel B). Densitometric values shown in the bargraph are the mean of 8 samples per group and are given as percentage of control, set as 100%. On the top a representative blot image with protein bands is shown (*$p < 0.05$).

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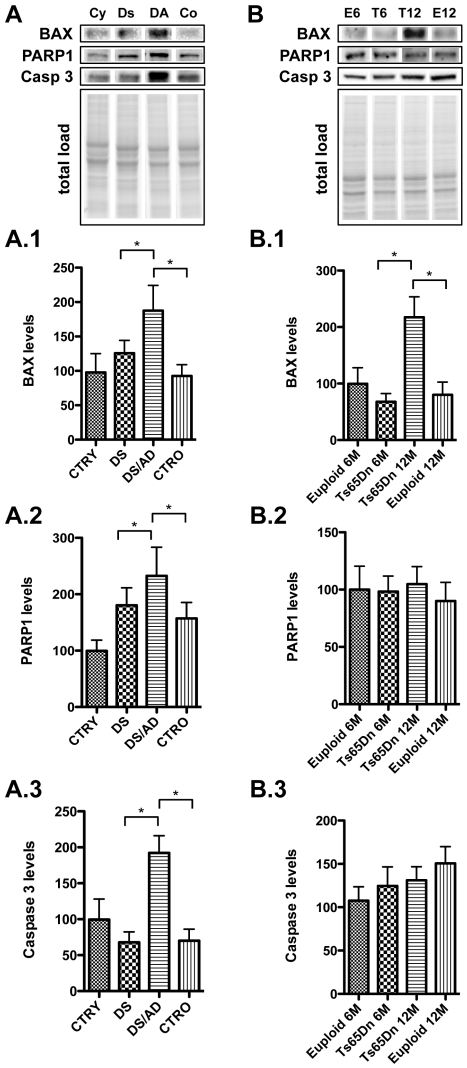

Figure 4. Bax, PARP1 and caspase-3 down stream targets of p53

Proteins associated with the apoptotic pathway including bax (1), PARP1 (2) and Caspase-3 (3) levels were measured by Western Blot in the frontal cortex of controls and DS cases (Panel A) and in the frontal cortex of Ts65Dn mice at 6 and 12 months of age compared to age-matched euploid animals (Panel B). Densitometric values shown in the bargraph are the mean of 8 samples per group normalized per total protein load and are given as percentage of control, set as 100%. On the top a representative blot image with protein bands is shown (*$p < 0.05$).

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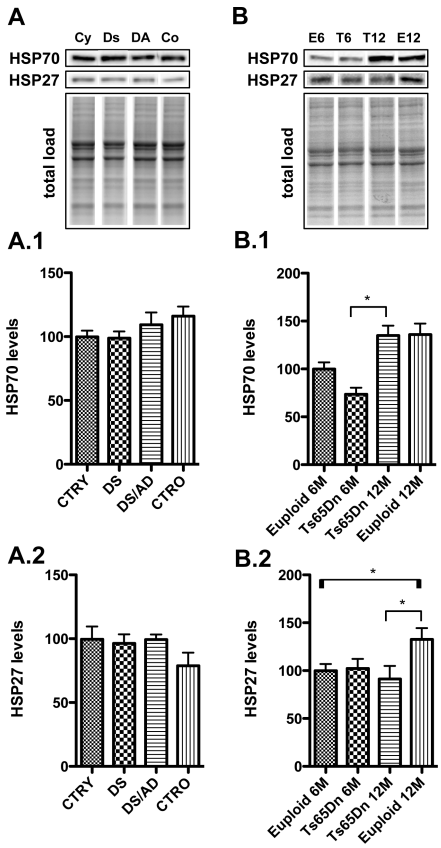

Figure 5. Heat shock protein in response to p53 activation

Two heat shock proteins Hsp70 (1) and Hsp27 (2) were measured by Western Blot in the frontal cortex of controls and DS cases (Panel A) and in the frontal cortex of Ts65Dn mice at 6 and 12 months of age compared to age-matched euploid animals (Panel B). Densitometric values shown in the bargraph are the mean of 8 samples per group normalized per total protein load and are given as percentage of control, set as 100%. On the top a representative blot image with protein bands is shown (*$p < 0.05$).

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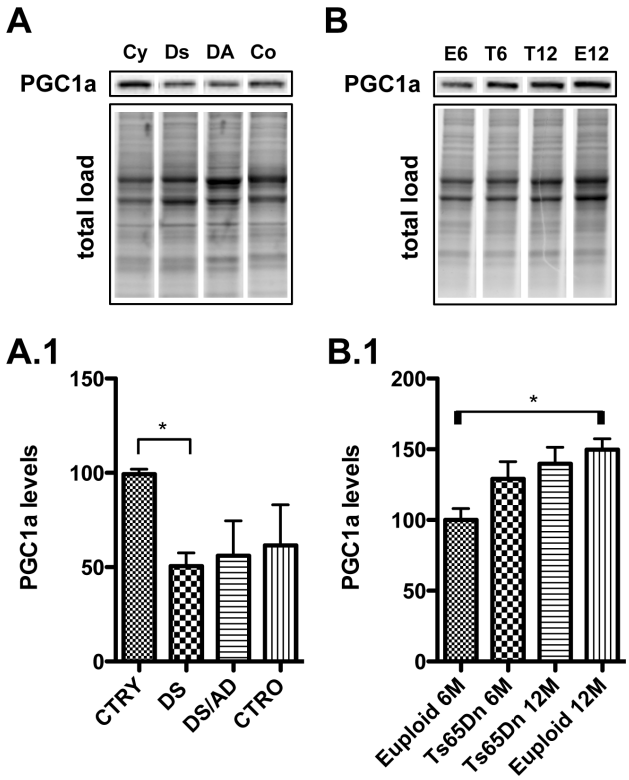

Figure 6. PGC1α, a down stream target of p53

PGC1α levels were measured by Western Blot in the frontal cortex of controls and DS cases (Panel A) and in the frontal cortex of Ts65Dn mice at 6 and 12 months of age compared to age-matched euploid animals (Panel B). Densitometric values shown in the bargraph are the mean of 8 samples per group normalized per total protein load and are given as percentage of control, set as 100%. On the top a representative blot image with protein bands is shown (*$p < 0.05$).

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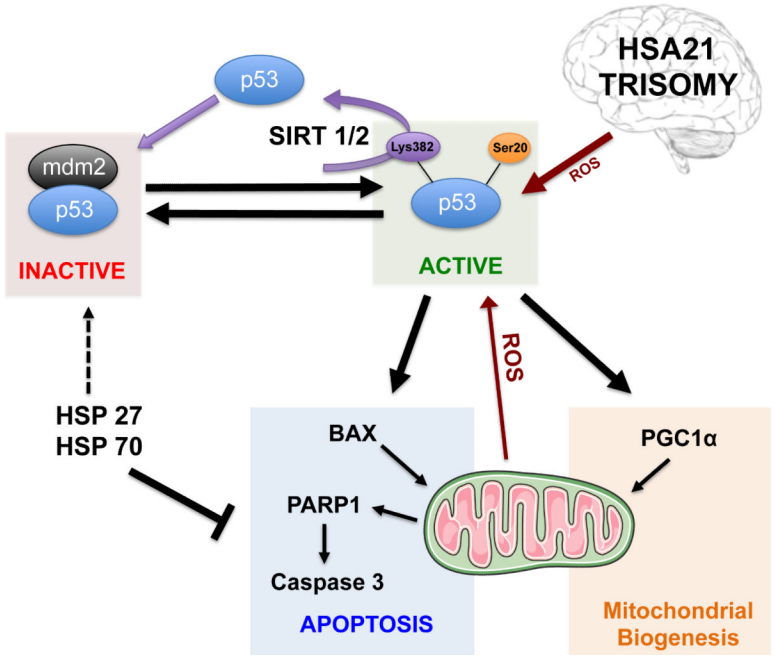

Figure 7. Putative scenario of p53 network in Down Syndrome

Accumulation and transcriptional activation of p53 occurs in response to a wide variety of insults such as DNA damage, oxidative stress, excitotoxicity, etc. p53 undergoes PTMs that critically control its stability and function, including phosphorylation, acetylation, ubiquitination, oxidation and others. Cellular stress affects the interaction between p53 and MDM2, an ubiquitin ligase responsible for p53 degradation. In parallel, transcriptional activity of p53 requires acetylation and/or phosphorylation that may promote cell death pathway through the activation of downstream targets (Bax, Puma, PARP1, Caspase 3, PGC-1α and others). HSPs (27 and 70) act as pro-survival signals by interacting with pro-apoptotic mediators and stabilizing p53 inactive form (p53/MDM2 complex).

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Table 1

Autopsy case demographics. A complete set of information is reported.

GROUPS	CTRY	DS	CTRO	DS/AD
n.	8	8	8	8
Gender (m/f)	4/4		5/3	5/3
Age years (avg. ± SD)	24.8 ± 11.6	23.3 ± 16.8	57.2 ± 7.6	59.0 ± 3.2
PMI (avg. ± SD)	12.1 ± 4.7	13.4 ± 2.1	11.3 ± 6.9	4.3 ± 1.5

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Table 2

Mice demographics

GROUPS	Euploid 6M	Ts65Dn 6M	Euploid 12M	Ts65Dn 12M
n.	8	8	8	8
Gender (m/f)	3/5	5/3	4/4	5/3
Age months (avg. ± SD)	6.3 ± 0.2	6.1 ± 0.1	12.1 ± 0.1	12.2 ± 0.2
Weight (avg. ± SD)	27.2 ± 2.3	28.8 ± 5.1	44.0 ± 6.4	34.3 ± 6.2