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Poly (ADP-ribose) polymerase 1 expression in fibroblasts of Down syndrome subjects

Communication

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Abstract: Down syndrome (DS) is the most common chromosomal disorder. It is featured by intellectual disability and is caused by trisomy 21. People with DS can develop some traits of Alzheimer disease at an earlier age than subjects without trisomy 21. Apoptosis is a programmed cell death process under both normal physiological and pathological conditions. Poly (ADP-ribose) polymerase 1 is a mediator of programmed-necrotic cell death and appears to be also involved in the apoptosis. The aim of the present work was to detect the intracellular distribution of PARP-1 protein using immunofluorescence techniques and the expression of *PARP-1* mRNA in culture of fibroblasts of DS subjects. The analysis of the intracellular distribution of PARP-1 show a signal at the nuclear level in about 75 % of the cells of DS subjects with a slight uniformly fluorescent cytoplasm. In contrast, in about 65% of the analyzed fibroblasts of the normal subjects only a slight fluorescent was found. These observations have been confirmed by *PARP-1* gene mRNA expression evaluation. The data obtained from this study strengthen the hypothesis that the over-expression of *PARP-1* gene could have a role in the activation of the apoptotic pathways acting in the neurodegenerative processes in DS.

Keywords: *PARP-1 gene • Histochemistry • Down syndrome • qRT-PCR • mRNA • Fibroblasts*

1. Introduction

Down syndrome (DS) is the most common chromosomal disorder. It is the single most common genetic cause of intellectual disability and is thus of major socioeconomic concern, the overall incidence of DS worldwide being reported between 1 in 600 to 1 in 800 live births [1]. DS can be caused by three types of chromosomal abnormalities: trisomy 21 (no disjunction), translocation, or mosaicism [2,3]. Trisomy is characterized by the presence of three copies of chromosome 21, generally resulting from no disjunction during maternal meiosis and is seen in about 95% of cases [3]. People with DS can develop some traits of Alzheimer disease at an earlier age than subjects without trisomy 21 [4].

Apoptosis is a programmed cell death process under both normal physiological and pathological conditions. Apoptotic pathways in neurodegenerative processes and in cancer proliferation are very important, when the apoptotic process is somewhat encouraged, neurodegenerative processes, such as those related to Alzheimer disease, will be prevailing [5].

Poly (ADP-ribose) polymerase 1 (*PARP-1*) gene is located to 1q42, split into 23 exons and is 43 kb long (OMIM 173870). Genetic studies found that overexpression of *PARP-1* is a key mediator of programmed-necrotic cell death and appears to be also involved in

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programmed cell death processes besides necrosis or apoptosis [6]. *PARP1* is proteolytically cleaved at the onset of apoptosis by caspase-3 [6]; *PARP-1* activation is also required for translocation of apoptosis-inducing factor (AIF) from the mitochondria to the nucleus [7]. Furthermore, *PARP-1* activity and poly (ADP-ribose) (PAR) polymer, mediate *PARP-1*-induced cell death [8,9].

A small number of gene expression studies using tissues from DS subjects, including fibroblasts, have been conducted [4,10]. The aim of the present work was to apply immunofluorescence protocols to detect the intracellular distribution of PARP-1 protein and to evaluate the possible differential expression of *PARP-1* mRNA in culture of fibroblasts from periodontal gingival tissue of DS subjects compared with culture of fibroblasts of normal subjects.

2. Materials and Methods

2.1. Patients and cell cultures

The DS cases and the controls were recruited after family and/or personal informed consent at the IRCCS Oasi of Troina (Italy), a specialized centre for mental retardation and brain aging studies. Human fibroblasts were obtained from oral biopsy of periodontal gingival tissue in four normal subjects (2 males and 2 females; age range 32-45 years) and four DS subjects (2 males and 2 females; age range 28-45 years). Human gingival fibroblasts were isolated from explants of human gingival and cultured in DMEM (Dulbecco Modified Eagles Medium) in 5% CO_2 humidified atmosphere supplemented with fetal bovine serum (FBS), 2 mM glutamine and 100 units/ml of streptomycin and penicillin. The cells growing out from the explants were mechanically scraped and subcultured until confluency (approximately 10^5 cells/cm^2). A second culture passage was carried out before the immunofluorescence and real time experiments. Twenty-four hours before the immunofluorescence experiments, the samples were fixed with 4% formalin for 30 min at 4°C and post fixed with 70% ethanol for 24 h at -20°C.

2.2. Immunofluorescence experiments

For immunofluorescence studies, 0,2 ml of a suspension containing $20 \cdot 10^6$ fibroblasts/mL in culture medium was incubated for 1 h at room temperature with a 1/500 dilution of the primary antibody PARP-(F-2), a mouse monoclonal antibody raised against PARP-1 protein, (Santa Cruz Biotechnology, Inc., Heidelberg, Germany) PARP-1 (F-2): sc-8007). The antibody was diluted in Tris-buffered saline (TBS) containing 3% bovine serum albumin. Following several washes in TBS containing 0.05% Tween-20, the suspension was incubated with a 1/30 dilution of FITC-labeled goat anti-mouse IgG (Sigma-Aldrich Corp., St Louis, MO, USA) for 1 h at room temperature. Before microscopic examination, nuclei were counter-stained with 100 ng/mL 4,6-diamidino-2-phenylindole (DAPI) (Cytocell, Bambury, UK).

2.3. RNA extraction

A suspension containing $5 \cdot 10^6$ fibroblasts/mL in culture medium was treated with the RNeasy Mini Handbook (QIAGEN Sciences, Germantown, PA), following the manufacturer's protocol. The RNA quality and quantity were checked by spectrophotometry.

2.4. qRT-PCR

To avoid any genomic DNA contamination during qRT-PCR, a brief incubation of the samples at 42 °C with a specific Wipeout buffer (QuantiTect Reverse Transcription Kit**,** QIAGEN Sciences, Germantown, PA) was carried out. Retro-transcription of 600 ng of total RNA from each sample was then performed in a final volume of 20 µl and generated cDNA was used as a template for real-time quantitative PCR analysis using gene expression products. For each sample real-time PCR reactions were carried out in duplicate using 2.5 µl of cDNA and QuantiTect Probe PCR Master Mix Kit (QIAGEN Sciences, Germantown, PA) in a total volume of 50 µl. *PARP1* and *GAPDH* assays were obtained from Applied Biosystems (Carlsbad, CA). The thermal cycling conditions consisted of one cycle for 2 min at 50°C, one cycle of 15 min at 95°C and 40 cycles for 15 s at 94°C followed by 1 min at 60°C. Real-time analysis was performed on Light Cycler 480 (Roche Diagnostics; Mannheim, Germany). The amplified transcripts were quantified using the comparative CT method [11] and relative quantification analysis data were played using the comparative ΔΔCt method included in the Software Version 1.5 supplied with the LightCycler 480. *PARP-1* gene expression level was normalized to *GAPDH* level.

2.5. Microscopic evaluation

Slides were observed and cells visually scored at 20X and 40X. The fraction of PARP-1 positive cells was evaluated independently in a blinded fashion by two co-authors (M.S. and C.S.) No significant difference was observed between the two observers.

3. Results

The analysis of the intracellular distribution of PARP-1 protein was carried out on about 350 fibroblasts of DS subjects and about 350 fibroblasts of normal subjects. The presence of the protein at the nuclear level has been highlighted in about 75 % of the cells of DS subjects with a slight uniformly fluorescent cytoplasm (Figure 1). In contrast, in about 65% of the analyzed fibroblasts of the normal subjects only a slight fluorescent was found (Figure 2). These data were confirmed by *PARP-1* gene mRNA expression evaluation. In fact, Table 1 shows an increased expression of *PARP-1* in fibroblasts from DS subjects compared to fibroblasts from normal subjects.

Figure 1. Immunofluorescence of fibroblasts obtained from oral biopsy of periodontal gingival tissue in DS male subject, PARP-1 protein green fluorescence, nuclei were counter-stained in blue with 100 ng/mL 4,6-diamidino-2-phenylindole (DAPI) .

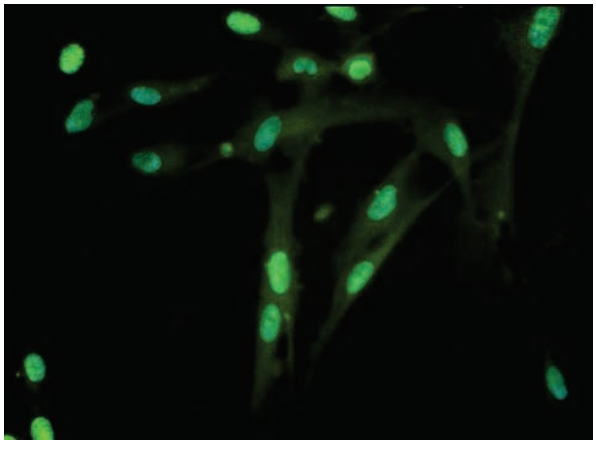

Figure 2. Immunofluorescence of fibroblasts obtained from oral biopsy of periodontal gingival tissue in normal male subject, PARP-1 protein green fluorescence, nuclei were counter-stained in blue with 100 ng/mL 4,6-diamidino-2-phenylindole (DAPI).

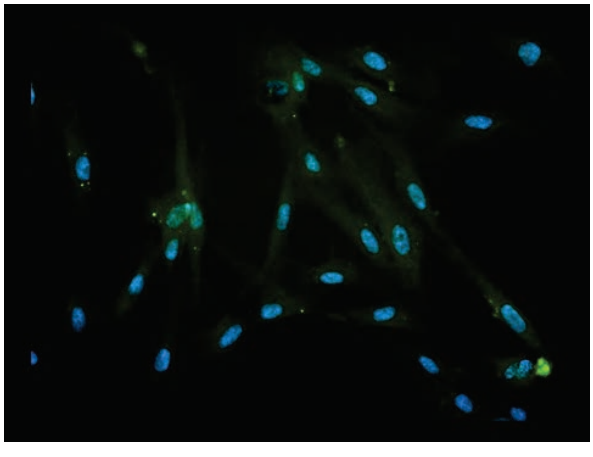

Table 1. Quantitative Real Time PCR analysis of the PARP-1 gene in fibroblasts of subjects with Down syndrome (DS) compared with normal subjects (controls).

Fibroblasts	Sex	Age	PARP-1 mRNA Fold Change
Control 1	F	32	1.00
DS 1	F	31	1.31
Control 2	M	45	1.00
DS 2	M	45	2.96
Control 3	M	32	1.00
DS 3	M	28	1.81
Control 4	F	44	1.00
DS 4	F	41	2.14

4. Discussion

PARP-1 is a highly abundant chromatin-associated enzyme present in all higher eukaryotic cells, where it plays key roles in maintenance of genomic integrity, transcriptional control and chromatin remodeling [12]. PARP-1 gene product binds to DNA single- and double-strand breaks through an N-terminal region containing two zinc fingers, F1 and F2. The C-terminal catalytic domain of PARP-1 protein is activated via an unknown mechanism, causing formation and addition of the polyadenosine-ribose (PAR) complex to acceptor proteins including PARP-1 itself [13]. PARP-1 activation has been connected to Retinal Degeneration, a neurodegenerative disease affecting photoreceptors and causing blindness in humans [14]. In fact, uncontrolled poly-ADP-ribosylation reactions can result in tissue damage and massive necrotic cell death, which in turn often leads to severe inflammatory or neurodegenerative disorders [9,15]. Recently a case-control study of *PARP-1* mRNA expression in leukocytes of peripheral blood of DS subjects compared with normal individuals reported a greater expression in DS subjects [16].

In addition, recent studies using DNA damaging agents such as N-methyl-N'-nitro-N-nitrosoguanidine (MNNG), hydrogen peroxide (H_2O_2) or peroxynitrite, which are well known to induce necrosis at high concentrations, showed that pharmacological inhibition of PARP-1 activity or knockout of the *PARP-1* gene blocks programmed-necrotic cell death induced by these agents [14,17,18]. All together these evidences lead to the hypothesis of using inhibitors of *PARP-1* gene as a protective therapy also to prevent neurodegeneration and premature aging in individuals with DS. The data obtained from this study strengthen the hypothesis that the overexpression of *PARP-1* gene could have a role in the activation of the apoptotic pathways acting both in early aging and in neurodegenerative processes in DS. On these bases, it's possible to hypothesize that in DS subjects pro-apoptotic mechanisms may trigger

a systemic process, and that inhibition of these mechanisms may be useful to modify the phenotype of this pathology.

To confirm the data obtained by this preliminary study further investigations on larger DS cohorts are certainly needed.

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