RESEARCH NOTE

Differential expression of *PARP1* mRNA in leucocytes of patients with Down's syndrome

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Introduction

Down's syndrome (DS) is one of the most common numerical chromosomal aberrations, usually caused by trisomy of chromosome 21, and is the most frequent genetic cause of mental retardation. People with DS can develop some traits of Alzheimer disease at an earlier age than subjects without trisomy 21 (Wisniewski *et al.* 1985). It may be assumed that these individuals would experience a neurodegenerative process because of the presence of an extra copy of amyloid precursor protein (*APP*) gene located on chromosome 21, even if this hypothesis has not been entirely confirmed by the literature (Arriagada *et al.* 2010). In addition, it has been widely demonstrated that genes related to apoptosis play a crucial role in neurodegenerative processes (Engidawork *et al.* 2001; Fromage and Anglade 2002). The role of the apoptotic pathways in neurodegenerative processes and in cancer proliferation is determinant. The latter is favoured when the apoptotic surveillance is, for any reason, decreased (Hu and Kavanagh 2003); conversely, when the apoptotic process is somewhat encouraged, neurodegenerative processes, such as those related to Alzheimer disease, will be prevailing (Elmore 2007).

Poly (ADP-ribose) polymerase 1 (*PARP1*) gene is located at 1q42 and is 43-kb long and splits into 23 exons (OMIM 173870). Grube and Bürkle (1992) suggested that higher *PARP1* action capacity may contribute to the efficient maintenance of genome integrity. Yu *et al.* (2006) showed that *PARP1* activation is required for translocation of apoptosis-inducing factor (AIF) from the mitochondria to the nucleus. *PARP1* is proteolytically cleaved at the onset of apoptosis by caspase-3 (Nicholson *et al.* 1995); further, *PARP1* activity and poly (ADP-ribose) (PAR) polymer, mediate *PARP1*-induced cell death (Yu *et al.* 2006).

Genetic and pharmacological studies found that overexpression of *PARP1* is a key mediator of programmed-necrotic cell death *in vivo*. *PARP1* appears to be also involved in programmed cell death processes besides necrosis, such as apoptosis or macroautophagocytotic cell death (Hassa and Hottiger 2008). The aim of the present study was to evaluate the possible differential expression of *PARP1* mRNA in leucocytes of peripheral blood of DS subjects compared with the normal population.

Materials and methods

A total of 36 subjects were enrolled for this study at the Unit of Pediatrics and Medical Genetics of the IRCCS Oasi Institute, Troina, Italy, a specialized centre for patients with mental disability mainly from Sicily. They included: 18 DS patients (7 males and 11 females) with a mean age of 38.44 ± 10.51 (range 20–55 years) and 18 normal subjects (7 males and 11 females) with a mean age of 38.33 ± 11.28 (range 19–55 years). The DS cases and controls were recruited after family and personal informed consent.

RNA extraction from leucocytes of peripheral blood was performed using RNeasy Mini Handbook (Qiagen, Germantown, USA), following the manufacturer's protocol. The RNA quality and quantity were checked by spectrophotometry.

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, Qiagen, Germantown, USA) was carried out. Retrotranscription of

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600 ng of total RNA from each samples 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, Germantown, USA) in a total volume of 50 μL. *PARP1* and *GAPDH* assays were obtained from Applied Biosystems (Carlsbad, USA). 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, Mannheim, Germany). The amplified transcripts were quantified using the comparative CT method (Livak and Schmittgen 2001) and relative quantification analysis data were played using the comparative $\Delta\Delta$Ct method included in the software version 1.5 supplied with the LightCycler 480. *PARP1* gene expression level was normalized to *GAPDH* level and target mean Cp definition was used to indicate the mean normalized cycle threshold.

Results and discussion

In our case–control study, *PARP1* gene expression was increased in 13 (72.22%) DS subjects compared to normal age and sex-matched control subjects (table 1). Four of them (DS8, DS13, DS15 and DS16 in table 1) have shown an expression at least doubled when compared to normal subjects. Further, 5 of those 13 DS subjects (DS1, DS3, DS9, DS10 and DS18 in table 1) have shown a *PARP1* expression greater than 1.5 compared to the corresponding control subject.

Overactivation of *PARP1* appears to be also a key mediator of cell death in low or nonproliferating cells *in vivo* (Hassa

Table 1. *PARP1* gene expression in DS and normal controls.

Sample name	Age (years)	Sex	Target mean Cp (*PARP1* gene)	Reference mean Cp (*GAPDH* gene)	Ratio normalized
N1	30	M	28.82	25.13	1.000
DS1	32	M	28.22	25.23	1.628
N2	19	F	24.26	20.96	1.000
DS2	20	F	24.74	20.92	0.695
N3	25	F	24.54	20.60	1.000
DS3	27	F	24.29	20.98	1.558
N4	55	F	25.95	22.84	1.000
DS3	55	F	26.63	23.80	1.205
N5	41	M	24.34	21.71	1.000
DS5	39	M	24.44	22.21	1.318
N6	31	F	26.91	24.52	1.000
DS6	31	F	25.74	22.87	0.783
N7	41	M	24.15	20.79	1.000
DS7	42	M	22.44	17.92	0.447
N8	32	M	34.05	41.61	1.000
DS8	35	M	26.43	35.06	2.122
N9	52	M	24.65	33.80	1.000
DS9	51	M	23.77	33.51	1.502
N10	48	F	25.50	37.47	1.000
DS10	50	F	28.33	41.14	1.801
N11	25	F	25.08	21.19	1.000
DS11	27	F	23.19	19.12	0.888
N12	43	F	23.44	19.71	1.000
DS12	45	F	26.80	23.60	1.439
N13	44	F	27.83	31.32	1.000
DS13	45	F	27.55	32.19	2.219
N14	44	F	27.80	31.30	1.000
DS14	44	F	27.46	31.32	1.291
N15	44	F	27.83	31.32	1.000
DS15	47	F	31.39	35.97	2.123
N16	52	M	25.64	30.23	1.000
DS16	47	M	24.83	33.75	2.111
N17	41	M	25.60	30.20	1.000
DS17	39	M	24.93	29.33	0.880
N18	19	F	28.82	25.13	1.000
DS18	20	F	28.22	25.23	1.620

Cp, crossing points; N, normal subject; DS, Down's syndrome patient.

et al. 2006; Schreiber *et al.* 2006). Uncontrolled poly-ADP-ribosylation reactions can result in massive necrotic cell death and tissue damage, which in turn often leads to severe inflammatory or neurodegenerative disorders (Hassa *et al.* 2006; Yu *et al.* 2006). Further, overexpression of *PARP1* has been implicated in the pathogenesis of several diseases, including stroke, myocardial infarction, diabetes, shock and allergy (D'Amours *et al.* 1999).

The use of *PARP1* inhibitors has been proposed as a protective therapy in decreasing cell death and other tissue damage in inflammatory and neurodegenerative disorders (Hassa *et al.* 2006; Yu *et al.* 2006).

Indeed, 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 *PARP1* activity or knockout of the *PARP1* gene blocks programmed-necrotic cell death induced by these agents (Jagtap and Szabo 2005; Hassa *et al.* 2006; Schreiber *et al.* 2006).

Eustermann *et al.* (2011) in their study concluded that it is clear that a full description of the activation mechanism of *PARP1* will require significant further work to characterize its interdomain interactions and their DNA-dependence, both at the structural and functional levels. Nonetheless, the results presented in the above mentioned study, provide valuable insights into recognition of chromosomal DNA single-strand breaks, the crucial first step of the activation process, by the *PARP1* DNA binding domain. In fact, *PARP1* is a highly abundant chromatin-associated enzyme present in all higher eukaryotic cell nuclei, where it plays key roles in maintenance of genomic integrity, chromatin remodelling and transcriptional control. *PARP1* gene product binds to DNA single-strand and double-strand breaks through an N-terminal region containing two zinc fingers, F1 and F2 (Langelier *et al*. 2010). The C-terminal catalytic domain of *PARP1* protein is activated via an unknown mechanism, causing formation and addition of the polyadenosine-ribose (PAR) complex to acceptor proteins including *PARP1* itself (Kannan *et al.* 2011).

Several reports from various laboratories indicate that inhibition or absence of *PARP1* provides remarkable protection in disease models such as septic shock, diabetes, stroke, myocardial infarction and ischaemia, which are characterized predominantly by programmed-necrotic cell death (Jagtap and Szabo 2005; Schreiber *et al.* 2006).

Kannan *et al*. (2011) showed the interaction of hairy/enhancer of split1 (*HES1*) and *PARP1* in B cell acute lymphoblastic leukaemia (B-ALL). In effect, they report that HES1 regulates proapoptotic signals via the novel interacting protein PARP1. This mechanism reveals a cell type-specific proapoptotic pathway which may lead to a new cancer therapy. Also, *PARP1* activation has been causally connected to photoreceptor cell death (Paquet-Durand *et al.* 2007) and this observation was confirmed by Sahaboglu *et al.* (2010) who showed a causal involvement of *PARP1* in retinal degeneration, a neurodegenerative diseases affecting photoreceptors and causing blindness in humans.

We tried to investigate a possible relationship between overexpression and neurophenotype of the subjects examined. The small number of subjects does not allow a statistical evaluation. Nonetheless, the group of patients with overexpression at least doubled when compared to normal, including people with profound degree of intellectual disability (ID) and drug-resistant epilepsy. All remaining groups include people with severe or modest ID.

In conclusion, the *PARP1* overexpression at least doubled in comparison to normal, seems to be associated with a severe ID phenotype. The data obtained from our experiments need to be verified finding a link between overexpression of the gene *PARP1* and activation of the apoptotic pathways both in early ageing and neurodegenerative processes in DS. The confirmation of these data and further studies on larger samples, could lead to the hypothesis of using inhibitors of *PARP1* gene as a protective therapy also to prevent neurodegeneration and premature ageing of individuals with DS. Currently, an evaluation of the expression of the *PARP1* gene in other tissues and in cell cultures of fibroblasts obtained from subjects with DS is in progress, to increase our knowledge and strengthen the evidences obtained.

Further, it would be very interesting to examine in subsequent studies whether the repair function of *PARP1* is implicated in Down's syndrome or other diseases associated with mental retardation and/or cerebral involution.

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