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LDOC1 expression in fibroblasts of patients with Down syndrome

Abstract: Down syndrome (DS) is characterised by intellectual disability and is caused by trisomy 21. Apoptosis is a programmed cell death process and is involved in neurodegenerative diseases such as Alzheimer. People with DS can develop some traits of Alzheimer disease at an earlier age than subjects without trisomy 21. The leucine zipper, down regulated in cancer 1 (LDOC1) appears to be involved in the apoptotic pathways. The aim of the present work was to detect the presence of intracellular synthesis of LDOC1 protein and LDOC1 mRNA in fibroblast cultures from DS subjects. The western blot shows the presence of LDOC1 protein in fibroblasts of DS subjects but no evidence of LDOC1 protein in fibroblasts of normal subjects. *LDOC1* gene mRNA expression is increased in fibroblasts from DS subjects compared to fibroblasts from normal subjects. The data obtained from this study strengthen the hypothesis that the over-expression of *LDOC1* gene could play a role in determining the phenotype of individuals with DS but does not exclude that this results from apoptotic mechanisms.

Keywords: Down syndrome; fibroblast; *LDOC1* gene; mRNA; qRT-PCR expression

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1 Introduction

Down syndrome (DS) is the most common human genetic disorder affecting 1 in every 700-800 live births [1]. More than 99% of the individuals with DS have an extra copy of the entire chromosome 21, 95% of which are free trisomies and the remainder are mosaics or result from translocations [2,3]. The phenotype of Down syndrome is characterized by distinctive physical phenotype, hypotonia, immunological defects, endocardial, hematological and endocrinal alterations, intellectual disability, and behavioral and cognitive deficits [4]. The leucine zipper down regulated in cancer 1 (LDOC1) gene was mapped at Xq27 and consists of only one exon (OMIM 300402). The Xq27 region is characterized by large segmental duplications (SDs) [5]. The existence of complex genomic architecture that may create instability in the genome predisposes Chromosomal rearrangements [6].

LDOC1 gene codes for a nuclear protein of 146-amino acid LDOC1 [7], which has been detected in normal tissues with high expression in brain and thyroid [7]. Also, *LDOC1* may play an important role in the development and/or progression of some cancers [8]. *LDOC1* gene is known as regulator of nuclear factor kappa B (NF-KB) in the apoptosis pathway, favoring apoptosis [8,9,10].

In our previous study, we investigated the expression of the *LDOC1* gene in leukocytes of DS subjects and the results obtained showed a higher expression of this gene in DS subjects LDOC1 compared to normal controls [11]. Considering the above evidence, the aim of this study was to evaluate the possible differential expression of *LDOC1* mRNA and protein in fibroblasts obtained from oral biopsy of periodontal gingival tissue in DS subjects compared with normal subjects.

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2 Experimental Procedures

2.1 Patients and cell cultures

The fibroblasts from DS and control subjects were recruited at the IRCCS Oasi of Troina (Italy), a specialized centre for intellectual disability and brain aging studies. Human fibroblasts of 2 males and 1 female with DS (age range 28-45 years), were obtained from oral biopsy of periodontal gingival tissue. Normal Human fibroblasts of 2 males and 1 female (age range 32-45 years) were obtained from oral biopsy of periodontal gingival tissue. The DS cases and controls were recruited after family and/or personal informed consent. Human gingival fibroblasts were isolated and treated as described by Salemi et al. 2013 [12].

2.2 Western blot analysis

Fibroblast proteins from normal donors and subjects with DS were solubilized in Laemmli buffer, at the concentration of about 1–2 • 102 fibroblast/lL in the presence of 5% b-mercaptoethanol at 100 °C for 3 min, electrophoresed on 15% polyacrylamide/SDS gel, then electroblotted onto nitrocellulose membrane (Bio-Rad, Philadelphia, PA, USA) for 2 h at 0.24 mA/cm2. Protein bands were detected on the membrane using LDOC1 antibody primary (LDOC-1 Antibody, N. NBP1-80323, rabbit polyclonal antibody, Novus Biologicals, Atlanta, GA,USA) and b Actin antibody primary as control (b Actina Antibody, N. # 4967, rabbit polyclonal antibody, Cell Signaling Technology, Beverly, MA, USA). A goat anti-rabbit antibody HRP conjugate (Goat-Anti- Rabbit secondary Antibody , N. 31460, Thermo Fisher Scientific Inc., Rockford, IL, USA) was used as the secondary antibody.

2.3 RNA extraction and qRT-PCR

Total mRNA was obtained by a suspension of fibroblasts ($5 \cdot 10^6$ fibroblasts/mL) [12]. 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. *LDOC1* (ID TaqMan Assay LDOC1 Hs00273392-sl) and *GAPDH* (ID TaqMan Assay GAPDH Hs99999905-ml) assays were obtained from Applied Biosystems (Carlsbad, CA, 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 Diagnostics; Mannheim, Germany). The amplified transcripts were quantified using the comparative CT method and relative quantification analysis data were played using the comparative ΔΔCt method included in the Software Version 1.5 supplied with the LightCycler 480. *LDOC1* gene expression level was normalized to *GAPDH* level.

3 Results

Western blot analysis shows the presence of LDOC1 protein in fibroblasts of DS subjects but no evidence of LDOC1 protein in fibroblasts of normal subjects (Fig.1). QRT-PCR showed an increased expression of *LDOC1* in fibroblasts from DS subjects compared to fibroblasts from normal subjects (Table 1).

4 Discussion

Over-expression of LDOC1 causes externalization of the cell membrane phosphatidylserine, which is characteristic for early-phase apoptotic events and reduced cell viability in some human cell lines [8, 9]. Mizutani *et al.* (2005) [10] have shown that ectopically expressed LDOC1 is localized in the nucleus and induces apoptosis, accompanied by an increase in the tumor protein p53 (p53) protein level, but not in p53 transcription, suggesting that LDOC1

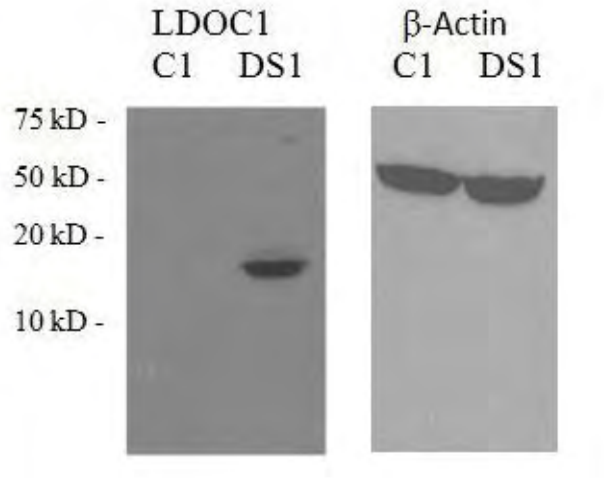

Figure 1 Western blot analysis of LDOC1 protein in fibroblasts of Down syndrome and normal subjects* *Western blot of a normal fibroblast cell line (C1) and a Down Syndrom cell line (DS1), LDOC1 protein has a molecular mass of approximately 17 kD, b-Actin protein has a molecular mass of approximately 45 kD.

Table 1: *LDOC1* mRNA expression in fibroblast of Down syndrome subjects and normal controls

Sample Name	Age (yars)	Sex	Target Mean Cp (LDOC1gene)	Reference Mean Cp (GAPDH gene)	Ratio Normalized
C1	32	M	30.68	31.92	1.000
DS1	28	M	26.47	28.98	2.397
C2	32	F	29.05	30.13	1.000
DS2	31	F	27.92	30.02	2.014
C3	45	M	27.39	28.89	1.000
DS3	45	M	29.42	31.56	1.559

Cp: crossing points; C: normal subject; DS: Down syndrome patient

inhibits the degradation of p53. Lynn *et al.* (2009) [13] have suggested that innate immune response and cell-proliferation regulation may play important downstream roles in development of hypertension and specifically that *LDOC1* gene plays a key role in the regulatory mechanisms related to apoptosis in hypertension.

We hypothesize that the presence of LDOC1 mRNA and absence of LDOC1 protein in fibroblasts of normal subjects could be explained either by a block of LDOC1 mRNA translation into protein in normal subjects, or by protein degradation.

In conclusion, the set of data obtained in this preliminary study, confirm our previous results obtained in leukocytes of DS [11]. It also reinforces the hypothesis that the over-expression of LDOC1 gene could play a role in determining the phenotype of individuals with DS but the possibility that apoptotic mechanisms are activated in DS cannot be excluded either.

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Conflict of interest: There are no conflicts of interest for any of the authors.

References

[1] Capone G.T., Down syndrome: advances in molecular biology and the neurosciences, J. Dev. Behav. Pediatr., 2001, 22, 40-59

[2] Warburton D., Dallaire L., Thangavelu M., Ross L., Levin B., Kline J., Trisomy recurrence: a reconsideration based on North American data, Am. J. Hum Genet., 2004, 75, 376-85

[3] Newberger D.S., Down Syndrome: Prenatal risk assessment and diagnosis, Am. Fam. Physician., 2000, 62,825-832

[4] Elmore S., Apoptosis: a review of programmed cell death, Toxicol. Pathol., 2007, 35, 495-516

[5] Sharp A.J., Locke D.P., McGrath S.D., Cheng Z., Bailey J.A., Vallente R.U., et al., Segmental duplications and copy-number variation in the human genome, Am. J. Hum. Genet., 2005, 77, 78-88

[6] Shaw C.J., Lupski J.R., Implications of human genome architecture for rearrangement-based disorders: the genomic basis of disease, Hum .Mol. Genet., 2004, 1,57-64

[7] Nagasaki K., Manabe T., Hanzawa H., Identification of a novel gene, LDOC1, down-regulated in cancer cell lines., Cancer. Lett., 1999,140, 227-234

[8] Nagasaki K., Schem C., von Kaisenberg C., Biallek M., Rösel F., Jonat W., Maass N., Leucine-zipper protein, LDOC1, inhibits NF-kappaB activation and sensitizes pancreatic cancer cells to apoptosis, Int. J. Cancer., 2003, 105,454-458

[9] Inoue M, Takahashi K., Niide O., LDOC1, a novel MZF-1-interacting protein, induces apoptosis, FEBS Lett., 2005,579,604-608

[10] Mizutani K., Koike D., Suetsugu S., Takenawa T.,WAVE3 functions as a negative regulator of LDOC1, J Biochem., 2005, 138,639-646