VERSITA

Cent. Eur. J. Med. • 8(2) • 2013 • 204-207
DOI: 10.2478/s11536-012-0121-x

Central European Journal of **Medicine**

LDOC-1 and *PARP-1* mRNA expression in leukocytes of father and son with cutaneous malignant melanoma

Case Report

Michele Salemi[1], Pier Franco Soma[2], Rosita A. Condorelli[1], Domenico Recupero[1], Aldo E. Calogero[1]

1 Section of Endocrinology, Andrology and Internal Medicine, Department of Internal Medicine and Systemic Diseases, University of Catania, via S. Sofia 78, 95123 Catania, Italy

2 Plastic Surgery and Burns Centre, Cannizzaro Hospital, Catania, Italy

Received 18 September 2012; Accepted 9 November 2012

Abstract: Apoptosis is central to the biology of cutaneous malignant melanoma (CMM). The leucine zipper, down regulated in cancer 1 (*LDOC-1*) gene, is known to be a regulator of the nuclear factor kappa B (NF-kB) through inhibition of the same NF-kB. The poly (ADP-ribose) polymerase-1 (*PARP1*) gene plays an important role for the efficient maintenance of genome integrity. *PARP-1* protein is required for the apoptosis-inducing factor (AIF) translocation from the mitochondria to the nucleus. We report here two interesting cases of family melanoma, a father and son 84 and 40 years old, respectively. The histological evaluation of the lesions of both men revealed diffused superficial melanoma with epithelioid cells. We evaluated the differential expression of *LDOC-1* and *PARP-1* mRNA in peripheral blood leukocytes of both the father and son. We found that both *LDOC-1* and *PARP-1* genes were down-regulated in both patients compared with those of controls. These data suggest that low levels of expression of *LDOC-1* and *PARP-1* mRNA may be associated with familial melanoma.

Keywords: *Cutaneous malignant melanoma • Apoptosis • LDOC-1gene • PARP-1 gene • qRT-PCR*

1. Introduction

The incidence of cutaneous malignant melanoma (CMM) has increased in many countries [1]. CMM is a malignant tumor of the cells that produce and transport melanin pigment; it can occur on any skin surface. In Sicily, the incidence of melanoma is of 4.5 cases per 100,000 inhabitants, with a mortality of 1.2% [2]. Apoptosis plays an important role in cancer proliferation; at present, the mechanisms responsible for cell life or death outcome in CMM remains unclear [3]. Recent application of molecular techniques has revealed the involvement of several genes, whose fine-tuned balance dictates the final destiny of CMM cells, coding for apoptotic and anti-apoptotic regulators [3]. Apoptosis is important in the tumor biology of CMM; it is regulated by several key molecules [3].

We investigated the expression of two important genes involved in apoptosis: leucine zipper, down regulated in cancer 1 (*LDOC-1*), and poly (ADP-ribose) polymerase 1 gene (*PARP-1*). The *LDOC-1* gene has been mapped in chromosome X at q27; it encodes for a nuclear 146-amino acid long protein with a molecular weight of approximately 17 kD and splits into one exon (OMIM 300402). *LDOC-1* protein contains a leucine zipper-like motif in its N-terminal region and also a proline-rich region that shares marked similarity with a SH-binding domain. The loss of *LDOC-1* expression in most pancreatic and gastric cancer cell lines and the wide expression of *LDOC-1* mRNA in normal tissues indicate that down-regulation of *LDOC-1* may play an important role in the development and progression of some tumors. Furthermore, *LDOC-1* is a regulator of nuclear factor kappa B (NF-kB). The NF-kB gene can affect the phorbol 12-myristate 13-acetate (PMA) or tumor necrosis factor-α (TNFα)-mediated apoptosis path-

* *E-mail: micezia@tiscali.it*

way through the inhibition of NF-kB. The transcription factor MZF-1 has been shown to interact with *LDOC-1* and to enhance the apoptosis-inducing activity of *LDOC-1* [4,5].

The poly (ADP-ribose) polymerase-1 (PARP1) gene, located at 1q42, is 43 Kb-long and splits into 23 exons (OMIM 173870). Some authors have shown that *PARP-1* activation is required for translocation of the apoptosis-inducing factor (AIF) from the mitochondria to the nucleus [6]; other authors have also suggested that higher *PARP-1* action may contribute to the efficient maintenance of the genome integrity [7]. *PARP-1* is proteolytically cleaved at the onset of apoptosis by caspase-3 [6,7] . Up-expression of *PARP-1* is a key mediator of programmed necrotic cell death; in programmed cell death processes not only necrosis, but also apoptosis or macroautophagocytotic cell death, can occur [8] .

2. Case report

This report describes two interesting cases admitted, without specific symptoms, to the Cannizzaro's Hospital (Catania, Italy): father and son aged 84 and 40 years, respectively. The father had a lesion of approximately 1.3 cm on his right leg, and the son had a lesion of approximately 0.9 cm on his left hemithorax. The histological evaluation of the father's lesion revealed a diffused superficial melanoma, infiltration of the reticular dermis, no ulceration, lymphocytic infiltration (Brisk), Breslow thickness of 0.37 mm, Clark's level II, and low mitotic index at <1 mitosis/10HPF; surgical margins were negative. The histological evaluation of the son's lesion revealed, with the exception of a Breslow thickness of 0.43 mm, the same characteristics. In both cases, the lymph nodes were not removed. There were no metastases to other areas of the skin and no visceral metastases were detected by PET/CT scan. The cases and controls were recruited after personal informed consent.

We found it of interest to investigate the expression of *LDOC-1* and *PARP-1* genes with qRT-PCR in these two cases of family melanoma. We evaluated the possible differential expression of *LDOC-1* and *PARP-1* mRNA in peripheral blood leukocytes in these cases compared to that in six healthy men chosen as controls, three associated with the father's age and three associated with the son's age. RNA extraction from peripheral blood leukocytes was performed using RNeasy Mini Handbook (QIAGEN Sciences, Germantown, PA, USA), following the manufacturer's protocol. RNA quantity and quality 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 Kit, QIAGEN Sciences) was carried out. Retrotranscription of 600 ng of total RNA from each sample was then performed in a final volume of 50 µl, and the generated cDNA was used as a template for real time quantitative PCR analysis (RT-PCR) using gene expression products. For each sample, RT-PCR reactions were carried out in duplicate using 3 µl of cDNA and QuantiTect Probe PCR MasterMix Kit (QIAGEN Sciences, Germantown, PA) in a total volume of 50 µl. Target genes *LDOC-1*, *PARP-1* and reference gene *GAPDH* 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 42 cycles for 15 s at 94°C followed by 1 min at 60°C. Real Time analysis was performed on an ABI PRISM 5700 Sequencer Detector (Applied Biosystems). The amplified transcripts were quantified using the comparative CT method [9] and relative quantification analysis data were played using the comparative Delta-Delta Ct method included in the Software Version 1.3 supplied with the Applied Biosystems. *LDOC-1* and *PARP-1* gene expression level was normalized to the *GAPDH* expression gene level, and target mean Cp definition was used to indicate the mean normalized cycle threshold. We found that both *LDOC-1* and *PARP-1* genes were down-regulated in the patients with CMM compared to the six controls (Table 1).

3. Discussion and conclusion

In a previous study, we found that both *LDOC-1* and *PARP-1* genes were down-regulated in a patient with melanoma and repeated in-transit metastases [10]. These data suggest that *PARP-1* and *LDOC-1* cell death pathways could be inhibited in familial melanoma and that in these cases of melanoma, the apoptotic pathways are inhibited at different levels. These results support the assumption that in most pancreatic and gastric cancer cell lines the pro-apoptotic gene *LDOC-1* is down-regulated and confirms that *LDOC-1* down-regulation may have an important role in the development of some cancers [5,11]. Some authors have shown that ectopically expressed *LDOC-1* is localized in the nucleus and induces apoptosis, which is accompanied by an increase in the tumor p53 protein content but not in p53 transcription; this suggests that *LDOC-1* inhibits the degradation of p53 [11,12]. Moreover, the activation of *PARP-1* gene expression in response to DNA damage is an important mechanism to keep homeostasis or to trigger apoptosis [6-8,13].

Table 1. Expression of LDOC-1 and PAR-1 mRNA in two patients (father and son) with cutaneous malignant melanoma compared with six normal healthy controls.

	Case Father/Control 1				Case Son/Control 4			
	Control 1		Case study		Control 4		Case study	
	M. Cp	RT	M. Cp	RT	M. Cp	RT	M. Cp	RT
Target gene *LDOC-1* epxession	32.97	1.000	29.83	0.6068	39.11	1.000	35.37	0.4719
Target gene *PARP-1* expression	28.72	1.000	27.53	0.3626	30.52	1.000	26.48	0.5818
Reference gene *GAPDH* expression	30.83	-------	28.17	-------	32.04	-------	27.22	-------
	Case Father/Control 2				Case Son/Control 5			
	Control 2		Case study		Control 5		Case study	
	M. Cp	RT	M. Cp	RT	M. Cp	RT	M. Cp	RT
Target gene *LDOC-1* epxession	29.04	1.000	34.10	0.8023	37.59	1.000	35.29	0.8797
Target gene *PARP-1* expression	30.83	1.000	36.36	0.5797	36.24	1.000	33.83	0.9411
Reference gene *GAPDH* expression	30.22	-------	34.96	-------	36.99	-------	34.50	-------
	Case Father/Control 3				Case Son/Control 6			
	Control 3		Case study		Control 6		Case study	
	M. Cp	RT	M. Cp	RT	M. Cp	RT	M. Cp	RT
Target gene *LDOC-1* epxession	34.38	1.000	32.94	0.4733	35.60	1.000	32.50	0.9295
Target gene *PARP-1* expression	35.27	1.000	32.96	0.8631	31.05	1.000	28.76	0.5304
Reference gene *GAPDH* expression	35.48	-------	32.96	-------	30.57	-------	27.36	-------

M. Cp: Mean crossing point; RT: Ratio normalization

In conclusion, our data suggest that the reduced expression of *LDOC-1* and *PARP-1* genes may be associated also with familial melanoma. Furthermore, *LDOC-1* and *PARP-1* mRNA expression may be useful to evaluate the apoptotic pathways in family members of patients with CMM. These data would surely be useful to individualize the risk of CMM in the healthy relatives. This hypothesis has to be obviously confirmed by an adequately powered study of familial melanoma.

Conflict of interest

The authors declare no conflict of interest.

References

[1] de Vries E., Coebergh JW. : Melanoma incidence has risen in Europe, B.M.J., 2005,331, 698

[2] Tumino R., Frasca G., Palli D., Masala G., Tagliabue G., Evangelista A., Giurdanella MC., Vineis P., Veglia F., Celentano E., Galasso R., Cascone G.: Cancer incidence in EPIC-ITALY at first follow-up, Tumori., 2003,89,656-664

[3] Hu W., Kavanagh JJ.: Anticancer therapy targeting the apoptotic pathway, Lancet. Oncol., 2003,4, 721-729

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

[5] Inoue M., Takahashi K., Niide O., Shibata M., Fukuzawa M., Ra C.: LDOC1, a novel MZF-1-interacting protein, induces apoptosis, FEBS. Lett., 2005,579,604-608

[6] Yu SW., Wang H., Poitras MF., Coombs C., Bowers WJ., Federoff HJ., Poirier GG., Dawson TM., Dawson VL.: Mediation of poly (ADP-ribose) polymerase-1-dependent cell death by apoptosis-inducing Factor, Science., 2002,297,259-263

[7] Yu SW., Andrabi SA., Wang H.: Apoptosis-inducing factor mediates poly (ADP-ribose) (PAR) polymer-induced cell death, Proc. Natl. Acad. Sci. USA., 2006,103,18314-18319

[8] Hassa PO., and Hottiger MO.: The diverse biological roles of mammalian PARPs, a small but powerful family of poly-ADP-ribose polymerases, Front. Biosci., 2008,13,3046-3082

[9] Livak KJ., Schmittgen TD.: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method, Methods., 2001,4,402-408

[10] Salemi M., Giuffrida D., Soma PF., Giuffrid MC., Galia A., Calogero AE.: Two proapoptotic genes are downregulated in a patient with melanoma and repeated in-transit metastases, Am. J.

Dermatopathol., 2012, 34,454-455
[11] Mizutani K., Koike D., Suetsugu S., Takenawa T.: WAVE3 functions as a negative regulator of LDOC1. J. Biochem, 2001,38,639-646
[12] 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
[13] Andrabi SA., Kim NS., Yu SW. Poly(ADP-ribose) (PAR) polymer is a death signal, Proc. Natl. Acad. Sci. USA, 2006,103,18308-18313