Two Proapoptotic Genes Are Downregulated in a Patient With Melanoma and Repeated In-Transit Metastases

To the Editor:

Melanoma is a cancer of the cells producing and transporting melanin pigment, which may occur on any skin surface. The cutaneous malignant melanoma (CMM) has dramatically increased in many countries over the past decades.[1] In Sicily, the incidence of melanoma is of 4.5 cases for 100,000 inhabitants with a mortality of 1.2%.[2]

This letter describes an interesting case of a 53-year-old man without specific symptoms who, in 2004, underwent the surgical excision of a nodular lesion in the right leg. The histological evaluation of this lesion revealed features of a nodular, pigmented, acantholytic melanoma with epithelioid cells; infiltration of the reticular dermis; presence of vascular invasion; no ulceration, regression, and lymphocytic infiltration (nonbrisk); Breslow thickness of 2.25 mm; Clark level IV; and the presence of 2 microsatellites in the dermis.

Two months later, the patient underwent inguinal lymphadenectomy for the presence of lymph node metastases. From September 2005 to April 2010, 16 months after the initial excision of the melanoma, 25 in-transit metastases were excised from legs and thighs, whereas no visceral metastases were detected by positron emission tomography/computed tomography scan during this period.

The apoptotic pathway plays a pivotal role in cancer proliferation.[3] The mechanisms responsible for cell life or death outcome in CMM remains unclear.[4] However, recent application of the molecular techniques has revealed the involvement of several genes, including apoptotic and antiapoptotic regulators, whose fine-tuned balance dictates the final destiny of CMM cells.[4] Apoptosis is central to the tumor biology of CMM, and it is regulated by several key molecules, including p53, tumor necrosis factor-α, nuclear factor κB (NF-κB), Bcl-2, and the caspase family proteins.[4] Therefore, we found of interest to investigate the expression of 2 important genes involved in apoptosis by quantitative real-time polymerase chain reaction (qRT-PCR) in this patient. The genes chosen were *LDOC1* and *PARP1*.

LDOC1 gene has been mapped on the chromosome X at q27 and splits into 1 exon (OMIM 300402). It encodes for a nuclear 146-amino acid–long protein with a molecular weight of approximately 17 kD. LDOC protein contains a leucine zipper–like motif in its N-terminal region and a proline-rich region that shares marked similarity with an SH_3-binding domain. Northern blot analysis revealed a ubiquitous expression of *LDOC1* in normal tissues, which is elevated in brain and thyroid and low in placenta, liver, and leukocytes.[5] *LDOC1* messenger RNA (mRNA) is downregulated in a series of human pancreatic and gastric cancer cell lines.[6] The wide expression of *LDOC1* mRNA in normal tissues and the loss of *LDOC1* in most pancreatic and gastric cancer cell lines indicates that downregulation of *LDOC1* may have an important role in the development and/or progression of some tumors.[6] In addition, *LDOC1* is a novel regulator of NF-κB that can affect the phorbol 12-myristate 13-acetate (PMA)–mediated or tumor necrosis factor-α–mediated apoptosis pathway through the inhibition of NF-κB.[6] Furthermore, the transcription factor MZF-1 has been shown to interact with *LDOC1* and enhance the activity of *LDOC1* for inducing apoptosis.[7]

Poly (ADP-ribose) polymerase 1 (*PARP1*) gene, located at 1q42, is 43 kb long and splits into 23 exons (OMIM 173870). Grube and Burkle[8] suggested that higher *PARP1* action may contribute to the efficient maintenance of the genome integrity. Yu et al[9] showed that *PARP1* activation is required for the translocation of the apoptosis-inducing factor from the mitochondria to the nucleus. *PARP1* is proteolytically cleaved at the onset of apoptosis by caspase-3[10]; furthermore, *PARP1* activity and poly (ADP-ribose) (PAR) polymer mediate *PARP1*-induced cell death.[11,12] Genetic and pharmacological studies reported that overexpression of *PARP1* is a key mediator of programmed necrotic cell death in vivo. *PARP1* seems to be also involved in programmed cell death processes besides necrosis, such as apoptosis or macroautophagocytotic cell death.[13]

Therefore, we found of interest to investigate the expression of *LDOC1* and *PARP1* genes by qRT-PCR in this patient. On this account, we evaluated the possible differential expression of *LDOC1* and *PARP1* mRNAs in peripheral blood leukocytes in this men compared with 3 healthy men (controls).

RNA extraction from peripheral blood leukocytes was performed using RNeasy mini handbook (QIAGEN Sciences, Germantown, PA), 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 Kit; QIAGEN Sciences) was carried out. Retrotranscription of 600 ng of total RNA from each samples was then performed in a final volume of 50 μL, and generated complementary DNA was used as a template for qRT-PCR analysis using gene expression products. For each sample, RT-PCR reactions were carried out in a duplicate using 2.5 μL of complementary DNA and QuantiTect Probe PCR master mix kit (QIAGEN Sciences) in a total volume of 50 μL. Target genes *LDOC1* and *PARP1* and reference gene *HPRT1* (hypoxanthine phosphoribosyltransferase 1) assays were obtained from Applied Biosystems (Carlsbad, CA). The thermal cycling conditions consisted of 1 cycle for 2 minutes at 50°C, 1 cycle for 15 minutes at 95°C, and 40 cycles for 15 seconds at 94°C followed by 1 minute at 60°C. Real-time analysis was performed on an ABI PRISM 5700 Sequencer Detector (Applied Biosystems). The amplified transcripts were quantified using the

Conflicts of interest: None declared

TABLE 1. Real-Time PCR Analysis of the Proapoptotic LDOC1 and PARP1 Genes in a Patient With Melanoma and Repeated In-Transit Metastases Compared With 3 Healthy Normal Men (Controls)

	Case/Control 1				Case/Control 2				Case/Control 3			
	Control 1		Case Study		Control 2		Case Study		Control 3		Case Study	
	M. Cp	RT	M. Cp	RT	M. Cp	RT	M. Cp	RT	M. Cp	RT	M. Cp	RT
Target gene *LDOC1* expression	26.96	1.000	27.16	0.269	40.05	1.000	37.09	0.127	27.11	1.000	27.85	0.582
Target gene *PARP1* expression	24.42	1.000	25.95	0.107	31.56	1.000	28.68	0.120	24.76	1.000	25.61	0.540
Reference gene *HPRT1* expression	32.65	—	30.95	—	35.78	—	29.85	—	20.96	—	20.92	—

M. Cp, Mean Cp; RT, ratio normalized.

comparative C_t method,[14] and relative quantification analysis data were played using the comparative $\Delta\Delta C_t$ method included in the software version 1.3 supplied with the Applied Biosystems. *LDOC1* and *PARP1* gene expression level was normalized to *HPRT1* level, and target mean C_p definition was used to indicate the mean normalized cycle threshold.

We found that both *LDOC1* and *PARP1* genes were downregulated in the patient with CMM compared with the 3 controls evaluated (Table 1). This finding suggests that both *PARP1*- and *LDOC1*-mediated cell death pathways are inhibited. This molecular mechanism may explain the clinical manifestation found in this patient. Moreover, these results support the hypothesis that in some tumors, the apoptotic pathways are inhibited at different levels.

Michele Salemi, PhD*†
Domenica Giuffrida*
Pier Franco Soma‡
Maria C. Giuffrida*§
Antonio Galia¶
Aldo E. Calogero*
*Section of Endocrinology, Andrology, and Internal Medicine, Department of Biomedical Sciences, University of Catania, Catania, Italy
†Oasi Institute for Research on Mental Retardation and Brain Aging, Troina (EN), Italy
‡Plastic Surgery and Burns Centre, Cannizzaro Hospital, Catania, Italy
§Fondazione Fulvio Frisone, Catania, Italy
¶Unit of Pathology, Cannizzaro Hospital, Catania, Italy

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Histologic Assessment of Perilesional Skin in Vesiculobullous Diseases: Giving Its Due

To the Editor:

Direct immunofluorescence of perilesional skin (PS) is performed in most vesiculobullous dermatoses (VBL). Although immunopathologic and ultrastructural changes are described in PS, there is no data on histopathologic changes.[1–3] The few studies available affirm the absence of changes on morphology.[1,2]

We studied hematoxylin and eosin–stained frozen sections of perilesional skin biopsies from 36 VBL, the majority of which were pemphigus (19) and bullous pemphigoid (9). In cases of pemphigus, we observed incipient acantholysis (18) (Fig. 1A), clefts between strata basale and spinosum (18) (Fig. 1B), adnexal involvement (9) (Fig. 1C), and suprabasal vesicles (5) (Fig. 1D). PS in pemphigoid showed spongiosis (4) (Fig. 2A), sub/intraepidermal clefts (4), eosinophils in the papillary dermis (3) (Fig. 2D), and frank subepidermal vesicles (4) (Fig. 2C). Eosinophils sticking to the basement membrane were noted in 2 cases of pemphigoid (Fig. 2B). Dermal melanophages were noted in most sections of both pemphigus and pemphigoid. We had 2 cases of bullous vasculitis that showed spongiotic vesicles and leukocytoclastic vasculitis. Freezing artifacts were noted in some cases but did not hamper interpretation. The only

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