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Poly(adenosine diphosphate-ribose) polymerase 1 expression in malignant melanomas from photoexposed areas of the head and neck region☆

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Summary The family of the poly(adenosine diphosphate-ribose) polymerase (PARP) proteins is directly involved in genomic stability, DNA repair, and apoptosis by DNA damage. In this study, we evaluated the role of PARP-1 in melanoma and its prognostic importance. We studied by immunohistochemistry and Western blot analysis PARP-1 expression in a selected series of 80 primary melanoma of the head and neck region. The results were correlated with tumor thickness and patient's outcome. A follow-up of at least 3 years was available. Fifteen cases of benign melanocytic nevi were used as controls. Normal melanocytes showed only scattered, focal nuclear positivity and were considered as negative for PARP-1 expression by immunohistochemistry (score, 0). Thirty cases of melanoma (37.5%) showed nuclear expression of PARP-1 in both radial and vertical growth phases. Western blot analysis showed the presence of a high signal for full-length PARP-1 only in the cases with high immunohistochemical (nuclear) expression of protein (score, ++/+++) in both radial and vertical growth phase. A significant correlation was present between PARP-1 expression in vertical growth phase and the thickness of tumor lesion (P = .014); all but one tumor measuring less than 0.75 mm

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showed no or low PARP-1 expression. No correlation was found between PARP-1 expression in radial growth phase and tumor thickness (P = .38, data not shown). These data suggest that PARP-1 overexpression is a potential novel molecular marker of aggressive cutaneous malignant melanoma and a direct correlation between PARP-1–mediated inhibition of the apoptosis and biologic behavior of cutaneous malignant melanoma.

1. Introduction

In the last 2 decades, the incidence of all skin cancers is increasing in most Western countries and in Australia. Cutaneous malignant melanoma (CMM) death incidence is increasing faster than most of other skin malignancies at present, with an incidence that has tripled during the last 40 years and continues to grow [1-4].

In the United States, 32 868 white subjects have been diagnosed with melanoma during 1975 through 1990, covering approximately 10% of the American population [5]. In Italy, the incidence of CMM in the hospital referral population is steadily rising, and at present, this tumor represents one of the few causes of premature death with unfavorable trends [1,6,7].

The most significant prognostic factor (at the time of the diagnosis) is the extent of tumor invasion expressed by thickness rather than anatomic structure [2]. Patients with melanoma measuring 1.00 mm or less have a 5-year survival of 96%; thicker tumors are associated with a poor prognosis. In the last decade, there has been a positive trend of thin lesions at diagnosis [8]; however, some of these cases, often cured by the excisional biopsy with conservative surgical margins, show an unfavorable outcome that is unpredictable based on the classic clinicopathologic parameters [9].

Individual risk factors play an important part in the development of malignant melanoma [10]. The most important phenotypic risk factors are the number of total body acquired melanocytic nevi and the occurrence of previous epithelial skin cancer [11-15].

Solar damage is the major environmental causal factor in all skin cancers, and intermittent intense exposures to sunlight and/or severe sunburn are also the most important environmental risk factors for CMM [9].

A history of sunburn up to 12 years is one of the primary sun-related factors associated with an increased risk for CMM [16].

Even if the carcinogenic effect of UV rays has been attributed predominantly to short-wavelength (290-320 nm) UV radiation (UV-B), a carcinogenic role has been reported also for the long-wavelength (320-400 nm) UV light (UV-A) [17,18].

UV-A radiation exerts penetrating effects on the skin, causing DNA damage and increasing the risk for cancer. Recently, basic research on UV radiation showed that DNA repair via different pathways causes a cascade of cellular phenomena ranging from induction of pigmentation to immunomodulation, acid radical defense, apoptosis, and oncogene expression [19].

The members of the poly(adenosine diphosphate-ribose) polymerase (PARP) family proteins are directly involved in genomic stability, DNA repair, and cell death triggered by DNA damage. However, their potential role in carcinogenesis has not been well evaluated [20].

We evaluated the immunohistochemical expression of PARP-1 in a series of CMM from photoexposed areas, and the correlation between PARP-1 expression and tumor thickness and the patient's outcome. Western blot (WB) analysis was also performed on a limited number of cases to confirm the immunohistochemical data.

The aim of the study was to evaluate the role of PARP-1 in tumor progression and its prognostic importance in cutaneous melanomas.

2. Materials and methods

2.1. Selection of cases

All the cases of primary cutaneous melanomas diagnosed between January 1985 and December 1998 at the Precancerous Unit, Department of Dermatology, University Federico II of Naples, Italy, were reviewed. All the cases of CMM that have arisen in photoexposed areas of the head and neck region without a hereditary history of skin cancer and/or prior physical or chemical predisposing environmental factors and with a follow-up of at least 3 years were considered suitable for the present analysis. The paraffin-embedded blocks of these selected patients, stored in the archive of the Pathology Section, Department of Biomorphological and Functional Sciences, University Federico II of Naples, Italy, were used for the immunohistochemical determination of PARP-1 expression. Moreover, 5 specimens of human normal skin were obtained from patients who had undergone surgical procedures for reconstructive surgery (with the informed consent of the donors) and 15 cases of benign melanocytic nevi (5 junctional, 5 compound, and 5 intradermal nevi) were used as controls.

2.1.1. Immunohistochemistry

Four-micrometer serial sections from routinely formalin-fixed, paraffin-embedded blocks were cut for each case of cutaneous melanomas, melanocytic nevi, and

Table 1 Expression of PARP in CMM and follow-up

Case	Age (y)	Sex	New staging system, AJCC	PARPr	PARPv	Follow-up (y)	
1	58	Female	≤1.00	0	+	12	
2	36	Male	≤1.00	0	+	12	
3	67	Male	≤1.00	0	+	12	
4	45	Female	≤1.00	0	+	12	
5	50	Female	≤1.00	+	+	11	
6	49	Female	≤1.00	0	0	11	
7	42	Male	≤1.00	0	+	10	
8	43	Female	≤1.00	+	+	9	
9	43	Male	≤1.00	0	0	9	
10[a]	49	Male	≤1.00	0	0	7	
11[a]	40	Male	≤1.00	+	+	7	
12[a]	41	Male	≤1.00	+	+	7	
13[a]	39	Male	≤1.00	++	+++	6	R
14[a]	37	Female	≤1.00	0	+	6	
15	66	Female	1.01-2.00	++	+++	12	N
16	65	Female	1.01-2.00	0	0	12	
17	56	Male	1.01-2.00	0	0	12	
18	49	Male	1.01-2.00	0	0	12	
19	48	Male	1.01-2.00	0	+	12	
20	39	Female	1.01-2.00	0	0	12	
21	47	Female	1.01-2.00	+++	+++	12	N, M, D
22	51	Male	1.01-2.00	0	+	11	
23	53	Male	1.01-2.00	+	++	11	
24	56	Male	1.01-2.00	0	++	11	
25	51	Female	1.01-2.00	0	+	10	
26	45	Female	1.01-2.00	+	++	10	
27	53	Male	1.01-2.00	0	++	10	
28	67	Male	1.01-2.00	0	+	10	
29	54	Male	1.01-2.00	0	0	10	
30	34	Male	1.01-2.00	+	++	9	
31	44	Female	1.01-2.00	0	++	9	
32	43	Female	1.01-2.00	0	0	9	
33	71	Female	1.01-2.00	0	++	9	
34	34	Male	1.01-2.00	0	0	9	
35	43	Female	1.01-2.00	0	0	9	
36	51	Female	1.01-2.00	0	++	9	
37	50	Male	1.01-2.00	0	0	9	
38	39	Female	1.01-2.00	+++	+++	9	N
39	29	Female	1.01-2.00	0	+	9	
40[a]	42	Male	1.01-2.00	0	++	7	
41[a]	37	Female	1.01-2.00	+++	+++	7	R, N
42[a]	65	Male	1.01-2.00	0	+	7	
43[a]	43	Male	1.01-2.00	+++	+++	7	N
44[a]	44	Female	1.01-2.00	+	+	6	
45[a]	18	Female	1.01-2.00	+	++	4	
46[a]	22	Male	1.01-2.00	0	0	4	
47[a]	24	Male	1.01-2.00	0	+	4	
48[a]	32	Male	1.01-2.00	0	+	4	
49[a]	30	Female	1.01-2.00	0	0	4	
50[a]	38	Female	1.01-2.00	+	+	4	
51[a]	37	Male	1.01-2.00	0	+	3	
52[a]	40	Male	1.01-2.00	0	0	3	
53	40	Male	2.01-4.00	0	+	12	
54	36	Male	2.01-4.00	0	0	12	
55	39	Male	2.01-4.00	0	0	11	
56	50	Female	2.01-4.00	+	++	11	
57	48	Female	2.01-4.00	0	++	11	
58	45	Male	2.01-4.00	+++	+++	11	N, M, D
59	43	Female	2.01-4.00	0	+	10	
60	53	Female	2.01-4.00	+	++	10	
61	47	Male	2.01-4.00	++	+++	10	N
62	54	Male	2.01-4.00	+	++	10	
63	50	Male	2.01-4.00	+	++	10	
64	69	Female	2.01-4.00	0	0	10	
65	54	Male	2.01-4.00	0	+	10	
66	53	Male	2.01-4.00	0	+	9	
67[a]	42	Female	2.01-4.00	+++	+++	9	N, M, D
68[a]	37	Female	2.01-4.00	+++	+++	9	N, M
69[a]	45	Female	2.01-4.00	0	+	9	
70[a]	49	Female	2.01-4.00	+++	+++	9	N, M, D
71[a]	32	Male	2.01-4.00	+++	+++	7	N
72[a]	38	Female	2.01-4.00	++	+++	6	N
73[a]	38	Male	2.01-4.00	0	+	3	
74[a]	32	Male	2.01-4.00	++	+++	3	N, M
75[a]	40	Male	2.01-4.00	0	+	3	
76[a]	46	Female	2.01-4.00	+	+++	3	N
77[a]	50	Male	2.01-4.00	0	0	2	
78[a]	24	Female	2.01-4.00	+	++	2	
79[a]	30	Male	2.01-4.00	0	+	2	
80[a]	32	Female	2.01-4.00	0	+	2	

Abbreviations. PARPr, radial growth; PARPv, vertical growth; R, recidivation; N, lymph node metastasis; M, metastasis; D, death for tumor.

[a] WB.

normal skin, and were mounted on poly-L-lysine–coated glass slides.

Deparaffinized sections were boiled 3 times for 3 minutes in a 10^{-3} mol/L sodium citrate buffer (pH 6.0) as an antigen retrieval method. To prevent the nonspecific binding of the antibody, sections were preincubated with nonimmune mouse serum (1:20; Dakopatts, Hamburg, Germany) diluted in phosphate-buffered saline–bovine serum albumin (1%) for 25 minutes at room temperature. After quenching of endogenous peroxidases with 0.3% hydrogen peroxide in methanol, followed by 2 rinses with Tris-HCl buffer, the sections were incubated with the anti–PARP-1 primary antibody (PARP F2, mouse monoclonal IgG2a, raised against a recombinant protein corresponding to amino acids 764-1014 mapping at the carboxyl terminus of PARP of human origin; Santa Cruz Biotechnology, Inc, Santa Cruz, Calif) diluted 1:50 overnight at 4°C. The standard streptavidin-biotin-peroxidase complex technique, using sequential 20-minute incubation with biotinylated linking antibody and peroxidase-labeled streptavidin (DAKO labeled streptavidin-biotin-complex kit horse radish peroxidase; Carpinteria, Calif), was performed. 3,3′ -Diaminobenzidine (3-3′ diaminobenzidine tetrachloride; Vector Laboratories, Burlingame, Calif) was used as a substrate chromogen solution for the development of the peroxidase activity. Hematoxylin was used for nuclear counterstaining; then, the sections were

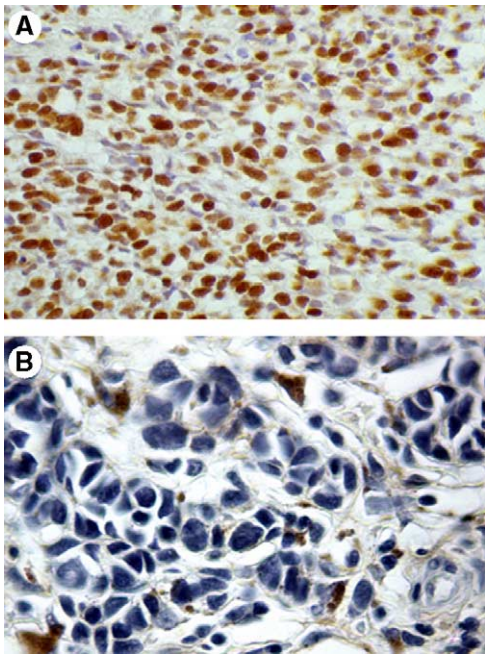

Fig. 1 A, Strongly immunoreactive for PARP-1 (cutaneous superficial spreading malignant melanoma, worse prognosis, vertical growth phase) (avidin-biotin complex technique, original magnification ×150). B, Absent immunostaining for PARP-1 in a case of cutaneous superficial spreading malignant melanoma (vertical growth phase, node negative, disease-free 10 years after surgery) (avidin-biotin complex technique, original magnification ×250).

mounted and coverslipped with a synthetic mounting medium (Entellan; Merck, Darmstadt, Germany).

As positive controls, the immunoreactivity of normal squamous epithelium next to the tumor was evaluated. Negative controls were performed using an antibody with irrelevant specificity but with the same isotype as the primary antibody and were included in each staining run. The nuclear expression of PARP-1 was evaluated semiquantitatively according to an arbitrary scale as follows: 0 (<5% of positive cells), + (≤25%), ++ (26%-50%), and +++ (>50% of positive cells).

2.2. Western blot analysis

Frozen tissues from 10 compounds and intradermal melanocytic nevi and from 30 cases of CMM were homogenized directly into lysis buffer containing 50 mmol/L 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, 150 mmol/L NaCl, 1 mmol/L EDTA, 1 mmol/L ethyleneglycotetraacetic acid, 10% glycerol, 1% Triton X-100 (weight/volume, 1:2), 1 mmol/L phenylmethylsulfonyl fluoride, 1 μg aprotinin, 0.5 mmol/L sodium orthovanadate, and 20 mmol/L sodium pyrophosphate (Sigma, St Louis, Mo) and were clarified by centrifugation at 14000g for 10 minutes. Protein concentrations were estimated using a modified Bradford assay (Bio-Rad, Melville, NY).

Fifty micrograms of total protein extracts were boiled in Laemmli buffer for 5 minutes before electrophoresis. The samples were subjected to sodium dodecyl sulfate–polyacrylamide gel electrophoresis (14% polyacrylamide) under reducing conditions. After electrophoresis, proteins were transferred to nitrocellulose membrane (Immobilon; Millipore, Bedford, Mass); complete transfer was assessed using prestained protein standards (Bio-Rad). The membranes were treated for 2 hours with blocking solution (5% nonfat powdered milk in 25 mmol/L Tris, pH 7.4; 200 mmol/L NaCl; 0.5% Triton X-100; Tris-buffered saline [TBS]–Tween 20), and then, the membranes were incubated for 12 hours at 4°C with the primary anti-PARP antibody. After washing with TBS–Tween 20 and TBS, membranes were incubated with the horseradish peroxidase–conjugated secondary antibody (1:5000) for 1 hour (at room temperature), and the reaction was detected with enhanced chemiluminescence system (Amersham Life Science, Piscataway, NJ).

2.3. Statistical analysis

PARP-1 expression, scored in 4 classes (negative, 0; low, +; moderate, ++; strong, +++), was grouped in 2 categories (negative/low, 0/+, and moderate/strong, ++/+++). The thickness of the tumor lesions was coded according to new staging system by the American Joint Committee on Cancer (AJCC, 2001; T1, ≤1.00 mm; T2, 1.01-2.00 mm; T3, 2.01-4.00 mm; T4, >4.00 mm) [21,22].

The correlation between PARP-1 expression and the thickness of the tumor lesion was evaluated by the χ^2 test.

Disease-free survival (DFS) was calculated from the date of surgery to the date of the first locoregional recurrence or distant metastases. DFS curves were drawn by the Kaplan-Meier method, and the statistical significance of the differences was calculated by log-rank test at univariate analysis.

All analyses have been performed using the S-Plus 2000 software (MathSoft Inc, Cambridge, UK).

3. Results

3.1. Patients

Following the aforementioned selection criteria, 80 cases of CMM were considered suitable for the analysis. The

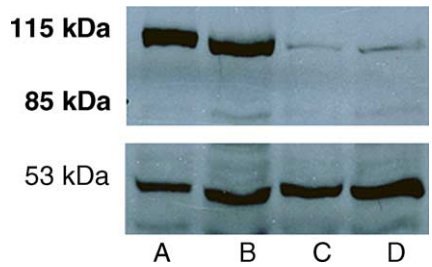

Fig. 2 Western blot showing full-length line positive in A and B (A, node positive, brain metastasis at 3 years after surgery; B, death for disease 5 years after surgery) and line positive in C and D, with a light presence of cleaved form (good prognosis at 8 and 12 years after surgery) (normalized for β-tubulin).

Table 2 PARP expression in radial and vertical growth phase of CMM with poor prognosis

New staging system, AJCC	Cases	Cases with poor prognosis	PARPr/PARPv	Follow-up
≤1.00	14	1	++/+++	R
1.01-2.00	38	1	++/+++	N
		1	+++/+++	N, M, D
		1	+++/+++	R, N
		2	+++/+++	N
2.01-4.00	28	3	+++/+++	N, M, D
		2	++/+++	N
		1	+++/+++	N, M
		1	+++/+++	N
		1	++/+++	N, M
		1	+/+++	N
>4.00	0	0	–	0

Abbreviations. PARPr, radial growth; PARPv, vertical growth; R, recidivation; N, lymph node metastasis; M, metastasis; D, death for tumor.

study population consisted of 43 men and 37 women, with a mean age of 44.5 years (range, 18-71 years). All the patients had undergone surgical treatment with curative intention at the Plastic Surgery Department of the University Federico II of Naples, Italy. The extent of invasion was assessed on hematoxylin-eosin–stained sections from formalin-fixed, paraffin-embedded tissue according to new staging system by the AJCC (2001) [21,22]: 14 (17.5%) patients had less than 1.00 mm; 38 (47.5%), between 1.01 and 2.00 mm; and 28 (35%), between 2.01 and 4.00 mm. No patients had CMM of greater than 4.00 mm.

3.2. PARP-1 expression in normal melanocytes

Normal skin specimens showed positivity for PARP-1 only in epithelial cells of basal and, less frequently, parabasal layers; normal melanocytes showed only scattered, low nuclear immunostaining, with a ratio of about 1:5 melanocytes, and this value was defined as "normal PARP-1 expression" (score, 0).

3.3. PARP-1 expression in melanocytic nevi

By immunohistochemistry, positive expression of PARP-1 was observed in all the 15 cases of melanocytic nevi as strong nuclear staining in up to 30% of melanocytes (mean positivity, 20% of melanocytes), and this was defined as "low intensity" (score, +).

The WB analysis showed the presence of both full-length and cleaved PARP-1 expression in all the 10 cases examined (5 compound and 5 intradermal nevi).

3.4. PARP expression in malignant melanomas

The expression of PARP-1 in the vertical (invasive) and in the radial growth phase of CMM correlated with follow-up data is reported in Table 1. Thirty cases of CMM (37.5%) showed nuclear expression (between + and +++) of PARP-1 in both radial and vertical growth phase (Fig. 1A). Several cases showed absent immunostaining for PARP-1 (Fig. 1B); cases were characterized by node negative and good behavior. Among the 30 cases of CMM examined by WB analysis (Fig. 2), the presence of high signal for full-length PARP-1 was found only in the cases with high immunohistochemical (nuclear) expression of the protein (score, ++/+++) in both radial and vertical growth phase. Overexpression of full-length PARP-1 and low levels of cleaved PARP-1 were found in CMM with high immunohistochemical expression of PARP-1 restricted to the cells of the vertical phase of growth; the cases of CMM with low/absent expression of PARP-1 in the vertical growth phase and absent PARP-1 expression in the radial growth phase showed a low/moderate amount of both cleaved and full-length PARP-1.

A significant correlation was present between PARP-1 expression in vertical growth phase and the thickness of the tumor lesion ($P = .014$); all but one tumor measuring less than 1.00 mm showed no or low PARP-1 expression. No correlation was found between PARP-1 expression in radial growth phase and tumor thickness ($P = .38$, data not shown).

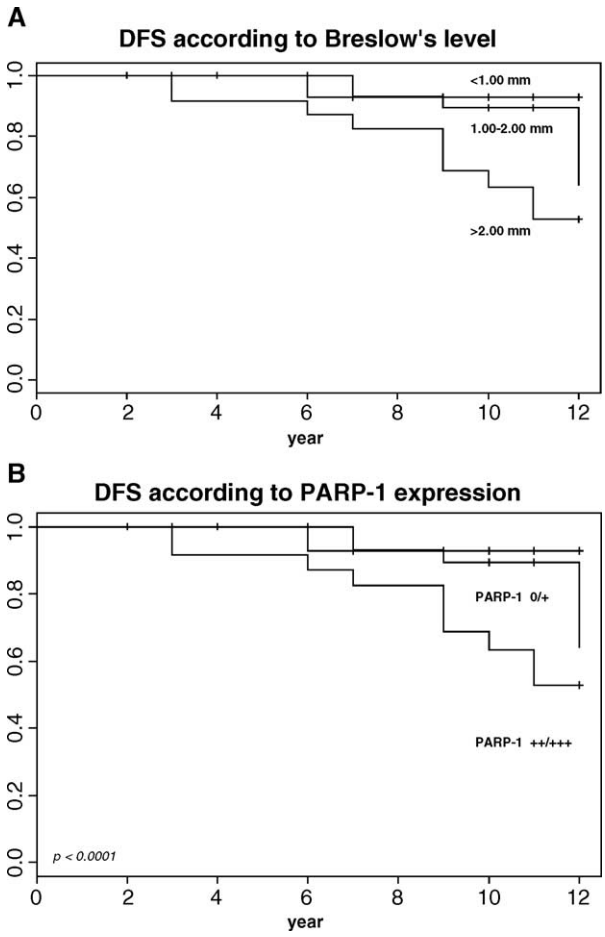

Fig. 3 DFS according to Breslow level (A) and PARP expression (B).

3.5. Patient's outcome

Overall, 15 patients relapsed: 7 patients at locoregional lymph nodes, 1 at skin, 1 at locoregional lymph nodes and skin, and 6 patients presented concomitant locoregional lymph nodes and distant metastases (Table 2).

As expected, DFS was affected by tumor's thickness; among the 15 relapsed patients, 9 were between 2.01 and 4.00 mm, 5 were between 1.01 and 2.00 mm, and only 1 had 1.00 mm or less. Thus, the actual proportion of relapse was 32.1% (9/28 patients) for T3 cases, 13.1% (5/38 patients) for T2, and only 7% (1/14 patients) for T1 patients.

Interestingly, PARP-1 overexpression demonstrated to be a strong predictor of relapse; all the 15 relapsed patients had tumors with ++/+++ PARP-1 expression in vertical growth phase, and 9 of them also had ++/+++ PARP-1 expression in radial growth phase.

Patients with ++/+++ PARP overexpression (either in vertical or in radial growth phase) showed a significantly short DFS than patients with low or negative PARP-1 overexpression (Fig. 3A and B).

4. Discussion

Long-term exposure to sunlight causes photoaging, immunosuppression, and skin cancer. Failure of the DNA repair processes constitutes one of the major molecular events underlying UV-related skin carcinogenesis. The UV radiation normally induces pyrimidine dimer formation, which leads either to DNA repair or apoptosis. However, UV radiation may determine multiple mutations in genes regulating apoptosis and DNA repair, leading to the uncontrolled cellular proliferation.

In the present study, we found a significant correlation between the nuclear overexpression of PARP-1 and the vertical (invasive) growth phase of melanomas, whereas the neoplastic melanocytes of the radial phase were characterized by a slight nuclear expression of the PARP-1.

As it is well-known, activation of PARP-1 is an immediate cellular reaction to DNA strand breakage induced by alkylating agents, ionizing radiation, or oxidants. The resulting formation of protein-coupled poly (adenosine diphosphate-ribose) facilitates survival of proliferating cells under conditions of DNA damage, probably via its contribution to DNA base-excision repair [23]. Furthermore, recent data indicate that PARP-1 acts as a negative regulator of genomic instability in cells under genotoxic stress [24]. The p53 tumor suppressor protein is activated by a variety of cellular insults, including UV radiation, to become a transcription factor for downstream markers such as the cyclin kinase inhibitor p21CIP1/WAF1 or caspase transactivation, which cleaves PARP-1 as an early step in apoptosis [25,26]. In fact, during apoptosis, CPP32 (caspase 3) cleaves the 116-kd death substrate PARP into a stable 85-kd fragment containing the carboxyl terminal and a 25-kd fragment [27,28]. The degradation of nuclear PARP-1 then is suggestive of caspase-mediated early apoptotic events.

Although the involvement of the PARP family proteins in the control of genomic stability, in DNA repair, and in the regulation of apoptosis program has been outlined before, their potential role in carcinogenesis has not been well evaluated [29].

In bronchogenic tumorigenesis, for example, cigarette smoke augments asbestos-induced bronchogenic carcinoma in a synergistic manner by mechanisms that are not established. One important mechanism may involve alveolar epithelial cell injury resulting from oxidant-induced DNA damage that subsequently activates PARP-1 [30].

The findings of the present study suggest that the neoplastic progression toward the invasive (vertical) growth phase of melanocytes in CMM is characterized by the loss of cleavage of PARP-1, probably signaling an imbalance of the apoptotic process in these cells and therefore predisposing them to acquire alteration(s) of other gatekeeping genes, leading to further gain to aggressivity.

More interestingly, in our series of cases, the presence of overexpression of full-length PARP-1 in both (radial and vertical) growth phases was correlated with recurrence and/or progression of the disease.

The overexpression of full-length PARP-1 in both radial and vertical growth phase appears then as a promising new marker of worse prognosis for CMM of photoexposed areas.

Previous studies showed a deregulation of the apoptotic process in malignant melanomas. In particular, BCL-2 protein expression has been reported in most CMM [31]. As it is well-known, BCL-2 is the principal member of a family of proteins with either positive or negative activity on the apoptotic process [32,33].

It constitutes the prototype of the antiapoptotic proteins and has been found overexpressed in most human malignancies [34].

To the best of our knowledge, our study represents the first evidence of a direct correlation between the inhibition of the apoptotic process (PARP-1–mediated) and the biologic behavior of CMM. A strong reactivity with BCL-2 antibody is observed in melanocytes of normal skin. In nevocellular nevi, immunoreactivity gradually decreases or even disappears toward the deeper dermal component. In malignant melanomas of all stages and histological subtypes, the neoplastic cells express BCL-2 oncoprotein, the most intense positivity being restricted to cells in the radial growth phase. Expression of the protein in the great majority of malignant melanomas seems to exclude its prognostic significance in these tumors, even if cutaneous and lymph node metastases of malignant melanomas have been found often negative or only weakly and focally reactive for BCL-2 [35,36]. The expression of BCL-2 oncoprotein by malignant melanomas adds these neoplasms to a growing list of tumors expressing this oncoprotein. BCL-2 in malignant melanoma may play a role in tumor development

by sparing the cells from apoptotic death (and thereby exposing those to secondary events) or through cooperation with other oncogenes [37]. The lack of reactivity in metastatic melanoma suggests that mechanisms other than BCL-2 are involved in the survival and growth of metastatic melanoma cells [38].

Moreover, the analysis of PARP-1 expression may offer useful information concerning the pharmacological treatment of CMM.

Conventional anticancer drugs, in fact, kill susceptible cells through induction of apoptosis. Alteration of the pathways leading to apoptosis deficiency might represent a potent mechanism conferring drug resistance. Recent studies demonstrated that PARP-1 cleavage is strongly reduced in highly cisplatin-resistant melanoma cells sublines [39]. In addition, metastatic malignant melanoma is notoriously resistant to chemotherapeutic agents, but the exact mechanisms involved in this drug resistance are still unknown [40]. The imbalance of the apoptotic process provides a broad cytoprotective mechanism to cancerous cells, counteracting apoptosis induced by various chemotherapeutic drugs [37]. The survival advantage due to the full-length PARP-1 hyperaccumulation in melanoma cells, related to a loss of susceptibility to apoptosis and to defects in checkpoint pathways, may be responsible for the chemoresistance of this tumor.

Recently, it has been reported that the inhibition of PARP-1 activity is a promising strategy to improve the outcome of cytotoxic therapies in different tumor models [41,42].

In conclusion, the findings of the present study indicate that the analysis of PARP-1 expression in CMM may be potentially relevant for implementation of closer follow-up protocols and/or alternative therapeutic regimens, reiterating the importance of deregulation of apoptosis as a critical pathogenetic component of tumor progression, and identify PARP-1 overexpression as a potential novel molecular marker of aggressive neoplasia.

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References

[1] Cabrera R, Silva S, Diaz de Medina J, et al. Clinical study of 113 cases of malignant melanoma. Rev Med Chil 1994;122(8):900-6.
[2] Muller WA, Erlanger M. Malignant melanoma in life insurance—thickness or anatomic layer? Versicherungsmedizin 1994;46(6):193-5.
[3] MacKie RM, Bray CA, Hole DJ, et al. Incidence of and survival from malignant melanoma in Scotland: an epidemiological study. Lancet 2002;360(9333):587-91.
[4] Boni R, Schuster C, Nehrhoff B, et al. Epidemiology of skin cancer. Neuroendocrinol Lett 2002;23(Suppl 2):48-51.
[5] Braun MM, Tucker MA, Devesa SS, et al. Seasonal variation in frequency of diagnosis of cutaneous malignant melanoma. Melanoma Res 1994;4(4):235-41.
[6] Cavalieri R, Macchini V, Mostaccioli S, et al. Time trends in features of cutaneous melanoma at diagnosis: central-south Italy, 1962-1991. Ann Ist Super Sanita 1993;29(3):469-72.
[7] Franceschi S, Bidoli E, Prati S, et al. Mortality from skin melanoma in Italy and Friuli-Venezia Giulia region, 1970-1989. Tumori 1994;80(4):251-6.
[8] Yeh KA, Wei JP. An overview of cutaneous malignant melanoma. J Med Assoc Ga 1994;83(11):635-8.
[9] Von der Maase H, Osterlind A, Drzewiecki KT, et al. Malignant melanoma of the skin in Denmark—epidemiology, diagnosis and treatment. Ugeskr Laeger 1992;154(28):1949-53.
[10] Lawson DD, Moore DH, Schneider JS, et al. Nevus counting as a risk factor for melanoma: comparison of self-count with count by physician. J Am Acad Dermatol 1994;31(3 Pt 1):438-44.
[11] Marghoob AA, Kopf AW, Rigel DS, et al. Risk of cutaneous malignant melanoma in patients with 'classic' atypical-mole syndrome. A case-control study. Arch Dermatol 1994;130(8):993-8.
[12] Garbe C. Risk factors for the development of malignant melanoma and identification of risk groups in German-speaking regions. Hautarzt 1995;46(5):309-14.
[13] Abadir MC, Marghoob AA, Slade J, et al. Case-control study of melanocytic nevi on the buttocks in atypical mole syndrome: role of solar radiation in the pathogenesis of atypical moles. J Am Acad Dermatol 1995;33(1):31-6.
[14] Jackson A, Wilkinson C, Hood K, et al. Does experience predict knowledge and behavior with respect to cutaneous melanoma, moles, and sun exposure? Possible outcome measures. Behav Med 2000;26(2):74-9.
[15] Bakos L, Wagner M, Bakos RM, et al. Sunburn, sunscreens, and phenotypes: some risk factors for cutaneous melanoma in southern Brazil. Int J Dermatol 2002;41(9):557-62.
[16] Jansen C. Effect of sunlight on the skin—what have we learned? Nord Med 1995;110(3):85-7.
[17] Schmitz S, Garbe C, Tebbe B, et al. Long-wave ultraviolet radiation (UVA) and skin cancer. Hautarzt 1994;45(8):517-25.
[18] Autier P, Dore JF, Lejeune F, et al. Cutaneous malignant melanoma and exposure to sunlamps or sunbeds: an EORTC multicenter case-control study in Belgium, France and Germany. EORTC Melanoma Cooperative Group. Int J Cancer 1994;58(6):809-13.
[19] Adimoolam S, Ford JM. p53 and DNA damage–inducible expression of the xeroderma pigmentosum group C gene. Proc Natl Acad Sci U S A 2002;99(20):12985-90.
[20] Nomura F, Yaguchi M, Togawa A, et al. Enhancement of poly-adenosine diphosphate-ribosylation in human hepatocellular carcinoma. J Gastroenterol Hepatol 2000;15(5):529-35.
[21] Balch CM, Buzaid AC, Atkins MB, et al. Final version of the American Joint Committee on Cancer staging system for cutaneous melanoma. J Clin Oncol 2001;19:3635-48.
[22] Balch CM. Cutaneous melanoma. In: Greene FL, Page DL, Fleming ID, et al, editors. AJCC cancer staging manual. 6th ed. New York (NY): Springer Verlag; 2002. p. 209-17.
[23] Tong WM, Cortes U, Wang ZQ. Poly(ADP-ribose) polymerase: a guardian angel protecting the genome and suppressing tumorigenesis. Biochim Biophys Acta 2001;1552(1):27-37.
[24] Decker P, Muller S. Modulating poly(ADP-ribose) polymerase activity: potential for the prevention and therapy of pathogenic situations involving DNA damage and oxidative stress. Curr Pharm Biotechnol 2002;3(3):275-83.
[25] Wang X, Ohnishi K, Takahashi A, et al. Poly(ADP-ribosyl)ation is required for p53-dependent signal transduction induced by radiation. Oncogene 1998;17(22):2819-25.

[26] Bernstein C, Bernstein H, Payne CM, et al. DNA repair/pro-apoptotic dual-role proteins in five major DNA repair pathways: fail-safe protection against carcinogenesis. Mutat Res 2002;511(2):145-78.
[27] Lazebnik YA, Kaufmann SH, Desnoyers S, et al. Cleavage of poly(ADP-ribose) polymerase by a proteinase with properties like ICE. Nature 1994;371(6495):346-7.
[28] Kaufmann SH, Desnoyers S, Ottaviano Y, et al. Specific proteolytic cleavage of poly(ADP-ribose) polymerase: an early marker of chemotherapy-induced apoptosis. Cancer Res 1993;53(17): 3976-85.
[29] Vaculova A, Hofmanova J, Soucek K, et al. Tumor necrosis factor–alpha induces apoptosis associated with poly(ADP-ribose) polymerase cleavage in HT-29 colon cancer cells. Anticancer Res 2002;22(3): 1635-9.
[30] Kamp DW, Srinivasan M, Weitzman SA. Cigarette smoke and asbestos activate poly-ADP-ribose polymerase in alveolar epithelial cells. J Investig Med 2001;49(1):68-76.
[31] van den Oord JJ, Vandeghinste N, De Ley M, et al. Bcl-2 expression in human melanocytes and melanocytic tumors. Am J Pathol 1994; 145(2):294-300.
[32] Boise LH, Gonzalez-Garcia M, Postema CE, et al. bcl-x, a bcl-2–related gene that functions as a dominant regulator of apoptotic cell death. Cell 1993;74(4):597-608.
[33] Steller H. Mechanisms and genes of cellular suicide. Science 1995; 267(5203):1445-9.
[34] Larsen CJ. The BCL2 gene, prototype of a gene family that controls programmed cell death (apoptosis). Ann Genet 1994;37(3): 121-34.
[35] Leiter U, Schmid RM, Kaskel P, et al. Antiapoptotic bcl-2 and bcl-xL in advanced malignant melanoma. Arch Dermatol Res 2000; 292(5):225-32.
[36] Reed JC. Regulation of apoptosis by bcl-2 family proteins and its role in cancer and chemoresistance. Curr Opin Oncol 1995;7(6):541-6.
[37] Tang L, Tron VA, Reed JC, et al. Expression of apoptosis regulators in cutaneous malignant melanoma. Clin Cancer Res 1998;4(8):1865-71.
[38] Vlaykova T, Talve L, Hahka-Kemppinen M, et al. Immunohistochemically detectable bcl-2 expression in metastatic melanoma: association with survival and treatment response. Oncology 2002; 62(3):259-68.
[39] Helmbach H, Kern MA, Rossmann E, et al. Drug resistance towards etoposide and cisplatin in human melanoma cells is associated with drug-dependent apoptosis deficiency. J Invest Dermatol 2002; 118(6):923-32.
[40] Keilholz U, Martus P, Punt CJ, et al. Prognostic factors for survival and factors associated with long-term remission in patients with advanced melanoma receiving cytokine-based treatments: second analysis of a randomised EORTC Melanoma Group trial comparing interferon-alpha2a (IFNalpha) and interleukin 2 (IL-2) with or without cisplatin. Eur J Cancer 2002;38(11):1501-11.
[41] Feleszko W, Mlynarczuk I, Olszewska D, et al. Lovastatin potentiates antitumor activity of doxorubicin in murine melanoma via an apoptosis-dependent mechanism. Int J Cancer 2002;100(1):111-8.
[42] Shyong EQ, Lu Y, Lazinsky A, et al. Effects of the isoflavone 4′,5,7-trihydroxyisoflavone (genistein) on psoralen plus ultraviolet A radiation (PUVA)–induced photodamage. Carcinogenesis 2002; 23(2):317-21.