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Poly(ADP-ribose) in the skin and in melanomas

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Summary. Cutaneous melanoma (CM) and uveal melanoma (UM) represent the most aggressive pigment cell tumor types. Our investigation examined the signaling molecule poly(ADP-ribose) (PAR) in CM and UM. We have demonstrated PAR in keratinocytes, sebocytes, hair follicles, endothelial cells and in subcutaneous adipocytes in the normal skin indicating that PAR may regulate physiological functions in these cell types. Furthermore, CM cells were PAR positive and tumor invasion level/thickness of CM correlated with the PAR content of the cell nuclei, with higher Clark and Breslow indices and AJCC scores associating with higher PAR content. This correlation was especially marked in the samples of female patients. In UM tumors (n=12) a slight overall and strong perivascular PAR staining was observed with considerable individual variations. In view of recent successful clinical trials with PARP inhibitors as adjuvant chemotherapeutic agents, our results suggest that melanomas may display differential sensitivity towards this novel therapeutic modality which should be considered for the selection of patients.

Key words: Melanoma, Poly(ADP-ribose), Poly(ADP-ribose) polymerase, Skin

Introduction

Cutaneous melanoma (CM) is the most aggressive form of skin cancer among the Caucasian population. It is the sixth most common cancer and its incidence is on the rise in western populations. In the United States an estimated 68,720 new cases (39,080 men and 29,640 women) were reported in 2009 causing 8650 deaths (5550 men and 3100 women). The number of total body acquired melanocytic nevi and the occurrence of previous epithelial skin cancer represent major risk factors for the development of CM (Lawson et al., 1994; Marghoob et al., 1996; Jackson et al., 2000; Bakos et al., 2002). Solar damage is the major environmental causal factor in all skin cancers, and intermittent intense exposures to sunlight and/or severe sunburn especially during childhood (Hedges and Scriven, 2008) are also important environmental risk factors for CM.

The prognosis of melanoma depends on the stage at which it is detected as patients who are diagnosed early and treated surgically with excision of the tumors have the highest chance for a complete cure. Unresectable or advanced metastatic diseases, however, have a poor prognosis (Garbe et al., 1995; Ahmed, 1997). Therapeutic strategies to combat CM include chemotherapy, bio-chemotherapy, immune adjuvants, cancer-specific vaccines, cytokines, monoclonal antibodies and specific immunostimulants (Guerry and Schuchter, 1992, Schadendorf, 2002).

Another pigment cell-derived malignancy, uveal melanoma is the most common adult primary intraocular tumor (Sato et al., 2008) however it appears to be unlinked to photodamage (Singh et al., 2004). Uveal melanoma can be classified as spindle-A, spindle-B, mixed-type and epitheloid melanomas with the order of degradation of the prognosis marked by increasing proportion of mitotic cells, metastatic capacity and extraocular extension. Possible treatments range from local laser photocoagulation, radiation therapy to enucleation dependent on the size of the melanoma.

Inhibition of poly(ADP-ribose) polymerases (PARPs) recently emerged as a novel therapeutic modality in the treatment of certain types of cancer.

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From the 17 member PARP enzyme family, the nuclear enzymes PARP-1 and PARP-2 are DNA nick sensors (De Murcia and Menissier de Murcia, 1994; Ame et al., 2004). Their binding to damaged DNA induces enzyme activation whereby PARPs cleave NAD^+ to nicotinamide and ADP-ribose and polymerize the latter to form branched poly(ADP-ribose) (PAR) polymer (Schreiber et al., 2006). PARylation regulates many processes including chromatin organization, replication, transcription, metabolism and cell death (Virag and Szabo, 2002; Erdelyi et al., 2005). PAR synthesis is involved in the initiation of the DNA damage response facilitating DNA base excision repair (Schreiber et al., 2006; Hassa and Hottiger, 2008). PARP inhibitors were proposed to be useful as adjuvant therapeutic agents in cancer patients including melanoma patients receiving chemotherapy or irradiation (Tentori et al., 2003; Kasper et al., 2007; Plummer et al., 2008; Chalmers, 2009).

The expression of PARP-1 has been investigated in CM (Staibano et al., 2005), however the signaling molecule PAR itself was not detected. PARP-1 is usually expressed abundantly in most cell types and its function is regulated primarily at the level of activity. PARP activity is affected by DNA breaks, special DNA structures, phosphorylation and acetylation. Thus PAR levels do not necessarily correlate with protein level of PARP-1 or other PARP enzymes. Here we set out to investigate the differences in PAR content in various types of cutaneous and uveal melanomas.

Materials and methods

Tissue samples

Studies involving human tissue samples were conducted in accordance with the declaration of Helsinki and were approved by the Ethical Committees of the Medical and Health Science Center of the University of Debrecen and Semmelweis University (for cutaneous and uveal melanoma samples, respectively).

Retrospective analysis was performed on formalin-fixed, paraffin embedded routine histology sections from the archive of the Department of Dermatology (University of Debrecen, Hungary). Melanoma patients (age 10-92 years) treated between 2002-2007 were selected. Clark stage I-V primary melanomas (Breslow 0,06-11,00 mm, T1a-4b) and cutaneous melanoma metastases were analyzed. Specimens were classified by histology as superficial spreading melanoma (17 subjects), lentigo melanoma (17 subjects), nodular melanoma (17 subjects), skin metastasis (15 subjects). Healthy skin (around melanocytic naevi) and melanocytic naevi were used as controls.

Uveal melanoma samples used in this study were from patients undergoing enucleation due to UM at the Department of Ophthalmology (Semmelweis University, Budapest, Hungary) between 2005 and 2006. We analyzed 12 formalin fixed, paraffin embedded tumors (n=12; 5 spindle, 3 epitheloid and 4 mixed).

Materials

The anti-poly(ADP-ribose) antibody (clone 10H) was purchased from Alexis Biochemicals, (Lausanne, Switzerland) (Erdelyi et al., 2009). The Vector Elite ABC kit (Vector Laboratories, Burlingame, CA, USA) was used for the immunostaining procedure. Unless stated otherwise, all materials were obtained from Sigma-Aldrich.

Immunohistochemistry

The immunohistochemical localization of PAR was performed as follows. After deparaffinization, 5 μm sections were treated with H_2O_2 [3% (v/v) in methanol] for 20 min in order to block endogenous peroxidase activity. After washing in PBS for 5 min, sections were subjected to antigen retrieval by heating for 5 min in a pressure cooker in sodium citrate buffer (0.01 M, pH 6.0). Sections were rinsed in distilled water and washed for 5 min in PBS followed by incubation with a mixture of 1% (w/v) bovine serum albumin and 1 % (v/v) horse serum at room temperature for 20 min, to prevent non-specific reactions. Subsequently, sections were incubated overnight at 4°C with anti-PAR monoclonal antibody diluted in blocking solution (1% BSA, horse serum). After washing with PBS (3x10 min), sections were further incubated for 45 min at room temperature with biotinylated horse anti-mouse IgG secondary antibodies (provided in the Vector kit and used at 1:600 dilution in blocking solution). The sections were then washed (3x10 min) with PBS, and treated with 2% avidin-biotin-peroxidase complex (ABC) reagent for 30 min. Afterwards, sections were washed with PBS (3x10 min) and reacted with Ni-DAB substrate (1.6 mM 3,3'-diaminobenzidine tetrachloride, 140 mM NaCl, 90 mM $NiSO_4$, 100 mM Na-acetate, 3 mM H_2O_2, pH 6.6) for 4 minutes. After rinsing sections in 0.1M TBS (pH 7.2), the color was enhanced by incubating the sections for 3 min in 0.5% cobalt chloride (in 0.1 M TBS, pH 7.2). After rinsing in distilled water, sections were counterstained with Chromotrop 2R (*Chroma*, Stuttgart, Germany) solution (500 mg/l Chromotrop 2R, 0.005% (v/v) acetic acid). Negative immunohistochemical controls (isotype control antibody) were included in each staining run. Initially, PAR detection was performed after removal of melanin from the sections but the presence of melanin did not appear to interfere with the immunodetection, therefore this was omitted from the final procedure.

Scoring and statistical analysis

Nuclear staining was scored semiquantitatively by two experienced independent researchers on an arbitrary scale as follows: 0 (negative), 1 (low), 2 (moderate), 3 (partly low to moderate, partly strong), 4 (strong). Immunostained slides were compared to H&E stained counterparts in order to ensure that melanocytes were

scored.

PAR staining scores of the two independent investigators were averaged and then correlated with Breslow index, Clark stage and AJCC scores using Graphpad and SPSS17 softwares. The Spearman's coefficient of correlation was calculated and its significance was determined at $p<0.05$.

Results

PAR formation in healthy skin and CM

In normal skin, PAR formation was observed in various areas (Fig. 1A-G). Sebocytes and hair follicle cells, epidermal keratinocytes, endothelial cells and subcutaneous adipocytes all demonstrated positivity for PAR.

In cutaneous melanoma sections we observed nuclear PAR staining in the tumor cells. However, the intensity of the staining was highly variable (Fig. 1H-J and Table 1). The staining intensity correlated with Breslow index, Clark staging and AJCC scores of the melanomas (Fig. 2). The Spearman's rank coefficients for the correlation between the PAR signal and Breslow index, Clark stage and AJCC scores for all samples were r=0.4125 (p=0.0025), r=0.3257 (p=0.0197) and r=0.3657 (p=0.0083), respectively. Interestingly, this correlation was more pronounced in the case of female patients: Spearman's rank correlation coefficients and their statistical significance values for the female samples were (in the same order as above) r=0.6678 (p=0.0004), r=0.5584 (p=0.0046), r=0.5486 (p=0.0055) and for the male patients the values were r=0.3227 (p=0.1006), r=0.2283 (p=0.2520) and r=0.3575 (p=0.067).

Specificity of the PAR staining was further confirmed by staining WM35 human melanoma cells (Fig. 3A), that had been treated with H_2O_2 (5 min, 400 μM) in the absence (Fig. 3B) or presence of the PARP

Table 1. Summary of PAR expression in CM patients.

Age	Sex	Breslow	Clark	AJCC	Histology	PAR	Age	Sex	Breslow	Clark	AJCC	Histology	PAR
62	M	0.45	3	1	SSM	1	44	M	2.5	5	1.5	NM	1.5
33	M	0.24	3	1	SSM	1	53	M	4.5	4	2	NM	2
69	M	1.12	3	1	SSM	1.5	39	F	2	3	2	NM	2.5
44	F	0.48	3	4	SSM	1	68	F	6.5	4	4	NM	3
44	F	6	3	2	SSM	3	65	F	5.5	5	4	NM	3
38	F	0.12	2	1	SSM	2	42	F	2	4	2	NM	3
73	F	1.75	4	2	SSM	2	51	F	3	2.5	3	NM	2
63	F	1.1	3	2	SSM	3	51	F	2.5	4	3	NM	2
40	F	7	4	4	SSM	3	64	F	2.45	3	3	NM	3
69	F	2	4	2	SSM	2	75	F	11	5	4	NM	4
58	M	0.61	3	1	SSM	1	35	F	8.5	5	4	NM	2.5
44	M	0.88	3	1	SSM	2	55	M	0.65	3	1	NM	1.5
63	M	0.25	2	1	SSM	2	69	M	1.61	4	2	NM	3.5
51	M	0.65	3	1	SSM	3	40	M	4	4	3	NM	3
38	M	0.55	3	1	SSM	3	66	M	3.8	4	3	NM	4
50	M	0.31	2	1	SSM	1	56	M	1	3	1	NM	4
45	M	1.12	3	2	SSM	3.5	44	M	2.25	4	3	NM	4
67	M	0.12	2	1	LM	1	83	M				MET	4
71	M	0.06	2	1	LM	2	66	M				MET	3
79	F	0.42	3	1	LM	1	10	M				MET	2.5
66	F	0.2	1.5	1	LM	2	83	M				MET	3
81	F	0.07	2	1	LM	2	92	F				MET	3
80	F	0.18	2	1	LM	2	45	F				MET	2
85	F	0.3	2	1	LM	2.5	46	F				MET	2
73	F	0.22	3	1	LM	2	60	F				MET	4
65	F	0.28	2	1	LM	1	64	F				MET	3.5
45	F	0.2	1	1	LM	1	10	F				MET	2
69	M	0	1	1	LM	3	52	M				MET	3
82	M	0	1	1	LM	3	62	M				MET	4
70	M	0.85	3	1	LM	4	68	M				MET	3
77	M	1.4	3	2	LM	2	77	M				MET	3.5
75	M	0.3	2	1	LM	2	51	M				MET	2
71	M	0.43	2	1	LM	3							
68	M	0.56	3	1	LM	4							

CM cases are presented in groups of different histological classes (SSM: superficial spreading melanoma; LM: lentigo melanoma; NM nodular melanoma; MET metastasis). Age, sex, Clark stage and Breslow index, AJCC scores and PAR staining scores are shown.

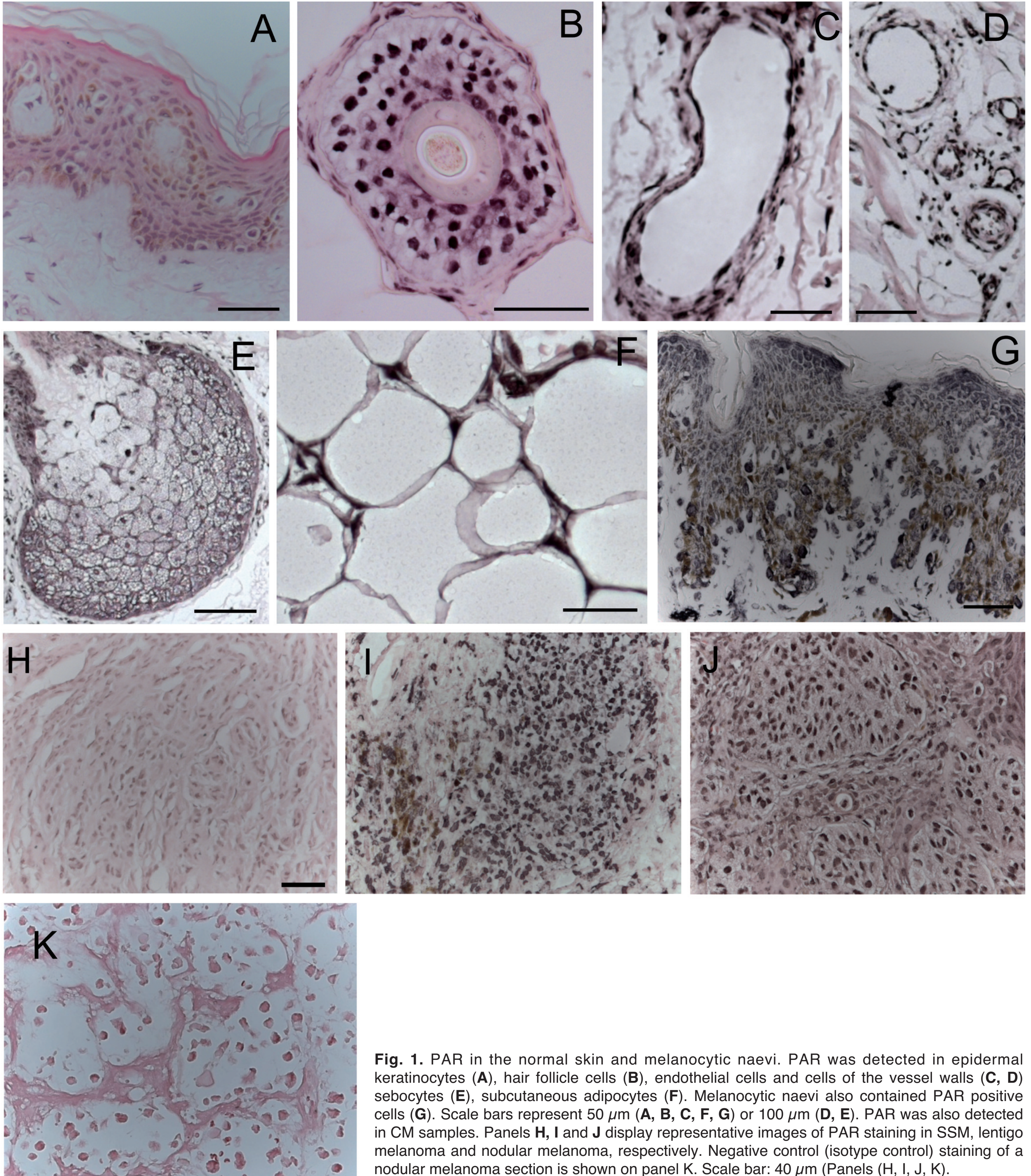

Fig. 1. PAR in the normal skin and melanocytic naevi. PAR was detected in epidermal keratinocytes (**A**), hair follicle cells (**B**), endothelial cells and cells of the vessel walls (**C, D**) sebocytes (**E**), subcutaneous adipocytes (**F**). Melanocytic naevi also contained PAR positive cells (**G**). Scale bars represent 50 μm (**A, B, C, F, G**) or 100 μm (**D, E**). PAR was also detected in CM samples. Panels **H, I** and **J** display representative images of PAR staining in SSM, lentigo melanoma and nodular melanoma, respectively. Negative control (isotype control) staining of a nodular melanoma section is shown on panel K. Scale bar: 40 μm (Panels (H, I, J, K).

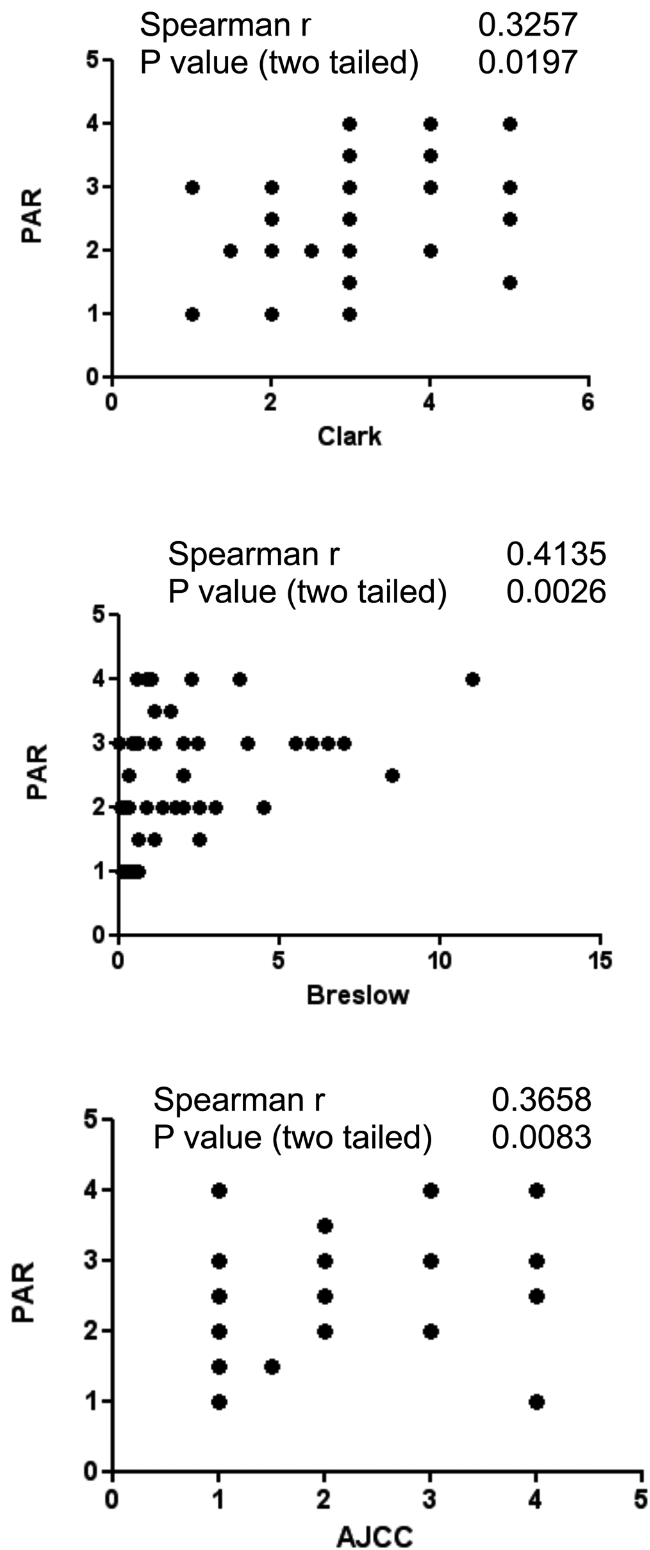

Fig. 2. Statistical evaluation of the PAR staining. PAR staining scores were correlated with Clark, Breslow and AJCC scores. For the correlation of these indices and PAR staining, the Spearman's rank correlation coefficient was calculated which revealed a statistically significant correlation (p values are shown on the figure).

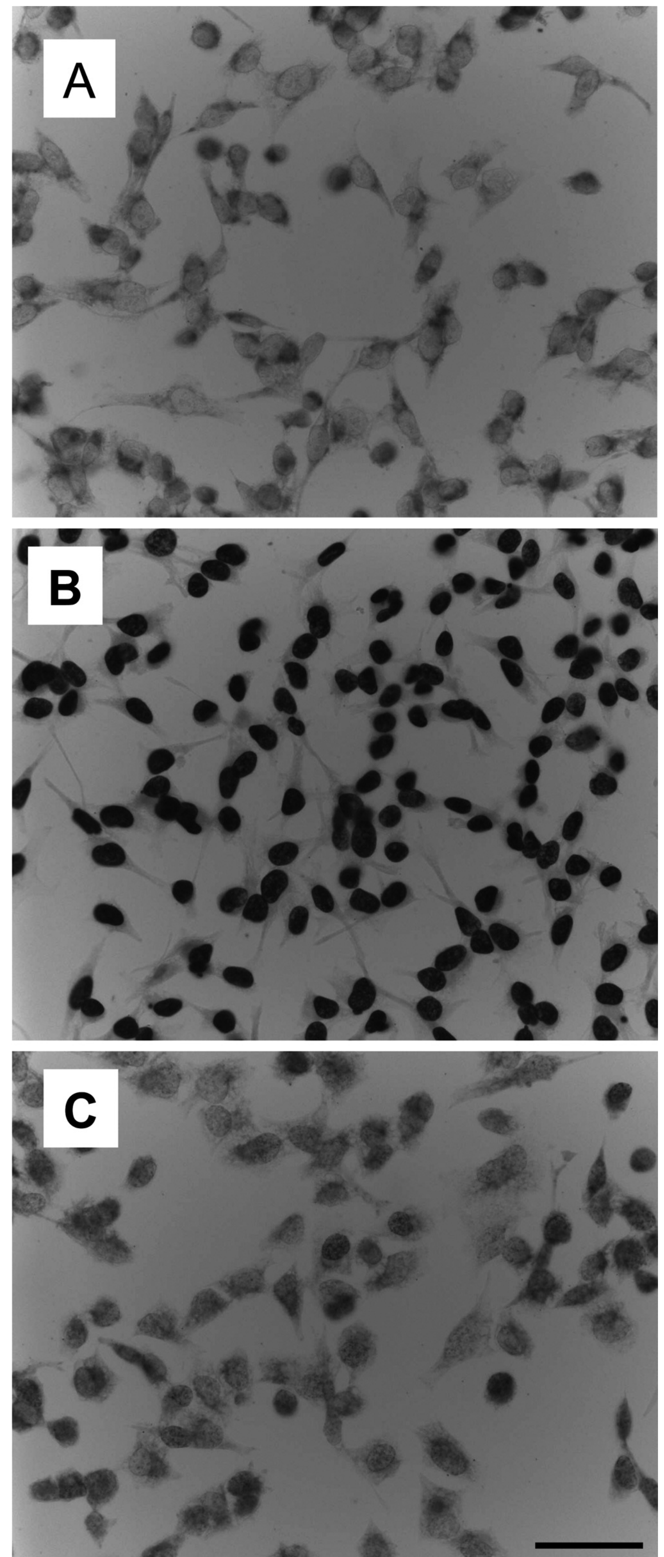

Fig. 3. PAR staining in cultured melanoma cells. In order to demonstrate the specificity of the PAR staining with the 10H antibody, we stained control **(A)**, and H_2O_2-treated (5 min, 400 μM) (**B, C**) melanoma cells with the anti-PAR specific 10H antibody. Melanoma cells on panel C were also pretreated with the PARP inhibitor PJ34 (30 min, 10 μM). Scale bar: 30 μm

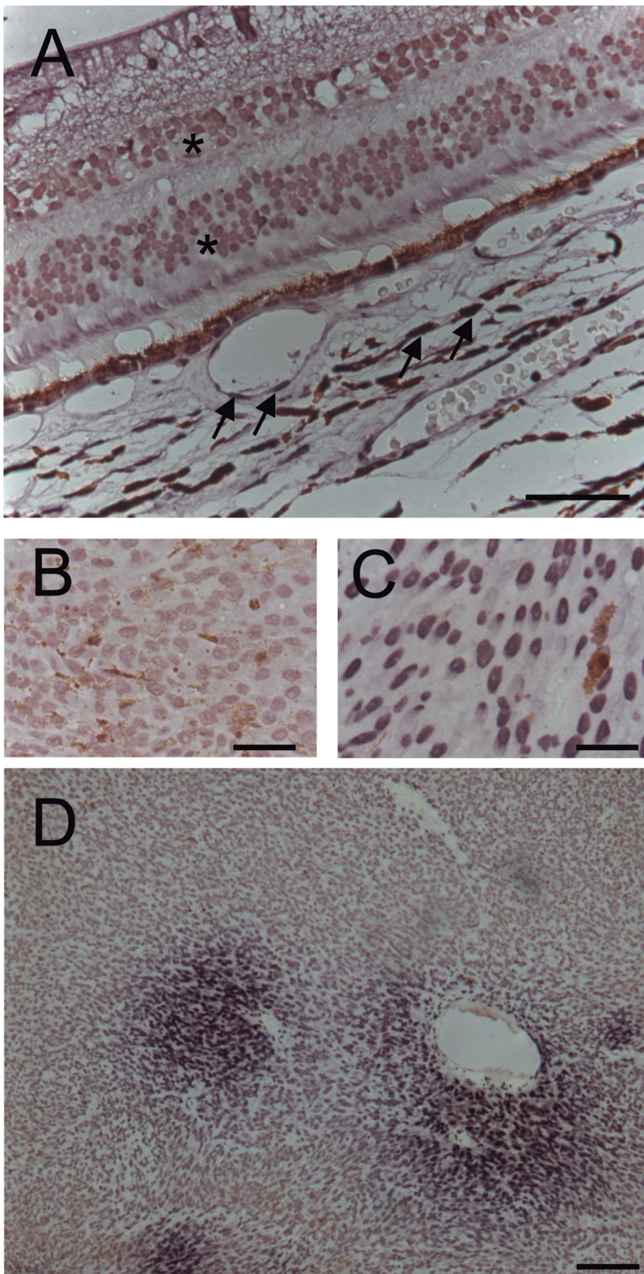

Fig. 4. PAR staining in the retina and in uveal melanomas. PAR was detected in the nuclear layers of the retina (A; stars) and strong immunopositivity could be seen around blood vessels of the choroid (**A**; arrows). In UM tumors (**B and C**) PAR positivity varied in intensity with perivascular regions often showing more intense staining than other tumor areas (**D**). Scale bars: A, 100 μm; B, C, 15 μm; D, 200 μm

inhibitor PJ34 (10 μM) (Fig. 3C).

PAR formation in uveal melanoma

In the unaffected (normal) area of the eye, nuclear PAR staining was detected in the nuclear layers of the retina (Fig. 4) and around blood vessels in the choroid (A). By comparison, PAR staining varied in location within the tumors (Fig. 4B,C). In some samples, staining was most intense around the blood vessels (Fig. 4D). Although we observed clear differences in PAR staining between individual tumors, we were unable to correlate PAR immunopositivity with the grade of the tumors due to the low number of cases in the study.

Table 2. Summary of PAR expression in UM patients.

Age	Histopathologic cell type	Extraocular extension	PAR staining
64	spindle	no	1
55	mixed	no	2
55	epitheloid	scleral infiltration	2
71	epitheloid	opticus infiltration	2
76	spindle	no	4
46	spindle	no	4
53	mixed	scleral infiltration	3
46	mixed	vortex vein	3
39	spindle	no	3
81	spindle	scleral infiltration	3
56	epitheloid	no	3
54	mixed	no	2

Discussion

Our present study provides strong evidence that poly(ADP-ribose) is synthesized in the normal human skin and can also be detected in CM and UM samples.

In normal skin, PAR positive cells were found in the epidermis. Keratinocytes that represent the majority of cells in the epidermis have already been described as expressing PARP-1 (Ross et al., 1998; Szabo et al., 2001). Moreover, several groups have reported a dominant expression of PARP-1 in cultured primary keratinocytes or HaCaT cells (Malanga and Althaus, 1994; Szabo et al., 2001; Bakondi et al., 2002, 2003, 2004; Pachernik et al., 2002; Cals-Grierson and Ormerod, 2004). In keratinocytes, PARP-1 activation has been linked to inflammatory skin diseases and sunburn (Szabo et al., 2001; Farkas et al., 2002; Bakondi et al., 2004; Bai et al., 2009). PARP1 has also been shown to regulate the expression of proinflammatory cytokines and chemokines (IL-1, TNFa, MIP-1a, MIP-2, MCP-1, etc.) (Szabo et al., 2001; Bakondi et al., 2003; Cals-Grierson and Ormerod, 2004,; Bai et al., 2009) and oxidative stress-induced cell death (Bakondi et al., 2003, 2004). Our current study indicates that PAR may also serve as a signaling molecule in keratinocytes as the polymer could be detected in keratinocytes in the healthy skin. However, whether PAR synthesis in keratinocytes is induced by DNA breaks (e.g. generated by topoisomerases) or by DNA break-independent processes, requires further investigation. Intense PAR staining was found in hair follicle cells. This may be due to the rapid cycling of cells as PARylation has been implicated in the regulation of proliferation (Virag and

Szabo, 2002).

We also detected PAR polymer in sebocytes and adipocytes suggesting a new role for PAR in these lipid accumulating cell types. The role of PARP-1 in adipocytes has already been proposed (Janssen and Hilz, 1989; Hsu and Yen, 2006), however, its presence in mature adipocytes has not yet been reported. PARP-1 has been shown to interact with the retinoid X-receptor (RXR) in the RXR - peroxisome proliferator activated receptor γ (PPARγ) nuclear receptor heterodimer (Miyamoto et al., 1999) that controls sebocyte lipid accumulation during sebocyte differentiation (Rosenfield et al., 1999; Kim et al., 2001). Recent data suggests that recruitment of PARP-1 to active nuclear receptors is initiated by DNA strand breaks during receptor activation (Ju et al., 2006) thus possibly accounting for the presence of PAR in these cells.

PARP-1 expression has previously been detected in human melanomas with correlation established between the intensity of expression and the clinical stage of the tumor (Staibano et al., 2005). However, PAR content may reflect the intensity of cellular PAR metabolism better than PARP-1 expression. Therefore, our findings that PAR content correlates with the Breslow index, Clark stage and AJCC score of the tumor may have therapeutic implications in patients treated with PARP inhibitors as adjuvant chemotherapeutic agents.

The question is what may cause PAR accumulation in melanoma cells. Melanoma cells have a higher cell division rate than the normal surrounding tissue. We have previously reported increased PARP activity in mitotic cells (Bakondi et al., 2002) which may help explain PAR synthesis in melanoma cells. However, the intensity of PAR staining was rather uniform in the CM specimens even though most cells are obviously not in mitosis suggesting that PAR synthesis may not be mitosis-related in melanoma cells. However, increased oxidative stress has also been reported in both CM (Sander et al., 2003) and UM (Blasi et al., 1999) leading to an increased risk of DNA damage that in turn may lead to PARP activation. PAR synthesis may also be related to tumor oxygenation as indicated by intense PAR staining around the tumor blood vessels in UM sections. In tumor tissues, *de novo* angiogenesis is important for providing nutrient and oxygen supply for tumors. Angiogenesis requires the concerted expression of VEGF and HIF-1 with the transcription of both of these factors requiring PARP-1 (Obrosova et al., 2004; Martin-Oliva et al., 2006,).

Our finding that the level of correlation between PAR levels and clinical scores was more pronounced in females than in males raises new questions. Gender has been reported to be an etiological and prognostic factor in melanoma and was found to affect various other skin diseases as well (Dao and Kazin, 2007). Furthermore, gender differences in the role of PARylation have previously been reported in animal models of stroke and endotoxin shock (Hagberg et al., 2004; McCullough, 2005; Mabley et al., 2005) and were proposed to be due to the protective effect of estrogen and to distinct sex-based cell death programs (Mabley et al., 2005; Szabó et al., 2006; Yuan et al., 2009). As several questions including the pathways leading to PAR production in melanoma and the role of PARylation in melanoma cell death are not precisely understood, the implications and significance of this finding requires further investigation.

Since inhibition of PARP-1 impairs base excision repair, targeting PAR metabolism has become a therapeutic opportunity in the treatment of CM (Tentori et al., 2005; Plummer et al., 2008; Fong et al., 2009). Adjuvant chemotherapy using PARP inhibitors (e.g. in combination with temozolomide) for the treatment of solid tumors is currently in different phases of clinical trials (Fong et al., 2009). Our results demonstrating individual differences in PAR content in patients with different stages of melanoma suggest that CM patients should be carefully selected for PARP inhibitor treatment.

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References

Ahmed I. (1997). Malignant melanoma: prognostic indicators. Mayo Clin. Proc. 72, 356-361.

Ame J.C., Spenlehauer C. and de Murcia G. (2004). The PARP superfamily. Bioessays 26, 882-893.

Bai P., Hegedus C., Szabo E., Gyure L., Bakondi E., Brunyanszki A., Gergely S., Szabo C. and Virag L. (2009). Poly(ADP-ribose) polymerase mediates inflammation in a mouse model of contact hypersensitivity. J. Invest. Dermatol. 129, 234-238.

Bakondi E., Bai P., Szabo E., Hunyadi J., Gergely P., Szabo C. and Virag L. (2002). Detection of poly(ADP-ribose) polymerase activation in oxidatively stressed cells and tissues using biotinylated NAD substrate. J. Histochem. Cytochem. 50, 91-98.

Bakondi E., Gonczi M., Szabo E., Bai P., Pacher P., Gergely P., Kovacs L., Hunyadi J., Szabo C., Csernoch L. and Virag L. (2003). Role of intracellular calcium mobilization and cell-density-dependent signaling in oxidative-stress-induced cytotoxicity in HaCaT keratinocytes. J. Invest. Dermatol. 121, 88-95.

Bakondi E., Bai P., Erdelyi K., Szabo C., Gergely P. and Virag L. (2004). Cytoprotective effect of gallotannin in oxidatively stressed HaCaT keratinocytes: the role of poly(ADP-ribose) metabolism. Exp. Dermatol. 13, 170-178.

Bakos L., Wagner M., Bakos R.M., Leite C.S., Sperhacke C.L., Dzekaniak K.S. and Gleisner A.L. (2002). Sunburn, sunscreens, and phenotypes: some risk factors for cutaneous melanoma in southern Brazil. Int. J. Dermatol. 41, 557-562.

Blasi M.A., Maresca V., Roccella M., Roccella F., Sansolini T., Grammatico P., Balestrazzi E. and Picardo M. (1999). Antioxidant

pattern in uveal melanocytes and melanoma cell cultures. Invest. Ophthalmol. Vis. Sci. 40, 3012-3016.

Cals-Grierson M.M. and Ormerod A.D. (2004). Nitric oxide function in the skin. Nitric Oxide 10, 179-193.

Chalmers A.J. (2009). The potential role and application of PARP inhibitors in cancer treatment. Br. Med. Bull. 89, 23-40.

Dao H. Jr and Kazin R.A. (2007) Gender differences in skin: a review of the literature. Gend. Med. 4, 308-328.

De Murcia G. and Menissier de Murcia J. (1994). Poly(ADP-ribose) polymerase: a molecular nick-sensor. Trends. Biochem. Sci. 19, 172-176.

Erdelyi K., Bakondi E., Gergely P., Szabo C. and Virag L. (2005). Pathophysiologic role of oxidative stress-induced poly(ADP-ribose) polymerase-1 activation: focus on cell death and transcriptional regulation. Cell. Mol. Life Sci. 62, 751-759.

Erdelyi K., Bai P., Kovacs I., Szabo E., Mocsar G., Kakuk A., Szabo C., Gergely P. and Virag L. (2009). Dual role of poly(ADP-ribose) glycohydrolase in the regulation of cell death in oxidatively stressed A549 cells. FASEB J. 23, 3553-3563.

Farkas B., Magyarlaki M., Csete B., Nemeth J., Rabloczky G., Bernath S., Literati N.P. and Sumegi B. (2002). Reduction of acute photodamage in skin by topical application of a novel PARP inhibitor. Biochem. Pharmacol. 63, 921-932.

Fong P.C., Boss D.S., Yap T.A., Tutt A., Wu P., Mergui-Roelvink M., Mortimer P., Swaisland H., Lau A., O'Connor M.J., Ashworth A., Carmichael J., Kaye S.B., Schellens J.H. and de Bono J.S. (2009). Inhibition of poly(ADP-ribose) polymerase in tumors from BRCA mutation carriers. N. Engl. J. Med. 361, 123-134.

Garbe C., Buttner P., Bertz J., Burg G., d'Hoedt B., Drepper H., Guggenmoos-Holzmann I., Lechner W., Lippold A., Orfanos C.E., Peters A., Rassner G., Stadler R. and Stroebel W. (1995). Primary cutaneous melanoma. Identification of prognostic groups and estimation of individual prognosis for 5093 patients. Cancer 75, 2484-2491.

Guerry D.T. and Schuchter L.M. (1992). Disseminated melanoma--is there a new standard therapy? N. Engl. J. Med. 327, 560-561.

Hagberg H., Wilson M.A., Matsushita H., Zhu C., Lange M., Gustavsson M., Poitras M.F., Dawson T.M., Dawson V.L., Northington F. and Johnston M.V. (2004) PARP-1 gene disruption in mice preferentially protects males from perinatal brain injury. J. Neurochem. 90, 1068-75.

Hassa P.O. and Hottiger M.O. (2008). The diverse biological roles of mammalian PARPs, a small but powerful family of poly-ADP-ribose polymerases. Front. Biosci. 13, 3046-3082.

Hedges T. and Scriven A. (2008). Sun safety: what are the health messages? J. R. Soc. Promot. Health 128, 164-169.

Hsu C.L. and Yen G.C. (2006). Induction of cell apoptosis in 3T3-L1 pre-adipocytes by flavonoids is associated with their antioxidant activity. Mol. Nutr. Food Res. 50, 1072-1079.

Jackson A., Wilkinson C., Hood K. and Pill R. (2000). Does experience predict knowledge and behavior with respect to cutaneous melanoma, moles, and sun exposure? Possible outcome measures. Behav. Med. 26, 74-79.

Janssen O.E. and Hilz H. (1989). Differentiation of 3T3-L1 pre-adipocytes induced by inhibitors of poly(ADP-ribose) polymerase and by related noninhibitory acids. Eur. J. Biochem. 180, 595-602.

Ju B.G., Lunyak V.V., Perissi V., Garcia-Bassets I., Rose D.W., Glass C.K. and Rosenfeld M.G. (2006). A topoisomerase IIbeta-mediated dsDNA break required for regulated transcription. Science 312, 1798-1802.

Kasper B., D'Hondt V., Vereecken P. and Awada A. (2007). Novel treatment strategies for malignant melanoma: a new beginning? Crit. Rev. Oncol. Hematol. 62, 16-22.

Kim M.J., Deplewski D., Ciletti N., Michel S., Reichert U. and Rosenfield R.L. (2001). Limited cooperation between peroxisome proliferator-activated receptors and retinoid X receptor agonists in sebocyte growth and development. Mol. Genet. Metab. 74, 362-369.

Lawson D.D., Moore D.H. 2nd, Schneider J.S. and Sagebiel R.W. (1994). Nevus counting as a risk factor for melanoma: comparison of self-count with count by physician. J. Am. Acad. Dermatol. 31, 438-444.

Mabley J.G., Horváth E.M., Murthy K.G., Zsengellér Z., Vaslin A., Benko R., Kollai M. and Szabó C. (2005) Gender differences in the endotoxin-induced inflammatory and vascular responses: potential role of poly(ADP-ribose) polymerase activation. J. Pharmacol. Exp. Ther. 315, 812-820.

Malanga M. and Althaus F.R. (1994). Poly(ADP-ribose) molecules formed during DNA repair in vivo. J. Biol. Chem. 269, 17691-17696.

Marghoob A.A., Slade J., Kopf A.W., Salopek T.G., Rigel D.S. and Bart R.S. (1996). Risk of developing multiple primary cutaneous melanomas in patients with the classic atypical-mole syndrome: a case-control study. Br. J. Dermatol. 135, 704-711.

Martin-Oliva D., Aguilar-Quesada R., O'Valle F., Munoz-Gamez J.A., Martinez-Romero R., Garcia Del Moral R., Ruiz de Almodovar J.M., Villuendas R., Piris M.A. and Oliver F.J. (2006). Inhibition of poly(ADP-ribose) polymerase modulates tumor-related gene expression, including hypoxia-inducible factor-1 activation, during skin carcinogenesis. Cancer Res. 66, 5744-5756.

McCullough L.D., Zeng Z., Blizzard K.K., Debchoudhury I. and Hurn P.D. (2005). Ischemic nitric oxide and poly (ADP-ribose) polymerase-1 in cerebral ischemia: male toxicity, female protection. J. Cereb. Blood Flow. Metab. 25, 502-512.

Miyamoto T., Kakizawa T. and Hashizume K. (1999). Inhibition of nuclear receptor signalling by poly(ADP-ribose) polymerase. Mol. Cell. Biol. 19, 2644-2649.

Obrosova I.G., Minchenko A.G., Frank R.N., Seigel G.M., Zsengeller Z., Pacher P., Stevens M.J. and Szabo C. (2004). Poly(ADP-ribose) polymerase inhibitors counteract diabetes- and hypoxia-induced retinal vascular endothelial growth factor overexpression. Int. J. Mol. Med. 14, 55-64.

Pachernik J., Hampl A., Soucek K., Kovarikova M., Andrysik Z., Hofmanova J. and Kozubik A. (2002). Multiple biological effects of inhibitors of arachidonic acid metabolism on human keratinocytes. Arch. Dermatol. Res. 293, 626-633.

Plummer R., Jones C., Middleton M., Wilson R., Evans J., Olsen A., Curtin N., Boddy A., McHugh P., Newell D., Harris A., Johnson P., Steinfeldt H., Dewji R., Wang D., Robson L. and Calvert H. (2008). Phase I study of the poly(ADP-ribose) polymerase inhibitor, AG014699, in combination with temozolomide in patients with advanced solid tumors. Clin. Cancer Res. 14, 7917-7923.

Rosenfield R.L., Kentsis A., Deplewski D. and Ciletti N. (1999). Rat preputial sebocyte differentiation involves peroxisome proliferator-activated receptors. J. Invest. Dermatol. 112, 226-232.

Ross R., Gillitzer C., Kleinz R., Schwing J., Kleinert H., Forstermann U. and Reske-Kunz A.B. (1998). Involvement of NO in contact hypersensitivity. Int. Immunol. 10, 61-69.

Sander C.S., Hamm F., Elsner P. and Thiele J.J. (2003). Oxidative stress in malignant melanoma and non-melanoma skin cancer. Br. J.

Dermatol. 148, 913-922.
Sato T., Han F. and Yamamoto A. (2008). The biology and management of uveal melanoma. Curr. Oncol. Rep. 10, 431-438.
Schadendorf D. (2002). Is there a standard for the palliative treatment of melanoma? Onkologie 25, 74-76.
Schreiber V., Dantzer F., Ame J.C. and de Murcia G. (2006). Poly(ADP-ribose): novel functions for an old molecule. Nat. Rev. Mol. Cell Biol. 7, 517-528.
Singh A.D., Rennie I.G., Seregard S., Giblin M. and McKenzie J. (2004). Sunlight exposure and pathogenesis of uveal melanoma. Surv. Ophthalmol. 49, 419-428.
Staibano S., Pepe S., Lo Muzio L., Somma P., Mascolo M., Argenziano G., Scalvenzi M., Salvatore G., Fabbrocini G., Molea G., Bianco A.R., Carlomagno C. and De Rosa G. (2005). Poly(adenosine diphosphate-ribose) polymerase 1 expression in malignant melanomas from photoexposed areas of the head and neck region. Hum. Pathol. 36, 724-731.
Szabo C., Pacher P., Swanson R.A. (2006) Novel modulators of poly(ADP-ribose) polymerase. Trends Pharmacol. Sci. 27, 626-630.
Szabo E., Virag L., Bakondi E., Gyure L., Hasko G., Bai P., Hunyadi J., Gergely P. and Szabo C. (2001). Peroxynitrite production, DNA breakage, and poly(ADP-ribose) polymerase activation in a mouse model of oxazolone-induced contact hypersensitivity. J. Invest. Dermatol. 117, 74-80.
Tentori L., Leonetti C., Scarsella M., D'Amati G., Vergati M., Portarena I., Xu W., Kalish V., Zupi G., Zhang J. and Graziani G. (2003). Systemic administration of GPI 15427, a novel poly(ADP-ribose) polymerase-1 inhibitor, increases the antitumor activity of temozolomide against intracranial melanoma, glioma, lymphoma. Clin. Cancer Res. 9, 5370-5379.
Tentori L., Leonetti C., Scarsella M., Muzi A., Vergati M., Forini O., Lacal P.M., Ruffini F., Gold B., Li W., Zhang J. and Graziani G. (2005). Poly(ADP-ribose) glycohydrolase inhibitor as chemosensitiser of malignant melanoma for temozolomide. Eur. J. Cancer 41, 2948-2957.
Virag L. and Szabo C. (2002). The therapeutic potential of poly(ADP-ribose) polymerase inhibitors. Pharmacol. Rev. 54, 375-429.
Yuan M., Siegel C., Zeng Z., Li J., Liu F. and McCullough L.D. (2009). Sex differences in the response to activation of the poly (ADP-ribose) polymerase pathway after experimental stroke. Exp Neurol. 217, 210-218.

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