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Original Article

Melanomas suppress lipid peroxidation in host mice

Jan Borovansky[1], Jirina Crkovska[2], Zuzana Schwippelova[3], Stanislav Stipek[2]

[1]Institute of Biochemistry and Experimental Oncology,
[2]Institute of Medical Biochemistry and Laboratory Diagnostics, 1st Medical Faculty;
[3]Institute of Medical Chemistry and Biochemistry, 2nd Medical Faculty, Charles University, Prague, Czech Republic

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Corresponding Author
Jan Borovansky
Institute of Biochemistry and Experimental Oncology,
1st Faculty of Medicine,
Charles University, U nemocnice 5, 128 53 Prague 2, Czech Republic.
jborov@lf1.cuni.cz

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Free radical balance; Lipid peroxidation; Melanoma; Oxidative stress; Vitamin E

Abstract
Tumor growth can often induce signs of oxidative stress in host organism. To assess the situation as for melanoma, the oxidative stress markers (specific malondialdehyde-thiobarbituric acid complexes: MDA-TBA; and less specific thiobarbituric acid reactive substances: TBARS) were measured in sera, liver and tumors of B16- and Cloudman S91- bearing mice and compared to those of control animals. The MDA-TBA levels (unlike TBARS) in the sera and liver of melanoma-bearing mice were significantly lower compared to controls. In addition, a significantly higher concentration of vitamin E was found in the blood and liver of both melanoma models compared to controls. Contrary to expectation, it appears that melanoma-bearing mice are able to suppress the level of lipid peroxidation. The free radical balance in melanoma-bearing hosts is unique and differs from other tumor types. This should be taken into consideration when designing a human melanoma therapy.

INTRODUCTION

Tumor cells can exhibit increased intrinsic oxidative stress due in part to oncogenic stimulation, increased metabolic activity and mitochondrial malfunction [1, 2]. Persistent oxidative stress in tumor cells induces free radical damage of lipids, carbohydrates, proteins, amino acids and nucleic acids [3]. The modified molecules and/or their degradation products may serve as surrogate markers of oxidative stress. These substances have been tested as markers of tumor burden and/or the efficiency of tumor therapy. From the long list of such markers much attention has been paid to the malondialdehyde (MDA), a product of lipid peroxidation, which forms color product with thiobarbituric acid (TBA) that is easy to determine [3] as TBARS (TBA-reactive substances) or as more specific MDA-TBA adducts.

Melanoma differs from all other tumors by a special differentiation, *i.e.* by an ability to produce melanins in the process of melanogenesis during which reactive species including radicals but also antioxidants are produced and radicals scavenged [4, 5]. Hence, the free radical balance in melanoma cells and in melanoma-bearing hosts is quite complex [5, 6] (see also Table 1).

Increased levels of TBARS have been reported in the plasma of melanoma patients [7], in the liver of B16 melanoma-bearing mice [8] and confirmed in the sera, brain, liver and lungs [9] of the same melanoma model. On the other side of the equation, Sander *et al* [10] demonstrated increased expression and activity of antioxidant enzymes in human melanoma compared to basal cell- and squamous cell-carcinomas, and another study [11] postulated that the antioxidant defence of seven human melanoma cell lines was sufficient to prevent oxidative stress, in contrast to cell lines from five other human tumors.

To solve the discrepancy mentioned above, the free radical balance was assessed by measurements of TBA-reactive material and vitamin E levels in standard melanoma models. We measured both MDA-TBA

complexes devoid of intefering substances and TBARS levels in sera, liver and tumors of melanoma-bearing mice. Since we focused to manifestations of lipid peroxidation, the process intimately associated with membranes, the levels of vitamin E as a representative of hydrophobic antioxidants and the major lipid-soluble chain-breaking compound in vertebrates associated with melanoma growth were also monitored.

MATERIAL AND METHODS

Animal experiments were approved by the Ethics Committee of the 1st Medical Faculty, Charles University in Prague and were confirmed to comply with the article 18a , paragraph 2b of the Act No. 246/1992 of the Czech Republic. B16 and Cloudman S91 melanomas were grown intraperitoneally in approx. 10 week old female inbred C57BL/6J and DBA2 mice, respectively. Mice were supplied by VELAZ, Lysolaje, Czech Republic and were maintained in a temperature and light controlled environment with free access to tap water and standard pellet food *ad libitum*. Blood, liver and tumor tissue were obtained from animals euthanised in ether anesthesia by decapitation.

TBARS were measured spectrophotometrically at 532 nm by the method of Yagi [12]. Concentration of the TBA-MDA complex was determined spectrophotometrically at 532 nm [13] after its HPLC separation on a Separon column (SGX C18, 7 μm, 4 x 250 mm, TESSEK, Czech Republic); mobile phase: methanol/phosphate buffer pH 6.3, 40:60 (v/v) as recommended by Peuchant *et al* [14]. The concentration of proteins was determined by the method of Bradford [15].

Vitamin E concentration was analyzed after its extraction from sera and tissues by the method of Bucher and Roberts [16] by using isocratic HPLC as suggested by Bui [17]**.** Technical details: HPLC Shimadzu LC-9A, integrator Shimadzu CR-5A Chromatopac, column Separon SGX C18, 5 μm, 3 x 150 mm (TESSEK, Czech Republic); mobile phase: acetonitrile/tetrahydrofurane/methanol 680:220:70, UV detection at 290 nm.

Statistical analysis was performed using Student´s t-test. Significance was accepted at $P < 0.05$.

RESULTS

Specific MDA measurements revealed that the MDA-TBA adduct levels in the sera of melanoma-bearing mice were significantly lower than those found in control animals. The MDA-TBA levels in the liver of melanoma hosts were also lower (Fig.1).

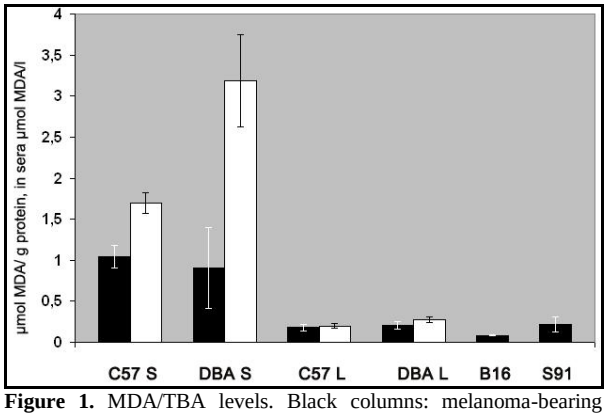

Figure 1. MDA/TBA levels. Black columns: melanoma-bearing mice; white columns: control animals. C57 S = sera of C57BL mice; DBA S = sera of DBA2 mice; C57 L = liver of C57 mice; DBA L = liver of DBA2 mice; B16 = B16 melanoma; S91 = Cloudman S91 melanoma. The results are expressed as x + SD.

There was no significant increase of less specific TBARS in sera ($P = 0.62$) and liver ($P = 0.57$) of B16 melanoma-bearing mice in early phase of the disease; tumor weight < 5% of the body weight. The TBARS measurements showed a significant increase in the sera ($P = 0.02$) and in the liver ($P = 0.04$) of B16 melanoma-bearing mice in advanced stage of the disease; melanoma weight > 10% body weight (not shown) in accord with previous reports [8, 9]. This is not an unexpected result, because the colour reaction with thiobarbituric acid is not specific for malondialdehyde and many other compounds are also TBA positive [3]. Sialic acid is one of these interfering substances [3, 18] and an increase of serum sialic acid during the tumor progression has been demonstrated both in melanoma patients [19] and in melanoma-bearing animals [20]. In addition, a special sialoform of gamma-glutamyltransferase (GGT) released by B16 and S91 melanoma into the blood stream [21] can contribute to the increase of non-specific TBARS in the sera of melanoma-bearing mice.

Tumor tissue MDA-TBA concentration in B16 melanoma was lower than that in S91 melanoma (Fig.1), possibly due to the scavenging properties of eumelanin in a more pigmented tumor type [4, 22-24].

Serum and liver concentrations of vitamin E in B16 melanoma-bearing mice and in S91 melanoma-mice (Fig.2) were significantly higher compared to control animals. Similarly, elevated levels of vitamin E have been noted in melanoma cells [25, 26] in other studies.

DISCUSSION

Transplantable pigmented B16 melanoma and hypopigmented Cloudman S91 melanoma have been used as excellent standard models of human melanoma for more than six decades [27].

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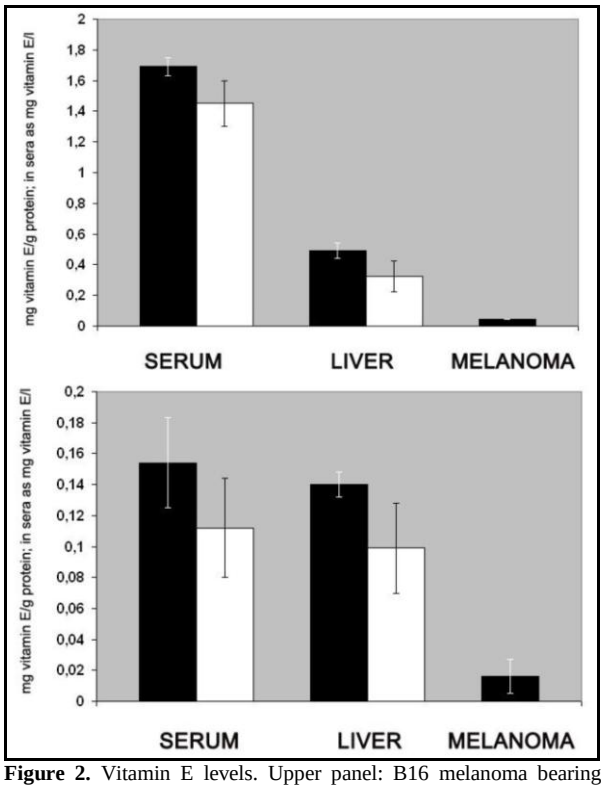

Figure 2. Vitamin E levels. Upper panel: B16 melanoma bearing mice; black columns: melanoma-bearing mice; white columns: control animals. Lower panel: Cloudman S91 melanoma bearing mice; black columns: melanoma-bearing mice; white columns: control animals. The results are expressed as x + SD.

Our data indicate that, in the model systems examined, unlike other non-melanoma tumor models [1, 2, 28], the presence of tumor cells results in a reduction of levels of lipid peroxidation in the host animals. This phenomenon appears to be confined to melanoma-bearing animals and suggests that the suppressive mechanism is associated with the melanin content, which is consistent with the differences we observed between B16 and S91 melanomas.

It is important to emphasise that the data relate specifically to lipid peroxidation and do not necessarily imply any general diminution in free radical processes in melanoma-bearing hosts. A challenge for novel anti-melanoma therapeutic strategies is to adjust the metabolic balance towards radical-induced apoptotic signalling [2, 26]. Some suitable agents have already been tested [5, 28, 29]. Thiostrepton has been found very promising because it selectively impairs viability of melanoma cells in contrast to melanocytes [30].

Table 1 summarizes the complex system of production of pro- and antioxidant species during the process of melanogenesis [5, 6, 24, 25, 31-33] specific to melanoma cells. Tyrosinase catalyses synthesis of reactive quinones and semiquinones associated with reactive oxygen species (ROS) formation [4, 5, 31]. Tyrosinase reaction produces also diphenols acting as inhibitors of lipid peroxidation (*e.g.* 5,6-dihydroxy-indole was shown to be as potent as α-tocopherol [32]). Eumelanin as pseudosuperoxide dismutase produces hydrogen peroxide (H_2O_2) [22]. Melanins belong to the group of stable free radicals [4, 5, 29]. Hence, particularly eumelanin can act as a sink for diffusible free radicals [4, 5, 24]. Carboxylic groups of melanin can bind redox active metals and diminish the synthesis of free radicals [4, 5]. A recent study demonstrated that the presence of melanin in melanoma cells prevented the generation of mitochondrial damage by H_2O_2 as an oxidative stressor [34]. Upon irradiation eumelanin and particularly pheomelanin become strong radical producers with fatal consequences for the cell [4, 5, 23]. Pheomelanogenesis requires cysteine as a substrate which can deplete the cell of cysteine and glutathione (GSH) [5, 6].

Although the synthesis of potentially toxic melanin precursors is strictly compartmentalized into melanosomes, the occurrence of aberrant melanosomes in melanoma cells, often with membrane defects [5, 8, 29], does not prevent leakage of toxic species into other compartments [5, 8] exposing melanoma cells to radical and toxic species attack in relation to the extent of melanosomal membrane damage. Pigment cells defend themselves by physiological scavenging mechanisms. When their capacity is overcome, pathological reactions ensue [5, 8]. The possibility of amplifying the generation of toxic melanogenic intermediates has long been viewed as a basis for rational approach to melanoma therapy [5, 29].

Table 1. Free radical situation in pigment cells

	Pro-oxidant action	**Antioxidant action**
Tyrosinase	catalyzes synthesis of (semi)quinones and reactive oxygen species [4, 5, 31]	produces diphenols [32]; consumes superoxide radicals [33]
Eumelanin	as pseudosuperoxide dismutase produces hydrogen peroxide [22]; producer of free radicals [4, 5, 23]	scavenger of free radicals [4, 5, 24, 29]; binds redox active metals [4, 5]; prevents mitochondrial DNA damage [34]
Pheomelanin	high synthesis can deplete the cell of cysteine and glutathione [6]; strong producer of radicals [4, 5, 23]	

Free radical situation in melanoma cells is unique [4-6, 10] and different from other tumor cells [1-3, 10, 11]. The inhibition of lipid peroxidation *in vivo* described above corresponds to biochemical experiments of Bustamante *et al* [22] *in vitro*: Both spontaneous and 2,2-azobis/2-amidinopropan-induced lipid peroxidation of rat liver homogenate could be inhibited by the addition of synthetic or from tumor isolated melanins to the reaction mixture.

The present observed changes induced by melanoma growth are also in accord with a recent concept that tumors as organs can interface with the entire organism [35].

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COMPETING INTERESTS

The authors declare that they have no conflict of interests.

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