Pathology - Research and Practice 215 (2019) 21–28

Contents lists available at ScienceDirect

Pathology - Research and Practice

journal homepage: www.elsevier.com/locate/prp

The role of oxidative stress in the biology of melanoma: A systematic review

Serafinella Patrizia Cannavò[a], Alessandro Tonacci[b], Lucrezia Bertino[a], Marco Casciaro[c], Francesco Borgia[a], Sebastiano Gangemi[c,*]

[a] Section of Dermatology, Department of Clinical and Experimental Medicine, University of Messina, 98125, Messina, Italy
[b] Pisa Unit, National Research Council of Italy (CNR), Institute of Clinical Physiology (IFC), 56124 Pisa, Italy
[c] School and Operative Unit of Allergy and Clinical Immunology, Department of Clinical and Experimental Medicine, University of Messina, 98125, Messina, Italy

ARTICLE INFO

Keywords:
Melanoma
Oxidative stress
Reactive oxygen species
Antioxidant system
Skin tumour
Immune system

ABSTRACT

Melanoma is the most aggressive skin tumour, which incidence is rising fast over the year. The metastatic stage of disease is extremely difficult to treat and the mortality rate is still high. Emerging evidence suggested that oxidative stress (OS) is involved in the pathophysiological pathways of several chronic diseases and in the transformation and progression of many common cancers, including melanoma. In particular, it has emerged that OS interacts with inflammatory and immune response, all taking part in the melanomagenic process. In light of the interest shown by the scientific community for this topic, it was analysed the association between melanoma and oxidative stress. A systematic review was performed according to PRISMA guideline employing PubMed database. It identified n = 29 articles which investigated this aspect. Melanoma cells resulted to have adaptive mechanisms to overcome effects of high reactive oxygen species (ROS) levels. Furthermore, OS influences the metastatic ability of melanoma cells and their resistance to therapy. Nonetheless, the included studies were conducted on heterogeneous patient population and with differences in the design of the studies and in the protocols. Therefore, it is mandatory performing further studies which analyze all the aspect of OS pathways: ROS imbalance, its effect to proliferation and metastasis, role of microenvironment, ROS effect to drug resistance. All this in order to understand the role of oxidative stress in the complex biology of melanoma and to provide possibilities of defining new strategy of therapy.

1. Background

Melanoma is the most aggressive type of skin tumour and extremely difficult to treat in the metastatic stage of disease. Its incidence rises fast with an increasing global rate per year at 2–7% annually [1]. Before 2010, the mortality was high due to a less effective therapy [2]. Recent development of target therapies has offered the first improvement in response rate, duration of disease control and overall survival [2,3]. Nonetheless, a great number of patients is still succumbing to metastatic disease, making imperative to continue the research of new therapeutic strategies.

Over the years, the scientific research has suggested that the melanomagenic process is very complex [4]. There is a crosstalk of melanoma cells with inflammatory and immune reactions which significantly influences the biology of melanoma in terms of proliferation, differentiation and progression [5,6]. The inflammatory response induces the recruitment of innate and adaptive immune cells which could indirectly cause oxidative stress (OS) [7]. OS might itself be involved in the melanoma development. Many recent studies have confirmed the important role of ROS in different step of this process. First of all, the melanin synthesis involves redox reaction and accumulation of reactive oxygen species (ROS). Moreover, the involvement of OS is confirmed by

Abbreviations: AGEs, advanced glycation end products; AOPP, advanced oxidation protein products; AOS, antioxidant system; CAT, catalase; GPX, glutathione peroxidase; GSH, glutathione; GCLC, glutamate-L-cysteine ligase catalitic subunit; GSSG, oxidized glutathione; GST, glutathione-S-transferase; GGT, membrane-bound ecto-enzyme gamma-glutamyltransferase; HNE, 4-hydroxy-2-nonenal; H_2O_2, hydrogen peroxide; IDH2, Isocitrate dehydrogenase; LPO, lipid peroxidation; MDA, malondialdehyde; MM, malignant melanoma; NOX, NADPH oxidase; Nrf2, Nuclear factor erythroid-2-related factor; 8 − OHdG, 8-hydroxydeoxyguanosine; pt, patients; OS, oxidative stress; O_2^-, superoxide; ROS, reactive oxygen species; SOD, superoxide dismutase; TBARS, thiobarbituric acid reactive substances; TEM, transendothelial migration; TRP-2, dopachrome tautomerase; TRX, thioredoxin; VEGF, vascular endothelial growth factor

* Corresponding author at: School and Operative Unit of Allergy and Clinical Immunology, Department of Clinical and Experimental Medicine, University of Messina, 98125, Messina, Italy.

E-mail addresses: cannavop@unime.it (S.P. Cannavò), atonacci@ifc.cnr.it (A. Tonacci), bertino.lucrezia@gmail.com (L. Bertino), mcasciaro@unime.it (M. Casciaro), fborgia@unime.it (F. Borgia), gangemis@unime.it (S. Gangemi).

https://doi.org/10.1016/j.prp.2018.11.020
Received 10 October 2018; Received in revised form 22 November 2018; Accepted 23 November 2018

the evidence of several mutated melanoma-associated genes resulting from ROS activity [8,9]. ROS might also be associated with melanoma cell metabolism, hypoxia, metastasis and as seen before in the immune response [7].

OS is defined as an imbalance between the overproduction of ROS, such as superoxide (O_2^-) and hydrogen peroxide (H_2O_2), and the reduction of antioxidant system (AOS) [9]. OS is involved in the pathophysiological pathways of several chronic diseases, such as cardiovascular disease, retinal degeneration, benign prostatic hyperplasia, diabetic disease, asthma, neurodegenerative disease and also skin diseases [10–14]. Moreover, OS plays a role in the transformation and progression of many common cancers, including melanoma [15]. In fact, elevated levels of ROS lead to genomics instability and DNA damage, which have a pro-tumorigenic effect [12,16].

Mitochondria and NADPH oxidase (NOX) are the major source of endogenous ROS. In addition to these, other sources are cyclooxigenases, lipooxygenases and cythocrome P450. On the other hand, in the skin exogenous ROS production could be stimulated by sun exposure and by inflammation, as a result of post inflammatory hyperpigmentation [9]. In particular, UV induce in melanocytes the production of H_2O_2, a reduction of catalase activity and of HO-1 expression [17].

Direct impact of ROS in biological systems, like modification of DNA, lipids and proteins, could be evaluated by the measure of different biomarkers. Malondialdehyde (MDA) and 4-hydroxy-2-nonenal (HNE) are end products of lipid peroxidation, while advanced oxidation protein products (AOPPs) and advanced glycation end products (AGEs) derived from protein oxidation.

There is a natural protective mechanism to prevent oxidative damage and to promote cell survival, known as the antioxidant response, which is achieved by non-enzymatic and enzymatic molecules. The first ones include glutathione (GSH), which belongs to the glutathione system, also involving glutathione reductase, glutathione peroxidase (GPX) and glutathione-s-transferase (GST). The enzymatic ones include superoxide dismutase (SOD), superoxide reductase, catalase (CAT) and thioredoxin (TRX).

Therefore, the aim of this systematic review is to analyse published studies investigating the role of OS on survival, growth and metastasis of melanoma. This systematic review has been registered with PROSPERO (the NIHR International Prospective Register of Systematic Reviews) under the registration number CRD42018096546.

2. Materials and methods

2.1. Research strategy

This systematic review has been conducted according to PRISMA guideline [18] employing PubMed database. On this website, we searched until November 18, 2018 using “melanoma” keyword and four key terms related to oxidative stress (“oxidative stress”, “glycation end products, advanced”, “malondialdehyde”, “advanced oxidation protein products"). Table 1 describes the electronic search strategy. All titles and abstract were initially screened to include only articles that examined the association between melanoma and oxidative stress. The authors read the entire article only if the abstract indicates that the article potentially met the inclusion criteria.

Table 1
Search terms entered into the PubMed search engines for identification of the studies used in this systematic review.

Number	Search terms	Number of studies
1	Melanoma “Mesh terms”	88198
2	Oxidative stress “Mesh terms”	117253
3	Glycation end products, advanced “Mesh terms”	6956
4	Malondialdehyde “Mesh terms”	32146
5	Advanced oxidation protein products “Mesh terms”	274
6	2 OR 3 OR 4 OR 5	141793
7	1 AND 6	271

2.2. Study selection

Articles included in the review followed these inclusion criteria: (I) English language, (II) explicit reference to the evaluation of the association between melanoma and OS through marker of OS. Instead, articles were excluded by title, abstract or full-text for irrelevance to the topic of the review. Further exclusion criteria are reviews, books and documents.

2.3. Data extraction

Two authors (LB, AT) independently performed the initial search and selected the articles based on the inclusion and exclusion criteria. The data extracted included (I) study author names, (II) publication date, (III) sample size, (IV) group studies, (V) clinical and biological variables and (VI) outcome of interest of the study.

Principal outcome of interest included studies about OS markers evaluation in melanoma cells.

In view of the considerable heterogeneous patient populations, differences in the design of the studies and in the protocols, a meta-analysis was not deemed to be appropriate.

3. Results

Fig. 1 reports the flow of articles retrieved for the review. The search in PubMed provided 271 citations, and, after adjusting for duplicates, 270 of such citations were screened. Of these, 219 articles were excluded by language as they are not English written (n = 4), by title because not meeting the criteria, being reviews or books and documents (n = 22). The majority of articles (n = 193) were excluded as their title or abstract were not relevant to the outcome of interest for the review, meaning they don’t analyze the association between OS and melanoma proper. The remaining 51 articles were examined by reading the full text. A total of 22 records were excluded not meeting the inclusion criteria (n = 16) or not having available full-texts (n = 6). Finally, 29 articles were assessed for eligibility and all of them were included in the systematic review. Table 2 summarizes the records selected, in particular highlighting species employed, main outcomes and main properties of melanoma cells.

3.1. Melanoma cells are resistant to oxidative stress

Commonly, ROS have elevated levels in cancer cells. On one hand, they promote cancer survival, proliferation and metastasis, but on the other side, at higher level, they induce apoptosis and senescence of cancer via DNA damage. Therefore, melanoma should have adaptive mechanisms to overcome effects of high ROS levels [12].

One of the first studies in this domain was the one published by Applegate et al., in which the authors decided to determine the levels of two antioxidant compounds, GSH and ferritin, in human melanoma cells. Such levels were found low in the cell lines, however without being related to the sensitivity of melanoma cell lines to OS [19]. Therefore, authors concluded that melanoma cells are resistant to OS.

Bracalente et al. examined which group of genes are up or down regulated in melanoma cells in antioxidant response. There were 19 downregulated, co-expressed genes in metastatic cells, revealing a disruption in the antioxidant response. As a result, intracellular ROS increased triggering dedifferentiation and malignant metastatic progression. However, in the non-metastatic melanoma cell lines, 10 genes of the AOS were upregulated, underlining the increasing ability of these cells to respond to OS [20].

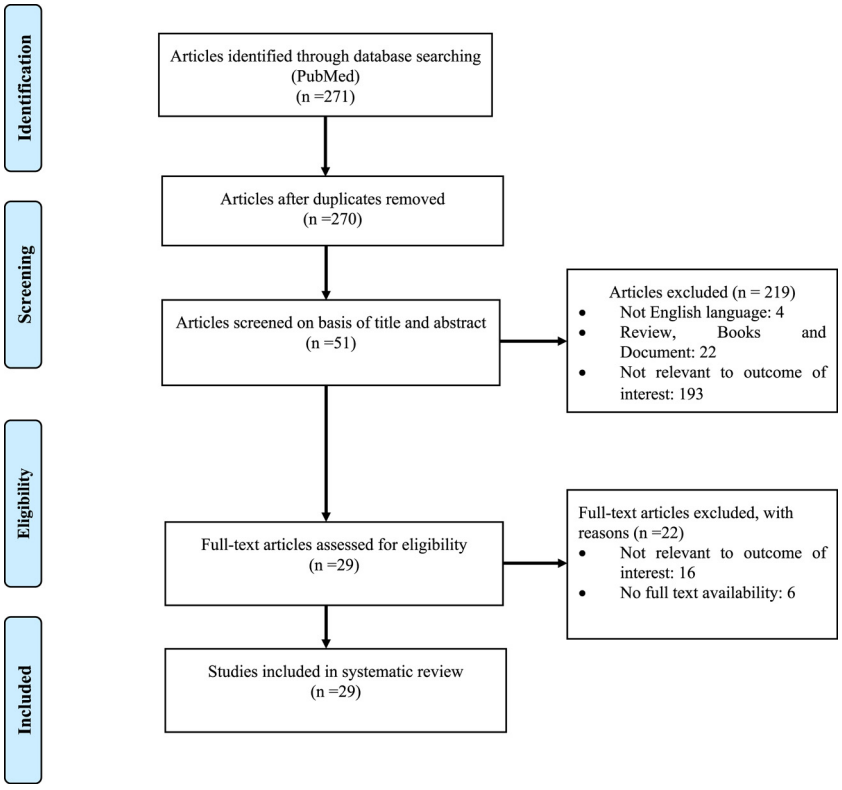

Fig. 1. Flow diagram of studies assessed in the systematic review. Diagram shows the several step of the studies selection in the systematic review, describing also the exclusion criteria and the final number of included articles for each step.

Three articles described elevated levels of enzymes involved in the defense from ROS, including GSH, CAT and SOD in addition to MDA [21–23]. In one study MDA, SOD and CAT levels were determined in blood and tissue of mice. The authors found that there is an age-associated increase of these enzymes [21]. Ortega et al. found that high GSH levels in melanoma cells promote survival to OS. They proposed that high levels could implemented metastatic growth too [22]. Finally, in the third study authors determined high levels of MDA, SOD and CAT, in human melanoma biopsy [23].

Furthermore, Meyskens et al. showed that some transcriptional factors play an important role in protecting melanomas from ROS damage. Melanoma cells exposed to ROS presented an increased AP-1 activation and of NF-kB pathway. Moreover, O_2^- level raised in melanoma compared to melanocytes and it directly correlated with AP-1. On the other hand, H_2O_2 level was decreased in melanoma cells and it exhibited a correlation with NF-kB. However, the recruitment of these two transcriptional factors did not provide a control of apoptosis, suggesting a general mechanism by which melanoma cells can escape by noxious injury [24].

Wang et al. demonstrated that the activation of MAPK pathway played a role in protecting melanoma cells from OS through the Skp2/MTH1 axis. Skp2 limited DNA damage upregulating MTH1 that, in turn, prevented misincorporation of oxidized dNTPs into genomic DNA. MTH1 expression was upregulated in melanoma cells and in patients specimen, compared with nevi, and It was also in metastatic melanoma compared to primary melanomas, suggesting that increase of MTH1 is related to disease development and progression [25].

Another study by Pieri et al. suggested that ROS contribute to affect the redox regulation of protein phosphorylation, an essential mechanism of intracellular signal transduction, impacting on the cellular protein kinase/phosphatase balance. The membrane-bound ecto-enzyme gamma-glutamyltransferase (GGT) may participate in the process promoting ROS production, such as H_2O_2 [26].

Also, Giommarelli et al. focused on GGT role in antioxidant response in melanoma cells lines. GGT overexpression was associated with resistance to OS and with an increased expression of CAT [27].

CD147 is a cell surface receptor for cyclophilin A, which is overexpressed in various tumour cells protecting them from OS. Li et al. demonstrated that CD147 silencing sensitized MM cell to ROS. They also found that CD147 silencing exacerbate H_2O_2-induced damage, increasing ROS and MDA generation and decreasing SOD activity. Therefore, they propose that CD147 depletion may enhance chemio- and radiotherapy effects [28].

Mougiakakos et al. investigated the expression of glutamate-l-cysteine ligase catalytic subunit (GCLC), a key factor of GSH synthesis, in malignant melanoma cell lines. High GCLC levels were high and they correlated with lower intracellular-level of ROS as well as with lower rates of cell proliferation. Additionally, in patients with malignant melanoma, GCLC levels correlated with a better 5-year overall survival [29].

3.2. Oxidative stress influences metastatic ability of melanoma cells

Cutaneous melanoma is an aggressive malignancy due to its high metastatic ability. Piskounova et al. found that OS, albeit increasing in metastasizing cells, limits the metastasis of melanomas. The GSH-to-oxidized glutathione (GSSG) ratio was in fact lower in metastatic nodules or circulating melanoma cells. This suggested that metastatic cells consumed GSH in an effort to maintain redox homeostasis. The authors also demonstrated that anti-oxidant treatment promoted distant metastasis [30].

In two studies, the role of PG1α, a transcriptional coactivator, was investigated, which protects against OS and influences melanoma drug sensitivity and survival. Vazquez et al. showed that PG1α positive melanoma cells have increased ROS detoxification capacities [31]. Luo et al. found that elevated levels of PG1α in melanoma cells inversely correlate with vertical growth in human melanoma. In addition, it also suppresses metastasis, acting on the regulation of transcriptional

Table 2
Studies exploring the association between oxidative stress and melanoma. AOPP advanced oxidation protein products; AOS antioxidant system; CAT catalase; GPx glutathione peroxidase; GSH glutathione; GCLC glutamate-l-cysteine ligase catalitic subunit; GSSG oxidized glutathione; GST glutathione-S-transferase; GGT membrane-bound ecto-enzyme gamma-glutamyltransferase; LPO lipid peroxidation; MDA malondialdehyde; Nrf2Nuclear factor erythroid-2-related factor; 8 − OHdG 8-hydroxydeoxyguanosine; pt patients; OS oxidative stress; ROS reactive oxygen species; SOD superoxide dismutase; TBARS thiobarbituric acid reactive substances.

Study	Species employed	Main outcomes	Main properties of melanoma cells
Wang et al. [25]	Human Animal Cell lines	Melanoma cells are resistant to OS	Upregulation of MAPK-Skp2-MTH1 axis
Coelho et al. [46]	Cell lines	ROS contributes to melanoma treatment resistance	Upregulation of CAT
Luo et al. [32]	Cell lines	OS promotes melanoma metastasis	Downregulation of PCG1α
Bracalente et al. [20]	Cell lines	Melanoma cells are resistant to OS	Downregulation of 19 AOS genes in metastatic cells, upregulation of 10gens in non-metastatic one
Hintsala et al. [36]	Human (N = 121pt) Cell lines	AOS correlate with Breslow thickness	Nrf2 is more expressed in metastatic cells, while 8-OHdG is less expressed
Kaur et al. [42]	Animal	OS promotes melanoma metastasis	Loss of APE-1
Bernardes et al. [33]	Human (N = 30 cases and N = 30 controls)	OS promotes melanoma metastasis	Less levels of TGF-1β and increased TRAP, thiol, AOPP and LPO in metastatic cells
Herraiz et al. [43]	Animal Cell lines	OS inhibits progression	Inverse correlation between OS and actomyosin contractility
Piskounova et al. [30]	Human (N = 8pt) Animal	OS inhibits progression	Higher level of OS and lower GSH/GSSG ratio in metastatic cells
Bernardes et al. [37]	Human (N = 43 cases and N = 50 controls)	OS correlates with Breslow thickness	Elevated levels of MDA
Xie et al. [38]	Animal	OS promotes melanoma metastasis	Elevated levels of ROS
Moretti et al. [39]	Cell lines	OS inhibits progression	Calpain-3 increases ROS production
Kim SH et al. [35]	Animal Cell lines	OS inhibits progression	Elevated levels of ROS in IDH2(-) melanoma cells
Vazquez et al. [31]	Cell lines	OS inhibits progression	AOS increased in PGC1α(+) melanoma cell
Lin et al. [44]	Cell lines	ROS promote tumor invasiveness	High-level ROS in primary melanoma
Mougiakakos et al. [29]	Human (28 pt) Animal Cell lines	Melanoma cells are resistant to OS	High expression of GCLC
Li et al. [28]	Cell lines	Melanoma cells are resistant to OS	Higher level of SOD, Lower of MDA then H_2O_2 MM cells treated
Giommarelli et al. [27]	Cell lines	Melanoma cells are resistant to OS	High GGT levels
Michard et al. [47]	Cell lines	ROS contributes to melanoma treatment resistance	High GSH levels in melanoma cells overexpressing TRP-2
Baldi et al. [40]	Human (11 pt) Animal Cell lines	Ferritin expression enhanced OS sensitivity	Increased SOD and decreased CAT in ferritin (-) metastatic cells In vivo ferritin correlated with T and M status
Cheng et al. [45]	Cell lines	ROS promote tumor invasiveness	High ROS levels in metastatic cells
Wheeler et al. [41]	Animal Cell lines	OS promotes melanoma metastasis	High SOD levels in non-metastatic cells
Sander et al. [21]	Human (N = 18 cases vs N = 28 controls)	Melanoma cells are resistant to OS	Elevated CAT, SOD, MDA levels
Pieri et al. [26]	Cell lines	ROS damage protein phosphorylation	Elevated GGT levels
Ortega et al. [22]	Animal Cell lines	Melanoma cells are resistant to OS	Elevated GSH levels
Wozniak et al. [23]	Animal Cell lines	Melanoma cells are resistant to OS	Elevated level of MDA, SOD, CAT, GPx
Nogués et al. [34]	Human (N = 34 cases vs N = 101 controls)	ROS levels are correlated with Clark levels	GST increased, SOD and TBARS decreased
Meyskens et al. [24]	Cell lines	Melanoma cells are resistant to OS	O_2^- and H_2O_2 upregulated NF-kB and AP1
Applegate et al. [19]	Cell lines	Melanoma cells are resistant to OS	Low levels of ferritin and GSH

programs relating to AOS. Moreover, metastatic cells have lower amounts of PG1α [32].

An important regulator of growth and melanoma progression is TGF-1β, which specifically inhibits tumor relapse, as pointed out by Bernardes et al. [33]. More in depth, they explored the relationship between TGF-1β levels and OS in patients with metastatic melanoma. Such subjects had less circulating levels of TGF-1β and increased TRAP, thiol, AOPPs and lipid peroxidation (LPO). Moreover, a negative correlation was observed between TGF-1β levels and systemic levels of MDA, as well as with AOPPs, while a positive correlation was revealed between TGF-1β and GSH levels [33].

Nogués et al. found that GST was increased in melanoma samples, whereas SOD and thiobarbituric acide reactive substances (TBARS) were decreased in direct relation to Clark levels. Furthermore, GST

activity correlated to malignity and a decrease of SOD linked to progression of melanoma [34].

Isocitrate dehydrogenase (IDH2) is one of the major enzymes of AOS. Kim et al. investigated the role of IDH2 in tumor progression, observing that a downregulation of IDH2 inhibits tumor growth, presumably inducing apoptosis and a reduction of angiogenesis-related factors [35].

Hintsala et al. measured the levels of Nrf2, a marker for AOS, and 8-OHdG, a marker for oxidative lesion in human tissue samples. They found that Nrf2 expression is a powerful negative prognostic factor of primary tumour. Indeed, in melanoma cells it correlates positively with deeper Breslow, which is an indicator of aggressiveness and risk of recurrence. Nrf2 expression presents also a positive correlation with invasive phenotype (Clark III-V), nodular histology and worse melanoma-specific survival. These results may reflect a role for Nrf2 in radial-to-vertical growth transformation of melanoma. On the other hand, the study revealed that 8-OHdG is significantly less expressed in malignant melanoma. Its nuclear endothelial expression is associated with the presence of ulceration and correlates with worse prognosis [36].

Bernardes et al. evaluated the relationship between systemic OS and Breslow thickness in 43 patients with cutaneous melanomas. MDA and thiol levels were higher in these patients' tissue samples compared to controls, while GSH was lower. MDA levels correlated positively with the Breslow thickness, indicating that the greater the thickness, the greater the systemic OS [37].

Other authors investigated the role of Atg7 gene, which controls macro-autophagy, promoting melanoma survival and growth. Atg7-deficient mice model melanomas presented high levels of 8-OxodG and an elevated ROS production [38].

Moretti et al. focused on Calpain-3, an intracellular cysteine protease, which has a diminished expression in melanomas on a vertical growth phase and, on an even greater extent, in metastasis. They found in melanoma cells lines that an overexpression of Calpain-3 leads to a modulation of OS-related genes and to an increased formation of ROS, resulting in DNA damage. Besides, a downregulation of this enzyme is linked to metastatic melanoma, due to an impaired cell growth [39].

Another study pointed out the role of ferritin in the progression of melanoma. High levels of L-ferritin were strongly associated to the metastatic phenotype. In fact, L-ferritin downregulation in metastatic melanoma cells reduced the proliferation rate and enhanced sensitivity to OS, with increased SOD and decreased CAT activities. Finally, analysis of human melanomas showed that ferritin expression in non-metastatic tumors was correlated with T and M status [40].

Wheeler et al. also evaluated the effects of OS on tumor growth in melanoma cells lines. High levels of SOD blunted oxidant-dependent vascular endothelial growth factor (VEGF) expression. These data suggest that OS may facilitate tumor vascularization, thus promoting melanoma growth [41].

Some authors even analyzed the effect of aged-microenvironment on melanoma progression. Aged dermal fibroblasts secreted a Wnt antagonist, sFRP2, which in turn activated a pathway ultimately resulting in loss of function of a key redox effector, APE-1. The loss of this effector reduced the response of melanoma cells to DNA damage induced by ROS. Furthermore, upregulation of sFRP2 increased both angiogenesis and metastasis of melanoma cells [42].

Another study explored the links between actomyosin, a key regulator for tumor invasion, and OS DNA damage. The authors found an inverse correlation between actomyosin contractility and OS. In particular, an upregulation of genes increasing ROS was showed in melanoma cell lines, suggesting a beneficial role for ROS in the suppression of tumor invasion [43].

In one article, it was explored the role of tumor-associated macrophages (TAMs), which played a part in tumor growth, invasion and metastasis. In mice high-levels of ROS in non-metastatic melanoma environment resulted to have ability to promote tumor invasiveness by influencing TAMs behavior [44].

Finally, some authors explored in melanoma cells lines how OS could promote the intravasation of melanoma cells to make metastasis occurring. More in depth, Cheng et al. tested the hypothesis that ROS could influence melanoma cell reverse trans-endothelial migration (TEM). ROS may enhance TEM and this mechanism could be triggered by an ultraviolet radiation through the expression of two other proteins, thioredoxin interacting protein and thioredoxin [45].

3.3. Oxidative stress and treatment resistance

Resistance to OS is a key mechanism of tumor treatment resistance. Coelho et al. investigated the influence of adipocyte secretome in melanoma cell radio-sensitivity. Indeed, obesity is a risk factor for melanoma, but also high adiposity is associated with a pro-oxidative status. The activity of CAT is higher in irradiated cell lines exposed to adipocyte secretome, whereas CAT usually presents a diminished basal activity after radiation [46].

Two studies also revealed how the loss of function of AOS attenuate the response of melanoma cells to DNA damage induced by ROS. As a result, this response makes the cells more resistant to target therapy [28,42].

Dopachrome tautomerase (TRP-2) is a melanogenic enzyme, whose expression improves resistance to cytotoxic treatment. Michard et al. found that TRP-2 overexpression in melanoma cell lines reduced their sensitivity to OS and consequently to DNA damage. Furthermore, they reported an 80% increase in GSH content following TRP-2 expression [47].

4. Discussion

In this systematic review, 29 studies were taken into account and examine the relationship between OS and melanoma. 11 of them focus on the ability of melanoma cells to overcome the effects of high levels of ROS. On the other hand, 16 studies analyse the role of OS to influence metastasis. Finally, 4 studies showed how OS improves melanoma treatment resistance.

Emerging evidences suggest that OS seems to play an important role in cancer cell survival, proliferation, invasion and migration [48]. Moderate ROS levels have been showed to activate pro-tumorigenic signalling through the regulation of different pathways as well as mutations in transcription factors. However, excessive ROS accumulation is antitumorigenic, triggering severe cellular damage and apoptosis [12]. As a consequence, melanoma should have adaptive mechanisms to be resistant to OS: tumour increased antioxidant response, which keeps ROS levels below a critical threshold but still remaining higher than occurs in normal cells (Fig. 2a).

Two studies analysed the important role of NF-kB and MAPK/Skp2 pathways [24,25]. NF-kB is a transcriptional factor that regulate the cell cycle and also the development of drug resistance in many tumours [12]. Meyskens et al. demonstrated that the recruitment of NF-kB did not provide a control of apoptosis and in this way melanoma cells could escape from ROS injury. As regards Wang et al. showed that the MAPK pathway played a role in protecting cells from OS. MAPK activates the Skp2/MTH1 axis, which limits DNA damage [25]. In particular, levels of MTH1 progressively increased from nevi to primary tumour and, secondly, in metastatic melanoma cells, underling its protective role from OS.

Moreover, six studies conducted on humans demonstrated the presence of elevated levels of antioxidant molecules, such as SOD and CAT, in melanoma cells. In addition, MDA, AOPP, thiols and LPO levels were high [21,30,33,34,37,40] and this aspect was even confirmed by 10 studies conducted on animals or cell lines [22,23,26–28,38,41,42,44,45], pointing out that moderate ROS levels have however a pro-tumorigenic role.

One study also revealed that 10 genes involved in AOS were upregulated in the primary tumour. On the other hand, 19 co-expressed

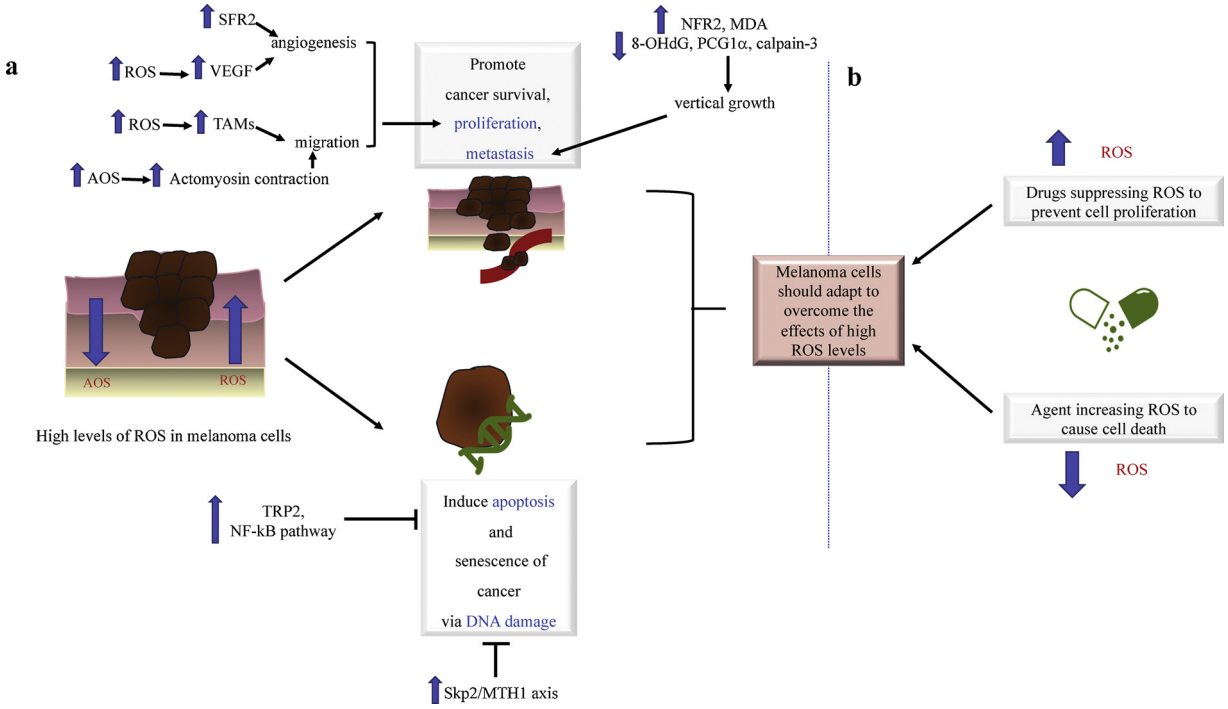

Fig. 2. ROS in the biology of melanoma. (a) ROS have a dual role in melanoma cells: both pro and antitumorigenic one. b) The potential targets of therapy in melanoma: drugs which suppressing ROS and others which increasing them.

genes involved in antioxidant response were downregulated in metastatic melanoma cells [20]. All these aspects underline the capacity of melanoma to be resistant to OS, in order to survive, to proliferate and to migrate. Furthermore, the downregulation of antioxidant response genes enhance the data of Piskounova et al. [30]. They pointed out that melanomas are inefficient to form distant metastasis entering in the circulation, compared to subcutaneous metastasis.

However, the metastatic ability of melanoma cells is pivotal to be taken into account because metastasis are the main cause of death in cancer patients. Nevertheless, this review shows that only few studies focused their attention on this subject. In detail, five articles analysed the link between OS and melanoma vertical growth [31,32,36,37,39], which is one of the most accepted indicator of aggressiveness and recurrence. It is named the Breslow thickness: high thickness strongly correlates to melanoma recurrence and poor survival.

Two of the studies were conducted on humans. Hintsala et al. focused their attention on two markers, Nrf2 and 8-OHdG. Nrf2 is a transcriptional factor and also a marker of AOS that increases the production of antioxidant proteins. Nfr2 correlated positively with deeper Breslow and also with a more invasive phenotype. 8-OHdG, a marker of OS DNA damage, was less expressed in malignant melanomas, confirming the OS unbalance in these cells [36]. Similar data was showed by the second study, which found high levels of MDA and thiols and low levels of GSH in melanoma. Furthermore, MDA levels correlated positively with vertical growth [37].

The other three studies were conducted on animals or cell lines. Two of them evidenced a role of a transcriptional coactivator, PCG1α, which protects from OS and influences the survival and drug sensitivity of melanoma cells [31,32]. PCG1α levels resulted lower in melanoma cells, with an inverse correlation with Breslow thickness. The last study focused on an intracellular cysteine protease, calpain-3, which modulates OS-related gene in an antitumorigenic sense [39]. Its expression resulted to be diminished in the vertical growth phase and also in the metastatic phase of melanomas.

Therefore, all the five articles pointed out the importance of the antioxidant response to promote the proliferation of melanoma. Although the extracellular matrix and also the immune cells round tumour play an important role in tumour progression [49,50].

The review shows that only two studies evidenced that the microenvironment contributes in that sense [42]. The first one evidenced a role for dermal fibroblasts, which secreted sFRP2, a Wnt antagonist. It indirectly led to a loss of function of a key redox effector, APE-1, which in turn reduced the response of melanoma cells to DNA damage. SFR2, which resulted to be upregulated in melanoma, also increased angiogenesis. Indeed, in another study, the authors showed that high levels of ROS in melanoma cells promoted VEGF expression [41].

Instead, the second study explored the role of OS on tumour associated macrophages. High levels of ROS in primary tumour promoted tumour invasion by influencing macrophages activity [44]. In addition, another study evidenced that ROS could influence the reverse transendothelial migration of melanoma cells [45].

In contrast to all these studies that underline the role of ROS in the invasion, Herraiz et al. evidenced that a key regulator of invasion phase, actomyosin, is inversely correlated with OS [43]. This data is not, therefore, so strange because, as pointed out previously, melanoma the survive and proliferates thanks to imbalance between ROS and AOS [12].

Finally, this systematic review underlines that OS even influences tumour treatment resistance. One study pointed out that a melanogenic enzyme (TRP2) was overexpressed reducing sensitivity to OS and, as a consequence, improving resistance to cytotoxic treatment [47]. Furthermore high adiposity, which correlated with a pro-oxidant status, negatively influences the melanoma cell radio-sensitivity [46]. Two other studies also showed that a loss of AOS made melanoma cells more resistant to target therapies [28,42].

Concerning these evidences, in the last years two different strategies were proposed for the treatment of melanoma [51]. The first one uses agents that increase ROS levels to drive OS-induced cell death, whereas the second one utilizes drugs which suppress ROS levels to prevent tumour cell proliferation [9,12] (Fig. 2b). For example, L-buthionine-S-sulfoximine increase ROS level through depletion of GSH and down-regulation of GST expression, inhibiting cell proliferation [52]. Additionally, high levels of ROS were measured during chemotherapy, such as cisplatin e doxorubicin, and radiotherapy, inducing as a result

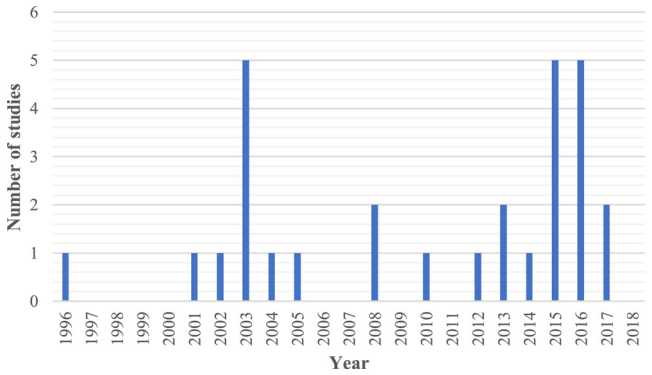

Fig. 3. Graph of the number of included studies. The graph shows the number of included studies per year of publication, from 1996 to 2018.

cells death [12]. On the other hand, the antioxidant N-acetylcysteine and selenium are examples of suppressing ROS drug. Both could be administered to protect nevi from UV induced [53,54].

5. Conclusion

One limitation of this review is that, to date, there are few articles in literature about the association between melanoma and OS (Fig. 3), sometimes with different outcome. Nevertheless, the evidences obtained in this review highlight OS seems to play an important role in the biology of melanoma.

According to all the selected studies, even though their heterogeneity about sample populations and techniques applied, OS acts at every step of melanoma life, from the primary one to the metastatic one. Different pathways are involved in melanoma: they promote escape from ROS injury and limit DNA damage. Moreover, AOS is necessary to melanoma vertical growth and migration. It is emerged also that OS in the microenvironment influences these aspects. The review even shows that AOS get involved in the tumour drug resistance.

Overall, the data suggesting that it is necessary to conduct studies which perform an accurate analysis of all the aspect that are selectively analyse by the included articles: ROS imbalance, its effect to proliferation and metastasis, role of microenvironment, ROS effect to drug resistance. All this in order to understand the complexity of OS pathways regulating melanoma. Furthermore, the data point out that new therapeutic options for melanoma could act on the OS, to contrast every step of progression. Finally, in the near future, it should also be examined if the marker of OS an AOS could be used as biomarker of prognosis and drug response.

Founding sources

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflicts of interest

The authors have no conflict of interest to declare.

Acknowledgement

None.

References

[1] P. Gladfelter, N.H.E. Darwish, S.A. Mousa, Current status and future direction in the management of malignant melanoma, Melanoma Res. 27 (2017) 403–410, https://doi.org/10.1097/CMR.0000000000000379.
[2] V. Atkinson, Recent advances in malignant melanoma, Intern. Med. J. 47 (2017) 1114–1121, https://doi.org/10.1111/imj.13574.
[3] A. Najem, M. Krayem, A. Perdrix, J. Kerger, A. Awada, F. Journe, G. Ghanem, New drug combination strategies in melanoma: current status and future DirectionsNew drug combination strategies in melanoma: current status and future directions, Anticancer Res. 37 (2017) 5941–5953, https://doi.org/10.21873/anticanres.12041.
[4] W.E. Damsky, N. Theodosakis, M. Bosenberg, Melanoma metastasis: new concepts and evolving paradigms, Oncogene 33 (2014) 2413–2422, https://doi.org/10.1038/onc.2013.194.
[5] K. Margolin, Introduction to the role of the immune system in melanoma, Hematol. Oncol. Clin. North Am. 28 (2014) 537–558, https://doi.org/10.1016/j.hoc.2014.02.005.
[6] Y. Yang, A.V. Bazhin, J. Werner, S. Karakhanova, Reactive oxygen species in the immune system, Int. Rev. Immunol. 32 (2013) 249–270, https://doi.org/10.3109/08830185.2012.755176.
[7] H.G.M. Wittgen, L.C.L.T. van Kempen, Reactive oxygen species in melanoma and its therapeutic implications, Melanoma Res. 17 (2007) 400–409, https://doi.org/10.1097/CMR.0b013e3282f1d312.
[8] S. Meierjohann, Oxidative stress in melanocyte senescence and melanoma transformation, Eur. J. Cell Biol. 93 (2014) 36–41, https://doi.org/10.1016/j.ejcb.2013.11.005.
[9] L. Denat, A.L. Kadekaro, L. Marrot, S.A. Leachman, Z.A. Abdel-Malek, Melanocytes as instigators and victims of oxidative stress, J. Invest. Dermatol. 134 (2014) 1512–1518, https://doi.org/10.1038/jid.2014.65.
[10] P.L. Minciullo, A. Inferrera, M. Navarra, G. Calapai, C. Magno, S. Gangemi, Oxidative stress in benign prostatic hyperplasia: a systematic review, Urol. Int. 94 (2015) 249–254, https://doi.org/10.1159/000366210.
[11] J. Frijhoff, P.G. Winyard, N. Zarkovic, S.S. Davies, R. Stocker, D. Cheng, A.R. Knight, E.L. Taylor, J. Oettrich, T. Ruskovska, A.C. Gasparovic, A. Cuadrado, D. Weber, H.E. Poulsen, T. Grune, H.H.H.W. Schmidt, P. Ghezzi, Clinical relevance of biomarkers of oxidative stress, Antioxid. Redox Signal. 23 (2015) 1144–1170, https://doi.org/10.1089/ars.2015.6317.
[12] J.N. Moloney, T.G. Cotter, ROS signalling in the biology of cancer, Semin. Cell Dev. Biol. (2017), https://doi.org/10.1016/j.semcdb.2017.05.023.
[13] F. Guarneri, A. Asmundo, D. Sapienza, S.P. Cannav??, Glutathione S-transferase M1/T1 gene polymorphisms and vitiligo in a Mediterranean population, Pigm. Cell Melanoma Res. 24 (2011) 731–733, https://doi.org/10.1111/j.1755-148X.2011.00872.x.
[14] M. Vaccaro, G. Bagnato, M. Cristani, F. Borgia, G. Spatari, V. Tigano, A. Saja, F. Guarneri, S.P. Cannavò, S. Gangemi, Oxidation products are increased in patients affected by non-segmental generalized vitiligo, Arch. Dermatol. Res. 309 (2017) 485–490, https://doi.org/10.1007/s00403-017-1746-z.
[15] F. Guarneri, A. Asmundo, D. Sapienza, F. Borgia, V. Papaianni, S.P. Cannavo, Glutathione S-transferase M1/T1 genotype and melanoma in a Southern Italian population: a case-control study, G. Ital. Dermatol. Venereol. 151 (2016) 140–144 (accessed September 3, 2018), http://www.ncbi.nlm.nih.gov/pubmed/25588060.
[16] S. Imbesi, C. Musolino, A. Allegra, A. Saija, F. Morabito, G. Calapai, S. Gangemi, Oxidative stress in oncohematologic diseases: an update, Expert Rev. Hematol. 6 (2013) 317–325, https://doi.org/10.1586/ehm.13.21.
[17] M. Venza, M. Visalli, C. Beninati, G.V. De Gaetano, D. Teti, I. Venza, Cellular mechanisms of oxidative stress and action in melanoma, Oxid. Med. Cell. Longev. 2015 (2015) 1–11, https://doi.org/10.1155/2015/481782.
[18] A. Liberati, D.G. Altman, J. Tetzlaff, C. Mulrow, P.C. Gøtzsche, J.P.A. Ioannidis, M. Clarke, P.J. Devereaux, J. Kleijnen, D. Moher, The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate healthcare interventions: explanation and elaboration, BMJ 339 (2009) b2700. Accessed July 18, 2018 http://www.ncbi.nlm.nih.gov/pubmed/19622552.
[19] L.A. Applegate, C. Scaletta, F. Labidi, G.F. Vile, E. Frenk, Susceptibility of human melanoma cells to oxidative stress including UVA radiation, Int. J. Cancer 67 (1996) 430–434, https://doi.org/10.1002/(SICI)1097-0215(19960729)67:3<430 AID-IJC19>3.0.CO;2-B.
[20] C. Bracalente, I.L. Ibañez, A. Berenstein, C. Notcovich, M.B. Cerda, F. Klamt, A. Chernomoretz, H. Durán, Reprogramming human A375 amelanotic melanoma cells by catalase overexpression: upregulation of antioxidant genes correlates with regression of melanoma malignancy and with malignant progression when downregulated, Oncotarget 7 (2016), https://doi.org/10.18632/oncotarget.9273.
[21] C.S. Sander, F. Hamm, P. Elsner, J.J. Thiele, Oxidative stress in malignant melanoma and non-melanoma skin cancer, Br. J. Dermatol. 148 (2003) 913–922, https://doi.org/10.1046/j.1365-2133.2003.05303.x.
[22] A.L. Ortega, J. Carretero, E. Obrador, J. Gambini, M. Asensi, V. Rodilla, J.M. Estrela, Tumor cytotoxicity by endothelial cells: impairment of the mitochondrial system for glutathione uptake in mouse B16 melanoma cells that survive after in vitro interaction with the hepatic sinusoidal endothelium, J. Biol. Chem. 278 (2003) 13888–13897, https://doi.org/10.1074/jbc.M207140200.
[23] A. Woźniak, G. Drewa, B. Woźniak, D.O. Schachtschabel, Activity of antioxidant enzymes and concentration of lipid peroxidation products in selected tissues of mice of different ages, both healthy and melanoma-bearing, Z. Gerontol. Geriatr. 37 (2004) 184–189, https://doi.org/10.1007/s00391-004-0229-y.
[24] F.L. Meyskens, S.E. McNulty, J.A. Buckmeier, N.B. Tohidian, T.J. Spillane, R.S. Kahlon, R.I. Gonzalez, Aberrant redox regulation in human metastatic melanoma cells compared to normal melanocytes, Free Radic. Biol. Med. 31 (2001) 799–808 http://www.ncbi.nlm.nih.gov/pubmed/11557318.
[25] J.Y. Wang, G.Z. Liu, J.S. Wilmott, T. La, Y.C. Feng, H. Yari, X.G. Yan, R.F. Thorne, R.A. Scolyer, X.D. Zhang, L. Jin, Skp2-mediated stabilization of MTH1 promotes survival of melanoma cells upon oxidative stress, Cancer Res. 77 (2017) 6226–6239, https://doi.org/10.1158/0008-5472.CAN-17-1965.
[26] L. Pieri, S. Dominici, B. Del Bello, E. Maellaro, M. Comporti, A. Paolicchi,

A. Pompella, Redox modulation of protein kinase/phosphatase balance in melanoma cells: the role of endogenous and γ-glutamyltransferase-dependent H2O2production, Biochim. Biophys. Acta - Gen. Subj. 1621 (2003) 76–83, https://doi.org/10.1016/S0304-4165(03)00048-5.

[27] C. Giommarelli, A. Corti, R. Supino, E. Favini, A. Paolicchi, A. Pompella, F. Zunino, Cellular response to oxidative stress and ascorbic acid in melanoma cells overexpressing γ-glutamyltransferase, Eur. J. Cancer 44 (2008) 750–759, https://doi.org/10.1016/j.ejca.2008.02.010.

[28] J. Li, L. Peng, L. Wu, Y. Kuang, J. Su, M. Yi, X. Hu, D. Li, H. Xie, T. Kanekura, X. Chen, Depletion of CD147 sensitizes human malignant melanoma cells to hydrogen peroxide-induced oxidative stress, J. Dermatol. Sci. 58 (2010) 204–210, https://doi.org/10.1016/j.jdermsci.2010.03.022.

[29] D. Mougiakakos, R. Okita, T. Ando, C. Dürr, J. Gadiot, J. Ichikawa, R. Zeiser, C. Blank, C.C. Johansson, R. Kiessling, High expression of GCLC is associated with malignant melanoma of low oxidative phenotype and predicts a better prognosis, J. Mol. Med. 90 (2012) 935–944, https://doi.org/10.1007/s00109-012-0857-4.

[30] E. Piskounova, M. Agathocleous, M.M. Murphy, Z. Hu, S.E. Huddlestun, Z. Zhao, A.M. Leitch, T.M. Johnson, R.J. DeBerardinis, S.J. Morrison, Oxidative stress inhibits distant metastasis by human melanoma cells, Nature 527 (2015) 186–191, https://doi.org/10.1038/nature15726.

[31] F. Vazquez, J.-H. Lim, H. Chim, K. Bhalla, G. Girnun, K. Pierce, C.B. Clish, S.R. Granter, H.R. Widlund, B.M. Spiegelman, P. Puigserver, PGC1α expression defines a subset of human melanoma tumors with increased mitochondrial capacity and resistance to oxidative stress, Cancer Cell 23 (2013) 287–301, https://doi.org/10.1016/j.ccr.2012.11.020.

[32] C. Luo, J.-H. Lim, Y. Lee, S.R. Granter, A. Thomas, F. Vazquez, H.R. Widlund, P. Puigserver, A PGC1α-mediated transcriptional axis suppresses melanoma metastasis, Nature 537 (2016) 422–426, https://doi.org/10.1038/nature19347.

[33] S. Santos Bernardes, F.P. de Souza-Neto, G. Pasqual Melo, F.A. Guarnier, P.C. Marinello, R. Cecchini, A.L. Cecchini, Correlation of TGF-β1 and oxidative stress in the blood of patients with melanoma: a clue to understanding melanoma progression? Tumor Biol. 37 (2016) 10753–10761, https://doi.org/10.1007/s13277-016-4967-4.

[34] M.R. Nogués, M. Giralt, I. Cervelló, D. Del Castillo, O. Espeso, N. Argany, A. Aliaga, J. Mallol, Parameters related to oxygen free radicals in human skin: a study comparing healthy epidermis and skin cancer tissue, J. Invest. Dermatol. 119 (2002) 645–652, https://doi.org/10.1046/j.1523-1747.2002.00077.x.

[35] S.H. Kim, Y.H. Yoo, J.H. Lee, J.W. Park, Mitochondrial NADP+-dependent isocitrate dehydrogenase knockdown inhibits tumorigenicity of melanoma cells, Biochem. Biophys. Res. Commun. 451 (2014) 246–251, https://doi.org/10.1016/j.bbrc.2014.07.105.

[36] H.-R. Hintsala, E. Jokinen, K.-M. Haapasaari, M. Moza, A. Ristimäki, Y. Soini, J. Koivunen, P. Karihtala, Nrf2/Keap1 pathway and expression of oxidative stress lesions 8-hydroxy-2'-deoxyguanosine and nitrotyrosine in melanoma, Anticancer Res. 36 (2016) 1497–1506 http://www.ncbi.nlm.nih.gov/pubmed/27069125.

[37] S.S. Bernardes, F.P. de Souza-Neto, L.N.Z. Ramalho, D.R. Derossi, F.A. Guarnier, C.F.N. da Silva, G.P. Melo, A.N.C. Simão, R. Cecchini, A.L. Cecchini, Systemic oxidative profile after tumor removal and the tumor microenvironment in melanoma patients, Cancer Lett. 361 (2015) 226–232, https://doi.org/10.1016/j.canlet.2015.03.007.

[38] X. Xie, J.Y. Koh, S. Price, E. White, J.M. Mehnert, Atg7 overcomes senescence and promotes growth of BrafV600E-driven melanoma, Cancer Discov. 5 (2015) 410–423, https://doi.org/10.1158/2159-8290.CD-14-1473.

[39] D. Moretti, B. Del Bello, G. Allavena, A. Corti, C. Signorini, E. Maellaro, Calpain-3 impairs cell proliferation and stimulates oxidative stress-mediated cell death in melanoma cells, PLoS One 10 (2015) 1–22, https://doi.org/10.1371/journal.pone.0117258.

[40] A. Baldi, D. Lombardi, P. Russo, E. Palescandolo, A. De Luca, D. Santini, F. Baldi, L. Rossiello, M.L. Dell'Anna, A. Mastrofrancesco, V. Maresca, E. Flori, P.G. Natali, M. Picardo, M.G. Paggi, Ferritin contributes to melanoma progression by modulating cell growth and sensitivity to oxidative stress, Clin. Cancer Res. 11 (2005) 3175–3183, https://doi.org/10.1158/1078-0432.CCR-04-0631.

[41] M.D. Wheeler, O.M. Smutney, R.J. Samulski, Secretion of extracellular superoxide dismutase from muscle transduced with recombinant adenovirus inhibits the growth of B16 melanomas in mice, Mol. Cancer Res. 1 (2003) 871–881.

[42] A. Kaur, M.R. Webster, K. Marchbank, R. Behera, A. Ndoye, C.H. Kugel, V.M. Dang, J. Appleton, M.P. O'Connell, P. Cheng, A.A. Valiga, R. Morissette, N.B. McDonnell, L. Ferrucci, A.V. Kossenkov, K. Meeth, H.Y. Tang, X. Yin, W.H. Wood, E. Lehrmann, K.G. Becker, K.T. Flaherty, D.T. Frederick, J.A. Wargo, Z.A. Cooper, M.T. Tetzlaff, C. Hudgens, K.M. Aird, R. Zhang, X. Xu, Q. Liu, E. Bartlett, G. Karakousis, Z. Eroglu, R.S. Lo, M. Chan, A.M. Menzies, G.V. Long, D.B. Johnson, J. Sosman, B. Schilling, D. Schadendorf, D.W. Speicher, M. Bosenberg, A. Ribas, A.T. Weeraratna, SFRP2 in the aged microenvironment drives melanoma metastasis and therapy resistance, Nature 532 (2016) 250–254, https://doi.org/10.1038/nature17392.

[43] C. Herraiz, F. Calvo, P. Pandya, G. Cantelli, I. Rodriguez-Hernandez, J.L. Orgaz, N. Kang, T. Chu, E. Sahai, V. Sanz-Moreno, Reactivation of p53 by a cytoskeletal sensor to control the balance between DNA damage and tumor dissemination, J. Natl. Cancer Inst. 108 (2016) 1–14, https://doi.org/10.1093/jnci/djv289.

[44] X. Lin, W. Zheng, J. Liu, Y. Zhang, H. Qin, H. Wu, B. Xue, Y. Lu, P. Shen, Oxidative stress in malignant melanoma enhances tumor necrosis Factor-α secretion of tumor-associated macrophages that promote Cancer cell invasion, Antioxid. Redox Signal. 19 (2013) 1337–1355, https://doi.org/10.1089/ars.2012.4617.

[45] G.C. Cheng, P.C. Schulze, R.T. Lee, J. Sylvan, B.R. Zetter, H. Huang, Oxidative stress and thioredoxin-interacting protein promote intravasation of melanoma cells, Exp. Cell Res. 300 (2004) 297–307, https://doi.org/10.1016/j.yexcr.2004.07.014.

[46] P. Coelho, L. Silva, I. Faria, M. Vieria, A. Monteiro, G. Pinto, C. Prudêncio, R. Fernandes, R. Soares, Adipocyte secretome increases radioresistance of malignant melanocytes by improving cell survival and decreasing oxidative status, Radiat. Res. 187 (2017) 581–588, https://doi.org/10.1667/RR14551.1.

[47] Q. Michard, S. Commo, J.P. Belaidi, A.M. Alleaume, J.F. Michelet, E. Daronnat, J. Eilstein, D. Duche, L. Marrot, B.A. Bernard, TRP-2 specifically decreases WM35 cell sensitivity to oxidative stress, Free Radic. Biol. Med. 44 (2008) 1023–1031, https://doi.org/10.1016/j.freeradbiomed.2007.11.021.

[48] M. Peiris-Pagès, U.E. Martinez-Outschoorn, F. Sotgia, M.P. Lisanti, Metastasis and oxidative stress: are antioxidants a metabolic driver of progression? Cell Metab. 22 (2015) 956–958, https://doi.org/10.1016/j.cmet.2015.11.008.

[49] G. Botti, M. Cerrone, G. Scognamiglio, A. Anniciello, P.A. Ascierto, M. Cantile, Microenvironment and tumor progression of melanoma: new therapeutic prospectives, J. Immunotoxicol. 10 (2013) 235–252, https://doi.org/10.3109/1547691X.2012.723767.

[50] J.M. Brandner, N.K. Haass, Melanoma's connections to the tumour microenvironment, Pathology 45 (2013) 443–452, https://doi.org/10.1097/PAT.0b013e328363b3bd.

[51] J.P. Fruehauf, F.L. Meyskens, Reactive oxygen species: A breath of life or death? Clin. Cancer Res. 13 (2007) 789–794, https://doi.org/10.1158/1078-0432.CCR-06-2082.

[52] J.P. Fruehauf, S. Zonis, M. al-Bassam, A. Kyshtoobayeva, C. Dasgupta, T. Milovanovic, R.J. Parker, A.C. Buzaid, Melanin content and downregulation of glutathione S-transferase contribute to the action of L-buthionine-S-sulfoximine on human melanoma, Chem. Biol. Interact. 111–112 (1998) 277–305 (accessed November 20, 2018), http://www.ncbi.nlm.nih.gov/pubmed/9679561.

[53] A.G. Goodson, M.A. Cotter, P. Cassidy, M. Wade, S.R. Florell, T. Liu, K.M. Boucher, D. Grossman, Use of oral N-acetylcysteine for protection of melanocytic nevi against UV-induced oxidative stress: towards a novel paradigm for melanoma chemoprevention, Clin. Cancer Res. 15 (2009) 7434–7440, https://doi.org/10.1158/1078-0432.CCR-09-1890.

[54] P.B. Cassidy, H.D. Fain, J.P. Cassidy, S.M. Tran, P.J. Moos, K.M. Boucher, R. Gerads, S.R. Florell, D. Grossman, S.A. Leachman, S.A. Leachman, Selenium for the prevention of cutaneous melanoma, Nutrients 5 (2013) 725–749, https://doi.org/10.3390/nu5030725.