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Review

Oxidative stress in melanocyte senescence and melanoma transformation

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ABSTRACT

Melanoma is a severe type of skin cancer with a high metastasis potential and poor survival rates once metastasized. The causes of melanoma formation are multifactorial and not fully understood. Several signaling cascades such as the RAS/RAF/ERK1/2 pathway, the PI3K/AKT pathway, RAC1 and NF-κB are involved in melanoma initiation and progression. Reactive oxygen species (ROS) are induced by these signal transduction cascades, and they play a fundamental role in melanomagenic processes. Cells derived from the melanocytic lineage are particularly sensitive to an increase in ROS, and thus, melanoma cells rely on efficient antioxidant measures. This review summarizes the causes and consequences of ROS generation in melanocytes and melanoma and discusses the potential of pro-oxidant therapy in melanoma treatment.

Reactive oxygen species can be considered as second messenger molecules. When they are present in low amounts, they first react with easily accessible cysteine residues which are e.g. found in protein tyrosine phosphatases including PTP1B, SHP1 and -2 and PTEN (Boivin et al., 2008; Salsman et al., 2005). This abolishes the phosphatase's activity and enhances intracellular receptor tyrosine kinase signaling and proliferation (Monteiro et al., 2008). Furthermore, ROS are activators of NF-κB signaling (Gloire et al., 2006; Trevisi et al., 2010), and result in the generation of oxidized lipids, which can also aid proliferation and prevent apoptosis (Trevisi et al., 2010). However, at higher concentrations, ROS damage cell membranes and organelle function and impact cellular vitality. As a consequence, cells undergo senescence or apoptosis (Wang and Yi, 2008).

Melanoma belongs to the most aggressive forms of skin cancer and its incidence is continually rising worldwide (Godar, 2011). Melanomas arise from melanocytes of cutaneous or extracutaneous origin. In approximately one third of cases, they develop from nevi (or "moles"), which are therefore considered as a benign precursor form of melanoma. Multiple oncogenes contribute to melanoma development, among them the serine/threonine kinase BRAF, the GTPases NRAS, RAC1, GNAQ, and GNA11 and the receptor tyrosine kinases c-KIT and ERBB4 (Mehnert and Kluger, 2012). Melanocytes are characterized by their capacity to produce melanin as means to protect the skin from damaging UV irradiation. Although this constitutes an important protection device, it also emerges as factor which renders cells of melanocytic origin particularly sensitive towards reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2) or the superoxide radical ($O_2^{\bullet -}$).

ROS in melanocytes

UV exposure leads to enhanced melanocytic pigment production which generally protects the skin from the deleterious DNA-damaging effects of UV-A and -B. However, when melanocytes are compared to non-pigmented cells, they display raised ROS levels which are attributed to the presence of melanin (Jenkins and Grossman, 2013). Melanins are complex pigments which are derived from tyrosine by repeated oxidation steps. They can occur in different polymerization forms and can either consist of the black eumelanin or the reddish/brownish pheomelanin (Meyskens et al., 2001). In contrast to eumelanin, pheomelanin production requires the incorporation of cysteine. Although melanin has been reported to aid in ROS neutralization (Bustamante et al., 1993), it can also mediate oxidative stress on the cellular level (Hill et al., 1997). The presence of melanin in cultured primary human melanocytes is associated with a higher degree of ROS accumulation and simultaneous reduction of the cellular antioxidant glutathione (GSH) (Smit et al., 2008). Consequently, the inhibition of melanin synthesis in melanocytes causes a reduction of ROS to levels comparable to fibroblasts (Jenkins and Grossman, 2013). The irradiation of eu- or pheomelanin with UV light can

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also enhance ROS production (Meyskens et al., 2001). In particular, UVA causes higher levels of oxidative DNA damage in melanocytes compared to fibroblasts (Wang et al., 2010). The tumor suppressor protein $p16^{INK4A}$ plays a major role in the cell cycle control of melanocytes, which is demonstrated by the fact that people with familial $p16^{INK4A}$ deficiency (also known as Leiden syndrome) have a characteristic accumulation of nevi and an increased melanoma risk (Hussussian et al., 1994). $p16^{INK4A}$ itself is involved in the prevention of ROS accumulation in a manner which can be uncoupled from its cell cycle regulatory function (Jenkins et al., 2011) (Jenkins et al., 2013). Although $p16^{INK4A}$ deficiency was found to raise ROS levels in several cell types, the effect was particularly strong in melanocytes in a melanin-dependent manner (Jenkins and Grossman, 2013).

As a consequence of their increased sensitivity towards ROS, melanocytes have developed mechanisms to counteract aberrant oxidative damage. As an example, the transcription factor MITF, which is both a master regulator of the melanocyte lineage and a melanoma oncogene, is not only involved in inducing pigmentation, but it also controls the cellular response to ROS by enhancing the oxidative DNA damage repair enzyme apurinic apyrimidinic endonuclease 1 (APE-1/Ref-1) (Liu et al., 2009). Similar observations were made for the melanocyte stimulating hormone (α-MSH)-dependent signaling cascade. Pigment production is generally induced by the binding of α-MSH to its receptor MC1R, which is expressed on the melanocyte plasma membrane. The group around Z. Abdel-Malek demonstrated recently that MC1R signaling also affects ROS-dependent damage independent of melanin production by increasing the levels of base excision repair enzymes 8-oxoguanine DNA glycosylase (OGG1) and APE-1/Ref-1 (Kadekaro et al., 2012).

The role of ROS in premature melanocyte senescence

Many human nevi carry common melanoma oncogenes such as $BRAF^{V600E}$ or $NRAS^{Q61K/R}$ (Bauer et al., 2007; Poynter et al., 2006). It is therefore assumed that oncogene-induced senescence is at least partly involved in the limitation of nevus growth. Many researchers have described oncogene-induced melanocyte senescence *in vitro* and *in vivo*. We have found that strong receptor tyrosine kinase (RTK)- or $NRAS^{Q61K}$ signaling leads to the accumulation of high ROS levels (Leikam et al., 2008). The ensuing senescence is mediated by the DNA damage response and can be entirely prevented by the antioxidant N-acetylcysteine (NAC). Interestingly, the magnitude of oncogene signaling determines whether ROS levels are strong enough to cause senescence (Leikam et al., 2008). Oncogene-induced ROS can be generated by several mechanisms. On the one hand, the superoxide-generating NADPH oxidases are induced by oncogenic RTKs or RAS, and their pharmacological or siRNA-mediated inhibition prevents the induction of senescence ((Kodama et al., 2013) and own unpublished observations). On the other hand, ROS can be caused by metabolic stress as in case of aberrant mitochondrial engagement. In line with this, $BRAF^{V600E}$-induced senescence was recently described to go along with activation of the mitochondrial gatekeeper enzyme pyruvate dehydrogenase. This results in the predominant usage of pyruvate in the citric acid cycle and causes increased mitochondrial respiration coupled with oxidative stress and a reduction in reduced/oxidized glutathione ratio (Kaplon et al., 2013).

Our group could recently demonstrate that ROS-induced melanocyte senescence is counteracted by an activated transsulfuration pathway, which causes a raise of glutathione levels and thereby increases the amount of ROS scavengers (Leikam et al., 2013). The transsulfuration pathway serves the production of cysteine from methionine, and the expression of the *CTH* gene encoding cystathionase – the last enzyme in the transsulfuration pathway, which is expressed in melanoma cells, but not in melanocytes – was sufficient to prevent ROS-induced melanocyte senescence.

The role of ROS in melanocyte apoptosis

The massive induction of melanocyte apoptosis is a characteristic feature of vitiligo, a benign chronic disease which describes the depigmentation of certain skin areas in affected patients (Glassman, 2011). The loss of functional melanocytes is the reason for the observed depigmentation. Although the underlying pathomechanisms are not completely understood, a role of ROS in the development of the disease is undisputed. The affected vitiligo skin has been shown to suffer from enhanced oxidative stress, mainly caused by H_2O_2 concentrations in the millimolar range (Schallreuter et al., 1999). At this concentration, H_2O_2 has deleterious effects and leads e.g. to lipid peroxidation and apoptosis (Boissy et al., 1991a,b; Zhang et al., 2013). The fact that catalase levels are very low in vitiligo skin contributes to the strong H_2O_2 increase (Schallreuter et al., 1991). Various exogenous ROS sources such as phenols/catechols and tetrahydrobiopterin were furthermore found to be increased in vitiligo patients (Chavan et al., 2009; Morrone et al., 1992; Schallreuter et al., 1994). In particular, phenols and catechols can serve as competitive targets of tyrosinase, which on the one hand hampers the production of melanin and on the other hand leads to the production of reactive quinones (Westerhof and d'Ischia, 2007). Elevated H_2O_2 can even further raise reactive quinone generation. In addition to the cell-autonomous action of oxidative species, ROS-dependent autoimmunity seems to be involved in the elimination of melanocytes (Laddha et al., 2013). Importantly, treatment of pediatric vitiligo patients with the pseudocatalase PC-KUS, which releases the oxidative stress in the skin, leads to repigmentation in large areas of the body (Schallreuter et al., 2008).

Altogether, melanocyte senescence and melanocyte apoptosis can be caused by an imbalance between ROS production and ROS detoxication.

ROS in melanoma

The fact that cells from the melanocytic lineage are particularly sensitive to ROS represents a double-edged sword with respect to melanoma development: While aberrant ROS levels easily lead to the described anti-tumor programmes, sublethal amounts allow the accumulation of potentially oncogenic mutations. During the process of melanoma development, melanoma cells have accumulated numerous of these oncogenic mutations which changes cellular signal transduction and metabolism as well as the interaction with neighboring cells. This again alters the cellular response towards UV and ROS compared to melanocytes.

The relevance of ROS in melanoma development was previously demonstrated in a UV-induced mouse melanoma model: In presence of N-acetylcysteine, UV-induced oxidative damage was reduced and the mean onset of melanocytic tumors was strongly delayed (Cotter et al., 2007). Oral administration of NAC was therefore proposed as preventive measure against UV-induced oxidative stress (Goodson et al., 2009).

In the following, the function of ROS in melanoma development as well as progression of the disease will be discussed.

ROS in melanoma initiation and progression

One of the factors which enhances the risk of developing melanoma during lifetime is the presence of the red hair/fair