NRF2-dependent stress defense in tumor antioxidant control and immune evasion
José Pedro Friedmann Angeli | Svenja Meierjohann

DOI: 10.1111/pcmr.12946
Volume 34, Issue 2, Pages 268–279

If you wish to order reprints of this article, please see the guidelines here

EMAIL ALERTS
Receive free email alerts and stay up-to-date on what is published in Pigment Cell & Melanoma Research – click here

Submit your next paper to PCMR online at http://mc.manuscriptcentral.com/pcmr

Subscribe to PCMR and stay up-to-date with the only journal committed to publishing basic research in melanoma and pigment cell biology

As a member of the IFPCS or the SMR you automatically get online access to PCMR. Sign up as a member today at www.ifpcs.org or at www.societymelanomaresearch.org

To take out a personal subscription, please click here
More information about Pigment Cell & Melanoma Research at www.pigment.org
NRF2-dependent stress defense in tumor antioxidant control and immune evasion

José Pedro Friedmann Angeli | Svenja Meierjohann

1Rudolf-Virchow Center for Integrative and Translational Bioimaging, University of Würzburg, Würzburg, Germany
2Institute of Pathology, University of Würzburg, Würzburg, Germany
3Comprehensive Cancer Center Mainfranken, University of Würzburg, Würzburg, Germany

Correspondence
Svenja Meierjohann, Institute of Pathology, University of Würzburg, Würzburg, Germany.
Email: svenja.meierjohann@uni-wuerzburg.de

Funding information
German Research Foundation, Grant/Award Number: FR3736/3-1 and ME1899/6-1; Interdisciplinary Center for Clinical Research (IZKF), Grant/Award Number: B-424 and Z-14

Abstract
The transcription factor NRF2 is known as the master regulator of the oxidative stress response. Tumor entities presenting oncogenic activation of NRF2, such as lung adenocarcinoma, are associated with drug resistance, and accumulating evidence demonstrates its involvement in immune evasion. In other cancer types, the KEAP1/NRF2 pathway is not commonly mutated, but NRF2 is activated by other means such as radiation, oncogenic activity, cytokines, or other pro-oxidant triggers characteristic of the tumor niche. The obvious effect of stress-activated NRF2 is the protection from oxidative or electrophilic damage and the adaptation of the tumor metabolism to changing conditions. However, data from melanoma also reveal a role of NRF2 in modulating differentiation and suppressing anti-tumor immunity. This review summarizes the function of NRF2 in this tumor entity and discusses the implications for current tumor therapies.

Keywords
immune evasion, KEAP1, Nrf2, oxidative stress

1 | INTRODUCTION

Exposure to oxidants is a widespread event in cells of every tissue origin. Cellular oxidants commonly grouped under the term reactive oxygen species (ROS) encompass an array of reduced forms of molecular oxygen, such as superoxide ($\text{O}_2^-$), hydrogen peroxide ($\text{H}_2\text{O}_2$), and hydroxyl radical (OH-). Most of these species are sufficiently reactive to undergo spontaneous reactions with lipids, DNA, or proteins. At low levels, ROS can serve as signaling modulators and impact an ever-growing list of cellular functions. ROS-dependent oxidation of proteins, including $\text{H}_2\text{O}_2$-dependent oxidation of accessible cysteine residues, results in altered activity of signaling proteins such as NF-kB, prolyl hydroxylases, phosphatases such as SHP-1/2 or PTEN, and many others, thereby acting as a stimulator of pro-survival and pro-proliferative pathways (Berra et al., 2003; Lee et al., 2002; Meierjohann, 2014; Oliveira-Marques et al., 2009; Weibrecht et al., 2007). ROS-dependent oxidation of lipids leads to the generation of lipid-derived electrophiles including 4-hydroxynonenal (4-HNE), the so-called second messenger of free radicals, which acts as pleiotropic modulator of several signaling pathways (Csala et al., 2015). Furthermore, by activating AMP kinase in response to glucose intake, ROS can drive proliferation, as shown previously for colorectal cancer cells (Gutierrez-Salmeron et al., 2020).

In contrast, ROS lose their beneficial cellular function at high concentrations. Elevated levels of radical ROS species can eventually lead to difficult to control chain reactions and cellular damage. ROS are commonly produced during oxidative phosphorylation in mitochondria, for example, when electrons are accidentally transferred to $\text{O}_2^-$, thus forming $\text{O}_2^-$ (Dan Dunn et al., 2015), but they can also be generated by cells of the innate immune system such as macrophages or neutrophil granulocytes (Morel et al., 1991). In the skin, there are additional sources of ROS. UV exposure, in particularly the long-wave UV-A, can invade the epidermal layer and can drive the transfer of electrons and

This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

© 2020 John Wiley & Sons A/S. Published by John Wiley & Sons Ltd

Received: 2 September 2020 | Revised: 23 October 2020 | Accepted: 12 November 2020
DOI: 10.1111/pcmr.12946
energy from cellular photosensitizers including melanin to oxygen (Panich et al., 2016; Ravanat et al., 2001). Furthermore, melanin, although very efficient in shielding the nucleus from UV-B irradiation, can serve as ROS amplifier under certain conditions. For example, UV exposure was shown to induce nitric oxide synthase (NOS), as well as NAPDH oxidases, resulting in the generation of nitric oxide (NO) and superoxide. Both free-radical species can easily react leading to the formation of the highly reactive peroxynitrite (ONOO−), which can subsequently cause DNA damage in a melanin-dependent manner (Premi & Brash, 2016). Accordingly, when compared to other cell lineages such as keratinocytes and fibroblasts, melanocytes harbor elevated basal ROS levels (Jenkins & Grossman, 2013). Next to the black-brownish eumelanin, which is the focus of most ROS-related studies, the reddish cysteine-containing pheomelanin is also an important source of ROS. Pheomelanin is prevalent in people with red hair and fair skin, a population group characterized by distinct inactivating mutations in the melanocortin receptor MC1R, which is crucial for the response to melanocyte-stimulating hormone MSH. While a high activity of MC1R leads to the predominant formation of eumelanin, low MC1R activity results in pheomelanin formation (Valverde et al., 1995). This phenotype is reflected in mouse models, where Mc1r mutations also lead to a red hair/fair skin phenotype. Interestingly, the mouse models revealed that this phenotype leads to elevated ROS levels, probably due to aberrant cysteine incorporation into pheomelanin, resulting in UV-independent melanoma development (Mitra et al., 2012).

Melanomas are transformed melanocytes with a highly malignant potential and belong to the most frequent malignant tumor entities in the Northern Hemisphere. Importantly, most melanomas are still able to produce melanin and are additionally prone to produce even more ROS as a result of increased proliferation, elevated mitochondrial activation or oncogenic BRAF or NRAS, which are also a source of ROS (DeNicola et al., 2011; Haq et al., 2013; Leikam et al., 2008). Furthermore, like many other cancer types, melanomas have a strongly elevated glucose consumption, a feature which is diagnostically employed by using 18F-fluorodeoxyglucose PET to detect metastases. The consumption of glucose in the glycolysis can also contribute to enhanced ROS, for example, as a result of increased production of lactate during anaerobic glycolysis (Doherty & Cleveland, 2013; Gutierrez-Salmeron et al., 2020; Tauffenberger et al., 2019). ROS also play an important role in determining the metastatic potential of tumor cells. Several lines of evidence indicate that melanoma cells experience elevated oxidative stress during the process of metastasis into the bloodstream or even distant organs. Consequently, the treatment with the antioxidant N-acetylcysteine or with inhibitors of ferroptosis can improve metastatic efficacy (Piskounova et al., 2015; Ubellacker et al., 2020). Thus, an efficient ROS defense system might make the difference between inefficient and efficient metastasizers in melanoma.

2 | ANTIOXIDANT DEFENSE MECHANISMS

To cope with the cellular sources of increased ROS, cells are equipped with a large set of enzymes and antioxidant cofactors that allow them to efficiently buffer their ROS levels and revert oxidative damage. The most abundant intracellular antioxidant is the tripeptide glutathione (GSH) or γ-glutamyl-cysteinyl-glycine. It is synthesized in an ATP-dependent two-step reaction. Due to the reactive SH group of the central cysteine, whose sufficient supply by import or de novo synthesis is crucial, GSH is very reactive and is involved in numerous redox and detoxification processes, where it serves as cofactor for a large set of enzymes, including glutathione peroxidases (GPX) and glutathione S transferases (GST). Thus, GSH is central for the reduction of H2O2, lipid peroxides, and mixed persulfides as well as for biotransformation. An analogous system is the thioredoxin system, where cysteine residues from reduced thioredoxin serve as cofactor for thioredoxin reductases. In both systems, the cofactors are recycled after oxidation by NADPH-dependent glutathione or thioredoxin reductase, respectively. Accordingly, NAPDH supply, for example by the oxidative branch of the pentose phosphate pathway or by malic enzyme, is crucial to maintain the cellular redox balance. In addition, the cytosolic Cu/Zn superoxide dismutase (SOD1), mitochondrial Mn SOD (SOD2), and catalase are further enzymes that detoxify superoxide to H2O2 (SOD1) or H2O2 to water and O2 (catalase; reviewed in Hayes et al., 2020). Many of these antioxidant systems were shown to be required for melanoma maintenance (Cassidy et al., 2015; Jessen et al., 2020; Khamari et al., 2018; Leikam et al., 2014; Lokaj et al., 2009; Sato et al., 2020; Schmitt et al., 2015). Stress-induced transcription factors are major contributors for triggering these antioxidant pathways in response to ROS stress. These include hypoxia-inducible factor 1α (HIF1α), activator protein 1 (AP-1), nuclear factor κB (NF-κB), p53, and activating transcription factor 4 (ATF4) (Marinho et al., 2014; Paul et al., 2018). However, the most potent effector of the oxidative stress defense is nuclear factor erythroid 2-like 2 (NFE2L2, more commonly termed NRF2).

3 | THE TRANSCRIPTION FACTOR NRF2

NRF2 is a member of the Cap “n” Collar basic leucine zipper transcription factor family. Together with NRF1 and NRF3, NRF2 belongs to a subgroup of this transcription factor family responding to cellular stress. All three have been associated with pro-tumorigenic processes (Kim et al., 2016; Kobayashi, 2020; Rojo de la Vega et al., 2018), but the function of NRF2 is by far the best understood.

NRF2 serves as major determinant for counteracting and preventing oxidative damage and is therefore deemed the master regulator of the oxidative stress response. It induces the transcription of genes involved in all major metabolic pathways that are instrumental in relieving oxidative or electrophilic stress and enabling detoxification, such as the glutathione and thioredoxin-dependent detoxification pathways, the pentose phosphate pathway, as well as phase I and phase II detoxification enzymes (reviewed in He et al., 2020; Figure 1).

As a transcription factor usually responding to emergencies, NRF2 is fine-tuned at a post-translational level and has a short half-life of 15–20 min. In the cytosol, NRF2 is bound by its interaction...
partner Kelch-like ECH-associated protein 1 (KEAP1), which recruits the cullin 3 (CUL3)/RBX E3 ubiquitin ligase complex, eventually leading to proteasomal degradation of NRF2 (Cullinan et al., 2004; Kobayashi et al., 2004). Consequently, events that impair the physical interaction between KEAP1 and NRF2 constitute the main switch for activating NRF2, as they allow stabilization and nuclear accumulation of newly synthesized NRF2. Oxidative or electrophilic stress leads to the modification of cysteine residues in KEAP1, in particular Cys151, Cys226, Cys273, and Cys288, leading to a conformational change and impaired interaction with NRF2 (Baird et al., 2013; Li et al., 2012; McMahon et al., 2010; Zhang, & Hannink, 2003). Interestingly, the immunometabolite itaconate, which plays an important role in macrophage reprogramming, also modifies Cys residues in KEAP1 via alkylation and serves as potent NRF2 activator (Mills et al., 2018). In addition, the interaction between KEAP1 and NRF2 can be blocked by other means, such as binding of the autophagy marker p62/SQSTM1 to KEAP1 (Komatsu et al., 2010) or binding of p21CIP1 to NRF2 (Chen et al., 2009). Inhibiting the KEAP1-NRF2 interaction is furthermore the basis for a number of pharmacological NRF2 activators such as sulforaphane, curcumin, or tert-butylhydroquinone (Abiko et al., 2011; Kensler et al., 2013; Shin et al., 2020).

In several tumor entities such as bladder cancer, esophageal carcinoma, and (non-) small-cell lung cancer, NRF2 is found constitutively activated, either due to genetic loss or due to point mutations in KEAP1 or NRF2, resulting in impaired interaction between both proteins (Kerins & Ooi, 2018; Kim et al., 2010; Shibata et al., 2008; Singh et al., 2006). In melanoma, such mutations are found only sporadically (Miura et al., 2014), with no oncogenic NRF2 mutation and only two oncogenic KEAP1 mutations reported in the TCGA skin cutaneous melanoma dataset (Akbani et al., 2015; www.cbioportal.org). When NRF2 is activated—for example, in response to oxidative stress or due to mutational activation—it still requires an interaction partner to be transcriptionally active. The small MAF (sMAF) proteins MAFG, MAFF, and MAFK serve as NRF2 dimerization partners that enable the binding to antioxidant response elements (ARE) on the promoters of a wide array of genes (Hirotu et al., 2012; Katsuoka et al., 2005). Additionally, MAFG was also reported to enhance the nuclear retention of NRF2 by masking the NESzip motif of NRF2, which encompasses the nuclear export signal (Li et al., 2008).

### 4 NRF2 ACTIVATION IN MELANOCYTES AND MELANOMAS

Given the important contribution of UV radiation for ROS formation, it is not surprising that NRF2 activity is induced by UV in...
melanocytes. The most prominent effect is caused by the ROS-generating UV-A, but UV-B has also been reported to stabilize NRF2, though to a lesser extent (Kim et al., 2017; Marrot et al., 2008; Sample et al., 2018; Zhu et al., 2018). UV-A leads to a ROS-dependent induction of autophagy (Zhao et al., 2013), resulting in the accumulation of the autophagy cargo adapter p62, also called sequestosome 1 (SQSTM1), in melanocytes and melanoma cells. This causes an increase in NRF2 (Sample et al., 2018), because p62 has the ability to bind KEAP1 and thereby blocks its inhibitory interaction with NRF2 (Komatsu et al., 2010). As SQSTM1 contains an ARE promoter element and is a direct target gene of NRF2, both are connected in a positive feedback loop (Jain et al., 2010; Sample et al., 2018). In conclusion, NRF2 has an important role in survival and stress tolerance of melanocytes, which frequently encounter cell-damaging stress due to their epidermal location. In addition to the relief of oxidative stress and the induction of autophagy, NRF2-mediated survival might also be a consequence of the ability of NRF2 to increase the expression of anti-apoptotic proteins such as BCL-2 (Jian et al., 2011; Niture & Jaiswal, 2012).

A central function of NRF2 for melanocyte resilience is also visible in vitiligo, a skin condition with progressive loss of melanocytes, leading to patches of depigmented skin. The onset of vitiligo can be triggered by exogenous oxidative stressors including sunburn (Picardo & Bastonini, 2015), and compared to melanocytes from control subjects, those from vitiligo patients suffer from hypersensitivity to oxidative stress such as H₂O₂ (He et al., 2017; Qiu et al., 2014). Interestingly, nuclear translocation and activation of NRF2 after H₂O₂-induced oxidative injury were impaired in melanocytes from vitiligo patients, and serum levels of heme oxygenase 1, an indicator for NRF2 activity, were significantly reduced in a large patient cohort of 114 vitiligo patients compared to controls (Jian et al., 2014). Along the same lines, a polymorphism in the promoter region of NRF2 was associated with elevated vitiligo risk in a Han Chinese population (Guan et al., 2008), thus implying that Nrf2 knockdown mice show normal pigmentation, NRF2 is not required for melanocyte survival under unstressed conditions, but rather seems to be relevant under conditions of exogenous stress such as UV exposure (Chan et al., 1996).

Melanomas harbor additional NRF2 triggers. Many melanomas express oncogenic BRAFV600E or NRASQ61K/R, which are present in approximately 50% and 20% of cutaneous melanomas, respectively (Appenzeller et al., 2019; Akbani et al., 2015). Oncogenic BRAFV619E, the murine counterpart of BRAFV600E, can elevate NRF2-dependent transcription of target genes, an observation that was confirmed for inducible BRAFV600E in melanocytes (DeNicola et al., 2011; Jessen et al., 2020). As oncogenic KRAVG12D has a similar effect (DeNicola et al., 2011), it is plausible to assume that an activation of the MAPK pathway by RAF or RAS isoforms could be generally regarded as bona fide NRF2 activator.

5 | LINK BETWEEN NRF2 AND PIGMENTATION

Next to protecting from UV stress, NRF2 activity is also linked to melanocyte and melanoma differentiation and pigmentation, which are largely regulated by the microphthalmia-associated transcription factor MITF. MITF is a basic helix-loop-helix leucine zipper protein specifically binding to E boxes (5′-TCACGTGA-3′) and M boxes (5′-TCATGTG-3′) that are found in the promoter region of genes involved in melanin synthesis and melanosome formation such as tyrosinase (TYR) and Melanoma Antigen Recognized by T cells 1 (MART1 or MLANA), respectively (Bentley et al., 1994; Du et al., 2003). Several lines of evidence indicate that NRF2 limits differentiation features in melanocytes. In neonatal human dermal melanocytes (NHEM), NRF2 overexpression decreased the protein expression of pigmentation markers including TYR, resulting in a reduction in melanin content, while KEAP1 overexpression had the opposite effect (Shin et al., 2014). In another study focusing on the role of autophagy for melanocyte biology, it was found that a lack of autophagy mediated by ATG7 knockout led to an increase in oxidative stress and the induction of NRF2 targets, which correlated with dedifferentiation (Qiao et al., 2020). As already mentioned, UV-A exposure leads to an induction of NRF2 in melanocytes as well as in melanoma cells (Chaiprasongsuk et al., 2016). UV-A exposure has also been associated with an increase in tyrosinase protein and consequently melanin content. This increase was further enhanced when NRF2 levels were reduced by shRNA, thereby supporting an inhibitory effect of NRF2 on pigmentation (Chaiprasongsuk et al., 2016). The biological benefit of the NRF2-mediated dedifferentiation in melanocytes is not yet understood. However, it is possible that it helps to support melanocyte proliferation under conditions of stress recovery, as lowered MITF levels in melanocytes were shown to enhance proliferation in a zebrafish model (Taylor et al., 2011).

An influence of NRF2 on pigmentation was also shown for melanoma. In a recent study comparing the transcriptome of human melanoma cells transfected with control and NRF2-specific siRNA, the process "pigmentation" was found to be upregulated in NRF2-silenced cells (Jessen et al., 2020). While differentiation genes TYR, DCT, and MLANA were increased, expression levels of MITF were unaltered, which was consistent with the observation that NRF2 does not bind to the MITF promoter. However, NRF2 inhibited MITF transcriptional activity in a TYR promoter-driven luciferase assay, indicating that NRF2 counteracts MITF by a yet to be defined mechanism (Jessen et al., 2020). This underscores the role of NRF2 for melanoma malignancy, as melanomas with dedifferentiated features have a less favorable outcome (Takeuchi et al., 2003), and fits the observation that high nuclear NRF2 correlates with worse overall survival in melanoma (Hintsala et al., 2016).
Many of the MITF-regulated pigmentation markers such as TYR, DCT, and MLANA give rise to strongly antigenic surface peptides that are readily presented by major histocompatibility complex type I (MHCI) membrane proteins (Coulie et al., 1994; Fassler et al., 2019). Dedifferentiated melanomas, characterized by a MITF<sup>low</sup> signature, can therefore more likely escape immune control by cytotoxic T cells (Landsberg et al., 2012). The cytokine tumor necrosis factor α (TNFα) was reported as a potent trigger of dedifferentiation, as shown in cell culture and mouse studies (Landsberg et al., 2012; Riesenberg et al., 2015), and this inflammation-induced dedifferentiation contributed to the resistance to adoptive T-cell transfer in melanoma patients (Mehta et al., 2018).

Falletta and colleagues have revealed that extended timespans of TNFα treatment coincided with activation of the transcription factor ATF4, the effector of the integrated stress response, which drives the MITF<sup>low</sup> signature and the dedifferentiating effect of TNFα (Falletta et al., 2017). ATF4 serves as hub for integrating the response to various cellular stressors including ER stress, amino acid depletion, heme depletion, and viral infection. These stressors lead to the activation of the eIF2α kinases PERK, GCN2, HRI, and PKR, respectively, resulting in phosphorylation of eIF2α on position serine 51. While this blocks translation initiation and thereby global protein synthesis, the translation of ATF4 is enabled by a concerted mechanism involving the usage of an upstream open reading frame, which allows the correct ribosomal scanning and translation of the ATF4 transcript (Pakos-Zebrucka et al., 2016). As ATF4 responds to these various stress sources, it becomes activated under many conditions. Interestingly, TNFα is not only a trigger for ATF4, but also serves as potent activator of NRF2, which furthermore contributes to the dedifferentiation effect (Jessen et al., 2020). ATF4 and NRF2 are reported to physically interact and activate a subset of downstream genes interdependently (DeNicola et al., 2015; He et al., 2001), thus both transcription factors seem to jointly coordinate the TNFα-initiated stress response in melanoma. As illustrated by the example of the cytokine TNFα, melanoma cells can be driven into an MITF<sup>low</sup> state by external stress. This is also observed under therapy pressure, for example, in case of sustained BRAF or BRAF/MEK inhibition (Kemper et al., 2014; Muller et al., 2014). In particular, MITF<sup>low</sup> melanomas are characterized by the increased expression of receptor tyrosine kinases (RTK), such as AXL and EGFR, that contribute to the resistance of BRAF<sup>V600E</sup>-mutant melanomas to BRAF/MEK inhibition (Ji et al., 2015; Muller et al., 2014). Notably, NRF2 supports the expression of EGFR in MITF<sup>low</sup> melanoma cells, thereby implicating that NRF2 is establishing and/or maintaining an MITF<sup>low</sup>/EGFR<sup>high</sup> state (Jessen et al., 2020). Although the causes and consequences of NRF2-mediated EGFR expression in melanoma are not yet understood, data from KEAP1-mutant NSCLC support a role of NRF2 in RTK signaling, as NRF2 leads to elevated levels and activation of several RTKs including IGF1R, ERBB3, and EGFR in this tumor subtype (Chio et al., 2016; Vartanian et al., 2019).

In addition to altering the availability of differentiation antigens, NRF2 also has the capacity to block tumor immunity by several other means. UV exposure of melanocytes triggers the expression of the immune checkpoint ligand programmed death-like 1 (PD-L1), which mediates inhibitory interactions between tumor cells and effector T cells in an NRF2-dependent manner (Zhu et al., 2018). Reversely, shRNA-mediated depletion of NRF2 increases CD4<sup>+</sup> and CD8<sup>+</sup> T cells and suppresses melanoma progression in vivo (Zhu et al., 2018). In our own study, the in vivo function of NRF2 in melanomas was addressed by knocking out endogenous NRF2 in BRAF-mutant murine melanoma cells before injecting them subcutaneously into immune-competent mice (Jessen et al., 2020). In accordance with the study by Zhu et al. (2018), reduced melanoma growth and increased immune cell infiltration were observed. However, the effect was stronger, and several mice injected with NRF2-ko melanomas did not develop tumors at all, which is most likely due to the complete lack of tumor NRF2. RNA sequencing analysis revealed a striking upregulation of the gene set "defense to virus," an innate immune response gene signature responding to cytosolic DNA, in NRF2-ko melanomas (Jessen et al., 2020). The presence of cytosolic DNA is typically observed in response to viral infections, where it triggers the cGAS/STING pathway, resulting in the induction of type I interferons, cytokines, helicases, and other virus defense genes (Ni et al., 2018). Interestingly, it was previously described that infection with DNA viruses such as Herpes simplex in NRF2-deficient MEFs (Gunderstofte et al., 2019) and shows that NRF2 also contributes to the suppression of the innate immune response. Similar observations were made in human cells, where NRF2 repressed STING RNA and protein expression (Olagnier et al., 2018). Importantly, tumor immunogenicity has been reported to be strongly enhanced by activation of the cGAS/STING pathway (Schadt et al., 2019) and STING activation can break resistance to PD-1 blockade in mice (Fu et al., 2015). It is therefore likely that by suppressing the cGAS/STING pathway, NRF2 promotes an immunecold microenvironment.

This theory is further supported by the observation that NRF2 serves as potent inducer of cyclooxygenase 2 (COX2), an enzyme that converts phospholipid-derived arachidonic acid into prostaglandin H2 (PGH2), which is the precursor for PGE2. PGE2 has been shown to block the activation of T cells by attenuating T-cell receptor (TCR) signaling and thereby provides an immune-averse tumor environment (Wiemer et al., 2011). In addition, the induction of the innate immune response is reduced by PGE2 (Zelenay et al., 2015). In mouse models of melanoma, successful tumor growth depends on the tumor's ability to secrete PGE2, which mediates immune
tolerance, for example, by impairing the infiltration of type 1 dendritic cells into the tumor and limiting T-cell-mediated tumor elimination (Bottcher et al., 2018; Zelenay et al., 2015). Basal as well as H_{2}O_{2}- and TNFα-induced COX2 induction is dependent on NRF2, and a strong reduction in PGE2 is observed in NRF2-depleted human melanoma cells and mouse melanomas (Jessen et al., 2020). COX2 is encoded by the gene prostaglandin endoperoxide synthase 2 (PTGS2). The PTGS2 promoter does not bind NRF2, but has a binding site for ATF4, which serves as strong activator of PTGS2 expression downstream of NRF2. Importantly, forced overexpression of NRF2 or ATF4 leads to a robust upregulation of PTGS2, but only in presence of the respective other partner (Jessen et al., 2020 and unpublished results). Thus, the joint activation of ATF4 and NRF2 is required for the induction of immune-suppressive COX2. These observations underline the tight linkage of these two stress-induced transcription factors. This is further supported by studies of the PERK-mediated ER stress response. Next to activating ATF4 translation by phosphorylating eIF2α, PERK also phosphorylates NRF2, resulting in dissociation from KEAP1 and NRF2 stabilization (Cullinan et al., 2003). Further, ATF4 can also induce the transcription of NRF2 under conditions of ER stress (Sarcinelli et al., 2020). Although this was not investigated, it is therefore highly likely that ER stress will also serve as potent trigger of COX2 in melanoma.

7 | POTENTIAL ROLE OF NRF2 IN TARGETED THERAPY RESISTANCE AND FERROPTOSIS

It is known from KEAP1 mutated lung cancer that activation of the NRF2 pathway enables the development of resistance to chemotherapy as well as EGFR-targeted therapy (Frank et al., 2018; Park et al., 2018). Elevated NRF2 activity was also detected in A375 melanoma cells with acquired resistance to BRAF inhibitor, where it contributed to vemurafenib resistance (Khamari et al., 2018). After extended BRAF/MEK inhibition for several days, a small fraction of melanoma cells is able to withstand targeted cancer therapy and forms a resilient cancer cell pool until acquired resistance develops. This initial drug-tolerant state has acquired a dedifferentiated and mesenchymal-like signature (Tsoi et al., 2018; Viswanathan et al., 2017), features reminiscent of the changes caused by NRF2. It was demonstrated that dedifferentiated melanoma cells are particularly sensitive to ferroptosis inducers such as inhibitors of the selenocysteine containing enzyme glutathione peroxidase 4 (GPX4) or the xCT cystine-glutamate antiporter system, an intracellular cysteine source for the generation of glutathione (GSH). Ferroptosis is caused by iron-dependent peroxidation of unsaturated membrane lipids, which triggers a toxic chain reaction ultimately leading to cell death (Friedmann Angeli et al., 2019; Nehring et al., 2020). GPX4 detoxifies oxidized lipids and is therefore one of the main players in preventing ferroptosis (Friedmann Angeli et al., 2014; Yang et al., 2014), along with pathways supplying the GPX4 cofactor GSH (Dixon et al., 2012). Furthermore, other lipid radical scavengers such as coenzyme Q10 as well as factors determining availability of cellular iron or unsaturated membrane lipids have been reported to contribute to ferroptosis sensitivity (Doll et al., 2017, 2019; Friedmann Angeli et al., 2019). NRF2 induces a large set of ferroptosis-relevant genes including GPX4 and the gene encoding the xCT cystine-glutamate antiporter SLC7A11 (Figure 1 and Dai et al., 2020), and NRF2-ko melanoma cells show a marked sensitivity to GPX4 inhibitors (unpublished observations). How can these contrasting roles of NRF2 be reconciled? On the one hand, NRF2 supports melanoma dedifferentiation, a state, which reportedly sensitizes cancer cells to ferroptosis. On the other hand, NRF2 is a potent inducer of genes enabling ferroptosis resistance. Possibly, NRF2 serves as a marker of permanent oxidative stress, indicating a state where cells are particularly close to a maximal pro-oxidant threshold and can be easily driven into ferroptosis. Although anti-ferroptotic processes are already running, these might not be sufficient to tolerate the additional stress caused by GPX4 inhibitors. Future studies will be required to clarify the role of NRF2 in ferroptosis-sensitive drug-tolerant cells.

8 | IMMUNE THERAPY: THERAPEUTIC IMPLICATIONS

The link between NRF2 and immune tolerance has already been indicated in lung adenocarcinoma, where KEAP1 mutations are present in up to 20%, leading to permanent NRF2 activation (Cancer Genome Atlas Research, 2014; www.cbioportal.org). RNA sequencing revealed that KEAP1 mutated non-small-cell lung cancers show a reduced expression of a T-cell-inflamed gene expression signature (GEP) independent of tumor mutational burden (Cristescu et al., 2018), thus supporting an immune-suppressive role of NRF2 in cancer. This is relevant for cancer therapy, as the T-cell-inflamed GEP correlated with responsiveness toward anti-PD-1 immune therapy (Cristescu et al., 2018). An inhibition of the immune-evasive effects of NRF2 might therefore have the potential to trigger the endogenous anti-tumor response or increase the responsiveness to existing checkpoint inhibitor therapy. NRF2 itself is currently not targetable, as available inhibitors lack specificity.

However, downstream effectors of NRF2 such as COX2 or STING are attractive targets, with inhibitors and agonists, respectively, already approved or in clinical trials. In a BRAFV600E mouse model, COX inhibition with aspirin strongly synergized with anti-PD-1 immune therapy (Zelenay et al., 2015). Similar observations were made with the COX2 inhibitor celecoxib, which sensitized pancreatic cancer to immune checkpoint blockade in mouse models (Markosyan et al., 2019). In humans, a retrospective study revealed an increased time to progression in checkpoint inhibitor-treated melanoma patients if they also co-administered COX inhibitors (Wang et al., 2020). Clinical phase II trials are underway to test the effect of aspirin in combination with PD-1 and CTLA4-targeting checkpoint inhibitors in melanoma (www.clinicaltrials.gov).

In addition, the negative effect of NRF2 on the cGAS/STING pathway might constitute a targetable Achilles heel. Although
reduced pathway activation correlates with reduced immunogenicity (Schadt et al., 2019), it sensitizes to viral infection, as already mentioned (Gunderstofte et al., 2019; Olagnier et al., 2018).

Interestingly, a recent report described the susceptibility of STING-deficient melanoma cells to Talmogene laherparepvec (T-VEC), an oncolytic Herpes simplex virus engineered to express granulocyte-macrophage colony-stimulating factor (GM-CSF), which is approved for melanoma therapy (Bommareddy et al., 2019). A T-VEC therapy might therefore serve as promising approach after previous stimulation of NRF2, for example, by oxidative stress inducers or cytokine treatment.

In summary, it emerges that NRF2 is a central hub, not only to counteract acute oxidative stress on the cellular level, but also to prevent immune recognition and cell clearance of the damaged cells (Figure 2). This protective system is hijacked in tumors like melanoma and non-small-cell lung cancer, where it contributes to immune evasion and therapy resistance.

ACKNOWLEDGEMENTS

JP F-A is supported by the junior group leader program of the Rudolf Virchow Center, University of Würzburg, the German Research Foundation grant FR3736/3-1 and the Interdisciplinary Center for Clinical Research (IZKF) B-424. SM is supported by the German Research Foundation grant FR3736/3-1 and the Interdisciplinary Center for Clinical Research (IZKF) Z-14 (Central Unit for Personalized Oncology grant ME1899/6-1 and the Interdisciplinary Clinical Research (IZKF) B-424. SM is supported by the German Foundation grant FR3736/3-1 and the Interdisciplinary Center for Clinical Research (IZKF) Z-14 (Central Unit for Personalized Oncology grant ME1899/6-1 and the Interdisciplinary Clinical Research (IZKF) B-424.

CONFLICT OF INTEREST

The authors have no conflict of interest in relation to this work.

ORCID

Svenja Meierjohann https://orcid.org/0000-0002-9058-7196

REFERENCES

Abiko, Y., Miura, T., Phuc, B. H., Shinkai, Y., & Kumagai, Y. (2011). Participation of covalent modification of Keap1 in the activation of Nrf2 by tert-butylbenzoquinone, an electrophilic metabolite of butylated hydroxyanisole. Toxicology and Applied Pharmacology, 255(1), 32–39. https://doi.org/10.1016/j.taap.2011.05.013

Akbani, R., Akdemir, K. C., Aksoy, B. A., Albert, M., Ally, A., Amin, S. B., Arachchi, H., Arora, A., Auman, J. T., Ayala, B., Baboud, J., Balasundaram, M., Balu, S., Barnabas, N., Bartlett, J., Bartlett, P., Bastian, B. C., Baylin, S. B., Behera, M., ... Zou, L. (2015). Genomic classification of cutaneous melanoma. Cell, 161(7), 1681–1696. https://doi.org/10.1016/j.cell.2015.05.044

Appenzeller, S., Gesierich, A., Thiem, A., Hufnagel, A., Jessen, C., Kneitz, H., Regentsburger, M., Schmidt, C., Zirkenbach, V., Bischler, T., Schilling, B., Siedel, C., Goebele, M.-E., Houben, R., Schrama, D., Gehrig, A., Rost, S., Maurus, K., Bargou, R., ... Meierjohann, S. (2019). The identification of patient-specific mutations reveals dual pathway activation in most patients with melanoma and activated receptor tyrosine kinases in BRAF/NRAS wild-type melanomas. Cancer, 125(4), 586–600. https://doi.org/10.1002/cncr.31843

Baird, L., Lieres, D., Swift, S., & Dinkova-Kostova, A. T. (2013). Regulatory flexibility in the Nrfl2-mediated stress response is conferred by conformational cycling of the Keap1-Nrf2 protein complex. Proceedings of the National Academy of Sciences of the United States of America, 110(38), 15259–15264. https://doi.org/10.1073/pnas.1305687110

Bentley, N. J., Eisen, T., & Goding, C. R. (1994). Melanocyte-specific expression of the human tyrosinase promoter: Activation by the microphthalmia gene product and role of the initiator. Molecular and Cellular Biology, 14(12), 7996–8006. https://doi.org/10.1128/mcb.14.12.7996

Berra, E., Benizri, E., Ginouves, A., Volmat, V., Roux, D., & Pouyssegur, J. (2003). HIF prolyl-hydroxylase 2 is the key oxygen sensor setting low steady-state levels of HIF-1alpha in normoxia. EMBO Journal, 22(16), 4082–4090. https://doi.org/10.1093/emboj/cdg392

Bommareddy, P. K., Zloza, A., Rabkin, S. D., & Kaufman, H. L. (2019). Oncolytic virus immunotherapy induces immunogenic cell death and overcomes STING deficiency in melanoma. Oncoimmunology, 8(7), 1591875. https://doi.org/10.1080/21626402X.2019.1591875

Böttcher, J. P., Bonavita, E., Chakravarty, P., Blees, H., Cabeza-Cabrerizo, M., Sammicheli, S., Rogers, N. C., Sahai, E., Zelenay, S., & Reis e Sousa, C. (2018). NK cells stimulate recruitment of cDC1 into the tumor microenvironment promoting cancer immune control. Cell, 172(5), 1022–1037 e1014. https://doi.org/10.1016/j.cell.2018.01.004

Cancer Genome Atlas Research, N. (2014). Comprehensive molecular profiling of lung adenocarcinoma. Nature, 511(7511), 543–550. https://doi.org/10.1038/nature13385

Cassidy, P. B., Honegger, M., Poerschke, R. L., White, K., Florell, S. R., Andtbacka, R. H. I., Tross, J., Anderson, M., Leachman, S. A., & Moos, P. J. (2015). The role of thioredoxin reductase 1 in melanoma metabolism and metastasis. Pigment Cell & Melanoma Research, 28(6), 685–695. https://doi.org/10.1111/pcmr.12398

FIGURE 2 Activators of NRF2 and immune-relevant effects in melanoma, as discussed in this review.
Dan Dunn, J., Alvarez, L. A., Zhang, X., & Soldati, T. (2015). Reactive oxygen species and mitochondria: A nexus of cellular homeostasis. *Redox Biology*, 8, 69–76. https://doi.org/10.1016/j.redox.2015.12.006

Chan, K., Lu, R., Chang, J. C., & Kan, Y. W. (1996). Nrf2, a member of the NF-E2 family of transcription factors, is not essential for murine erythropoiesis, growth, and development. *Proceedings of the National Academy of Sciences of the United States of America*, 93(24), 13943–13948. https://doi.org/10.1073/pnas.93.24.13943

Chen, W., Sun, Z., Wang, X. J., Jiang, T., Huang, Z., Fang, D., & Zhang, D. D. (2009). Direct interaction between Nrf2 and p21Cip1/WAF1 upregulates the Nrf2-mediated antioxidant response. *Molecular Cell*, 34(6), 663–673. https://doi.org/10.1016/j.molcel.2009.04.029

Chio, I. I. C., Jafarnejad, S. M., Ponz-Sarvise, M., Park, Y., Rivera, K., Palm, W., Wilson, J., Sangar, V., Hao, Y., Ohiund, D., Wright, K., Filippini, D., Lee, E. J., Da Silva, B., Schoepfer, C., Wilkinson, J. E., Buscaglia, J. M., DeNicola, G. M., Tiriach, H., & Tuveson, D. A. (2016). Nrf2 promotes tumor maintenance by modulating mRNA translation in pancreatic cancer. *Cell*, 166(4), 963–976. https://doi.org/10.1016/j.cell.2016.06.056

Coulie, P. G., Brichard, V., Van Pel, A., Wölfel, T., Schneider, J., Traversari, C., Mattei, S., De Plaen, E., Lurquin, C., Szikora, J. P., Renaud, J. C., & Boon, T. (1994). A new gene coding for a differentiation antigen recognized by autologous cytotoxic T lymphocytes on HLA-A2 melanomas. *Journal of Experimental Medicine*, 180(1), 35–42. https://doi.org/10.1084/jem.180.1.35

Cristescu, R., Mogg, R., Ayers, M., Albright, A., Murphy, E., Yearley, J., Sher, X., Liu, X. Q., Lu, H., Nebozyn, M., Zhang, C., Luncsemy, I., Gleason, C. E., Patel, D. N., Bauer, A. J., Cantley, A. M., Yang, W. I. A., & Tuveson, D. A. (2011). Oncogene-induced Nrf2 transcription promotes ROS detoxification and tumorigenesis. *Nature*, 475(7354), 106–109. https://doi.org/10.1038/nature10189

Dixons, S. J., Lemberg, K. M., Lamprecht, M. R., Skouta, R., Zaitsev, E. M., Gleason, C. E., Patel, D. N., Bauer, A. J., Cantley, A. M., Yang, W. S., Morrison, B., & Stockwell, B. R. (2012). Ferroptosis: An iron-dependent form of nonapoptotic cell death. *Cell*, 149(5), 1060–1072. https://doi.org/10.1016/j.cell.2012.03.042

Doherty, J. R., & Cleveland, J. L. (2013). Targeting lactate metabolism for cancer therapeutics. *Journal of Clinical Investigation*, 123(9), 3685–3692. https://doi.org/10.1172/JCI69741

Doll, S., Freitas, F. P., Shah, R., Aldrovandi, M., da Silva, M. C., Ingold, I., Goyo Grocin, A., Xavier da Silva, T. N., Panziluzi, E., Scheel, C. H., Mourão, A., Buday, K., Sato, M., Wanninger, J., Vignane, T., Mohana, V., Rehberger, F.,Flatley, A., Schepers, A., … Conrad, M. (2019). FSP1 is a glutathione-independent ferroptosis suppressor. *Nature*, 575(7784), 693–698. https://doi.org/10.1038/s41586-019-1707-0

Doll, S., Pronten, B., Tyurina, Y. Y., Panziluzi, E., Kobayashi, S., Ingold, I., Irmler, M., Beckers, J., Aichler, M., Walch, A., Prokisch, H., Trümbach, D., Mao, G., Qu, F., Bayir, H., Fülekrug, J., Scheel, C. H., Wurst, W., Schick, J. A., … Conrad, M. (2017). ACSL4 dictates ferroptosis sensitivity by shaping cellular lipid composition. *Nature Chemical Biology*, 13(1), 91–98. https://doi.org/10.1038/nchembio.2239

Du, J., Miller, A. J., Widlund, H. R., Horstmann, M. A., Ramaswamy, S., & Fisher, D. E. (2003). MLANA/MART1 and SILV/PME17/GP100 are transcriptionally regulated by MIF in melanocytes and melanoma. *American Journal of Pathology*, 163(1), 333–343. https://doi.org/10.1016/S0002-9440(10)63577-7

Falletta, P., Sanchez-del-Campo, L., Chauhan, J., Efferm, M., Kenyon, A., Kershaw, C. J., Siddaway, R., Lisle, R., Freter, R., Daniels, M. J., Lu, X., Tüting, T., Middleton, M., Buffa, F. M., Willis, A. E., Pavitt, G., Ronai, Z. A., Sauka-Spengler, T., Hözel, M., & Goding, C. R. (2017). Translation reprogramming is an evolutionarily conserved driver of phenotypic plasticity and therapeutic resistance in melanoma. *Genes & Development*, 31(1), 18–33. https://doi.org/10.1101/gad.290940.116

Fässler, M., Diem, S., Manganana, J., Hasan Ali, O., Berner, F., Bomze, D., Ring, S., Niederer, R., del Carmen Gil Cruz, C., Pérez Shibayama, C. I., Krolik, M., Siano, M., Joerger, M., Recher, M., Risch, L., Güesewell, S., Risch, M., Speiser, D. E., Ludewig, B., … Flatz, L. (2019). Antibodies as biomarker candidates for response and survival to checkpoint inhibitors in melanoma patients. *Journal for ImmunoTherapy of Cancer*, 7(1), 50. https://doi.org/10.1186/s40425-019-0523-2

Frank, R., Scheffler, M., Merkelbach-Bruse, S., Ihle, M. A., Kron, A., Rauer, M., & Wolf, J. (2018). Clinical and pathological characteristics of KEAP1- and NFE2L2-mutated non-small cell lung carcinoma (NSCLC). *Clinical Cancer Research*, 24(13), 3087–3096. https://doi.org/10.1158/1078-0432.CCR-17-3416

Friedmann Angeli, J. P., Krysko, D. V., & Conrad, M. (2019). Ferroptosis at the crossroads of cancer-acquired drug resistance and immune evasion. *Nature Reviews Cancer*, 19(7), 405–414. https://doi.org/10.1038/s41568-019-0149-1

Friedmann Angeli, J. P., Schneider, M., Pronten, B., Tyurina, Y. Y., Tyurin, V. A., Hammond, V. J., Herbach, N., Aichler, M., Walch, A., Eggenhofer, E., Basavarajappa, D., Rädmak, O., Kobayashi, S., Seibt, T., Beck, H., Neff, F., Esposito, I., Wanke, R., Förster, H., … Conrad, M. (2014). Inactivation of the ferroptosis regulator Gpx4 triggers acute renal failure in mice. *Nature Cell Biology*, 16(12), 1180–1191. https://doi.org/10.1038/ncb3064

Fu, J., Kanne, D. B., Leong, M., Glickman, L. H., McWhirter, S. M., Lommens, E., Mechette, K., Leong, J. J., Lauer, P., Liu, W., Svick, K. E., Zeng, Q. I., Soares, K. C., Zheng, L., Portnoy, D. A., Woodward, J. J., Pardoll, D. M., Dubensky, T. W., & Kim, Y. (2015). STING agonist formulated cancer vaccines can cure established tumors resistant to PD-1 blockade. *Science Translational Medicine*, 7(283), 283ra252. https://doi.org/10.1126/scitranslmed.aad4306

Guan, C. P., Zhou, M. N., Xu, A. E., Kang, K. F., Liu, J. F., Wei, X. D., & Hong, W. S. (2008). The susceptibility to vitiligo is associated with NF-E2-related factor 2 (Nrf2) gene polymorphisms: A study on Chinese Han population. *Experimental Dermatology*, 17(12), 1059–1062. https://doi.org/10.1111/j.1600-0625.2008.00752.x
Gunderstofte, C., Iversen, M. B., Peri, S., Thielke, A., Balachandran, S., Holm, C. K., & Orlagier, D. (2019). Nrf2 negatively regulates type I interferon responses and increases susceptibility to herpes genital infection in mice. *Frontiers in Immunology*, 10, 2101. https://doi.org/10.3389/fimmu.2019.02101

Gutiérrez-Salmeron, M., Garcia-Martinez, J. M., Martinez-Users, J., Fernandez-Acenero, M. J., Voltet, B., Olivier, S., Chauhan, J., Lucena, S. R., De la Vieja, A., Goding, C. R., Chocarro-Calvo, A., & Garcia-Jimenez, C. (2020). Paradoxical activation of AMPK by glucose drives selective EP300 activity in colorectal cancer. *PLoS Biology*, 18(6), e3000732. https://doi.org/10.1371/journal.pbio.3000732

Haq, R., Shaog, J., Andreu-Perez, P., Yokoyama, S., Edelman, H., Rowe, G. C., & Wldlund, H. R. (2013). Oncogenic BRAF regulates oxidative metabolism via PGC1alpha and MITF. *Cancer Cell*, 23(3), 302–315. https://doi.org/10.1016/j.ccr.2013.02.003

Hayes, J. D., Dinkova-Kostova, A. T., & Tew, K. D. (2020). Oxidative stress in cancer. *Cancer Cell*, 38(2), 167–197. https://doi.org/10.1016/j.ccell.2020.06.001

He, C. H., Gong, P., Hu, B., Stewart, D., Choi, M. E., Choi, A. M., & Alam, B. (2018). Keap1 knockdown is essential for the activation of antioxidant response element driven gene transcription. *Oncogene*, 37(2), 285–293. https://doi.org/10.1038/s41388-018-0113-y

He, C. H., Gong, P., Hu, B., Stewart, D., Choi, M. E., Choi, A. M., & Alam, B. (2018). Keap1 knockdown is essential for the activation of antioxidant response element driven gene transcription. *Oncogene*, 37(2), 285–293. https://doi.org/10.1038/s41388-018-0113-y

Jian, Z., Li, K., Song, P., Zhu, G., Zhu, L., Cui, T., & Li, C. (2014). Impaired activation of the Nrf2-ARE signaling pathway undermines H2O2-induced oxidative stress response: A possible mechanism for melanocyte degeneration in vitiligo. *The Journal of Investigative Dermatology*, 138(4), 2221–2230. https://doi.org/10.1038/jid.2014.152

Katsuoka, F., Motoshita, H., Ishi, T., Abarutani, H., Engel, J. D., & Yamamoto, M. (2005). Genetic evidence that small maf proteins are essential for the activation of antioxidant response element dependent genes. *Molecular and Cellular Biology*, 25(18), 8044–8051. https://doi.org/10.1128/MCB.25.18.8044-8051.2005

Kemper, K., de Goeje, P. L., Peeper, D. S., & van Amerongen, R. (2014). Phenotype switching: Tumor cell plasticity as a resistance mechanism and target for therapy. *Cancer Research*, 74(21), 5937–5941. https://doi.org/10.1158/0008-5472.CAN-14-1174

Kensler, T. W., Egner, P. A., Agyeman, A. S., Visvanathan, K., Groopman, J. D., Chen, J. G., & Talalay, P. (2013). Keap1-nrf2 signaling: A target for cancer prevention by sulforaphane. *Topics in Current Chemistry*, 329, 163–177. https://doi.org/10.1007/128_2012_339

Kerins, M. J., & Ooi, A. (2018). A catalogue of somatic Nrf2 gain-of-function mutations in cancer. *Scientific Reports*, 8(1), 12846. https://doi.org/10.1038/s41598-018-31281-0

Khamari, R., Trinh, A., Garbert, P. E., Coraza-Rosas, P., Riveros-Cruz, S., Balayssac, S., Malet-Martino, M., Dekiouk, S., Joncquel Chevalier, M., Maboudou, P., Garçon, G., Ravasi, L., Guerreschi, P., Mortier, L., Quesnel, B., Marchetti, P., & Kluzu, J. (2018). Glucose metabolism and Nrf2 coordinate the antioxidant response in melanoma resistant to MAPK inhibitors. *Cell Death & Disease*, 9(3), 325. https://doi.org/10.1038/s41419-018-0340-4

Kim, H. M., Han, J. W., & Chan, J. Y. (2016). Nuclear factor erythroid-2 like 1 (NFE2L1): Structure, function and regulation. *Gene*, 584(1), 17–25. https://doi.org/10.1016/j.gene.2016.03.002

Kim, J. Y., Lee, H., Lee, E. J., Kim, M., Kim, T. G., Kim, H. P., & Oh, S. H. (2017). Keap1 knockdown in melanocytes induces cell proliferation and survival via HO-1-associated beta-catenin signaling. *Journal of Dermatological Science*, 88(1), 85–95. https://doi.org/10.1016/j.jdermsci.2017.05.007

Kim, Y. R., Oh, J. E., Kim, S. M., Kang, M. R., Park, S. W., Han, J. Y., & Lee, S. H. (2010). Oncogenic Nrf2 mutations in squamous cell carcinomas of oesophagus and skin. *The Journal of Pathology*, 220(4), 446–451. https://doi.org/10.1002/path.2653

Kobayashi, A. (2020). Roles of Nrf3 in the hallmarks of cancer: Proteasomal inactivation of tumor suppressors. *Cancers (Basel)*, 12(9), 2681. https://doi.org/10.3390/cancers12092681

Kobayashi, A., Kang, M.-I., Okawa, H., Ohitsuji, M., Zenke, Y., Chiba, T., Igarashi, K., & Yamamoto, M. (2004). Oxidative stress sensor Keap1 functions as an adaptor for Cul3-based E3 ligase to regulate proteasomal degradation of Nrf2. *Molecular and Cellular Biology*, 24(16), 7130–7139. https://doi.org/10.1128/MCB.24.16.7130-7139.2004

Komatsu, M., Kurokawa, H., Sugita, S., Taguchi, K., Kobayashi, A., Ichimura, Y., Sou, Y.-S., Ueno, I., Sakamoto, A., Tong, K. I., Kim, M., Nishito, Y., Iemura, S.-I., Natsume, T., Ueno, I., Komimami, E., Motohashi, H., Tanaka, K., & Yamamoto, M. (2010). The selective autophagy substrate p62 activates the stress responsive transcription factor Nrf2 through inactivation of Keap1. *Nature Cell Biology*, 12(3), 213–223. https://doi.org/10.1038/nccb2011

Landsberg, J., Kohlmeier, J., Renn, M., Bald, T., Rogava, M., Cron, M., Fatho, M., Lennerz, V., Wölfel, T., Hözel, M., & Tütting, T. (2012). Melanomas resist T-cell therapy through inflammation-induced reversible dedifferentiation. *Nature*, 490(7420), 412–416. https://doi.org/10.1038/nature11538

Lee, S. R., Yang, K. S., Kwon, J., Lee, C., Jeong, W., & Rhee, S. G. (2002). Reversible inactivation of the tumor suppressor PTEN by H2O2. *Journal of Biological Chemistry*, 277(23), 20336–20342. https://doi.org/10.1074/jbc.M111899200
Leikam, C., Hufnagel, A., Schartl, M., & Meierjohann, S. (2008). Oncogene activation in melanocytes links reactive oxygen to multinucleated phenotype and senescence. Oncogene, 27(56), 7070–7082. https://doi.org/10.1038/onc.2008.323

Leikam, C., Hufnagel, A., Walz, S., Kneitz, S., Fekete, A., Müller, M. J., Ellers, M., Schartl, M., & Meierjohann, S. (2014). Cystathionase mediates senescence evasion in melanocytes and melanoma cells. Oncogene, 33(6), 771–782. https://doi.org/10.1038/onc.2012.641

Li, W., Yu, S., Liu, T., Kim, J. H., Blank, V., Li, H., & Kong, A. N. (2008). Heterodimerization with small Maf proteins enhances nuclear retention of Nrf2 via masking the NESzip motif. Biochimica et Biophysica Acta, 1783(10), 1847–1856. https://doi.org/10.1016/j.bbabio.2008.05.024

Li, Y., Paonessa, J. D., & Zhang, Y. (2012). Mechanism of chemical activation of Nrf2. PLoS One, 7(4), e35122. https://doi.org/10.1371/journal.pone.0035122

Lokaj, K., Meierjohann, S., Schultz, C., Teutschbein, J., Schartl, M., & Sickmann, A. (2009). Quantitative differential proteome analysis in an animal model for human melanoma. Journal of Proteome Research, 8(4), 1818–1827. https://doi.org/10.1021/pr800578a

Marinho, H. S., Real, C., Cyrne, L., Soares, H., & Antunes, F. (2014). Hydrogen peroxide sensing, signaling and regulation of transcription factors. Redox Biology, 2, 535–562. https://doi.org/10.1016/j.redox.2014.02.006

Markosyan, N., Li, J., Sun, Y. H., Richman, L. P., Lin, J. H., Yan, F., Quinones, L., Sela, Y., Yamazoe, T., Gordon, N., Tobias, J. W., Byrne, K. T., Rech, A. J., FitzGerald, G. A., Stanger, B. Z., & Von Zecherle, R. H. (2019). Tumor cell-intrinsic EPHA2 suppresses anti-tumor immunity by regulating PTGS2 (COX-2). Journal of Clinical Investigation, 129(9), 3594–3609. https://doi.org/10.1172/JCI127755

Marrot, L., Jones, C., Perez, P., & Meunier, J. R. (2008). The significance of Nrf2 pathway in (photo)-oxidative stress response in melanocytes and keratinocytes of the human epidermis. Pigment Cell & Melanoma Research, 21(1), 79–88. https://doi.org/10.1111/j.1755-148X.2007.00424.x

McMahon, M., Lamont, D. J., Beattie, K. A., & Hayes, J. D. (2010). Keap1 perceives stress via three sensors for the endogenous signaling molecules nitric oxide, zinc, and alkenals. Proceedings of the National Academy of Sciences of the United States of America, 107(44), 18838–18843. https://doi.org/10.1073/pnas.1007387107

Mehta, A., Kim, Y. J., Robert, L., Tsoi, J., Comín-Andúix, B., Berent-Maiz, B., Cochran, A. J., Economou, J. S., Tumeh, P. C., Puig-Saus, C., & Ribas, A. (2018). Immunotherapy resistance by inflammation-induced dedifferentiation. Cancer Discovery, 8(8), 935–943. https://doi.org/10.1158/2159-8290.CD-17-1178

Meierjohann, S. (2014). Oxidative stress in melanocyte senescence and melanoma transformation. European Journal of Cell Biology, 93(1–2), 36–41. https://doi.org/10.1007/s10045-013-0111-5

Mills, E. L., Ryan, D. G., Prag, H. A., Dikovskaya, D., Menon, D., Zaslona, A., Lujic, M., Samali, A., & Gorman, A. M. (2016). The integrated stress response. EMBO Reports, 17(10), 1374–1395. https://doi.org/10.15252/embr.201642195

Panich, U., Sittithumcharree, G., Rathiboon, N., & Jinawatnootai, S. (2016). Ultraviolet radiation-induced skin aging: The role of DNA damage and oxidative stress in epidermal stem cell damage mediated skin aging. Stem Cells International, 2016, 7370642. https://doi.org/10.1155/2016/7370642

Park, S.-H., Kim, J. H., Ko, E., Kim, J.-Y., Park, M.-J., Kim, M. J., Seo, H., Li, S., & Lee, J.-Y. (2018). Resistance to gefitinib and cross-resistance to irreversible EGFR-TKIs mediated by disruption of the Keap1-Nrf2 pathway in human lung cancer cells. The FASEB Journal, 32(11), 5862–5873. https://doi.org/10.1096/fj.201800011R

Paul, B. D., Sbodio, J. I., & Snyder, S. H. (2018). Cysteine metabolism in neuronal redox homeostasis. Trends in Pharmacological Sciences, 39(5), 513–524. https://doi.org/10.1016/j.tips.2018.02.007

Picard, M., & Bastonini, E. (2015). A new view of vitiligo: Looking at normal-appearing skin. The Journal of Investigative Dermatology, 135(7), 1713–1714. https://doi.org/10.1038/jid.2015.92

Piskounova, E., Agathocleous, M., Murphy, M. M., Hu, Z., Heddleston, S. E., Zhao, Z., Leitch, A. M., Johnson, T. M., DeBerardinis, R. J., & Morrison, S. J. (2015). Oxidative stress inhibits distant metastasis by human melanoma cells. Nature, 527(7577), 186–191. https://doi.org/10.1038/nature15726

Premi, S., & Brash, D. E. (2016). Chemical excitation of electrons: A dark path to melanoma. DNA Repair (Amst), 44, 169–177. https://doi.org/10.1016/j.dnarep.2016.05.023

Qiao, Z., Xu, Z., Xiao, Q., Yang, Y., Ying, J., Xiang, L., & Zhang, C. (2020). Dysfunction of ATG7-dependent autophagy dysregulates the
death by GPX4. Cell, 156(1–2), 317–331. https://doi.org/10.1016/j.cell.2013.12.010

Zelenay, S., van der Veen, A. G., Böttcher, J. P., Snelgrove, K. J., Rogers, N., Acton, S. E., Chakravarty, P., Girotti, M. R., Marais, R., Quezada, S. A., Sahai, E., & Reis e Sousa, C. (2015). Cyclooxygenase-dependent tumor growth through evasion of immunity. Cell, 162(6), 1257–1270. https://doi.org/10.1016/j.cell.2015.08.015

Zhang, D. D., & Hannink, M. (2003). Distinct cysteine residues in Keap1 are required for Keap1-dependent ubiquitination of Nrf2 and for stabilization of Nrf2 by chemopreventive agents and oxidative stress. Mol Cell Biol, 23(22), 8137–8151. https://doi.org/10.1128/mcb.23.22.8137-8151.2003

Zhao, Y. I., Zhang, C.-F., Rossiter, H., Eckhart, L., König, U., Karner, S., Mildner, M., Bochkov, V. N., Tschachler, E., & Gruber, F. (2013). Autophagy is induced by UVA and promotes removal of oxidized phospholipids and protein aggregates in epidermal keratinocytes. The Journal of Investigative Dermatology, 133(6), 1629–1637. https://doi.org/10.1038/jid.2013.26

Zhu, B. O., Tang, L., Chen, S., Yin, C., Peng, S., Li, X., Liu, T., Liu, W., Han, C., Stawski, L., Xu, Z.-X., Zhou, G., Chen, X., Gao, X., Goding, C. R., Xu, N., Cui, R., & Cao, P. (2018). Targeting the upstream transcriptional activator of PD-L1 as an alternative strategy in melanoma therapy. Oncogene, 37(36), 4941–4954. https://doi.org/10.1038/s41388-018-0314-0

How to cite this article: Friedmann Angeli JP, Meierjohann S. NRF2-dependent stress defense in tumor antioxidant control and immune evasion. Pigment Cell Melanoma Res. 2021;34:268–279. https://doi.org/10.1111/pcmr.12946