Trends in Cell Biology

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Review

The Complex Interplay between Antioxidants and ROS in Cancer

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Reactive oxygen species (ROS) play important roles in tissue homeostasis, cellular signaling, differentiation, and survival. In this review, we discuss the types of ROS, their impact on cellular processes, and their pro- and antitumorigenic effects. Further, we discuss recent advances in our understanding of both endogenous and exogenous antioxidants in tumorigenic processes. Finally, we discuss how aberrant activation of antioxidant programs by the transcription factor NFE2-related factor 2 (NRF2) influences tumorigenesis and metastasis, and where the current gaps in our knowledge remain.

Highlights

New tools allow *in vivo* measurements of ROS in tumors.

Mouse modeling and genetic screening approaches have revealed novel complexities and redundancies in endogenous antioxidant systems.

Exogenous antioxidants may promote cancer through complex mechanisms.

Aberrant NRF2 activation has diverse, and sometimes contradictory, impacts on tumor growth and metastasis.

Tissue of origin, tumor stage, and the microenvironment greatly influence the influence of ROS on cancer.

Introduction

ROS play important roles in tissue homeostasis, from the regulation of signaling and differentiation to the promotion of cellular damage and death. Unsurprisingly, their levels are tightly regulated by cellular antioxidant defenses to prevent unwanted consequences of their actions. While generally grouped together, ROS are a diverse class of molecules with distinct effects on cellular components. Consequently, their influence on cellular processes is complex and they have both pro- and antitumorigenic effects. In this review, we discuss the role of both oxidants and antioxidants in tumorigenic processes.

Types of ROS

ROS are defined as molecules that contain and engage in the transfer of electrons from reactive oxygen. It is challenging to measure ROS directly, and consequently many tools have been developed for the indirect measure of ROS in cells and tissues (Box 1). Here we outline the different forms of ROS, their sources, and their primary targets (Figure 1).

Superoxide

Superoxide (O_2^-) is primarily produced as a consequence of electron reaction with molecular oxygen at complex I/III of the mitochondrial electron transport chain. O_2^- is moderately reactive but short lived. It is easily dismutated to hydrogen peroxide (H_2O_2) by superoxide dismutases or nonenzymatically. Its anionic charge prevents its diffusion through membranes. Substantial extracellular O_2^- is also produced by certain cell types (e.g., neutrophils) by NADPH oxidase enzymes. In the cell, it targets iron–sulfur (Fe-S) clusters to release iron [1]. O_2^- can also form peroxynitrite ($ONOO^-$) through a reaction with nitric oxide (NO). $ONOO^-$ reacts with proteins to cause oxidation or nitration of amino acids, DNA to induce double-strand breaks, and lipids to induce lipid peroxidation.

Hydrogen Peroxide

H_2O_2 is formed from O_2^- and is moderately reactive but long lived. H_2O_2 is also generated by Ero1 as a consequence of oxidative protein folding in the endoplasmic reticulum (ER) [2]. It can diffuse through membranes and consequently can have effects distal from its site of production. It is the primary ROS responsible for protein oxidation. While low levels (1–10 nM) play an important role in signaling via redox signaling via oxidation [protein tyrosine phosphatases (PTPs), insulin signaling], higher levels (>100 nM) cause 'oxidative stress' [3].

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Box 1. Tools for Measuring ROS

Many methods exist for the measurement of ROS and they have distinct advantages and disadvantages [147,148]. Many studies rely on indirect measurements of ROS. These include redox-active probes that yield fluorescent or luminescent products on oxidation by ROS, but most can be oxidized by several ROS species so specificity is a problem. Spin trapping with electron paramagnetic resonance (EPR) detection is the most unambiguous method for free radical detection, but cellular antioxidants may scavenge ROS before they can react with spin traps. The use of multiple complementary methods, including evaluation of redox ratios (e.g., GSH/GSSG), protein oxidation states, and ratiometric reporters (e.g., HyPER, roGFP) is suggested. Further, ROS are generated in distinct subcellular compartments and consequently their location should be considered when interpreting their effects. Importantly, distinct cell states including loss of ECM attachment can alter cellular metabolism to support mitochondrial ROS metabolism [149]. While ratiometric reporters have been targeted to subcellular compartments, recently tools to generate localized ROS have also been developed [150]. As the oxidation states of the mitochondria and ER are quite different from the cytosol, these tools will greatly improve our understanding of how different compartments respond to changes in ROS.

Until recently, the evaluation of ROS levels *in vivo* has remained elusive. The development of redox-active positron emission tomography (PET) tracers for *in vivo* ROS imaging has the potential to greatly expand our toolkit. These include: [^{18}F]ROStrace [151], an analog of the O_2^- probe dihydroethidium; [^{18}F]PC-FLT [152], which measures extracellular and intracellular levels of H_2O_2; and [^{18}F]ROS1 [153], which measures O_2^- and OH• radicals.

Peroxyl Radical

Because O_2^- and H_2O_2 are only moderately reactive, most ROS-induced cellular damage is due to their conversion to other species. The peroxyl radical (OH•) is formed from H_2O_2 and is the most reactive of all ROS. OH• is formed when H_2O_2 reacts with iron (Fe^{2+}) in the Fenton reaction [4]. O_2^- also contributes to OH• formation by reducing Fe^{3+} to Fe^{2+}.

Lipid Peroxides

Because of their carbon–carbon double bonds, polyunsaturated fatty acids (PUFAs) contain reactive hydrogen atoms that are highly susceptible to lipid peroxidation, which compromises the integrity of lipid bilayers in cells. Lipid peroxidation is initiated by OH•, leading to the formation of lipid radicals and lipid peroxyl radicals, which react with PUFAs in a propagation reaction to generate lipid peroxides. Excessive lipid peroxidation is associated with the iron-dependent form of cell death known as ferroptosis [5].

Tumor-Initiating/Promoting Effects of ROS

ROS induce DNA damage through their oxidation of nucleobases including guanine. Repair of these modified bases can result in errors leading to mutagenesis. Consistently, radiation is one of the most well-known sources of ROS [6] and has long been associated with tumor-initiating events [7]. ROS can also alter cellular processes through their effects on protein function (Figure 2). The effects of ROS are related to the degree of protein oxidation. Mild oxidation promotes cellular signaling and is typically reversible (disulfides, sulfenic acid, sulfinic acid), allowing rapid changes in protein activity and signaling networks. By contrast, excessive oxidation leads to terminal oxidation (sulfonic acid) and complete loss of protein function. While irreversible cysteine modifications can be detrimental to protein function, reversible modifications can be protective during stress. Protein modifications play a key role in adaptation to oxidative stress by activating antioxidant (KEAP1) or metabolic (GAPDH, PKM2) programs to facilitate ROS metabolism. Other reversible modifications can occur endogenously, including CoAlation [8,9] and glutathionylation [10], which can both protect proteins from terminal oxidation and alter their function to promote metabolic rewiring. The influence of ROS on tumor initiation and promotion is complex and related to the amount, duration, location, and context.

Endogenous Antioxidants

Antioxidant is a general term used to describe an enzyme or cofactor that participates in the elimination of ROS (Figure 3). The most abundant endogenous antioxidant is the metabolic

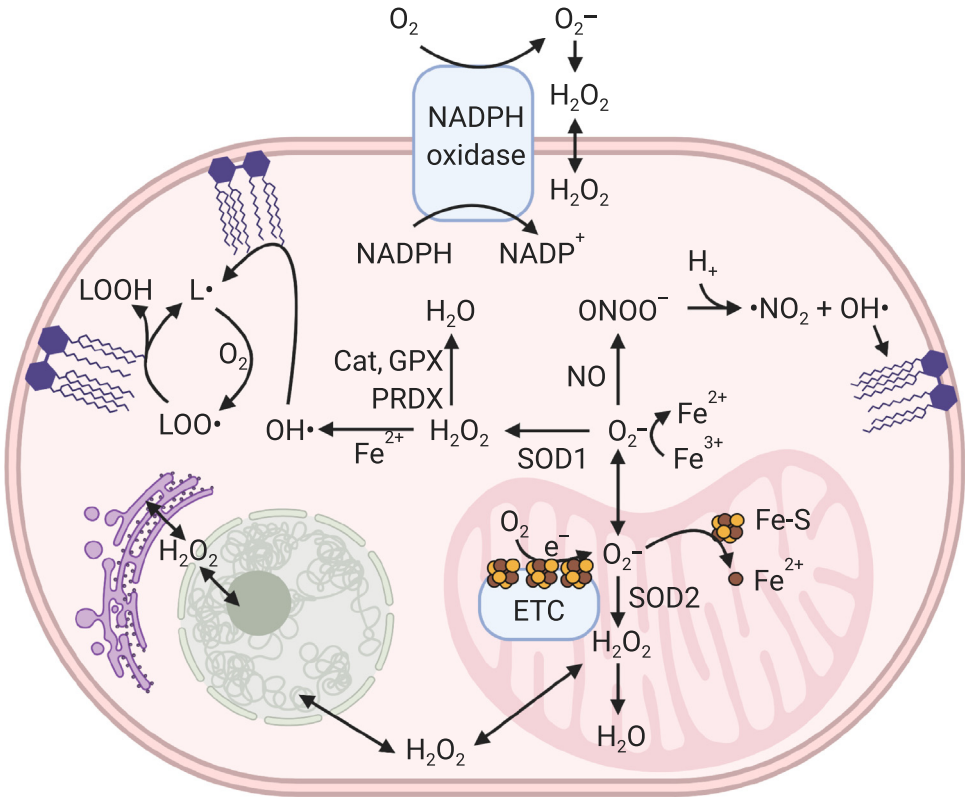

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Figure 1. Types of Reactive Oxygen Species (ROS). Superoxide (O_2^-) is produced extracellularly by NADPH oxidase or intracellularly by the mitochondrial electron transport chain (ETC). In the mitochondria, it targets iron–sulfur (Fe-S) clusters to release iron (Fe^{2+}) and reduces ferric iron (Fe^{3+}) to ferrous iron (Fe^{2+}). O_2^- is dismutated to hydrogen peroxide (H_2O_2) by superoxide dismutases (SOD1, SOD2). H_2O_2 diffuses through membranes to react with proteins and DNA and is detoxified to water by cellular peroxidases [catalase (Cat), glutathione peroxidase (GPX), peroxiredoxins (PRDX)]. O_2^- produces peroxynitrite ($ONOO^-$) through a reaction with nitric oxide (NO). The peroxyl radical (OH•) is formed from the reaction of H_2O_2 with Fe^{2+} and the decomposition of $ONOO^-$ and initiates the lipid peroxidation cascade. First, OH• reacts with lipids to form lipid radicals (L•), which react with oxygen to form lipid peroxide radicals (LOO•). LOO• reacts with lipids to reform L• plus lipid peroxides (LOOH) and the cycle continues. Excessive lipid peroxidation leads to ferroptosis.

cofactor glutathione (GSH) [11]. GSH was first discovered over a century ago [12] and has long been known to play a role in detoxifying reactions in cancer cells [13]. GSH is a tripeptide that is synthesized in a two-step process. In the first step, the condensation of glutamate and cysteine is catalyzed by glutamate–cysteine ligase catalytic subunit (GCLC). In the second, GSH synthetase (GSS) incorporates glycine to form the tripeptide. Although cysteine is the rate-limiting metabolite for this pathway in most contexts [14], both glutamate [15] and glycine [16,17] can also be limiting for GSH synthesis. GSH is used as a cofactor by GSH *S*-transferases (GSTs) and GSH peroxidases (GPXs) to eliminate ROS. GST and GPX enzymes comprise multiple families and isoforms [18,19] and the exact targets of each are unclear. Besides GSH-dependent antioxidant systems, the sulfaredoxin (SRX) and thioredoxin (TXN) antioxidant networks regenerate peroxiredoxins (PRDXs), a set of enzymes with high catalytic activity towards H_2O_2 [20,21]. Unlike the highly abundant GSH metabolites, TXNs are small protein antioxidants and less abundant [11]. While the TXN system can reduce PRDX disulfide bonds, SRX will reduce PRDXs that are overoxidized to sulfinic acid. Distinct, but highly homologous, PRDX and TXN proteins localize to either the mitochondria or the cytoplasm and the relative importance of each subcellular system, as well as the crosstalk between them, is unclear [21]. Finally, the detoxification of ROS by

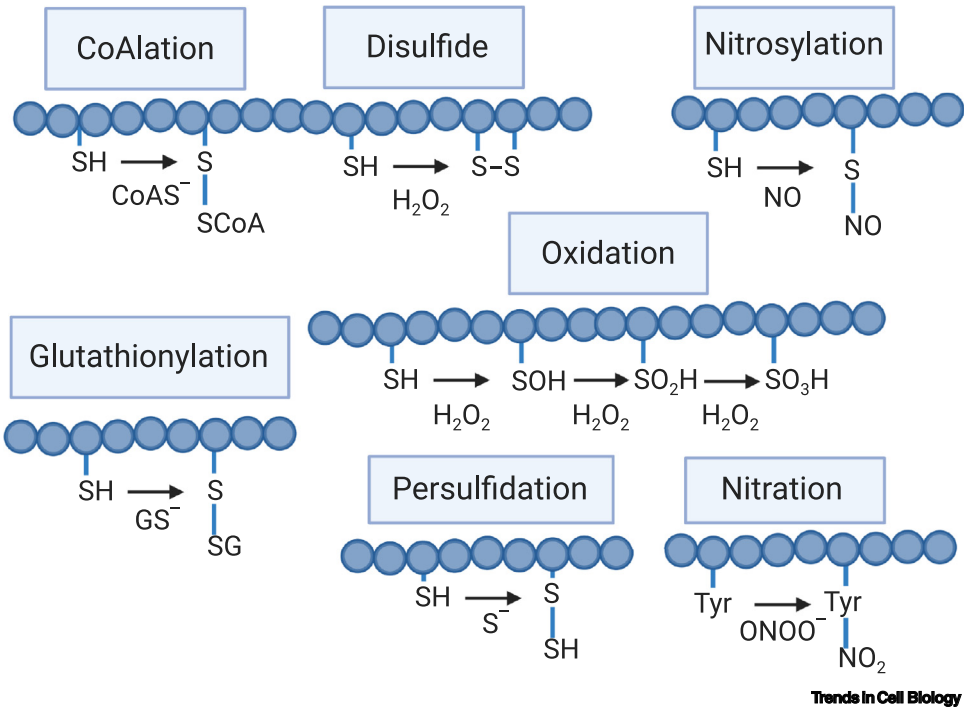

Figure 2. Oxidative Protein Modifications. Oxidative protein modifications have important impacts on cellular signaling and protein function. They include reversible modifications [CoAlation, disulfide (S–S) bond formation, nitrosylation, glutathionylation, and persulfidation]. CoAlation and glutathionylation are the consequence of the reaction of the thiol of coenzyme A or glutathione with the thiol (SH) of cysteine to form a disulfide bond. Nitrosylation is modification of the cysteine thiol by nitric oxide (NO). Thiols can also be oxidized. Mild oxidation to sulfenic acid (SOH) and sulfinic acid (SO_2H) is reversible. By contrast, irreversible modifications [sulfonic acid (SO_3H), tyrosine nitration (Tyr-NO_2) by peroxynitrate ($ONOO^-$)] are terminal oxidation states that result in loss of protein function.

GSH and TXN generates oxidized forms of these antioxidants, which must be regenerated for subsequent reactions. Oxidized GSH and TXN are both regenerated by reductases [GSH reductase (GR), TXN reductase 1 and 2 (TXNRD1/2)] using NADPH as an electron donor [22,23]. These pathways are complementary and redundancy between GSH and TXN systems exists both in normal and malignant tissue [24–27]. Furthermore, oxidative insults promote the expression of enzymes in both the GSH and TXN systems, suggesting that they may work in unison to buffer oxidative stress.

The importance of endogenous antioxidants in tumors depends heavily on the stage of tumorigenesis. Antioxidants play an important role in preventing tumor initiation by preventing ROS-induced oxidation of DNA and subsequent DNA damage. However, many studies rely heavily on carcinogen-induced tumor model systems and because antioxidant systems participate in carcinogen detoxification, their direct role in preventing ROS-induced tumor initiation is less clear. By contrast, there are clear roles for antioxidant proteins in tumor progression. Below, we provide an overview of these findings and highlight outstanding questions in the field.

Prevention of Tumor Initiation by Endogenous Antioxidants

Through the detoxification of ROS, antioxidants have the potential to prevent deleterious, and sometimes oncogenic, outcomes. Multiple isoforms of GSTs can prevent skin, liver, and colon tumor initiation in mice following exposure to carcinogens or loss of tumor suppressors [28–31]. Similarly, GPXs can protect against carcinogen- and ROS-induced tumor initiation in

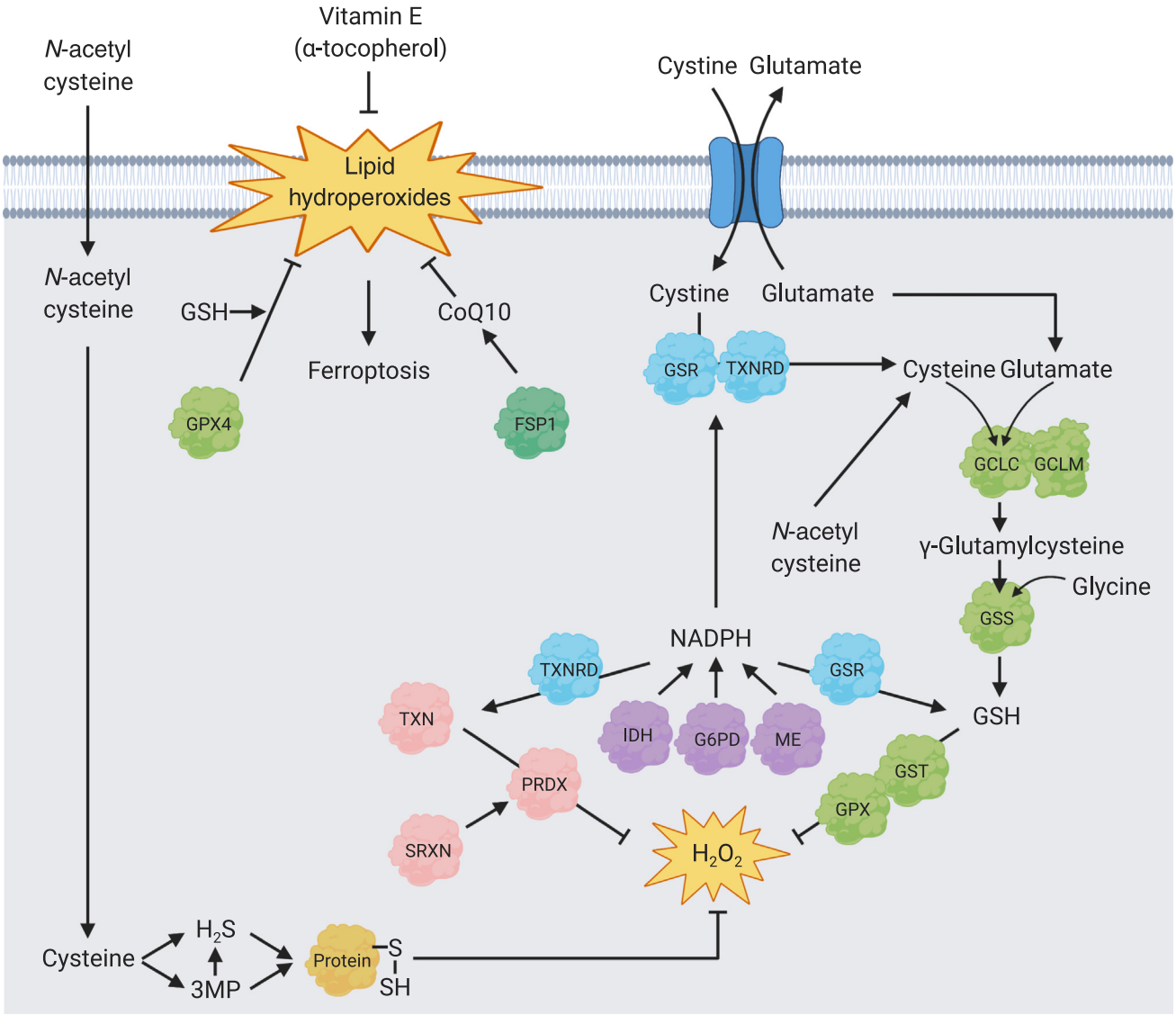

Figure 3. Types of Antioxidants. Glutathione (GSH) is synthesized from cysteine, glutamate, and glycine in a two-step reaction by glutamate-cysteine ligase catalytic (GCLC) and modifier (GCLM) subunits and GSH synthetase (GSS). Peroxidases and transferases (GPX and GST) use GSH as a cofactor to neutralize hydrogen peroxide (H_2O_2). Thioredoxin (TXN) and sulfiredoxin (SRXN) promote peroxiredoxin (PRDX)-mediated H_2O_2 detoxification. GSH reductase (GSR) and TXN reductase (TXNRD1) use NADPH to regenerate GSH and TXN as well as to reduce imported cystine to cysteine. NADPH is generated via multiple metabolic enzymes (IDH1/2, G6PD, ME1). Lipid peroxidation is controlled by GSH-dependent GPX4 and GSH-independent ubiquinone (CoQ10) with ferroptosis suppressor protein 1 (FSP1). Exogenous supply of vitamin E (α-tocopherol) buffers lipid peroxides. *N*-Acetyl cysteine promotes GSH production and protein persulfide-dependent ROS elimination.

multiple models. GPX3 suppresses tumor initiation in mouse models of colon cancer [32]. Similarly, mice with reduced expression of SOD2, either alone or in combination with loss of GPX1, exhibit increased DNA damage and tumor incidence [33,34]. Furthermore, treatment with SOD mimetics that localize to the mitochondria blocks cancer cell proliferation and tumor growth [35,36]. Evidence for the tumor suppressive abilities of antioxidants also exists in the TXN system. Loss of *Prdx1* leads to the accumulation of DNA damage and increased tumor incidence in older mice [37]. Further, PRDX1 inhibits cancer cell growth by acting as a reductant towards PTEN to promote its phosphatase activity towards AKT [38]. In addition, loss of PRDX6 accelerates HPV8-

induced skin carcinogenesis. Importantly, GSH and TXN can act together towards tumor prevention, as mice with combined loss of GR and liver-specific TXNRD1 have increased sensitivity to carcinogen-induced liver malignancies [39]. Finally, the accumulation of oxidized DNA is sufficient to promote tumor initiation in mice. Loss of OGG1, which repairs 8-oxo-deoxyguanine in DNA, results in spontaneous mouse lung tumors in the absence of any carcinogenic treatment [40]. Therefore, multiple lines of evidence indicate that endogenous antioxidants play a role in tumor prevention.

By contrast, *in vivo* genetic studies in mice have also demonstrated a role for endogenous antioxidants in promoting tumor initiation. $Gpx2^{-/-}$ mice are protected against azoxymethane-induced colorectal tumorigenesis [41], suggesting that Gpx2 may support survival during the early stages of transformation. Further, $Srx^{-/-}$ mice had fewer and smaller urethane-induced lung tumors [42] and DMBA/TPA-induced skin tumors [43]. In sum, while some studies provide clear evidence for certain antioxidants in the prevention of cancer initiation, others promote tumor initiation in similar contexts. Additional work is needed to dissect these seemingly contradictory results and their direct relation to ROS and protein antioxidant function. Further, work is needed to determine whether tumor suppressive antioxidant pathways can be selectively upregulated therapeutically for cancer prevention without inducing cancer promotion.

Support of Tumor Progression by Endogenous Antioxidants

On transformation, cells upregulate processes including mitochondrial metabolism [44] and protein translation that lead to increased generation of ROS, necessitating an increased reliance on antioxidants to maintain redox balance [45,46]. Recent studies have demonstrated a role for GSH in limiting DNA damage and maintaining protein homeostasis in tumors [26,47–49]. Without ample GSH synthesis, tumor cells reach a barrier in progression to more advanced and aggressive malignancies. The GST and GPX enzymes involved in the downstream utilization of GSH have also been implicated in tumor progression. GSTs metabolize chemotherapies, including cisplatin [50–52], and activate oncogenic signaling proteins such as Akt [53]. GPXs are required to buffer ROS generation during tumor progression [54], most notably GPX4 via its inhibition of lipid peroxidation and ferroptosis [55,56]. Ferroptosis implicates multiple processes, including GSH-independent pathways that use the antioxidant cofactor ubiquinone (CoQ10) [57–59]. Interestingly, therapy-resistant cancer cells that have undergone epithelial–mesenchymal transition (EMT) are more sensitive to ferroptosis [56,60,61]. Components of the TXN system, such as TXN and TXNRD1, promote tumor growth [62,63]. Expression of PRDX1 and PRDX4 is elevated in malignant tissues and supports tumor survival [64,65]. Further, PRDX6 overexpression accelerated malignant progression [66]. The metabolism of superoxide also plays a role in tumor progression. Inhibition of SOD1 through copper chelators blocked lung tumorigenesis [67], although copper chelation can also have SOD1-independent antitumorigenic properties [68]. Further, targeting the TXN or SOD1 antioxidant systems impaired lung cancer cell survival following O_2^- exposure [69]. Thus, endogenous antioxidant programs have the potential to support tumor progression and viability. The significant redundancy between the antioxidant systems remains a challenge for therapy [62] and more work is needed to understand compensatory mechanisms between the different protein components in these systems.

NADPH, which regenerates endogenous antioxidants in GSH and TXN systems, must be regenerated from $NADP^+$. Regeneration of NADPH is fueled by several metabolic processes, most notably the pentose phosphate pathway (PPP) and one-carbon metabolism [70,71]. Interestingly, glucose-6-phosphate dehydrogenase (G6PD), which generates NADPH in the first step of the PPP, is the most common enzyme defect in humans [72]. G6PD-deficient patients have reduced NADPH levels, resulting in resistance to malaria as well as susceptibility to hemolytic anemia [73].

Several studies have demonstrated the importance of NADPH generation and reductive capacity for tumorigenesis [74,75]. In line with these reports, G6PD-deficient patients show a lower risk for colorectal cancer [76]. It is difficult to ascertain the contribution of G6PD deficiency to cancer risk as the cellular role of G6PD extends well beyond the regeneration of GSH and TXN systems. Recent studies have demonstrated the importance of NADPH/$NADP^+$ control for the maintenance of folate metabolism [77]. In addition, local control of NADPH/$NADP^+$ can influence the oxidation of cellular components, such as Fe-S clusters, independent of the GSH and TXN system [78]. Additional studies are required to better understand the subcellular interplay between G6PD, NADPH, ROS, and GSH/TXN activity in tumorigenesis. Further, there is a lack of understanding on the relative importance of these antioxidant programs across tumor types and between differing environments.

Exogenous Antioxidants

Exogenous antioxidants are commonly used to treat animal models of cancer and to interrogate the causal role of intracellular ROS in various tumor processes. The most widely used tool for these studies is *N*-acetyl cysteine (NAC). Treatment of mice with NAC impaired p53-null lymphoma and lung cancer growth by preventing oxidation of DNA and subsequent mutagenic events [79]. NAC also blocks the stabilization of Hif1a and perturbs hepatocellular xenograft tumors [80]. Preclinical evidence supported clinical trials, but ultimately these showed no benefit for patients [81]. However, recent studies with NAC suggest it can promote tumorigenesis as well. NAC supplementation promoted the initiation, progression, and metastasis of multiple genetically engineered mouse models of cancer, including melanoma, and lung cancer [82–84]. Further, the exact mechanisms behind the effects of NAC supplementation on cellular redox status are also unclear. While NAC can contribute to GSH synthesis in some contexts, its major antioxidant function may be through the production of hydrogen sulfide and protein persulfidation [85]. Importantly, protein persulfides can be reduced to regenerate unmodified thiols [86,87].

Similar to NAC, vitamin E (alpha-tocopherol) was largely regarded as having antitumor potential as a supplement [88]. These beliefs were upheld, even when other exogenous antioxidants, such as beta-carotene, were found to increase cancer incidence [89]. Ultimately, a large-scale, multicenter clinical trial was initiated to investigate the ability of vitamin E supplementation to prevent prostate cancer incidence [90]. This trial was stopped because the vitamin E supplementation arm was incurring higher rates of prostate cancer [91,92]. Similar to NAC, vitamin E promotes lung tumor and melanoma growth and progression [82,83]. Further, vitamin E can directly prevent lipid oxidation and ferroptosis [55,93].

The interpretations of antioxidants are complicated by the ability of these molecules to be oxidized themselves or to produce antioxidant-independent effects. Vitamin C (or ascorbate) is an antioxidant that is absorbed through the diet and routinely supplemented exogenously in experiments. Recent studies have shown that vitamin C is autoxidized to dehydroascorbate (DHA) and can subsequently increase oxidative stress in cells [94,95]. Additionally, vitamin C can negatively regulate hematopoietic stem cell (HSC) function by promoting the activity of Tet2 [96]. As previously mentioned, NAC can produce H2S, which can influence metabolic and signaling pathways [97,98]. In summary, caution must be taken when using exogenous antioxidants to interrogate and interpret the impact of intracellular ROS on tumor biology.

Aberrant Activation of ROS Detoxification in Cancer

The tumor-promoting effects of cellular antioxidant programs are best evidenced by the aberrant activation of the antioxidant transcription factor NRF2 in multiple cancer types. Under basal conditions, NRF2 levels are constrained by its association with KEAP1, which targets NRF2 for

proteasomal degradation [99]. Following exposure of cells to oxidative or electrophilic stress, cysteine residues on KEAP1 are modified, leading to impaired NRF2 ubiquitination and NRF2 accumulation. NRF2 promotes the transcription of many genes in the antioxidant system, including those in the GSH and TXN antioxidant pathway [100]. Interestingly, NRF2 accumulation is common in cancer, suggesting that increased antioxidant defense contributes to one or multiple stages of the tumorigenic process. Various mechanisms exist for NRF2 accumulation across various cancer types. Mutations in NRF2 and KEAP1 that disrupt proper NRF2 degradation are common in multiple cancers, including lung [100]. NRF2 exon skipping to delete the KEAP1 binding domain has been described in lung cancer [101] and oncogene-driven transcription can increase the levels of NRF2 [102]. KEAP1 inactivation as a consequence of promoter methylation [103], p62-mediated sequestration [104,105], and modification by the oncometabolites fumarate and methylglyoxal [106–108] can also result in NRF2 accumulation.

Elegant animal studies have dissected the contribution of NRF2 to specific stages of tumorigenesis (Figure 4). Studies with NRF2 knockout mice have demonstrated that NRF2 contributes to the incidence and growth of oncogene-driven lung tumors [102,109] and p62-driven pancreatic tumorigenesis [110]. Further, KEAP1 deletion increases the tumor burden in lung tumor models driven by $Kras^{G12D}$/loss of p53 or loss of PTEN [111,112] and liver tumor burden driven by Myc [113]. NRF2 activation in these models was associated with decreased levels of ROS and oxidative DNA damage and the activation of metabolic processes. NRF2 regulates multiple metabolic pathways at the interface of antioxidant defense and proliferative processes, including the PPP and serine biosynthesis [17,114,115], which may play a dominant role over antioxidant processes at certain stages of tumor progression. Importantly, NRF2 activation causes widespread

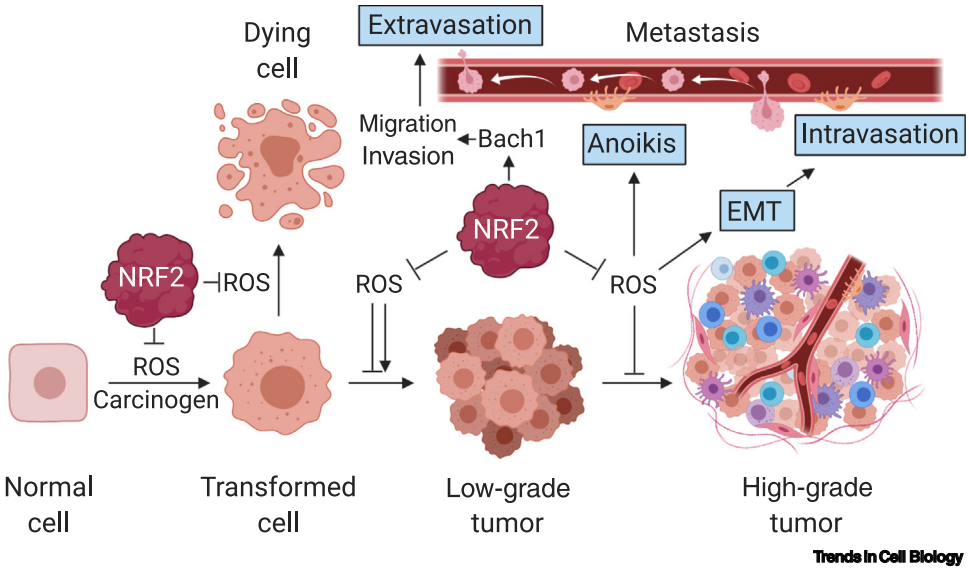

Figure 4. The Complex Role of NFE2-Related Factor 2 (NRF2) at Different Stages of Carcinogenesis. NRF2 plays dual roles in tumor initiation, progression, and metastasis. NRF2 protects against oxidation and carcinogen-induced DNA damage via the antioxidant and detoxification programs. By preventing excessive oxidative damage, NRF2 promotes the viability of transformed cells during early stages of tumorigenesis. Further, NRF2 promotes progression to higher-grade tumors. The role of NRF2 in metastasis is complex and tumor and tissue specific. Loss of NRF2 promotes epithelial–mesenchymal transition (EMT) via reactive oxygen species (ROS) to promote migration and invasion to support intravasation/extravasation. By contrast, NRF2 can promote migration and invasion through the transcription factor Bach1. ROS also promote the death of cells detached from the extracellular matrix (anoikis), which NRF2 may protect against. Consequently, NRF2 and ROS play complex roles at different tumor stages.

changes in global cysteine reactivity [116,117], suggesting that the effects on NRF2 on metabolism may not be limited to direct transcriptional targets.

The influence of ROS on metastasis is complex and seemingly contradictory. ROS have been shown to promote metastasis in multiple contexts [36,118–123]. By contrast, detachment of cells from the extracellular matrix (ECM) induces oxidative stress that limits survival in circulation and antioxidants have been shown to be protective and metastasis promoting [82–84,124–127]. It is therefore unsurprising that the influence of NRF2 on metastasis would be complex as well. NRF2 activation in $Kras^{G12D}$; $p53^{flox/flox}$ lung tumors indirectly promoted the stability of the transcription factor BACH1 via heme catabolism, which promoted metastasis via BACH1 transcriptional targets [124], an effect that could be recapitulated by antioxidant treatment [125]. However, in a mouse model of pancreatic cancer similarly driven by $Kras^{G12D}$ and p53 loss-of-function, ROS induced by deletion of TP53-induced glycolysis and apoptosis regulator (TIGAR) or NRF2 increased metastasis to the lung [128]. Interestingly, BACH1 was not affected by ROS in the pancreatic cancer model; rather, DUSP6 expression was lost, thereby leading to increased ERK activity and EMT. Notably, the difference between the lung and the pancreas is further exemplified by a recent study examining the consequence of *Keap1* deletion in the context of the $Kras^{G12D}$ and $Kras^{G12D}$; $p53^{R172H}$ pancreatic tumor models [129], which recapitulates the genetics of the lung tumor study. However, *Keap1* deletion in these pancreatic tumor models instead resulted in pancreatic atrophy [129]. Thus, the influence of NRF2 on metastasis is highly context dependent and may be influenced by the tissue type, the NRF2 dosage, and ROS-dependent and -independent effects.

The effects and degree of KEAP1 loss of function may also be context dependent within the same tissue and genetic context. In a competition model, KEAP1 deletion was not selected for in $Kras^{G12D}$, $Kras^{G12D}$; $p53^{flox/flox}$, or $Kras^{G12D}$; $LKB1^{flox/flox}$ models compared with other tumor suppressors [130]. Further, while a heterozygous $KEAP1^{R554Q}$ loss-of-function mutant modestly increased tumor size, in agreement with deletion studies in the $Kras^{G12D}$; $p53^{flox/flox}$ model, homozygous $KEAP1^{R554Q}$ expression actively antagonized tumor formation [131]. Additional work is needed to understand whether specific KEAP1 mutations or degrees of NRF2 activation have disparate effects on tumor growth and progression.

Concluding Remarks

Stepping away from a 'one size fits all' view of the effects of ROS and antioxidants on tumor biology will help to reconcile many of the seemingly contradictory effects of these molecules across studies (see Outstanding Questions). Recent studies support the idea that there are different pools of ROS with differing functions. While NADPH oxidase-derived ROS was shown to promote proliferation in the mouse intestine, ROS resulting from loss of TIGAR impaired proliferation in the same cells [132,133]. It is important to note that NADPH oxidase generates extracellular O_2^-, while TIGAR protects against ROS intracellularly by supporting the PPP [134]. In addition, the role of antioxidant programs in the microenvironment needs to be considered when interpreting antioxidant and whole-body gene knockout studies. These cell populations can rely on both antioxidant programs and ROS generation for function [135–139]. Further, the beneficial or detrimental roles of ROS in the cell do not necessarily need to be mutually exclusive. Improvement in technologies, including genetic screens that have brought increased clarity to enzymatic pathways [57,58,140–142], and large-scale profiling efforts of cancer models using omic technologies [143–146], will undoubtedly advance our understanding of the complexities of ROS and antioxidant pathways.

Outstanding Questions

Do antioxidants have additional effects on the tumor microenvironment compared with the activation of tumor antioxidant programs?

Do specific exogenous and endogenous antioxidants have unique effects on tumor biology?

Can we define the context-specific, compartment-specific, and concentration-dependent roles of ROS during different stages of tumor initiation, progression, and metastasis?

Are there roles for different types of ROS (H_2O_2 vs O_2^- vs $ONOO^-$)?

Can we better define the functional consequences of oxidative protein modifications?

Would targeting enzymes that use antioxidant cofactors, such as GSTs and GPXs, instead of targeting enzymes that synthesize or regenerate the antioxidants cofactors themselves provide a larger therapeutic window for cancer treatment?

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