

Cancer, Oxidative Stress, and Metastasis

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Reactive oxygen species (ROS) are highly reactive molecules that arise from a number of cellular sources, including oxidative metabolism in mitochondria. At low levels they can be advantageous to cells, activating signaling pathways that promote proliferation or survival. At higher levels, ROS can damage or kill cells by oxidizing proteins, lipids, and nucleic acids. It was hypothesized that antioxidants might benefit high-risk patients by reducing the rate of ROS-induced mutations and delaying cancer initiation. However, dietary supplementation with antioxidants has generally proven ineffective or detrimental in clinical trials. High ROS levels limit cancer cell survival during certain windows of cancer initiation and progression. During these periods, dietary supplementation with antioxidants may promote cancer cell survival and cancer progression. This raises the possibility that rather than treating cancer patients with antioxidants, they should be treated with pro-oxidants that exacerbate oxidative stress or block metabolic adaptations that confer oxidative stress resistance.

Reactive oxygen species (ROS), including superoxide radicals ($O_2^{\cdot-}$), hydroxyl radicals ($OH\cdot$), and hydrogen peroxide (H_2O_2), are generated as by-products of aerobic metabolism as well as from a number of other sources. These ROS display varying reactivities toward different targets but share the ability to damage cells by oxidizing proteins, lipids, and DNA (Martinez-Cayuela 1995). The ability of ROS to mutate DNA and to damage cells raised the possibility that cellular aging and cancer initiation reflect accumulated ROS damage over time (Harman 1956). Although damage from ROS does contribute to aging and cancer initiation, ROS damage does not seem to provide a general explanation for the morbidities of aging, and antioxidant administration has so far failed to increase longevity outside of certain mutant genetic backgrounds (Finkel and Holbrook 2000). Nonetheless, decades of work in this area gave rise to the idea that antioxidants from the diet or from dietary supplements might slow aging and reduce cancer incidence by neutralizing cellular ROS (Greenwald et al. 1990). Results from clinical trials generally have not supported this idea.

SOURCES AND EFFECTS OF ROS

ROS are produced in various subcellular compartments by nonenzymatic and enzymatic reactions (Hernandez-Garcia et al. 2010). Nonenzymatic mechanisms include single-electron reduction of O_2 to produce superoxide in the mitochondria. Enzymatic mechanisms are numerous and include NADPH oxidases, nitric oxide synthases,

xanthine oxidase, cytochrome P450 enzymes, cyclooxygenases, and lipoxygenases (Gorrini et al. 2013b). The endoplasmic reticulum also serves as a source of ROS during protein folding via protein disulfide isomerase, endoplasmic reticulum oxidoreductin, NADPH oxidase (especially NOX4), and other mechanisms (Malhotra et al. 2008; Bhandary et al. 2012; Higa and Chevet 2012). Peroxisomes produce ROS through β -oxidation of fatty acids and flavin oxidase activity (Schrader and Fahimi 2006). Hypoxia, sustained mitochondrial respiration, ER stress, and oncogenes all contribute to high ROS levels in some cancer cells (Szatrowski and Nathan 1991; Gorrini et al. 2013b). Because ROS are so highly reactive, ROS generally oxidize targets within, or adjacent to, the intracellular compartment in which they are generated.

Cellular ROS levels can also increase as a result of UV irradiation, ionizing radiation, toxins such as heavy metals, chemotherapy, and neighboring inflammatory cells, although the mechanisms vary widely (Federico et al. 2007; Azzam et al. 2012; Vera-Ramirez et al. 2012). For example, the chemotherapeutic doxorubicin forms a complex with topoisomerase and DNA that leads to double-strand breaks, increasing ROS levels and potentiating cellular damage (Lyu et al. 2007; Rowe et al. 2008; Zhang et al. 2012). Doxorubicin also promotes mitochondrial dysfunction through multiple mechanisms including changes in iron metabolism and releasing electrons from the electron transport chain (Granados-Principal et al. 2010).

At lower levels, ROS activate signaling that can be advantageous for cells, promoting proliferation, survival, or oxidative stress resistance (Ranjan et al. 2006; Rhee 2006; Schieber and Chandel 2014). Redox-sensitive signaling pathways include the PI3-kinase and MAP kinase signaling pathways in which ROS regulates signaling by epidermal growth factor (EGF) (Bae et al. 1997), Ras (Lander et al. 1997), platelet-derived growth factor (PDGF) (Sundaresan et al. 1995), and phosphatase and tensin homolog (PTEN) (Lee et al. 2002; Leslie et al. 2003). These pathways can be indirectly regulated by ROS or directly activated by “redox switches.” For example, nitric oxide production results in S-nitrosylation of p21^{Ras} at Cys118, increasing guanine nucleotide exchange and Ras signaling (Lander et al. 1997).

ANTIOXIDANT MECHANISMS FOR ROS DEFENSE

Cells must maintain homeostasis by limiting ROS production and having antioxidant mechanisms to neutralize ROS or mitigate oxidative stress. Antioxidant enzymes include superoxide dismutases (SODs), catalase, peroxiredoxins (PRDXs), thioredoxins, glutathione peroxidase, and heme oxygenase (Sabharwal and Schumacker 2014). Some of these enzymes, such as SOD, have different isoforms in mitochondria and in the cytoplasm. These enzymes work together to neutralize ROS (Fig. 1). For example, SOD can convert $O_2^{\cdot -}$ to O_2 or H_2O_2 . Catalase and glutathione peroxidase subsequently convert the H_2O_2 to H_2O and O_2 . Nonenzymatic antioxidants include

proteins and metabolites produced by cells, such as thioredoxin, glutathione, and nicotinamide adenine dinucleotide phosphate (NADPH), as well as dietary components, such as vitamins A, C, and E, selenium, and β -carotene. Thioredoxin and glutathione are abundant endogenous redox buffers that serve as electron donors to peroxidases, which convert H_2O_2 to H_2O (Fig. 1). They can then be regenerated from their oxidized, disulfide forms, using NADPH as an electron donor.

Nuclear factor-erythroid 2-related factor 2 (NRF2) is a transcriptional master regulator of cellular redox status. It promotes the transcription of genes that encode antioxidant and detoxification enzymes in response to redox stress (Hayes and McMahon 2009). NRF2 is negatively regulated by Kelch-like ECH-associated protein 1 (KEAP1), which sequesters NRF2 in the cytoplasm (Hayes and Dinkova-Kostova 2014). However, ROS can oxidize and inactivate KEAP1, allowing NRF2 levels to increase in the nucleus and triggering the transcription of genes with antioxidant response elements in their promoters (Rushmore et al. 1991; Nioi et al. 2003). These genes include key components of the endogenous antioxidant response systems that import cysteine into cells (Lewerenz et al. 2013) and promote the synthesis of glutathione (Higgins et al. 2009) and thioredoxin (Hayes and Dinkova-Kostova 2014). NRF2 also promotes the expression of glutathione peroxidase, glutathione reductase, and thioredoxin reductase (Fig. 1; Hayes and McMahon 2009; Abbas et al. 2011; Jeong et al. 2012; Hawkes et al. 2014; Lu and Holmgren 2014), as well as proteins such as ferritin, which blocks the formation of free radicals, and NADPH:quinone oxidoreductase 1, which inhibits the

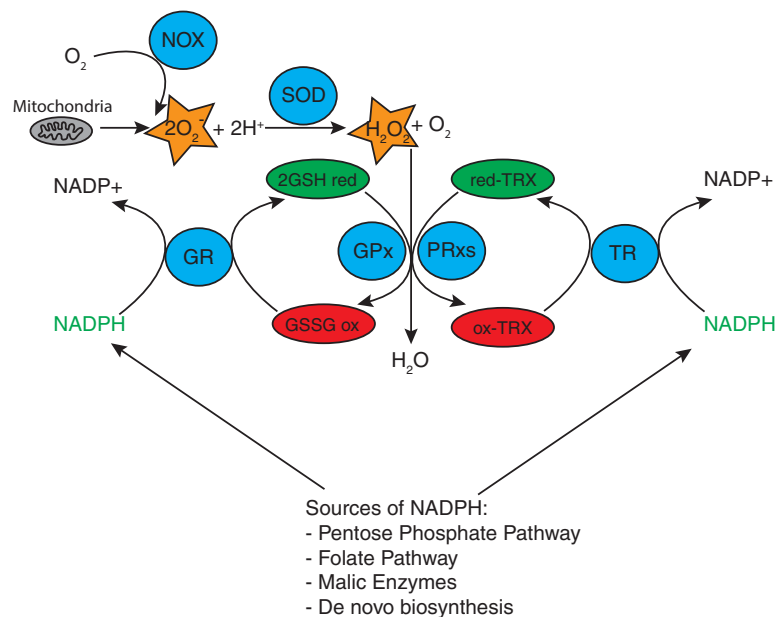


Figure 1. Endogenous antioxidant mechanisms. Reactive oxygen species (ROS) such as superoxide can be produced either in the cytoplasm or in mitochondria. Two main arms of the antioxidant response are shown: thioredoxin (TRX) and glutathione (GSH). Enzymes are highlighted in blue. GR, glutathione reductase; GSH red, reduced glutathione; GSSG ox, oxidized glutathione; GPx, glutathione peroxidase; SOD, superoxide dismutase; PRxs, peroxiredoxins; red-TRX, reduced thioredoxin; ox-TRX, oxidized thioredoxin; NOX, NADPH oxidase; NADPH, nicotinamide adenine dinucleotide phosphate.

formation of free radicals by quinones (Nioi and Hayes 2004). Finally, NRF2 promotes the expression of NADPH-generating enzymes to produce the NADPH required for glutathione and thioredoxin regeneration (Thimmulappa et al. 2002; Lee et al. 2003; Wu et al. 2011; Mitsuishi et al. 2012; Singh et al. 2013). This concerted upregulation of approximately 200 genes enables the adaptation of cells to increased ROS levels (Hayes and Dinkova-Kostova 2014).

Tumor suppressors also help to control ROS. For example, BRCA1 and fumarate hydratase both promote NRF2 function through different mechanisms (Gorrini et al. 2013b). p53 decreases ROS levels by inhibiting glycolysis and promoting the generation of NADPH via the pentose phosphate pathway (Green and Chipuk 2006). The increased DNA damage in the absence of p53 can be partially rescued by treatment with the antioxidant *N*-acetylcysteine (NAC), suggesting that the effects of p53 on redox homeostasis are a significant component of its functions that promote genomic stability (Liu et al. 2004; Sablina et al. 2005).

CLINICAL TRIALS OF ANTIOXIDANTS IN CANCER PREVENTION

Based on the hypothesis that cellular damage from ROS is a major driver of aging and oncogenic mutations, dietary supplementation with antioxidants was proposed to prevent and/or treat cancer (Greenwald et al. 1990). The Nutritional Prevention of Cancer Study Group tested whether dietary supplementation with selenium (a component of glutathione peroxidase and thioredoxin reductase) could reduce the incidence of skin cancers in patients with a history of basal and/or squamous cell carcinomas (Clark et al. 1996). Although selenium did not protect participants from the development of additional skin cancers, selenium supplementation was associated with a nearly 40% reduction in total cancer incidence, particularly in prostate cancer. The Linxian General Population Nutrition Intervention Trial found that a combination of selenium, vitamin E, and β -carotene reduced overall mortality as well as cancer rates (Blot et al. 1993; Qiao et al. 2009).

These promising findings spawned many additional trials to assess the efficacy of antioxidant use for cancer prevention (see Table 1 for a summary of randomized, placebo-controlled trials with more than 10,000 participants). Subsequent trials not only failed to reproduce these findings (Hennekens et al. 1996; Lee et al. 1999, 2005; Hercberg et al. 2004, 2007; Gaziano et al. 2009, 2012) but suggested that in some cases, antioxidants may actually promote cancer initiation and progression. The Alpha-Tocopherol Beta Carotene (ATBC) Cancer Prevention Study treated male smokers with vitamin E, β -carotene, both, or neither for 5–8 yr (ATBC 1994). Patients receiving β -carotene had an 18% increase in lung cancer incidence. These results were recapitulated in the CARET trial, which also found an increase in lung cancer incidence and mortality in participants taking β -carotene and vitamin A (Omenn et al. 1996a,b).

The SELECT trial was a randomized, double-blind, placebo-controlled trial of older males given vitamin E, selenium, both, or neither for 7–12 yr (Lippman et al. 2009; Klein et al. 2011). Men taking vitamin E alone were significantly more likely to develop prostate cancer, though overall cancer incidence did not significantly differ between groups. Among patients who received selenium, those with high baseline selenium levels had an increased risk of high-grade prostate carcinoma whereas those with low baseline selenium did not (Kristal et al. 2014).

Some trials also tested the ability of antioxidants to prevent the formation of new primary tumors in patients who had already been treated for cancer. The EURO-SCAN trial treated patients with a history of head and neck cancer or lung cancer for 2 yr with vitamin A (retinyl palmitate), NAC, both, or neither (van Zandwijk et al. 2000). Patients receiving neither vitamin A nor NAC had the lowest incidence of new primary tumors, although this was not statistically significant. A Phase III randomized, double-blind, placebo-controlled trial tested the effect of selenium versus placebo for patients with resected non-small cell lung cancer (Karp et al. 2013). The trial was stopped early as selenium treatment was associated with a trend toward increased second primary tumors.

The U.S. Preventative Services Task Force (USPSTF) reviewed many of the above trials and concluded that there was insufficient data for or against the use of most nutrient supplements for cancer prevention (Moyer and U.S. Preventive Services Task Force 2014). Two exceptions included vitamin E and β -carotene. Vitamin E consistently showed no impact on cancer prevention. β -carotene showed an increased risk for lung cancer in smokers.

The other important question is whether dietary supplementation with antioxidants benefits healthy people. Many clinical trials have explored whether antioxidant use affects mortality due to heart disease, aging, or diseases of aging. A meta-analysis examining all-cause mortality in 68 randomized trials of antioxidants for many different indications included 232,606 participants and found a significant increase in mortality in patients taking β -carotene, vitamin A, and vitamin E (Bjelakovic et al. 2007). An additional meta-analysis showed that antioxidant supplements significantly increased the risk of bladder cancer (Myung et al. 2010).

CLINICAL TRIALS OF ANTIOXIDANTS IN CANCER TREATMENT

In addition to cancer prevention, some trials have included antioxidants as adjuvant therapy in the treatment of patients with cancer. A systematic review found no benefit of supplemental ascorbate (vitamin C) for overall or progression-free survival in cancer patients, most of whom had breast or colorectal cancer (Jacobs et al. 2015). Another systematic review examined treatment efficacy and patient survival with the use of any adjuvant antioxidants during chemotherapy or radiotherapy (Yasueda et al. 2016). The trials showed contradictory results, but

Table 1. Large, randomized, controlled trials designed to assess antioxidant impact on cancer incidence

Clinical trial	Reference(s)	Patient cohort	# enrollees	Treatment arms	Results
LINXIAN	Blot et al. 1993; Qiao et al. 2009	40–69-yr-old Linxian residents with no history of cancer or major disease	29,584	1. Retinol (200% RDI) + zinc (150% RDI) 2. Riboflavin (188% RDI) + niacin (200% RDI) 3. Vitamin C (200% RDI) + molybdenum (40% RDI) 4. Selenium (71% RDI) + vitamin E (111% RDI) + β -carotene (100% RDI)	Decrease in mortality and lower cancer incidences (especially stomach) in those taking selenium + vitamin E + β -carotene. This finding was durable at follow-up 10 yr after the study's end.
ATBC	ATBC Study Group 1994	Male smokers 50–69 yr of age in Finland	29,133	1. Vitamin E (185% RDI) 2. β -carotene (133% RDI) 3. Vitamin E + β -carotene (above doses) 4. Neither	Increased incidence of lung cancer with β -carotene. Slight increase in mortality in those who received vitamin E.
CARET	Omenn et al. 1996a,b	Male and female current or former smokers OR male workers exposed to asbestos	18,314	1. β -Carotene (100%–200% RDI) 2. Vitamin A (1000% RDI) 3. β -carotene + vitamin A (above doses) 4. Placebo	Increased incidence of lung cancer and mortality in β -carotene + vitamin A group.
PHS I	Hennekens et al. 1996	U.S. male physicians, 40–84 yr old	22,071	1. β -carotene (167% RDI) 2. Placebo	No difference in cancer incidence or overall mortality.
WHS	Lee et al. 1999, 2005	Female health professionals >age 45	39,876	1. β -carotene (167% RDI) 2. Vitamin E (1000% RDI) 3. Placebo	No difference in cancer incidence or overall mortality.
SU.VI.MAX	Herberg et al. 2004, 2007	French females age 35–60 and males age 45–60	13,017	1. Vitamin C (200% RDI) + vitamin E (111% RDI) + β -carotene (40% RDI) + selenium (142% RDI) + zinc (133% RDI) 2. Placebo	Lower cancer incidence and mortality seen in men, but not women, taking supplements. Increased incidence of skin cancer in women taking supplements.
PHS II	Gaziano et al. 2009, 2012	Male physicians \geq 50 yr old	14,641	2. Placebo 1. Vitamin E (667% RDI) + vitamin C (833% RDI) 2. Vitamin E (667% RDI) + placebo, 3. Placebo + vitamin C (833% RDI), 4. Multivitamin daily 5. Placebo	No effect on prostate and total cancer incidence (primary outcome measure). No effect on lung, colorectal, or other cancers. Modest decrease in incidence of total cancer with multivitamin.
SELECT	Lippman et al. 2009, Klein et al. 2011	Males >50–55 yr old w/PSA \leq 4 ng/mL and normal DRE	35,533	1. Selenium (286% RDI) 2. Vitamin E (1333% RDI) 3. Selenium + vitamin E (above doses) 4. Placebo	No difference in prostate cancer incidence at 5.5 yr. At 7 yr (1.5 yr after trial ended), men taking vitamin E alone had a higher incidence of prostate cancer.
[META-ANALYSIS]	Bjelakovic et al. 2007	Varied; cohort consisted of 68 unique randomized trials	232,606	Varied; cohort consisted of 68 unique randomized trials	Statistically significant increase in mortality in patients taking β -carotene, vitamin A, and vitamin E.

β -Carotene and retinol do not have an official FDA recommended daily intake (RDI); for this table, RDI calculated is based on vitamin A RDI (5000 IU) with half of daily intake from β -carotene (2500 IU) and half from retinol (2500 IU).

Several of the above trials (e.g., LINXIAN and PHS II) had a factorial design, meaning that participants were assigned to more than one treatment arm. PSA, prostate-specific antigen; DRE, digital rectal exam.

one trial showed that use of vitamin E and β -carotene in head and neck cancer patients receiving radiotherapy increased the risk of recurrence (RR = 2.41, 95% CI: 1.25–4.64) and cancer mortality (RR = 3.38, 95% CI: 1.11–10.34) specifically in smokers (Meyer et al. 2008).

Despite strong designs and very large numbers of participants, the above clinical trials (and other smaller trials) failed to yield clear evidence that antioxidants could reduce cancer development or progression. Contrary to what was expected, antioxidant use often appeared to increase cancer incidence, particularly in individuals at high risk.

ROS IN CANCER INITIATION AND PROGRESSION

ROS is a mutagen that promotes tumor initiation. ROS can oxidize guanine in DNA and RNA to form 8-hydroxyguanine (8-OHG) (Floyd 1990). 8-OHG can pair with adenine during DNA replication, resulting in G to T and C to A substitutions, potentially introducing mis-sense mutations (Cheng et al. 1992). A large body of work has found a strong correlation between the formation of 8-OHG and carcinogenesis (Feig et al. 1994).

Consistent with the idea that ROS is carcinogenic, antioxidant enzymes are tumor suppressors. SOD is a family of three enzymes that are major scavengers of superoxide in the cytoplasm (SOD1), mitochondria (SOD2), and extracellularly (SOD3). *Sod1*-deficient mice develop liver cancer marked by extensive oxidative and DNA damage (Elchuri et al. 2005). Mice heterozygous for a null allele of *Sod2* also form tumors, particularly lymphoma and pituitary adenoma (Van Remmen et al. 2003). *Sod3*-deficient mice do not form tumors, but SOD3 overexpression reduces tumor formation by 50% in a skin carcinogenesis model (Kim et al. 2005). *Peroxiredoxin1* (*Prdx1*)-deficient mice develop lymphomas, sarcomas, and carcinomas (Neumann et al. 2003).

Some other tumor suppressors also act partly by suppressing ROS generation. Loss-of-function mutations in multiple tumor suppressors that promote genomic integrity, including ataxia telangiectasia mutated (ATM), P53, and BRCA1, lead to the generation of ROS (Bae et al. 2004; Reliene et al. 2004; Sablina et al. 2005; Reliene and Schiestl 2006; Esteve et al. 2010; Gorrini et al. 2013a). This may reflect the leakage of damaged DNA into the cytoplasm in these cells, inducing an interferon-mediated innate immune response (as would be stimulated by viral DNA) that promotes the generation of ROS (Santos et al. 2014; Tasdogan et al. 2016; A Tasdogan and H Fehling, pers. comm.). *Atm*-deficient cells show genomic instability, oxidative stress, hematopoietic stem cell depletion, and lymphoid neoplasia (Reliene et al. 2004; Reliene and Schiestl 2006). Treatment of *Atm*-deficient mice with NAC largely rescues these phenotypes, reducing ROS levels, DNA damage, and cancer incidence (Ito et al. 2004). NAC treatment of *p53*-deficient mice has similar effects (Sablina et al. 2005). Although clinical trials in patients at high risk of cancer tended to show

that antioxidants were often ineffective or deleterious for cancer risk, these results in mice raise the possibility that antioxidants might reduce cancer risk in certain sensitized genetic backgrounds.

Numerous studies have also demonstrated a link between oncogene signaling and oxidative stress, though the mechanisms by which oncogenes increase ROS levels are murky. Ras activation increases the generation of superoxide (Irani et al. 1997). BCR-ABL-transformed cells show increased intracellular ROS, as well as oxidative DNA damage and chromosomal fragmentation (Sattler et al. 2000; Nowicki et al. 2004). c-Myc overexpression increases ROS levels, DNA damage, and genomic instability (Felsher and Bishop 1999; Vafa et al. 2002). The increase in ROS levels as a result of oncogene signaling may contribute to ongoing mutagenesis and genomic instability in cancer cells, promoting cancer progression. To balance these potentially toxic effects of ROS, several oncogenes also promote the expression of NRF2, which reduces ROS levels and promotes tumorigenesis (DeNicola et al. 2011).

Consistent with the idea that ROS can promote cancer initiation by promoting mutagenesis but impair cancer progression by causing oxidative damage, antioxidant enzymes have bimodal effects on cancer initiation and progression. *Prdx6* overexpression in keratinocytes can reduce the initiation of skin tumors, but once they arise, cancer progression is accelerated by *Prdx6* overexpression (Rolfs et al. 2013). Similarly, NRF2 confers resistance to chemical carcinogens but also promotes cancer progression by protecting cancer cells from oxidative stress and DNA damage (Ramos-Gomez et al. 2001; Iida et al. 2004; Hayes and McMahon 2006; Hu et al. 2006; Xu et al. 2006; Ma 2013; Satoh et al. 2013). Increased NRF2 expression in human cancers correlates with a poor prognosis (Moon and Giaccia 2015). Deletion of *NRF2* in pancreatic cancer cells increases DNA damage and decreases tumorigenesis (DeNicola et al. 2011). High levels of ROS are thus detrimental to cancer cells and cancer progression depends on endogenous antioxidants that attenuate oxidative stress.

ROS AND METASTASIS

Metastasis is a multistep process involving invasion, migration, intravasation into the blood, survival in circulation, extravasation into distant organs, and proliferation (Vanharanta and Massague 2013). Circulating cancer cells are commonly observed in the blood of patients and mice with various cancers (Nagrath et al. 2007; Stott et al. 2010; Yu et al. 2013, 2014; Sullivan et al. 2014). Nevertheless, metastasis is a very inefficient process (Vanharanta and Massague 2013) as very few metastasizing cancer cells survive and even fewer proliferate to form micrometastases (Luzzi et al. 1998; Cameron et al. 2000; Kienast et al. 2010). Accumulating evidence suggests that oxidative stress kills cancer cells at multiple stages of the metastatic process, contributing to the inefficiency of the process.

Metastasis begins with detachment from the local extracellular matrix. Epithelial cells undergo cell death when they detach from extracellular matrix in culture as a result of reduced glucose uptake, ATP depletion, and oxidative stress (Debnath et al. 2002; Debnath and Brugge 2005). Oncogenic signaling promotes the survival of detached breast epithelial cells by increasing glucose uptake and flux through the pentose phosphate pathway, which generates NADPH and regenerates glutathione (Schafer et al. 2009). Multiple transcription factors also cooperate to induce an antioxidant response that promotes survival, including NRF2 and ATF4. NRF2 and ATF4 promote the expression of serine/glycine biosynthesis enzymes to increase glutathione synthesis (DeNicola et al. 2015) as well as heme oxygenase 1 (Dey et al. 2015), each of which reduces oxidative stress, blocks anoikis, and promotes survival during metastasis.

Cancer cells are more sensitive than normal cells to elevated ROS levels (Raj et al. 2011). Cancer cells rely on glutathione and thioredoxin to protect them from ROS during cancer initiation and cancer progression (Harris et al. 2015). Combined inhibition of glutathione and thioredoxin synergistically induces the death of cancer cells (Harris et al. 2015). Antioxidant treatment of mouse models of lung cancer increases tumor progression and reduces mouse survival by reducing ROS levels, DNA damage, and p53 expression in the cancer cells (Sayin et al. 2014). Oxidative stress also impairs cancer progression by globally suppressing protein translation: NRF2-deficient cancer cells show an increase in oxidized cysteine residues in components of the translational initiation complex, globally reducing translation (Chio et al. 2016). This phenotype can be rescued by antioxidant treatment. Consistent with the critical role of NRF2 in redox regulation, some cancers suppress ROS by mutations in *KEAP1* or *NRF2* that prevent NRF2 from being sequestered in the cytoplasm, constitutively activating NRF2 (Singh et al. 2006; Ohta et al. 2008; Shibata et al. 2008a,b).

Circulating melanoma cells in the blood of xenografted mice as well as metastatic nodules have higher levels of ROS relative to primary subcutaneous tumors (Piskounova et al. 2015). Oxidative stress is a barrier to distant metastasis in these melanomas as treatment with the antioxidant NAC increases the frequency of circulating melanoma cells in the blood, as well as metastatic disease burden, without significantly affecting the growth of primary subcutaneous tumors (Piskounova et al. 2015). The finding that distant metastasis is limited by oxidative stress is not an artifact of xenotransplantation into immunocompromised mice as similar results were observed in immunocompetent mice with autochthonous melanomas: Treatment with NAC or vitamin E promoted distant metastasis without affecting the growth of subcutaneous tumors (Le Gal et al. 2015). Consistent with this, cancer cells depend on NRF2 (Wang et al. 2016), thioredoxin-like 2 (Qu et al. 2011), superoxide dismutase (Kamarajugadda et al. 2013; Glasauer et al. 2014), and glutamate cysteine ligase (the rate-limiting step of glutathione synthesis) (Nguyen et al. 2016) to survive during metastasis.

A number of studies have thus indicated that reducing oxidative stress is critical for metastasis. Nonetheless, other studies have reported that ROS can promote metastasis. Antioxidants inhibit the metastasis of some cell lines (Ferraro et al. 2006; Ishikawa et al. 2008; Porporato et al. 2014). Mouse melanoma cells in an aged microenvironment show decreased APE1 expression as a result of changes in Wnt signaling, which increases ROS, metastasis, and therapy resistance (Kaur et al. 2016). Mouse lung carcinoma cells containing mitochondrial DNA with mutations in NADH dehydrogenase subunit 6 (ND6) displayed higher ROS levels and increased metastasis when compared with wild-type mitochondrial DNA and the increase in metastasis could be inhibited by NAC treatment (Ishikawa et al. 2008). ROS can also act cell-extrinsically in the tumor microenvironment to promote cancer progression (Jeziarska-Drutel et al. 2013), either by influencing the properties of tumor stromal cells (Cat et al. 2006; Toullec et al. 2010) or by attenuating the activity of inflammatory cells (Sato et al. 2010). These studies are a reminder that ROS can induce signaling that provides a selective advantage to cells in certain circumstances and that the net effect of ROS on cancer reflects a complex combination of adaptive and maladaptive consequences within the cells and their environment.

CANCER CELLS UNDERGO METABOLIC CHANGES TO MANAGE ROS

Because oxidative stress limits cancer progression, the rare cancer cells that successfully metastasize may undergo metabolic changes that allow them to cope with oxidative stress. Consistent with this, melanoma cells undergo reversible metabolic changes during metastasis that increase their fitness to form tumors after metastasis (Piskounova et al. 2015).

NADPH is central to oxidative stress resistance. In cells in which glutathione and thioredoxin have been depleted by oxidative stress, NADPH must be diverted to regenerate the reduced forms of these redox buffers (Fig. 2). Cancer cells use multiple metabolic pathways to generate NADPH, including the pentose phosphate (Patra and Hay 2014), folate (Fan et al. 2014), and malic enzyme pathways (Fig. 2). The pentose phosphate pathway is the first line of defense against oxidative stress in many human cells and can promote the survival of cells during neoplastic transformation and during detachment from extracellular matrix (Boada et al. 2000; Debnath et al. 2002; Debnath and Brugge 2005; Bensaad et al. 2006; Sukhatme and Chan 2012; Hu et al. 2013; Kuehne et al. 2015).

De novo serine synthesis and the folate pathway are another major source of NADPH for cancer cells (Fan et al. 2014; Lewis et al. 2014; Ye et al. 2014). De novo serine synthesis can be limiting for flux through the folate pathway, and reduced serine hydroxymethyltransferase expression reduces the cellular NADPH/NADP⁺ ratio while increasing ROS levels and cell death (Ye et al. 2014). Phosphoglycerate dehydrogenase (PHGDH) catalyzes the first step in serine biosynthesis. This enzyme is

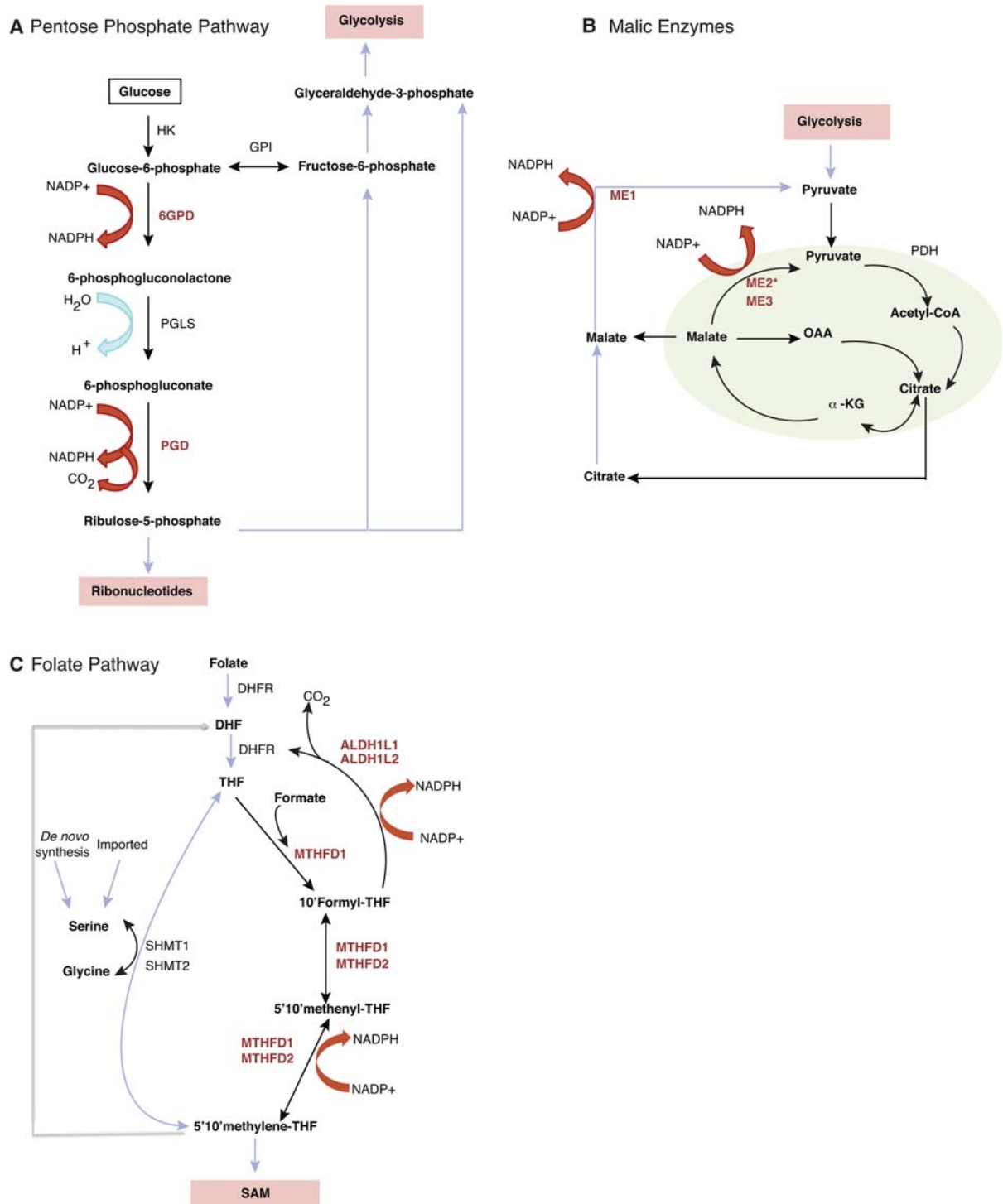


Figure 2. NADPH regenerating pathways. Cancer cells depend on different metabolic pathways to produce NADPH to regenerate endogenous antioxidants, such as GSH and TXN. (A) The pentose phosphate pathway. In the pentose phosphate pathway, NADPH is generated in two reactions catalyzed by glucose-6-phosphate dehydrogenase (G6PD) and 6-phosphogluconate dehydrogenase (PGD), shown in red. G6PD catalyzes the rate-limiting step in the oxidative branch of the pentose phosphate pathway. Its activity is regulated by the NADP⁺/NADPH ratio, where NADPH is the negative regulator and NADP⁺ is required for proper enzymatic function. (B) Malic enzymes. Three malic enzymes have been identified in mammalian cells. They differ with respect to their localization in the cytosol (ME1) versus mitochondria (ME2, ME3) and with respect to their use of NADP (ME1, ME3) versus NAD or NADP (ME2*) as electron acceptors. Malic enzymes mediate the conversion of malate to pyruvate, accompanied by the production of NADPH. (C) The folate pathway. The folate pathway uses one-carbon groups that come from serine to generate either nucleotides or NADPH. Serine can either be synthesized *de novo* by cells or imported. Several enzymes within the folate pathway, aldehyde dehydrogenase L1 (ALDH1L1) and 2 (ALDH1L2), and methylenetetrahydrofolate dehydrogenase 1 (MTHFD1) and 2 (MTHFD2) are able to regenerate NADPH.

increased in expression in many breast cancers and melanomas, and is necessary for the proliferation of those cells (Locasale et al. 2011; Mullarky et al. 2011; Possemato et al. 2011). The folate pathway uses one-carbon groups from serine to generate either nucleotides or NADPH. Several enzymes within the folate pathway generate NADPH including methyltetrahydrofolate dehydrogenases 1 and 2 (MTHFD1 and MTHFD2) and aldehyde dehydrogenase-like 1 and 2 (ALDH1L1 and ALDH1L2) (Fig. 2; Fan et al. 2014). ALDH1L2 expression reversibly increases in melanoma cells during metastasis and knock-down of ALDH1L2 or MTHFD1 reduces the metastasis of patient-derived melanoma xenografts in vivo without affecting the growth of primary tumors in the same mice (Piskounova et al. 2015).

Finally, malic enzymes mediate the conversion of malate to pyruvate, accompanied by NADPH production (Fig. 2). Malic enzymes promote the growth of several cancers (Son et al. 2013; Ren et al. 2014). ME2 expression is associated with reduced ROS levels in melanoma cells and promotes cutaneous melanoma proliferation and invasion in culture (Jiang et al. 2013).

Cancer cells sometimes benefit from metabolic changes that preserve NADPH for the regeneration of glutathione and thioredoxin. AMP-activated protein kinase (AMPK) is activated in response to ROS as well as during energy stress and can promote the survival of cells partly through redox regulation (Schafer et al. 2009). AMPK inhibits acetyl-CoA carboxylases, inhibiting the consumption of NADPH by fatty acid synthesis and promoting the generation of NADPH by fatty acid oxidation (Jeon et al. 2012). High ROS levels in cancer cells also decrease oxidative phosphorylation by promoting glycolysis by stabilizing hypoxia-inducible factor-1 (HIF-1) (Chandel et al. 2000; Semenza 2011) or by oxidizing and inhibiting pyruvate kinase M2, diverting glucose into the pentose phosphate pathway (Anastasiou et al. 2011). These changes may reflect a broader need to shut down anabolic pathways that generate ROS or consume NADPH in cancer cells experiencing oxidative stress.

Consistent with these observations, some cancer cells undergo metabolic changes during invasion in vitro and metastasis in vivo that would be expected to reduce the generation of ROS (Chen et al. 2007; Lu et al. 2010; Qu et al. 2011; Kamarajugadda et al. 2012, 2013; Dong et al. 2013; Shi et al. 2014). For example, HIF-1 activity is transiently increased during metastasis due to high ROS levels (Montagner et al. 2012; Vanharanta et al. 2013; Zhao et al. 2014). HIF-1 activation metabolically reprograms metastasizing cells away from oxidative phosphorylation to glycolysis and lactic acid production, through increased expression of lactate dehydrogenase and pyruvate dehydrogenase. These metabolic changes reduce ROS levels and promote survival during metastasis.

The metabolic plasticity of cancer cells allows them to undergo dynamic changes in mitochondrial mass and mitochondrial function that facilitate their ability to cope with energy stress and oxidative stress (Senft and Ronai 2016; Vyas et al. 2016). Both glycolysis and oxidative phosphorylation can be used in cancer cells in comple-

mentary strategies to enhance metabolic plasticity to overcome changes in the tumor environment or in energy demands (Jose et al. 2011). PGC-1 α , which promotes mitochondrial biogenesis, is dynamically expressed by cancer cells. Although metastasizing cells can increase mitochondrial biogenesis and respiration by increasing PGC-1 α expression (LeBleu et al. 2014), PGC-1 α inhibits metastasis in other contexts (Luo et al. 2016). Because PGC-1 α would be expected to increase mitochondrial mass and the generation of ROS, the observation that PGC-1 α ^{low} cells have more metastatic potential in some cancers (Luo et al. 2016) is consistent with the observation that oxidative stress limits distant metastasis (Piskounova et al. 2015). Cancer cells also dynamically control mitochondrial fusion and fission to regulate oxidative phosphorylation and ROS levels (Hagenbuchner et al. 2013) and to promote invasion (Zhao et al. 2013). The question of whether metastasizing cancer cells benefit from increased or decreased mitochondrial function may depend on the tissue of origin as mitochondrial DNA (mtDNA) copy numbers vary widely across tumor types (Reznik et al. 2016).

CONCLUSION

ROS promote cancer initiation by promoting mutagenesis and perhaps by activating signaling pathways that promote proliferation, survival, and stress resistance. However, ROS also limits cancer initiation and progression by causing oxidative stress that kills many cancer cells. For this reason, cancer cells depend on a variety of mechanisms to suppress ROS and to cope with oxidative stress. Antioxidants promote cancer initiation and progression in experimental mouse models as well as in clinical trials. Cancer may be more effectively treated with pro-oxidants that exacerbate the oxidative stress experienced by cancer cells or that prevent metabolic adaptations that confer oxidative stress resistance.

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