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The antioxidant paradox: what are antioxidants and how should they be used in a therapeutic context for cancer

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Abstract
So-called antioxidants have yet to make a clinical impact on the treatment of human cancer. The reasons for this failure are several. First, many agents that are called antioxidants are truly antioxidants at a given dose, but this dose may not have not been given in clinical trials. Second, many agents are not antioxidants at all. Third, not all tumors use reactive oxygen as a signaling mechanism. Finally, reactive oxygen inhibition is often insufficient to kill or regress a tumor cell by itself, but requires sequential introduction of a therapeutic agent for maximal effect. We hope to provide a framework for the logical use of these agents in cancer.

Definition of an antioxidant
Many compounds have been described as antioxidants. These include compounds with free sulfhydryl groups, including N-acetylcysteine (NAC) and lipoic acid, compounds with multiple double bonds and conjugation (e.g., carotenoids, tocopherols and retinoids, among others) and polyphenols (e.g., epicatechingallate and quercetin, among others). Compounds that inhibit reactive oxygen generation (i.e., NADPH oxidase inhibitors), xanthine oxidase inhibitors and compounds that induce oxidant defenses (e.g., Nrf2 activation-sulforaphane, among others) have also been allocated to this category \cite{fung2015,bramhall2012,lemasney2013}. Reactive oxygen species include superoxide, hydrogen peroxide, singlet oxygen and reactive nitrogen species derived by the reaction of other reactive oxygen species with nitric oxide. For the purposes of this article, we will focus predominantly on superoxide and hydrogen peroxide.

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Categories of antioxidants

Sulfhydryls

The antioxidant activity of compounds with free sulfhydryls has been described in thousands of references, but has not been used widely in clinical practice. At low concentrations, compounds with free sulfhydryls are capable of absorbing free electrons and reacting with short-lived reactive oxygen species, such as superoxide and peroxynitrite \[4\]. However, high concentrations of sulfhydryl groups can form adducts with p53 (p53 glutathionylation) and also form adducts with other proteins, resulting in endoplasmic reticulum stress, with the accumulation of misfolded proteins \[5,6\]. Finally, sulfhydryl-containing compounds upregulate members of the aldo-keto reductase family, which is involved in the detoxification of potential therapeutic agents, such as retinal and chemotherapeutic agents \[7\]. Interestingly, high-sulfur-containing melanins (phaeomelanin) have been shown to generate reactive oxygen and participate in the pathogenesis of melanoma \[8\]. Thus, the ratio of beneficial to non-beneficial activities of drugs containing free sulfhydryls is unclear (Figure 1).

Gao et al. evaluated the effect of NAC in two myc-dependent murine tumor models, including P493 lymphoma cells and a transgenic model of myc expression in the liver \[9\]. Both NAC and vitamin C decreased tumor growth in these models, and it was noted that hypoxia-induced HIF1α was blocked by these antioxidants, but baseline levels of HIF1α were not affected. These data indicate that hypoxia induces reactive oxygen, likely through mitochondrial dysfunction, and the elevated reactive oxygen induces HIF1α. Notably, the P493 lymphoma cell does not express HIF2α. The authors also found that the PC3 tumor cell line, which is not c-myc dependent, is more resistant to antioxidant treatment. PC3 may be more representative of the complexity of authentic human tumors. The myc-dependent tumors may be more sensitive to antioxidants than complex human tumors, and the expression of HIF2α may play a role in antioxidant resistance \[9,10\].

Further complexity is noted in that tumors that have elevated levels of glutathione may also have increased metastatic capabilities. Reduced glutathione serves as the major nonprotein thiol in the cell, and is actively taken up by mitochondria. The contrasting findings between NAC and glutathione may reflect preferential uptake of glutathione by mitochondria, allowing mitochondria to reduce reactive oxygen in the face of hypoxia, or a relatively ineffective inhibition of NF-κB by elevated levels of cytoplasmic glutathione \[11\]. Cytoplasmic glutathione may also play a role in resistance by forming adducts with chemotherapeutic agents with increased affinity compared with its ability to decrease NF-κB activation \[11\].

Highly conjugated systems

A second class of drugs that have been termed antioxidants are those with highly conjugated double bond systems. These can be oxygen containing (e.g., curcumin and retinoids) or nonoxygen containing (e.g., carotenoids). The highly conjugated structure leads to brightly colored compounds, with the greater the conjugation, the darker the compounds. Examples of highly conjugated colored compounds that can absorb electrons are gentian violet,
curcumin and indigo. Conjugated compounds are capable of absorbing electrons and forming adducts with oxygen species (e.g., epoxides, diols and other structures), thus acting like antioxidants. However, they can act as oxidants as well, in that they can form adducts with protein sulfhydryl groups. The most well-known example is the formation of adducts with the Nrf2/Keap cytoplasmic complex, in which Nrf2 subsequently undergoes nuclear translocation and Keap undergoes cytoplasmic degradation [12]. Thus, the cell interprets treatment with these so-called antioxidants as an oxidative insult and upregulates Nrf2-mediated transcription, which is often known as the antioxidant response. Virtually all compounds with an α–β unsaturated carbonyl can induce Nrf2. Similarly, sulforaphane, a compound from broccoli that gained prominence as a Nrf2 activator, is capable of forming covalent adducts with free sulfhydryl groups [13]. Finally, alkylating agents, which are capable of alkylating DNA, are also likely to alkylate proteins and induce an oxidant response.

Kensler et al. noted that Nrf2-deficient mice are more susceptible to chemical carcinogenesis [14]. Two major reasons may account for this. First, the induction of glutathione by Nrf2 activity may result in the preferential formation of glutathione adducts with carcinogens, preventing direct DNA damage. Second, loss of Nrf2 activation may result in increased Myd88/inflammasome activity, resulting in chronic oxidative stress, which is a known carcinogen [14]. Induction of glutathione is not the sole result of Nrf2 activation. Nrf2 induces multiple targets, including HO-1, ion channels and chaperone products. The role of these additional proteins in the Nrf2 response is poorly understood. Recently, the Dulak group demonstrated that HO-1 can be oncogenic in a manner independent of its catalytic activity, as constitutive induction of HO-1, both wild-type and enzymatically inactive, resulted in oncogenic transformation [15].

**Tocopherol & tocopherol esters**

Some of the most commonly used ‘antioxidants’ include tocopherol and tocopherol esters. These drugs are available as over-the-counter supplements. These compounds are thought to act as antioxidants through the formation of oxygen adducts and the absorption of free radicals and electrons. The highly branched structure of tocopherol likely predisposes it to form relatively stable compounds containing free radicals, such as is observed in highly branched structures such as tempol [16,17]. The formation of stable adducts does not mean that reactive oxygen is being neutralized, but may reflect a transfer of reactive oxygen to different cytoplasmic or nuclear locations. Despite enormous human exposure, tocopherol and tocopherol esters have not been definitively shown to be of benefit in a single human disease. On the other hand, they have been shown to be associated with bleeding complications.

**Proposed definition of an antioxidant**

In order to define antioxidant activity when focusing on cancer therapy, we would like to propose the following definition: an antioxidant is a compound that blocks the superoxide induction of NF-κB and Notch activation in cells in which this pathway is constitutively expressed. An additional measure includes that reactivation of the translational activity of p53 in cells in which wild-type p53 is oxidatively inactivated, resulting in the
phosphorylation of p53 at SER15 and the activation of p53 target genes, such as p21. This narrow definition will enable a coherent therapeutic application of antioxidants for the treatment of cancer. While we have characterized this response in our model systems, the vast majority of compounds described in this article have not been tested in such a manner. We thus cannot predict whether all of the compounds currently titled as antioxidants will fulfill this paradigm. In addition, there may be dose-dependent responses, with opposite responses seen at low and high doses.

**Definition of the reactive oxygen-driven tumor**

In order to use the same drugs for multiple tumors of different anatomic sites, it is necessary to classify tumors by signaling pathways. In our initial studies of the oncogenic role of MAPK, we found that MAPK activation is tumor suppressive in cells with deficient p53 function, while we and others have found that MAPK activation is tumorigenic in cells that have lost the tumor-suppressor gene p16ink4a [18]. We subsequently discovered that carcinogens that induce reactive oxygen (insoluble nickel) cause tumors with hypermethylation of p16ink4a [19]. Careful examination of the literature demonstrates that virtually all tumors that are caused by reactive oxygen, including chronic inflammation, results in hypermethylation of p16ink4a [20]. These include all tumors caused by Epstein–Barr virus (EBV; Burkitt’s lymphoma [BL], Hodgkin’s disease and gastric carcinoma), hepatitis C virus (hepatocellular carcinoma), ultraviolet A (melanoma), inflammatory bowel disease-induced colon carcinoma, schistosomiasis-induced bladder cancer, tobacco-induced oral and lung carcinoma and epidermolysis bullosa-associated squamous cell carcinoma [21–24]. As a corollary of this, most if not all of these tumors use reactive oxygen, especially superoxide, as a signaling pathway.

**Common signaling events in the reactive oxygen-driven tumor**

**Wild-type p53, loss of p16ink4a & EBV**

We have already noted that hypermethylation of p16ink4a is observed in tumors caused by reactive oxygen. In addition, many of these tumors also have wild-type p53. There is little selective pressure to mutate p53, as p53 is oxidatively inactivated and transcriptionally inactive [25–27]. Reactive oxygen has been shown to upregulate DNMT1, providing a plausible mechanism for the hypermethylation of tumor-suppressor genes as a response to reactive oxygen [28,29]. Moreover, reactive oxygen becomes a signaling mechanism in tumor cells initiated by reactive oxygen [30]. One classic example is that of BL. BL is a high-grade B-cell malignancy characterized by translocations of the immunoglobulin gene with the c-myc oncogene. BL is associated with EBV infection in Africa and patients with HIV, and occurs in a sporadic form associated with mutant p53. Interestingly, virtually all EBV-induced BLs contain hypermethylation of p16ink4a [31]. Thus, we predicted that EBV-associated BL would be an example of the reactive oxygen-driven tumor. EBV infection caused an induction of reactive oxygen, and reactive oxygen was sustained by the viral oncogenes LMP1 and EBER and autocrine IL-10 production. Virtually all EBV-positive BL cell lines exhibited high baseline levels of reactive oxygen, while EBV-negative cell lines did not [21]. Finally, treatment of EBV-positive BL cells with ebselen, a superoxide dismutase mimetic, resulted in downregulation of NF-κB, as demonstrated by
the decreased phosphorylation of IkB and a compensatory activation of MAPK. This could explain the apparent failure of antioxidants – in patients treated with these drugs as a monotherapy, one might observe faster-growing tumors as a result of antioxidant therapy, but these faster-growing tumors might be susceptible to chemotherapy and radiation as a result of decreased NF-κB activation [21]. Thus, we can introduce the concept of sequential antioxidant therapy, in that pretreatment with an antioxidant that downregulates NF-κB may sensitize tumors to chemotherapy and radiation.

**Loss of p16ink4a**

Melanoma is a common tumor and the leading cause of death due to a dermatologic condition. Notably, loss of p16ink4a is the major genetic cause of melanoma, and also predisposes individuals to pancreatic cancer [32,33]. The loss of p16ink4a has been seen with both Braf and Nras mutations, and is not preferentially associated with either of these mutations. Given that melanoma has frequent loss/mutation of p16ink4a and p53 mutations are uncommon in melanoma, we hypothesized that melanoma might use the reactive oxygen-driven tumor pathway [34]. Melanoma begins as a radial noninvasive form, with loss of p16ink4a and MAPK activation, and later progresses to an invasive form called ‘vertical growth melanoma’. This is associated with the ability of tumors to invade through basement membranes and undergo distant metastasis. We introduced Akt into a radial growth cell line, WM35, which is mutant for Braf [35]. Parental WM35 cell lines do not form tumors in immunocompromised mice. Upon the introduction of Akt, we noted upregulation of Nox4 and mitochondrial dysfunction with complex 1 mutations in tumor cells that were capable of forming tumors. Superoxide was highly elevated, and the introduction of Akt resulted in the development of aerobic glycolysis (i.e., the Warburg phenomenon). Akt introduction was also associated with the upregulation of VEGF and sirt1, and blockade of Akt with a small-molecule inhibitor resulted in the downregulation of VEGF and sirt1. Interestingly, treatment of melanoma with Braf inhibitors or MEK inhibitors does not address the reactive oxygen/Akt pathway, which may play a large role in why these inhibitors only exhibit temporary benefits in melanoma. Indeed, they have been observed to elevate reactive oxygen production and activation of Akt [36]. Furthermore, at least one of the known modes of resistance – amplification of IGF-1 – is a known upregulator of Akt [37].

**NADPH oxidase & the inflammatory response**

We have clinical proof of principle for the use of reactive oxygen inhibitors in human melanoma. Gentian violet is a topical drug that we found to be an NADPH oxidase inhibitor. In a patient with advanced, inoperable melanoma with metastasis, we debulked the lesions and treated the residual disease with gentian violet and imiquimod. The combination of these drugs with NADPH oxidase inhibition and TLR7 activation resulted in a vigorous inflammatory response. The patient was free of melanoma for 2 years, until his death from congestive heart failure at 94 years of age [38]. This treatment has been used successfully on patients with cutaneous ulcerating disease, but is less successful for subcutaneous disease, possibly due to poor delivery. The following synergistic mechanisms are proposed. First, high levels of VEGF cause a block in dendritic cell maturation and support the development of myeloid dendritic cells. These cause the promotion of a Treg response in the tumor.
microenvironment. Second, high levels of Akt and reactive oxygen support tumor expression of immunosuppressive molecules, such as B7H1, CD200 and IL-10, and the expression of these molecules causes cell death of cytotoxic CD8 lymphocytes and promotes immune exhaustion. High levels of IFN-α/β alone in the tumor microenvironment attract both Tregs and cytotoxic CD8 lymphoid cells, but the expression of B7H1 causes the preferential death of CD8 cytotoxic lymphocytes and immune evasion. Finally, the blockade of tumor-associated NF-κB and Akt allows greater apoptosis due to lymphocyte-mediated cell killing [39].

**Clinical recognition of the reactive oxygen-driven tumor**

The first tumors to be discovered as reactive oxygen driven were hemangiomas of infancy (HOI). These tumors are the most common benign tumors of childhood and have a life cycle of proliferation, regression and replacement with a connective tissue scar. The major growth factor of these lesions is angiopoietin-2 [40], and the infection of endothelial cells with *Bartonella* species causes HOI-like lesions in adults (verruga peruana) and the induction of angiopoietin-2 [41]. Our group was interested in US FDA-approved drugs that have a similar structure to diphenyleneiodonium, the most commonly used NADPH oxidase inhibitor. We found that the triphenylmethane structures of gentian violet and brilliant green, which are FDA-approved topical medications, were similar to diphenyleneiodonium in that they shared a central atom with a positive charge that could be delocalized among aromatic rings. These compounds inhibited Nox2 and Nox4 and blocked the growth of bend3 hemangiomas *in vivo* [42]. We then extended these findings to humans, demonstrating that eosin, a structurally similar compound of the triphenylmethane class, caused regression of HOI. We also demonstrated that eosin downregulates the production of angiopoietin-2, which is consistent with its activity against HOI [43]. Interestingly, the current treatment of choice of hemangiomas – propranolol – inhibits NADPH oxidase activity in addition to its activity as a β-blocker, and it is likely that β-blockade does not play a role in the activity of propranolol against HOI. Furthermore, we also found that cytoplasmic WT1 is highly expressed in HOI, but not in other vascular malformations [44]. WT1 is also expressed in other tumors that use reactive oxygen, such as melanoma and glioblastoma, and thus may serve as a biomarker for selecting tumors for clinical trials [45].

**Notch is implicated**

Our initial studies showed that triphenylmethanes were not well tolerated when given repeatedly intraperitoneally in mice. This was the impetus to develop another class of NADPH oxidase inhibitors, called fulvenes. Fulvenes are highly conjugated cyclopentadienes that take on aromatic characteristics. We synthesized a lead compound, fulvene 5, through a single-step reaction of indigo with sodium cyclopentadienide [46]. Fulvene 5 was well tolerated intraperitoneally and reduced the growth of bend3 hemangiomas in mice when given systemically. Gene chip analysis was performed, and among the genes that were most downregulated by fulvene 5 were *PLGF*, *Dll4* and *Nrarp*. This indicates that the reactive oxygen pathway is upstream of Notch signaling, and blockade of Nox2 and Nox4 may result in Notch pathway downregulation. Notch signaling is activated in many malignancies, including melanoma and leukemia; interestingly, it is also downregulated in tumors with mutant *p53* [47–49]. This is consistent with signaling...
pathways being protumorigenic in p16ink4a-deficient contexts and tumor suppressive in cells with mutant p53. Notch signaling may therefore be a useful biomarker for the reactive oxygen-driven tumor.

**Warburg phenomenon & reactive oxygen**

Decades ago, Otto Warburg noted that cancer cells used glycolysis as the predominant mode of glucose metabolism, generating only two ATP molecules per molecule of glucose, rather than the 38 molecules of ATP that could be generated by full respiration [50]. Pyruvate instead of oxygen served as the electron recipient, causing the accumulation of lactic acid in the tumor microenvironment, making it both acidic and hypoxic. We and others demonstrated that the introduction of Akt into cells caused the reversion of tumor cells to a predominantly glycolytic phenotype [35]. Cuezva et al. coined the term ‘bioenergetics index’ and demonstrated that tumors with a low bioenergetics index (low respiration) had a worse prognosis than tumors that were more respiratory [51]. In our hands, the introduction of Akt into respiratory melanoma tumor cells led to increases in reactive oxygen, either due to complex 1 mitochondrial dysfunction or the induction of NADPH oxidases [35]. Our hypothesis is that cancer cells use the Warburg phenomenon in order to allow them to use reactive oxygen as a signaling mechanism. The reactive oxygen generated enables IκB oxidation, causing NF-κB activation and increased survival [52]. Reactive oxygen can also activate Akt through the oxidation of PTEN [53].

**NADPH oxidase inhibition**

In our previous studies of BL, we demonstrated that the inhibition of reactive oxygen with ebselen led to a decrease in NF-κB and the activation of MAPK [21]. In vivo, this might manifest itself as rapidly growing tumors due to MAPK activation, but these tumors might become sensitized to conventional chemotherapy due to low levels of NF-κB activation. This might explain the failure of the vast number of antioxidant trials in humans, in that they might be effective at reducing NF-κB, but would increase MAPK activation, resulting in either no change or an increase in tumor volume. We thus propose sequential therapy, with NADPH oxidase inhibition preceding chemotherapy or radiation.

We have demonstrated the utility of the sequential treatment of NADPH oxidase blockade with chemotherapy. Imipramine blue is a novel NADPH oxidase inhibitor that is synthesized from the antidepressant imipramine, which crosses the blood–brain barrier, and Michler’s ketone [54]. Imipramine blue by itself crosses the blood–brain barrier and halts the invasion of glioblastoma into the brain parenchyma, although it does not significantly increase survival as a monotherapy. On the other hand, when administered prior to liposomal doxorubicin, imipramine blue resulted in long-term survival in eight out of eight rats, which is unprecedented for animal models of glioblastoma. We believe the reasons for this efficacy are as follows [54]: first, imipramine blue downregulates NF-κB, enabling downregulation of the multidrug resistance gene MDR and thus possibly increasing intracellular concentrations of doxorubicin; second, decreased NF-κB enables greater cell killing by doxorubicin; third, Akt may be downregulated, enabling the increased expression of tumor antigens and the decreased expression of immunoinhibitory molecules, such as B7H1, IL-10 and CD200 [55,56]; fourth, angiogenesis may be inhibited, which enables greater maturation...
of dendritic cells and decreased myeloid suppressor cells [57–59]; finally, p53 oxidation may be inhibited, enabling p53 to activate apoptosis in response to doxorubicin.

NADPH oxidase inhibitors and small molecules such as honokiol do not necessarily halt tumor growth [60–64], but may switch it from a glycolytic pathway to a PPAR-α/AMPK/PGC1α pathway (Figure 2) [65,66]. These tumors may be respiratory and, in fact, may be rapidly proliferating. However, PPAR-α, AMPK and PGC1α are all independently associated with NF-κB inhibition [67–72]. Honokiol is a known activator of AMPK, and triphenylmethanes such as gentian violet and proton sponge blue have been shown to activate PPAR-α [65,66,73]. In the case of honokiol, it is not currently known whether normalization of the Warburg phenomenon occurs before NF-κB activation, and it is theoretically possible that honokiol could inhibit NF-κB, enabling reactive oxygen in a tumor cell to kill the cell in the absence of protective NF-κB. Evidence in favor of this is the induction of p38 JNK in some tumor cells treated with honokiol [74,75]. Chronic honokiol treatment could then inhibit NF-κB in the face of normalized respiration and sensitize cells to apoptotic agents.

Antioxidants

Antioxidants are a diverse group of compounds that act to induce enzymes that provide glutathione (Nrf2/Keap interactions), scavenge reactive oxygen species, such as superoxide, reactive nitrogen species and hydrogen peroxide, normalize mitochondrial function or inhibit enzymes that generate reactive oxygen (NADPH oxidase inhibitors). We believe the following principles can be used to guide the use of antioxidants: first, not all tumors will benefit from antioxidant treatment. Only tumors with the reactive oxygen-driven phenotype will benefit. This phenotype includes high levels of Akt phosphorylation, high levels of NF-κB activation, wild-type p53 and often wild-type PTEN, which can be oxidatively inactivated in order to activate Akt. Additional enzymes such as PP2a and deubiquitinases, may also be oxidatively inactivated. Cytoplasmic WT1 is a biomarker that can identify tumors that may benefit from antioxidant treatment. Second, antioxidants have a conserved biochemical effect, resulting in the downregulation of NF-κB, downregulation of Akt, activation of wild-type p53 and downregulation of Notch signaling, especially the downstream target Nrarp. Third, antioxidants may convert glycolytic tumors to a respiratory phenotype by the induction of PPAR-α/AMPK/PGC1α pathway, which may not halt their proliferation, but may make them less glucose avid and more dependent on glutamine and fatty acid metabolism. In addition, the respiratory-induced phenotype in tumors may also lead to an alteration to the conserved biochemical effect of antioxidants. These respiratory-induced tumors may be relatively deficient in NF-κB, and while rapidly growing, may be increasingly sensitive to conventional chemotherapy, radiation and antiangiogenic activity. As a corollary of this, antioxidants used alone are unlikely to be effective as a monotherapy, and this may be the reason why virtually every antioxidant has failed in human clinical trials. It is our hope that this discussion will aid in the development of the sequential therapy of reactive oxygen blockade followed by chemotherapy in the treatment of cancer.
Conclusion & future perspective

While the prior trials of antioxidants in cancer have not yielded outstanding success, the prospects of incorporating antioxidants into cancer treatment are bright. Several natural products that have been termed antioxidants may work by promoting mitochondrial biogenesis. Promoting mitochondrial biogenesis in normal cells is predicted to have antioxidant activity, while in tumor cells with a fixed mitochondrial defect, increased biogenesis may promote increased levels of reactive oxygen. This may have the salutary effect of selectively killing cells with mitochondrial defects. Alternatively, compounds that induce Nrf2 or inhibit NADPH oxidase may augment chemotherapy and radiation by downregulating superoxide-induced NF-κB. The timing of the administration of these agents will be critical, as if these compounds are coadministered with chemotherapy and radiation, they may be inhibitory, but if they are administered prior to chemotherapy/radiation, they may enhance their therapeutic effects. The predicted sequence of events is as follows: a tumor that is excised is stained by the pathologist for signaling molecules that indicate the reactive oxygen-driven tumor, including positivity for cytoplasmic WT1 and negative expression of p53 (wild-type p53). Such tumors will be treated with reactive oxygen inhibitors, followed by chemotherapy. Tumors that express mutant p53 may be treated with a cocktail of drugs that selectively increase reactive oxygen in the tumor.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Key Terms

<table>
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<tr>
<th>Term</th>
<th>Definition</th>
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<tr>
<td>NADPH oxidases</td>
<td>Family of enzymes that convert molecular oxygen to superoxide.</td>
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<tr>
<td>Reactive oxygen species</td>
<td>Include superoxide, hydrogen peroxide and reactive nitrogen species.</td>
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<tr>
<td>NF-κB</td>
<td>Family of transcription factors that can be activated by reactive oxygen and stimulate resistance to chemotherapy, apoptosis and hypoxia.</td>
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<td>Warburg phenomenon</td>
<td>Phenomenon described by Otto Warburg to describe the predominant glycolytic metabolism of advanced cancer cells. One of the possible advantages that cancer cells derive from the Warburg phenomenon is the ability to survive under severe hypoxia, due to dysfunctional mitochondria driving reactive oxygen-NFκB signaling</td>
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<tr>
<td>Reactive oxygen driven tumor</td>
<td>A signaling phenotype in which high levels of superoxide inactivate tumor suppressor genes such as p53, PTEN, IκB and other protein phosphatases, leading to activation of NFκB, Akt, and oncogenic kinases.</td>
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References

Papers of special note have been highlighted as:

- of interest; ▪▪ of considerable interest.


Future Med Chem. Author manuscript; available in PMC 2015 June 01.


27. Garuti A, D’Orazi V, Arbiser JL, D’Orazi G. Gentian violet induces wtp53 transactivation in cancer cells. Int J Oncol. 2014; 44(4):1084–1090. Recent paper demonstrating that the commonly used dye gentian violet can reactivate wild-type p53 in tumor cells by reversing its oxidation. This provides proof of principle that NADPH oxidase inhibitors such as gentian violet can reactivate p53. [PubMed: 24535435]


Executive summary

Definition of an antioxidant

- Antioxidants are traditionally thought of as compounds that can combine or absorb oxygen, especially in activated states (e.g., superoxide, hydrogen peroxide and singlet radicals). A more complete definition would be compounds that can absorb and delocalize free electrons in a manner that prevents the formation of deleterious adducts.

Categories of antioxidants

- Categories of antioxidants include sulfhydryl groups, tocopherol and tocopherol esters, Nrf/glutathione inducers and NADPH oxidase inhibitors. The definition of some of these compounds as antioxidants may be dose dependent, as compounds that can induce Nrf2 (an antioxidant effect) can also form covalent bonds with glutathione and other sulfhydryls (a pro-oxidant effect).

Definition of the reactive oxygen-driven tumor

- Tumors caused by reactive oxygen carcinogens have a common phenotype, despite being in multiple anatomic sites. They tend to have wild-type $p53$, activation of Akt and MAPK and inactivation of $p16\text{ink}4a$. A common mechanism of inactivation is hypermethylation, caused by the reactive oxygen induction of DNMT1. Finally, they have high levels of NF-$\kappa B$, as a result of the oxidation of I-$\kappa B$.

Notch is implicated

- The treatment of reactive oxygen-driven tumors with NADPH oxidase inhibitors results in the downregulation of Notch signaling pathways. This may be used as a biomarker in order to assess the efficacy of candidate agents in vivo.

Warburg phenomenon & reactive oxygen

- Tumor cells preferentially use aerobic glycolysis to generate ATP, which is a highly ineffective way of generating ATP – this is termed the Warburg phenomenon. We propose that the gain that the tumor cell obtains from this inefficient mode of ATP generation is the ability to generate reactive oxygen and avoid reactive oxygen-induced apoptosis.

NADPH oxidase inhibition

- We propose that NADPH oxidase inhibition may reverse the Warburg phenomenon. As a monotherapy, this might lead to faster-growing tumors, but the resulting tumors might be more sensitive to hypoxia (i.e., central necrosis) and to chemotherapy and radiation due to a the downregulation of Akt and NF-$\kappa B$. Therefore, the optimal use of NADPH oxidase inhibitors is to enhance the efficacy of chemotherapy and radiation, not as a monotherapy.
Figure 1. Rheostat modulation of p53 transcriptional activity
With extremely high levels of free sulphydryl groups, p53 can be glutathionylated, with decreased p53 activity. With decreasing levels of reductive power, p53 can be at a physiologic level and can be activated by normal stimuli that activate p53-mediated transcription, including radiation and chemotherapy. With increasing levels of reactive oxygen, as seen in reactive oxygen-driven tumors, p53 sulphydryl groups are oxidized, blocking p53 binding to DNA. Finally, at extremely high levels of reactive oxygen, p53 can be activated as part of the cell death response.
ROS: Reactive oxygen species.
Figure 2. Transition between the glycolytic and respiratory states in solid tumors

Tumor cells can coexist between glycolytic cells, which exhibit the reactive oxygen-driven phenotype of elevated superoxide, elevated NF-κB from oxidative inactivation of IκB, elevated Akt as a result of oxidative inactivation of PTEN and oxidative inactivation of p53. The cells in this state exhibit stem-like characteristics. Cells may transition to a respiratory phenotype with PPAR-α/AMPK/PGC1α activation, and may be highly proliferative, but have low levels of NF-κB. Chemotherapy and radiation favors the transition to the glycolytic phenotype, while NADPH oxidase inhibitors can shift cells to the respiratory phenotype.

mt: Mitochondrial; ROS: Reactive oxygen species.