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Commentary

Antioxidants for prostate cancer chemoprevention: Challenges and opportunities

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ABSTRACT

Extensive research has led to the firm conclusion that antioxidants protect cells from damage caused by oxidative stress and its associated pathological conditions including inflammation. It has also been established that inflammation is a precursor in neoplastic transformation of the prostate. Although, a vast body of experimental and clinical evidence shows efficacy of antioxidants as preventive strategies for prostate cancer, there is a lack of consistent agreement in outcomes especially from recent large-scale randomized clinical trials. Despite these concerns, our understanding of the preventive mechanisms as well as clinical efficacy and safety data indicate that novel antioxidant therapeutics still hold great promise for prostate cancer chemoprevention. We propose that for effective use of antioxidants for prostate cancer prevention, further high impact translational research is needed with special attention on selecting those patients who will benefit from such intervention. Therefore, it is important to validate predictive biomarkers from successful trials and combine this with knowledge of preclinical characterization of antioxidants (and combinations) that will eventually facilitate the development of 'personalized prostate cancer chemoprevention'. In this review, we briefly describe some common and emerging antioxidants that have shown benefits in preclinical and clinical settings. Above all, we focus on summarizing the progress we made thus far in prostate cancer chemoprevention using antioxidants, the heightened interest and challenges in the future.

1. Introduction

Prostate cancer is the most commonly diagnosed cancer among men in the United States. Approximately 240,890 new cases and about 33,270 deaths are expected to occur in 2011 [1]. Until recently prostate cancer had been considered to be a major health problem in Western countries, however it is now reported as an emerging threat to the health of aging men in Asia [2]. In addition to aging, other factors such as genetic, epigenetic and environmental risk factors also increase the probability of developing prostate cancer. Recent studies have identified inherited variants (single nucleotide polymorphisms) as potential early markers for the risk of prostate cancer, particularly in disease aggressiveness [3–5]. Further, epidemiological and clinical studies suggest that several possible environmental factors such as carcinogens, hormone profile with age and inflammation are strongly associated with prostate carcinogenesis [6–8]. Diet is considered as a modifiable environmental factor that can influence prostate cancer incidence and clinical outcome [9,10].

Although little is known regarding etiology and factors that influence clinical outcome, 'elevated oxidative stress' in the cellular microenvironment is a common denominator in prostate cancer and aging. Oxidative stress causes damage to multiple cellular components such as DNA, proteins, and lipids, and is clearly implicated in prostate cancer. Cells have developed a robust antioxidant defense system to maintain cellular redox homeostasis and to protect from damage under conditions of oxidant attack. However, increased reactive species from inflammation or inhibition of defense mechanisms can easily overcome the capacity of the antioxidant system leading to perturbation of cellular redox balance. Knowledge of prostate cancer pathobiology gave rise to

Abbreviations: 8-oxo-dG, 8-oxo-2′-deoxyguanosine; ADT, androgen-deprivation therapy; AR, androgen receptor; ARE, antioxidant response element; ATBC, Alpha-Tocopherol, Beta-Carotene; BPH, benign prostatic hyperplasia; CARET, Carotene and Retinol Efficacy Trial; CRPC, castration-resistant prostate cancer; DRE, digital rectal exam; GPx, glutathione peroxidase; GSR, glutathione reductase; HGPIN, high-grade prostatic intraepithelial neoplasia; JPHC, Japan Public Health Center; NPC, Nutritional Prevention of Cancer; NQO, NADPH: quinone oxidoreductase; PCNA, proliferating cell nuclear antigen; PHS, Physicians' Health Study; PIA, proliferative inflammatory atrophy; PIN, prostatic intraepithelial neoplasia; PKC, protein kinase C; PSA, prostate-specific antigen; RNS, reactive nitrogen species; ROS, reactive oxygen species; SELECT, Selenium and Vitamin E Cancer Prevention Trial; SOD, superoxide dismutase; SU.VI.MAX, supplementation en vitamines et mineraux antioxydants; SWOG, Southwest Oncology Group; TRAMP, transgenic adenocarcinoma of the mouse prostate; TrxR, thioredoxin reductase.

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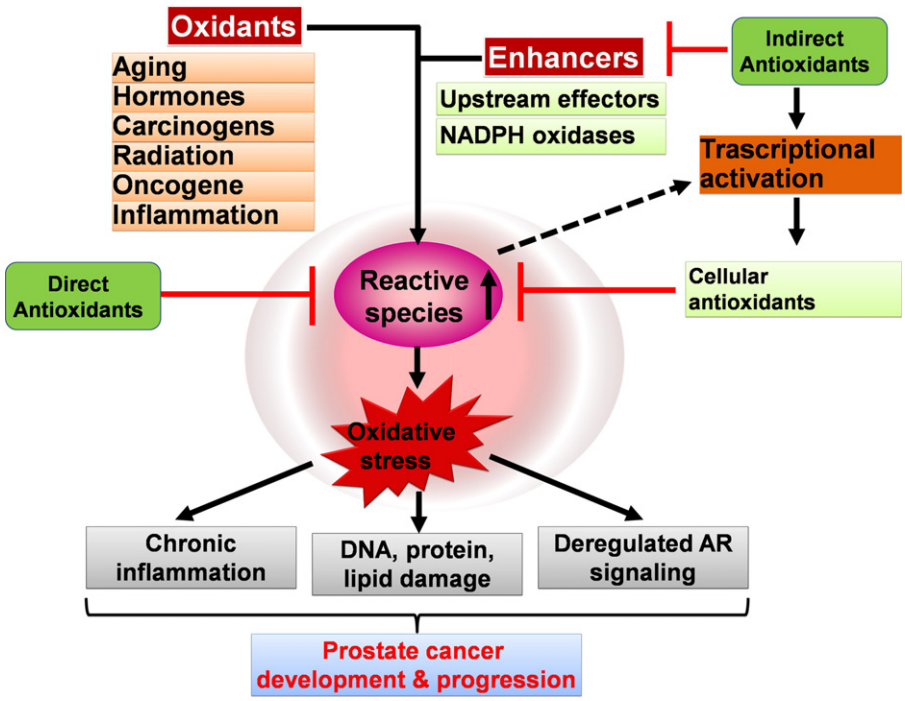

Fig. 1. Oxidative stress plays a central role in prostate cancer initiation and progression. Elevated oxidative stress is one of the hallmarks of prostate cancer. Although sources and nature of oxidants are heterogeneous, the most common sources include aging, genetic alterations, environmental insults, fluctuation in hormone and cellular metabolism. These changes in the prostatic microenvironment lead to recurrent and chronic inflammation, induction of DNA damage leading to genomic instability, induction of lipid and protein damage, and deregulation of cellular redox homeostasis via various important cellular pathways, which together lead to cancer progression. The modulation of increased oxidative stress by antioxidants may serve as novel biomarkers and chemopreventive targets for prostate cancer. (Solid arrow and broken arrow represent direct activation and redox balance in oxidative stress pathway, respectively. Red blunt arrow indicates inhibition of elevated oxidative stress by direct or indirect antioxidants.). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

the novel concept of antioxidants for its chemoprevention. Supplementation with antioxidants (direct antioxidants) or stimulation of cellular antioxidant systems (indirect antioxidants) can reduce oxidative injuries and thus prevent prostate cancer (Fig. 1). In this review, we briefly describe the role of antioxidants in prostate cancer prevention, the observed discrepancies between preclinical and clinical data regarding the use of antioxidants for prostate cancer chemoprevention and discuss challenges and opportunities that this system offers for its use as a prostate cancer chemoprevention strategy.

2. Antioxidants for prostate cancer chemoprevention

2.1. Significance of prostate cancer chemoprevention

Although surgery, radiation, chemo-, or hormonal-therapy, are used in the general management of prostate cancer; personalized treatment for prostate cancer still remains challenging. Many patients are either not cured by available therapeutic regimens and their cancer recurs, or cancers are diagnosed after it has metastasized. Long latency in the development of clinically significant prostate cancer combined with the indolent nature of its progression makes it a prime candidate for chemoprevention strategies. Therefore it is widely accepted that chemopreventive strategies not only reduce the risk of prostate cancer progression and mortality but can also reduce the need for invasive interventions.

The term chemoprevention was coined by Michael B. Sporn and is defined as the use of pharmacologic or natural agents to disrupt cancer [11]. The original term has since grown to include *primary* prevention in which the initiating DNA damage is blocked; *secondary* prevention whereby there is an arrest or reversal of the progression of initiated premalignant cells; and finally *tertiary* prevention in which metastatic progression of the primary tumor is blocked. Current chemopreventive strategies have empirically focused on two approaches: suppression of androgenic stimulation of the prostate and treatment with antioxidants to reduce damage to DNA [12]. The first strategy, androgen-deprivation therapy (ADT) is principally based on the pathological role of aberrant androgen receptor (AR) signaling which is clearly implicated in prostate carcinogenesis and prostate cancer progression [13]. The prostate and early-stage prostate cancers depend on androgens for growth and survival, therefore ADT causes tumor regression [14]. Recent chemoprevention attempts based on ADT, specifically using 5α-reductase inhibitors (finasteride and dutasteride), have shown significant benefits with an overall relative reduction of 23–25% in prostate cancer risk [15,16]. Benefit with the drugs was limited to low-grade prostate tumors with modified Gleason score of 6 or lower. In fact, in both trials, there was an absolute increase in the incidence of high-grade prostate cancers in the chemoprevention arm. It has been proposed that finasteride significantly enhances the ability of prostate specific antigen (PSA) to detect prostate cancer and high-grade prostate cancer [17]. However PSA has limited use and change in PSA is associated with other genitourinary conditions not related to prostate cancer. There are other concerns regarding the limitation and adverse effects of ADT including but not limited to the development of castration-resistant prostate cancer (CRPC), osteoporosis, neurodegenerative, and cardiovascular diseases [14,18–20]. Further finasteride is associated with hormonal and genitourinary side effects [21]. In addition there is no data available regarding the long-term effects of inhibiting 5α-reductase in otherwise healthy men. A combination of these reasons limits the use of finasteride as a primary chemoprevention agent for prostate cancer.

The second strategy using antioxidants holds abundant prospect for primary prevention of prostate cancer but still

remains controversial. A huge gap exists in the findings between laboratory-based preclinical observations and large-scale chemoprevention trials in the clinic. Despite the controversy, the public in general and the scientific and medical communities still have uninhibited interest in antioxidants. The optimistic hope is largely based on undeniable scientific facts and clinical safety of antioxidants. Since an estimated 30–50% of 30–50-year-old men show histological evidence of prostate cancer, an effective primary chemoprevention intervention prior to this age would be a good strategy since it could inhibit not only prostate carcinogenesis but also growth and progression of the disease [22].

2.2. Antioxidant defense system, aging and prostate cancer

Antioxidant defense system is an extensive network of molecules that eliminates the production of free radicals and reactive oxidants. Reactive oxygen species (ROS) are highly reactive molecules considered as central players in redox signaling. A variety of factors including aging, radiation, carcinogens, inflammation and/or activated oncogenes are known to increase production of cellular ROS, and ultimately increase the risk of prostate cancer initiation and progression. Normally, intracellular ROS levels are tightly controlled by inducible antioxidant defense system in a complex fashion. Antioxidant enzymes including superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GSR), NADPH: quinone oxidoreductase (NQO) and thioredoxin reductase (TrxR) constitute the backbone of the antioxidant defense system. SOD, CAT, and GPx are believed to play vital roles in prostate cancer development. SOD catalyzes the dismutation of superoxide into H_2O_2 and O_2, while CAT and GPx remove H_2O_2. The two major SOD enzymes in eukaryotic cells are manganese superoxide dismutase (MnSOD) found in mitochondria and copper zinc superoxide dismutase (Cu/ZnSOD) found primarily in the cytoplasm. Increased lipid peroxidation and a concomitant decrease in GPx and Cu/ZnSOD activities have been found in prostate cancer patients compared to non-cancerous controls [23]. On the other hand, SOD acts as a tumor suppressor gene and its overexpression has been found to inhibit growth of androgen-independent prostate cancer cells [24]. Peroxiredoxins (Prxn) are a family of multifunctional antioxidant thioredoxin-dependent peroxidases, which play an important role in protection against cellular oxidative stress. Work in animal models has shown that either low levels or lack of Prxn is associated with increased levels of ROS, elevated oxidative damage and increased susceptibility to tumor formation [25].

In addition, non-enzymatic antioxidants such as CoQ10, glutathione, non-protein thiols, vitamin C and vitamin E play important roles in cellular redox homeostasis. Non-protein thiols have a variety of functions in bio-reduction and detoxification processes. Vitamin E is recognized for protecting cells against lipid peroxidation while ascorbic acid interacts synergistically with lipoic acid to destroy many types of free radicals. Several reports have implicitly shown decreased level of antioxidants in prostate cancer than their normal cell counterparts [23,26,27]. Normally, ROS produced at low intracellular levels play vital roles in signal transduction and regulation of redox-sensitive transcription factors such as Nrf-2, NF-κB, HIF-1, AP-1, p53, PPARγ, and their antioxidant response elements (AREs) [28]. Sustained environmental stress such as aging or hormone exposure, leads to elevated ROS levels which as a result of chronic exposure causes significant DNA damage resulting in genetic alterations and neoplastic transformation [29,30]. Considerable evidence suggests that age-related increase in oxidative stress is associated with development and progression of prostate cancer [29,31]. Furthermore, older men have increased mean concentrations of the oxidative DNA damage marker, 8-hydroxyguanine, compared with younger men [32]. These biomarkers are statistically correlated with increased trend for prostate cancer progression with age [1].

2.3. Oxidative stress in the molecular pathogenesis of prostate cancer

Oxidative stress aids prostate cancer progression in several ways including triggering of cell survival pathways; stabilization of hypoxia-inducible factor; stimulation of growth signaling cascades that lead to more aggressive phenotypes; activation of inflammatory pathways; induction of DNA damage thereby accelerating the rate of cancer-causing mutations; development of hormone independence and castration resistance; and lowering cellular antioxidants [26,29,33–36]. The causal relationship between ROS and molecular pathogenesis of prostate cancer progression underscores the notion that use of antioxidants can reduce prostate cancer risk.

2.3.1. Oxidative stress-mediated signaling in prostate cancer progression

There has been substantial progress in identifying biochemical events associated with ROS production and cellular response to ROS in prostate cells [29]. We have a better understanding of how certain reactive species trigger various pathways (via regulating molecules such as upstream kinases/enhancers, DNA, transcription factors, and cell cycle regulators) and work together in a highly synchronized manner to facilitate prostate cancer progression. Despite these advancements, identification of predictive biomarkers and targets of chemoprevention for prostate cancer is still a work in progress.

Upstream kinases such as PI3K-Akt, MAPK, and protein kinase C (PKC) are frequently deregulated in prostate cancer, particularly in advanced disease. Components of the intracellular signaling network are vital in maintaining redox homeostasis in prostate cells in response to oxidative stress [37]. Abnormal or improper activation of these molecules has been associated with uncontrolled cell growth, and malignant transformation of prostate cells [38,39]. Kumar et al. showed that increased ROS generation along with activation of ERK, p38 and Akt in prostate cancer cells resulted in attenuation of growth regulatory proteins as compared to the normal cells and conditions of ROS inhibition [34]. The functional consequences of oxidative stress-induced Akt activation in prostate carcinogenesis has been observed in human cell lines, xenograft studies as well as in genetically engineered mouse models [25,34,40].

These upstream kinases activate redox sensitive transcription factors, such as Nrf-2, NF-κB, HIF-1, AP-1, p53, PPARγ, and their AREs that are activated in response to ROS accumulation and oxidative damage. For example, increased oxidative stress is directly associated with decrease in Nrf-2 and GST family genes in human prostate cancer [41]. Further, redox signaling can cross talk with other signaling pathways such as AR, Wnt, Hedgehog, and Notch resulting in uncontrolled transcription and proliferation of prostatic epithelial cells [42]. In the prostate, down-regulation of Nkx3.1 homeobox gene, Pten inactivation, Myc1 overexpression, FOXO activation, p53 mutation, and HIF-1 activation are few examples of molecular alterations in response to oxidative stress during carcinogenesis [29,43,44]. These lines of evidence strongly suggest that oxidative stress triggers multiple signaling molecules, transcriptional factors and their target genes. However, oxidative stress-mediated regulation is both complex and at times paradoxical.

2.3.2. Inflammation and oxidative stress in prostate cancer

Oxidative stress is thought to be intrinsic to inflammation. Inflammatory mediators either directly or indirectly promote oxidative injuries, which, in turn, contribute to inflammatory

pathology through the recruitment of inflammatory cells. Further, activated immune cells are responsible for elevated levels of ROS, reactive nitrogen species (RNS), cytokines, and growth factors, that lead to DNA damage, cell proliferation, and invasiveness [8,45]. In this milieu, the damaged epithelium generates proliferative inflammatory atrophy (PIA) lesions, which allows progression to prostatic intraepithelial neoplasia (PIN) or to prostate cancer [46]. In the last few years, various pathways that link chronic inflammation to oxidative stress have come to light. Therefore it is not surprising that anti-inflammatory drugs and antioxidants are used to attenuate prostatic carcinogenesis driven by chronic or recurrent prostate inflammation [47].

2.3.3. Cross-talk between oxidative stress and androgen receptor signaling

Recently, it has been suggested that blocking AR signaling may induce oxidative stress [36]. Prolonged exposure to reduced levels of androgen promotes prostate cancer progression and displays a molecular profile similar to androgen-independent prostate tumors in Nkx3.1; Pten mutant mice [48]. Castration induces oxidative stress in the rat prostate through upregulation of ROS-generating NADPH oxidases and suppression of cellular antioxidants [49]. Furthermore, gene expression analyses show increased oxidative stress marker genes in (androgen deficient) rat as well as human prostate cancer tissues [50,51]. On the other hand, there are several reports that show an association between increased oxidative stress and induced-or-altered androgen levels [45,52,53]. Tam et al. reported that administration of testosterone plus 17β-estradiol in noble rats induces oxidative stress in the prostate epithelium and promotes carcinogenesis [52]. Similarly, androgen exposure induces oxidative stress in AR-positive prostate cancer cells in vitro and in vivo [53]. Recent studies from our laboratory and others have shown that levels of NQO1 and several GST family genes are significantly suppressed in prostate tumors in preclinical models [25,41,45,54]. Specifically, we have found that testosterone and estradiol treatment decreased NQO1 levels, and increased proliferation in the prostate of noble rats that developed PIN [45]. However, little is known about the mechanism involved in androgen deprivation/exposure-induced oxidative stress. Taken together, it is noteworthy that therapeutic agents that reduce oxidative stress maybe useful as tertiary prevention agents to prevent the conversion of androgen-dependent prostate cancer into hormone resistant prostate cancer, as well as to reduce the adverse effects associated with the altered hormone milieu.

2.4. Promising antioxidant strategies for prostate cancer chemoprevention

Evidence discussed above presents a strong rationale for the use of antioxidants to combat oxidative stress and prostate cancer. Several antioxidants commonly cited in the literature can be broadly categorized into either direct or indirect antioxidants. Many of these antioxidants have been the focus of recent comprehensive review articles and are thus only briefly discussed here [10,42,55].

2.4.1. Direct antioxidants (antioxidants, diet and supplements that quench ROS)

Direct antioxidants participate either in the inactivation of free radicals or prevent chemical reactions initiated by these free radicals. Cellular antioxidant enzymes and dietary supplements that quench ROS and other reactive species generated via physiological, biochemical or cellular processes are promising targets as they are inversely linked to prostate cancer progression. A wide variety of natural or synthetic antioxidant compounds have been shown to interfere with prostate carcinogenesis at an early stage [42,55,56]. Epidemiological as well as clinical studies have highlighted the preventive role of diet-based antioxidants from tomatoes, soy products, carotenoids, cruciferous vegetables, green tea, etc. [10,57–59]. While most results are encouraging, some studies show insignificant effects. Differences in study design, heterogeneity of the chosen sample population, selection of antioxidants, sample size, dose administered, and/or concentrations achieved in the body may be possible reasons for the observed inconsistencies.

2.4.2. Indirect antioxidants (small molecule Nrf-2 activators, NADPH oxidase inhibitors, other upstream signaling such as inflammatory modifiers)

Indirect antioxidants block oxidative stress through many mechanisms without participating in direct quenching of reactive species or redox reactions. Small molecule Nrf-2 activators, NADPH oxidase inhibitors and anti-inflammatory agents that can balance cellular redox status are some examples. Nrf-2, a redox sensitive transcription factor, binds to ARE, and activates ARE regulated genes. These genes include many cellular antioxidants and play important roles in protecting cells from oxidative damage as well as pro-inflammatory insults [60]. A variety of thiol-reactive small molecules that stimulate ARE-driven transcription have been demonstrated to be protective in preclinical models and in clinical trials [61]. Nrf-2-activated transcriptional antioxidants are not directly consumed, have long half-lives, and are less likely to evoke strong pro-oxidant effects; therefore development and use of Nrf-2 activators as indirect antioxidants has been suggested as a promising approach to prevent prostate cancer [62]. NADPH systems present in prostate cancer cell lines are major sources of ROS generation and are required for cell proliferation and survival [34]. Increased NADPH oxidase expression-driven ROS generation in prostate cancer can lead to the generation of malignant phenotype by modulating various signaling cascades [29]. Furthermore, inflammation is intrinsic to oxidative stress and prostate cancer progression. Therefore, not only NADPH inhibitors but also anti-inflammatory drugs have been proposed for chemoprevention of prostate cancer [47].

3. Antioxidants and prostate cancer: current status

Several preclinical and clinical studies conducted in the last two decades support the proposition that antioxidant treatment results in decreased prostate cancer risk. In contrast, more recent large-scale randomized clinical trials resulted in contradictory outcomes raising doubts regarding the clinical use of antioxidants [63,64]. In Tables 1 and 2 we list some recent preclinical and clinical findings, respectively and discuss contentious issues related to the use of antioxidants for prostate cancer chemoprevention in the following sections.

3.1. Preclinical studies

Antioxidants have shown promising results in various preclinical models. Lycopene, selenium, vitamin E, genistein, green tea polyphenols and other novel synthetic compounds have been tested in various rodent models. Genetically modified mouse models that recapitulate human prostate cancer progression serve to help in the selection of appropriate agents for large-scale clinical trials [65]. In particular prostate cancer development and progression in the transgenic adenocarcinoma of the mouse prostate (TRAMP), Pten knockout and the Nkx3.1; Pten compound mutant mouse model as well as the hormone-induced rat model have been shown to have an oxidative damage component and thus validated as suitable models to study the role of antioxidants [25,66].

Table 1
Summary of antioxidant use in preclinical models of prostate carcinogenesis.

Antioxidants	Animal/model	Dose	Age	Mechanism of action	Major outcome	References
Indole-3-carbinol (I3C)	TRAMP mouse	1% I3C diet for 12–16 weeks	8 weeks	Induced Nrf-2, NQO-1 as well as cell cycle and apoptosis related biomarkers	I3C prevented prostate cancer in TRAMP mice via anti-oxidative activities	Wu et al. [74]
Soy and green tea	Noble rat	2% green tea; 200 g soy for various times	10 weeks	Suppressed NF-κB p50 via induction of IκBα and also decreased TNF-α, IL-6 and IL-1β	Combination of soy and green tea decreased PIN, and inflammatory markers	Hsu et al. [72]
Lycopene and β-carotene	Nude mouse/PC-3 tumor xenograft	lycopene (16 mg/kg) and β-carotene (16 mg/kg) twice a week for 7 weeks	6–8 weeks	Reduction of PCNA and interference with IGF-1 signaling	Lycopene and β-carotene strongly inhibited tumor growth	Yang et al. [70]
Whole tomatoes	TRAMP mouse	10% tomato powder for various times	5 weeks	Increase in serum antioxidant activity	Increased overall survival ($P < 0.01$), delayed progression from PIN to cancer	Pannellini et al. [67]
Tomato paste (TP) or lycopene beadlet (LB)	TRAMP mouse	100 mg lycopene per kg diet for 17 weeks	3 weeks	Oxidative DNA damage was significantly reduced in livers of mice fed LB and TP diets relative to the control group	Significantly decreased prostate cancer incidence in the LB group relative to the control group (60% vs. 95%, respectively, $P = 0.0197$)	Konijeti et al. [68]
γ-T-enriched mixed tocopherol	TRAMP mouse	0.1% mixed tocopherols	8 weeks	Upregulated expression of most detoxifying and antioxidant enzymes	Significantly suppressed the incidence of palpable tumor and PIN development	Barve et al. [54]
Curcumin and resveratrol	Prostate-specific PTEN knockout mice	50 mg/kg lipo-curcumin and lipo-resveratrol for 7 weeks	4 weeks	p-Akt, cyclin D1, mammalian target of rapamycin and AR were downregulated	Significantly decreased prostatic adenocarcinoma in vivo ($P < 0.001$)	Narayanan et al. [73]
RRR-α-TOCOPHERYLOXYBUTYL sulfonic acid	Nude mouse/tumor xenograft and TRAMP	6 weeks and 20 weeks oral intake nude and TRAMP mice, respectively	6–8 weeks	Induced apoptosis, repressed AR protein expression	Reduced tumor burden in xenografted prostate tumors in nude mice and inhibited tumor progression in TRAMP mice	Ni et al. [76]
γ-Tocopherol	TRAP rats	50 or 100 mg/kg α/γ tocopherol for 7–10 weeks	3–5 weeks	Activation of Caspase 3 and 7 signaling	Significantly suppressed sequential progression from PIN to adenocarcinoma in a dose-dependent manner	Takahashi et al. [77]
Silybin-phytosome	TRAMP mouse	0.5% and 1%, w/w, silybin-phytosome diets for 11 weeks	20 weeks	Strongly suppressed tumor microvessel density (up to 60%, $P < 0.001$), VEGF, and VEGFR-2 expression	Inhibited growth of prostate tumors (up to 60%, $P < 0.001$) and suppressed tumor progression from PIN to cancer	Singh et al. [101]
Epigallocatechin-3-gallate (EGCG)	TRAMP mouse	0.06% EGCG in tap water for 23 weeks	5 weeks	Attenuated AR, IGF-1, inflammation biomarkers, and decreased the MAPK signaling	EGCG inhibited early but not late stage prostate cancer	Harper et al. [71]
Vitamin E succinate	SCID mouse/tumor xenograft	50–100 mg/kg/d for 3 weeks	5–7 weeks	Induced apoptosis via Caspase-4 activation	Significantly suppressed tumor growth and lung metastases	Malafa et al. [75]
Vitamin D	Nkx3.1;Pten mutant mouse	46 ng/kg/d of 1,25 D_3 for 1–4 months	16–28 weeks	Upregulation of vitamin D receptor expression and no effect on AR signaling	Vitamin D significantly reduced PIN formation in Nkx3.1; Pten mice	Banach-Petrosky et al. [102]

Abbreviations: AR, androgen receptor; IGF, insulin-like growth factor; IL, interleukin; NQO, NADPH: quinone oxidoreductase; PCNA, proliferating cell nuclear antigen; PIN, prostatic intraepithelial neoplasia; Pten, phosphatase and tensin homolog; SCID, severe combined immunodeficiency; TNF, tumor necrosis factor; TRAMP, transgenic adenocarcinoma of the mouse prostate; VEGF, vascular endothelial growth factor.

Table 2
Summary of effects of antioxidants in clinical setting.

Antioxidants	Study design	Age, median (years)	Criteria	Outcome measure	Results	Study group/ references
Selenium	A double-blind, randomized, placebo-controlled trial	40+	Patients with HGPIN and no cancer	Progression of HGPIN to prostate cancer	Overall incidence in three-year was 36.6% (placebo) vs. 35.6% (selenium; $P=0.73$, adjusted)	SWOG [85]
Vitamin E, selenium, and soy protein	A randomized Phase III double-blind study	63	Confirmed HGPIN	Development of invasive prostate cancer	Incidence of Invasive prostate cancer: 26.3% vs. 26.5%	Fleshner et al. [86]
Lycopene	A randomized, double-blind, placebo-controlled	52–83	BPH and prostate cancer	Antioxidant biomarker: 8-oxo-dG	35% lower in the lycopene treatment group (125 ± 83 8-oxo-dG/106 dG) than in the placebo group (193 ± 341 8-oxo-dG/106 dG), but this difference was not significant ($P=0.22$)	van Breemen [91]
Selenium glycinate	Placebo-controlled	40–60	Healthy middle-aged men	GPx and PSA	Supplementation lowered PSA values in 28 of 30 subjects.	Zhang et al. [90]
Selenium and vitamin E	A randomized, placebo-controlled trial	50+	Relatively healthy men	Incidence of prostate cancer	Non-significant increased risk of prostate cancer in vitamin E group ($P=0.06$) but not in the selenium + vitamin E group.	SELECT [63]
Vitamin E and C	A randomized, double-blind, placebo-controlled factorial trial	50+	50+ years male physicians, including 1307 men with a history of prior cancer	Incidence of prostate cancer	Vitamin E had no effect on the incidence of prostate cancer (vitamin E and placebo, 9.1 and 9.5 events per 1000 person-years)	PHS II [64]
β-Carotene and retinylpalmitate	A randomized, double-blinded, placebo-controlled	58	Heavy smokers	Incidence of prostate cancer	High dose of β-carotene (30 mg/d) and retinylpalmitate (25,000 IU/d) plus at least one other dietary supplement may increase risk of aggressive prostate cancer.	CARET [82]
Genistein	A nested case-control study	40–69	Healthy Japanese men	Incidence of prostate cancer	Plasma genistein level tended to be inversely associated with the risk of total prostate cancer	JPHC [103]
Green tea	A prospective study	40–69	Healthy Japanese men	Incidence of prostate cancer	Green tea consumption was associated with a dose-dependent decrease in the risk of advanced prostate cancer; multivariate relative risk was 0.52 (95% CI: 0.28, 0.96)	JPHC [104]
Green Tea Catechins (GTCs)	A randomized, proof-of-principle trial	45–75	HGPIN	Incidence of prostate cancer	Incidence, approximately 3% in GTC-treated men and 30%in placebo-treated men	Bettuzzi et al. [89]
Lycopene	Phase II study	77	Patients with androgen-independent prostate cancer	Efficacy of a lycopene-rich tomato product in androgen-independent prostate cancer	One patient manifested a tumor response with a 50% or greater decline in serum prostate-specific antigen level, yielding a response rate of 2%	Jatoi et al. [88]
Lycopene	A prospective study	72	Metastatic HRPC	Prostate cancer progression	5% had complete response; 30% partial response stable disease in 50% and progression in 15% patients.	Ansari and Gupta [87]
Vitamin C, Vitamin E, β-Carotene, Selenium and Zinc	A randomized, placebo-controlled trial	45–60	Healthy middle-aged men	Incidence of prostate cancer	A moderate non-significant reduction in prostate cancer rate associated with the supplementation	SU.VI.MAX [83]
α-Tocopherol (vitamin E) and β-carotene	Post-intervention follow up	50–69	Male smokers	Incidence and mortality of prostate cancer	Beneficial and adverse effects of supplemental alpha-tocopherol and β-carotene disappeared during post-intervention follow-up. Smokers should avoid beta-carotene supplementation.	ATBC [81]

Table 2 (*Continued*)

Antioxidants	Study design	Age, median (years)	Criteria	Outcome measure	Results	Study group/ references
α-Tocopherol (vitamin E) and β-carotene	A randomized, placebo-controlled trial	50–69	Male smokers	Incidence and mortality of prostate cancer	A 32% decrease in the incidence of prostate cancer was observed among the subjects receiving alpha-tocopherol (n = 14,564) compared with those not receiving it (n = 14,569)	ATBC [79]
Selenium	Double-blind, placebo-controlled	64	Men with a history of basal cell or squamous cell carcinoma	Incidence of prostate cancer	A significant (63%) reduction in the secondary endpoint of prostate cancer incidence	NPC [78]

Abbreviations: 8-oxo-dG, 8-oxo-2′-deoxyguanosine; ATBC, Alpha-Tocopherol, Beta-Carotene; BPH, benign prostatic hyperplasia; CARET, Carotene and Retinol Efficacy Trial; CRPC, castration-resistant prostate cancer; GPx, glutathione peroxidase; HGPIN, high-grade prostatic intraepithelial neoplasia; JPHC, Japan Public Health Center; NPC, Nutritional Prevention of Cancer; PHS, Physicians' Health Study; PSA, prostate-specific antigen; SELECT, Selenium and Vitamin E Cancer Prevention Trial; SU.VI.MAX, supplementation en vitamines et minerauxantioxydants; SWOG, Southwest Oncology Group.

Several preclinical studies have demonstrated the efficacy of antioxidants as potential primary, secondary or tertiary chemopreventive agents as evidenced by decreased incidence, reduced tumor growth and metastasis, slower progression of PIN to adenocarcinoma in animal models (please see Table 1 and references therein). In the TRAMP model, tomato and lycopene supplementation significantly decreased incidence and delayed progression of prostate cancer [67,68]. Possible mechanisms include increase in serum antioxidant activity, inhibition of oxidative DNA damage, which is reflected in reduced serum inflammation/angiogenesis biomarkers such as VEGF, IL-6, and IL-11. These markers are often elevated in patients with benign prostatic hyperplasia and prostate cancer [69]. Lycopene has been shown to have antiproliferative effects by inhibiting proliferating cell nuclear antigen (PCNA) in tumor tissues and by increasing the levels of IGF-1 binding proteins in plasma [70]. A similar observation was noted in mice treated with β-carotene [70].

Natural dietary supplements, including green tea, soy, curcumin and resveratrol, have also emerged as promising antioxidants for prostate cancer chemoprevention. These compounds attenuate inflammation, decrease PIN lesions and subsequent adenocarcinoma via targeting NF-κB, AR, IGF-1 or mTOR signaling and inflammatory biomarkers [71–73]. Harper et al. reported that EGCG inhibited early but not late stage prostate cancer in TRAMP mice, suggesting that early intervention maybe critical for prostate cancer chemoprevention [71]. Recently, indole-3-carbinol (I3C), a dietary antioxidant found in crucifers, demonstrated preventive effects against prostate cancer. In this study, I3C-fed TRAMP mice were protected against prostate carcinogenesis via up-regulation of a novel Nrf-2-mediated anti-oxidative stress pathway [74]. Vitamin E has long been believed to reduce prostate cancer risk. Recent preclinical studies have also validated the notion that vitamin E or its active derivatives may reduce the risk of prostate cancer [54,75–77]. These lines of evidence show that elevated expression of antioxidant enzymes contribute to delayed progression of prostate cancer, in addition to their anti-proliferative effects via targeting other multiple pathways [54,76,77].

In summary numerous preclinical studies including many others not discussed here have demonstrated that antioxidants are potential candidates for prostate cancer prevention. However the translation of these preclinical data has been extremely challenging for a variety of reasons discussed below.

3.2. Clinical studies

We have summarized clinical studies published between 1998 and 2011 that include Phase I–III and post-intervention follow-up trials (Table 2 and references therein). Vitamins specifically vitamin E, C and A, selenium, lycopene, green tea, genistein, soy protein have been evaluated either alone or in combination in various clinical trials. Two randomized clinical trials firmly established strong inverse association between antioxidant supplementation and prostate cancer incidence. The Nutritional Prevention of Cancer (NPC) and the Alpha-Tocopherol, Beta-Carotene (ATBC) trial showed 63% and 32% incidence reduction with the use of selenized yeast and α-tocopherol (vitamin E), respectively [78,79]. In the NPC trial, administration of selenium (200 μg/d) to non-melanoma skin cancer patients was associated with significantly decreased prostate cancer incidence as evidenced by secondary end point analysis of total cancer including prostate, lung and colon. Complete analysis of the NPC trial showed that selenium significantly reduced prostate cancer incidence, although the effect was restricted to those with lower baseline PSA and plasma selenium concentrations [78,80]. Since the ATBC study was primarily designed to study the effect of antioxidants in lung cancer, men in the study (n = 290,406) aged between 50 and 69

years were all smokers. The cumulative incidence of prostate cancer in men receiving α-tocopherol decreased progressively from the second year relative to those who did not receive α-tocopherol and resulted in a 32% difference (95% CI = −47 to −12%) [79]. Post-intervention follow-up study of ATBC ×benefit on prostate cancer incidence with RR 0.88 (95% CI, 0.76–1.03) for participants receiving α-tocopherol compared with non-recipients [81]. However, a 23% increase in prostate cancer incidence and 15% increase in mortality were reported among subjects receiving β-carotene (n = 14, 560) in the same study. Recently, while assessing prostate cancer among smokers as a secondary outcome, the CARET study also suggested that additional supplements of carotene and/or excessive vitamins may increase the risk for aggressive prostate cancer [82].

The SU.VI.MAX (n = 5141) was a placebo-controlled trial to examine the role of vitamin and mineral supplementation on prostate cancer prevention. Men were given placebo or nutritional doses of vitamin C, vitamin E, β-carotene, selenium, and zinc daily and monitored for 8 years. Plasma samples (from all the enrolled men and follow up at the end from 3616 men) were examined for biomarkers of prostate cancer risk such as PSA and IGF. A marked statistically significant reduction in prostate cancer risk was seen in men receiving supplements with normal PSA (HR 0.52; 95% CI, 0.29–0.92); however, in men with elevated PSA at baseline, supplementation was associated with an increased incidence of prostate cancer of border line statistical significance [83].

The Selenium and Vitamin E Cancer Prevention Trial (SELECT), one of the largest (n = 35,533) and most widely discussed randomized clinical trials, tested selenium (200 μg/d) and vitamin E (400 IU/d), alone or in combination, for prostate cancer prevention. Apparently healthy men aged 50 years or older with a serum PSA level of 4 ng/ml or less, a digital rectal examination (DRE) not suspicious for cancer were used as eligibility criteria. The primary endpoint was biopsy-confirmed prostate cancer. Although the trial was planned to follow-up to 12 years, it was prematurely terminated at 7 years. The hazard ratios for prostate cancer were 1.13 (99% CI: 0.95–1.35) for vitamin E-alone, 1.04 (99% CI: 0.87–1.24) for selenium-alone and 1.05 (99% CI: 0.88–1.25) for selenium and vitamin E combination, compared with placebo. No statistically significant differences in the rates of prostate cancer were observed among the four groups receiving placebo or antioxidants (selenium, vitamin E, selenium + vitamin E). Further, a statistically non-significant increased risk of prostate cancer was seen in the vitamin E group (P = 0.06) [63]. Longer follow-up of SELECT showed significant increase in risk of prostate cancer. Compared with the placebo in which 529 men developed prostate cancer, 620 men in the vitamin E group developed prostate cancer (HR 1.17; 99% CI, 1.004–1.36, P = 0.008) [84]. SELECT clearly established that 200 μg/d selenium in the form of L-selenomethionine, when administered to apparently healthy men 50 years or older, does not prevent prostate cancer and 400 IU/d of vitamin E increases the risk of prostate cancer in apparently healthy men [84].

Another large scale randomized trial, PHS II (n = 14,641) also concluded that neither vitamin E nor C supplementation can reduce the risk of prostate cancer after a mean of 8 years of supplementation and follow up [64]. In a more recent study, Marshall et al. used selenomethionine (200 μg/d) in men with HGPIN and found no significant difference in overall incidence [85]. Fleshner et al. found a combination of vitamin E with selenium and soy prevented the progression from HGPIN to prostate cancer [86].

Small scale placebo-controlled trials (Phase I–II) of antioxidant supplementation though encouraging have generated contentious data [87–90]. For example 2 trials to examine the effect of lycopene in patients with hormone refractory prostate cancer published opposing findings. A study by Ansari et al. found lycopene therapy was effective and safe, while Jatoi et al. concluded that lycopene was ineffective [87,88]. A closer look suggests that the dose of lycopene (10 mg/d in the Ansari study vs. 30 mg/d in the Jatoi study); the composition of the antioxidant (lycopene as Lycored softules in the Ansari study vs. spaghetti sauce and tomato juice in the Jatoi study) and genetic variability of cytochrome P450 enzymes in the study population could have contributed to these differences. Bettuzzi et al. reported that Green Tea Catechins (GTCs; 600 mg/d) significantly decreased prostate cancer incidence (30% in placebo vs. 3% in GTCs group), which suggests that GTCs may be effective prostate cancer chemopreventive [89]. Work of Zhang et al. suggests that antioxidant selenium may be helpful in lowering oxidative stress markers in men who have low-prostate cancer risk [90]. van Breemen et al. recently observed that oral administration of lycopene caused moderate reduction in 8-oxo-dG levels in men with BPH but not in men with prostate cancer [91]. Overall, results from some clinical studies look promising, but no conclusive data have been generated to allow recommendation as a preventive agent.

4. Challenges for prostate cancer chemoprevention with antioxidants

4.1. Inconsistencies between preclinical and clinical outcomes

There has been a general failure of clinical translation despite the optimism regarding antioxidants in the preclinical setting. We propose that differences in intervention age, enrollment criteria, genetic variation, and the choice and dose of antioxidant are important contributing factors for the observed discrepancy. A majority of prostate cancer prevention studies with preclinical models used rodents in the primary prevention setting. The average age of intervention was 6–8 weeks, by which time animals are considered to have attained sexual maturity and are unlikely to have sustained hormone-induced oxidative stress. Age of men in most of the prostate cancer prevention trials on the other hand was 50 years or more. By this age hormone-induced disruption of redox balance is expected to cause oxidative stress-related changes. Therefore using antioxidants to prevent oxidative stress-mediated cellular damage that results in pre-neoplastic changes may not be useful at this stage. Commonly used enrollment criteria such as PSA and apparently normal DRE are not expected to reflect oxidative stress and the associated changes in signaling cascades. Therefore there is a great need to develop markers of oxidative damage that can be used to enroll men in primary chemoprevention trials that use antioxidants. Genetic variation and function of cytochrome P450 enzymes in a heterogeneous population of men could also account for some of the observed differences given that the preclinical models were inbred mouse strains. Most clinical trials used a single large dose of antioxidant over a long period of time. Antioxidants are micronutrients and are required in small quantities therefore gross overdose used in clinical trials such as SELECT is in sharp contrast to the dose escalation studies commonly used in preclinical studies. Last but not the least important is the nature of the form of antioxidant used. At this time we do not have a good understanding of the pharmacology of the various forms of potentially efficacious antioxidants therefore the choice of one form of antioxidant over another cannot be scientifically justified.

4.2. Do antioxidants increase cancer risk?

Antioxidants are micronutrients that are required in small quantities for physiological functions. However, antioxidants as dietary supplements are widely promoted in advertisements with claims of effectiveness, and are easily available without a prescription in the United States. Using large doses of antioxidants

can also lead to perturbations in redox balance and produce deleterious molecular changes. Lycopene was well tolerated in clinical trials, yet when used in high doses there were side effects [87,88]. Several studies have indicated that large doses of antioxidants can increase the risk of cancer and other diseases [63,81,84,92]. For example, ATBC revealed significantly increased lung cancer rates in smokers taking β-carotene, and SELECT demonstrated a trend for increased prostate cancer in men taking more than a 13-fold higher dose of the recommended daily dose of vitamin E [84]. Further, the SELECT study also showed a small trend for increased type 2 diabetes in men who were given 200 μg/d selenium [63]. Small quantity of selenium (recommended dosage of 55 μg/d for adults) is required for proper antioxidant functions of seleno-proteins, which in the United States (unlike Russia and China) is provided through dietary intake. However, clinical trials including SELECT used more than 3-fold higher dose of selenium. Although the SELECT trial showed that antioxidant overdose can increase prostate cancer risk, the molecular mechanisms involved are unknown. It is likely to be complex and context-dependent. For example, ROS detoxification by antioxidant supplementation has a positive effect on tumorigenesis in some genetic settings [93]. Therefore, the risk of cancer may critically depend on the delicate balance between oxidant and antioxidant pathways. At this time it is safe to conclude that there is no evidence that suggests that consuming recommended doses of antioxidants increases cancer risk. The question whether antioxidants increase cancer risk remains to be answered in the future through further mechanistic studies and targeted trials.

5. Antioxidants for prostate cancer chemoprevention: opportunities

5.1. Lessons learned

Antioxidants have great potential to be developed as chemopreventive agents for prostate cancer yet in their current form and dosage they have very limited effect. Following the recent failure of vitamin E and selenium in the SELECT trial antioxidants including vitamins have received more than their fair share of criticism for their potential use as prostate cancer chemopreventive agents. This combined with the inaccuracies noted in the literature lead us to believe that currently we do not have the knowledge basis to design large-scale prostate cancer chemoprevention trials. We argue that among other factors; time of intervention, dose and type of antioxidant chosen hold the key to the success or failure of antioxidants for prostate cancer chemoprevention. Smaller clinical studies are necessary to establish the optimal dose and choice of antioxidant that will be most beneficial and identify biomarkers of efficacy along the lines of randomized, double-blind, placebo-controlled trials of Hurst et al. [94]. Antioxidants are micronutrients therefore large doses of antioxidants can tip the delicate redox balance in cells and produce deleterious effects including increased cancer risk. Targeting the right sub-population that would benefit most from a given antioxidant is an important lesson learned. For example selenium is an important co-factor for antioxidant seleno-proteins, and the lack of selenium deficiency in the US makes selenium a poor choice as a chemopreventive agent in the US population. A large randomized trial carried out in Linxian County in north-central China (with a high percentage of subclinical deficiencies in several nutrients and a high incidence of epithelial cancers) showed that selenium, vitamin E and β-carotene intake reduced stomach cancer mortality and incidence [95,96]. Early intervention (age and stage of prostate cancer development) is also critical. As discussed above studies by van Breemen et al. found differences in effectiveness of lycopene in men without prostate cancer vs. men with prostate cancer. However, there are no supportive data at this time to suggest the time in life when the prostate epithelium is most at risk for damage from oxidative stress, which makes it difficult to judge the best time to intervene effectively with antioxidants. Clearly, additional preclinical studies are needed to understand mechanistic, genetic and other factors, which would be helpful in designing large-scale clinical trials in the future.

5.2. Future prospects

Evidence based on data generated from epidemiological, experimental and clinical studies show that oxidative stress is inherent to prostate carcinogenesis. The contribution of oxidants and antioxidants to prostate carcinogenesis should be extensively evaluated through all encompassing analyses of the pharmacology (including distribution of agents in target tissue), development of predictive biomarkers (redox biology in prostate carcinogenesis) for targeted chemoprevention alone or in combination (combination chemoprevention).

5.2.1. Combination of antioxidants and primary intervention

Various classes of reactive species are generated during cellular metabolism therefore thorough understanding of the types of reactive species that are commonly found in the prostate epithelium would allow use of specific quenchers. Alternatively rationally developed antioxidant combinations may be more useful in the chemoprevention of prostate cancer. Unpublished work from our laboratory has shown that an antioxidant combination is effective in inhibiting inflammatory foci in a hormone-induced rat model of prostate cancer. More antioxidant combination studies in preclinical models are needed to firmly establish their benefits as well as to identify biomarkers of response. Results from a preclinical study of vitamin E and selenium showed the ineffectiveness of this combination as chemopreventive agents for prostate cancer [97]. In this study antioxidant intervention was post carcinogen treatment suggesting that the antioxidant was used in a secondary prevention rather than primary prevention setting. It is our belief that oxidative stress may be a critical player in the initiation stages of carcinogenesis and therefore antioxidants would be most useful in the primary prevention setting.

5.2.2. Blocking ROS production targeting upstream signaling

The development of specific small molecules to activate cellular defense system and provide continuous supply of antioxidants maybe a successful strategy to prevent prostate cancer development. Current antioxidant strategies still simply propose to quench increased ROS or block subsequent events. However, given that conventional antioxidants exhibit poor reactivity with many endogenous ROS [98], we speculate that antioxidants may be unable to achieve clinical efficacy. In addition, some antioxidants such as α-tocopherol, vitamin E may behave as pro-oxidants in some instances [99]. Therefore, prevention of ROS formation by blocking the upstream signaling/enzymes or combination with other agent (that synergistically act on preventing ROS production) could be a more effective approach for combating oxidative stress than scavenging these highly reactive molecules post-formation. Since ROS and oxidative stress have long been considered the underlying causes of various chronic diseases including prostate cancer, modulating ROS generation to maintain the redox state in cells using NADPH oxidase inhibitors may prove to be an effective target for chemoprevention [29,98,100].

6. Conclusions

Prostate cancer is highly amenable to chemoprevention due to its indolent nature. Antioxidants have great prospect in prostate

cancer prevention due to their efficacy, safety, low cost and bioavailability. However to date no conclusive data have been produced to recommend any antioxidants as chemopreventive agents. Preclinical studies show that antioxidants slow or prevent prostate cancer progression in the primary prevention setting yet clinical proof of efficacy remains elusive. Dose and choice of antioxidants as well as criteria and age of intervention may hold the key to lack of clinical validity. A major problem is the lack of biomarkers of oxidative stress during prostate cancer progression. Identifying such biomarkers will allow stratification of individuals by risk, to improve early detection, diagnosis and monitoring of the disease and therefore provide more accurate prognosis of antioxidant treatment. Combination of agents (cocktails of antioxidants or combining antioxidant with other agents) should be carefully considered because mechanisms of action may be additive or synergistic. Extensive research is required in the pharmacology and molecular mechanism of antioxidant action before any large-scale prevention trials are undertaken.

Conflict of interest

The authors have no conflict of interest to report.

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