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FORUM EDITORIAL

Antioxidants Meet Molecular Targets for Cancer Prevention and Therapeutics

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Abstract

A fine balance between oxidants and antioxidants is required for the normal functioning of living systems. A deregulation of this balance has been implicated in many adverse effects and diseases, including cancer. Extensive research has been done in the area of cancer prevention and therapeutics by a wide range of antioxidants, especially naturally occurring and diet-based agents. However, additional efforts are still needed toward clinical development of the most promising antioxidant agents. For this purpose, it is important to focus our efforts toward (i) defining/validating new targets; (ii) identifying novel agents followed by assessments of their efficacy, safety/toxicity, metabolism, and bioavailability in appropriate model systems; and (iii) conducting clinical trials in an appropriate population. Although research with specific antioxidants is important, an emerging critical issue that is up for a debate is whether the "whole foods" concept is better for cancer prevention than a single agent. Recent work has suggested that the dietary phytochemicals can enhance the bioavailability of different nutrients and can target multiple molecular pathways to yield a better response. Another critical issue that is often ignored during target-based agent development is a lack of focus on the appropriate population for a specific target. It is possible that a specific target may not be appropriate for certain people. Further, we need to design quick "phase 0" clinical trials to eliminate the agents with little clinical potential. Thus, multidisciplinary efforts of researchers from diverse scientific disciplines are needed in order to take the most promising antioxidant agents from *the bench to the bedside*. *Antioxid. Redox Signal.* 19, 85–88.

A DELICATE BALANCE between oxidants and antioxidants is required for the normal functioning of living systems. A deregulation of this balance has been implicated in numerous diseases, including cancer, particularly in tumor initiation and tumor promotion. An understanding of redox signaling and its interactions with other biological processes during cancer development is important in identifying new targets toward developing novel means for cancer prevention and cancer therapeutics. At the same time, there is a crucial need to exploit the known redox-regulated pathways and targets to develop new agents for cancer management. The fundamental theme of this Forum revolves around (i) redox signaling-based molecular targets for cancer prevention and therapeutics, and (ii) novel antioxidants for cancer management. Three articles in this Forum (Bode and Dong, Kim *et al.*, Wilking *et al.*) have discussed several important well-known as well as some emerging potential targets based on redox signaling and interactions (1,3,8). The other five articles (Khan *et al.*, Li *et al.*, Park and Pezzuto, Steinbrenner *et al.*, Yum *et al.*) have discussed known and emerging antioxidants that may be useful in the prevention as well as treatment of cancer (2,4,5,7,9).

In the first original research communications article on pages 89–101 of this Forum (3), Kim and colleagues investigated a new interacting protein partner of NFE2-related factor 2 (Nrf2), which is a transcription factor that controls and integrates stress signals in mammalian cells and responds by modulating a variety of transcriptional programs. Employing the one-strep tag pull-down and LTQ Orbitrap liquid chromatography-tandem mass spectrometry, Kim *et al.* identified IQ motif containing GTPase activating protein 1 (IQGAP1) as a new Nrf2 interacting partner. They also assessed the functional role of IQGAP1 in Nrf2 by RNA *interference* mediated knock-down and demonstrated that IQGAP1 siRNA attenuated the

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expression of endogenous Nrf2, heme oxygenase-1 (HO-1) proteins, and Nrf2-target genes glutathione S-transferase-pi, glutamate cysteine ligase catalytic subunit, and nicotinamide adenine dinucleotide phosphate: quinone oxidoreductase 1. In addition, the stability of Nrf2 was found to be decreased in IQGAP1-deficient mouse embryonic fibroblasts. The authors provided evidence that IQGAP1 possibly plays an important role in Nrf2 stability. Further, the treatment of cells with calcium resulted in increases in Nrf2-mediated antioxidant responsive element-transcription activity and expression of the endogenous HO-1. Thus, this study identified a novel interaction and role of IQGAP1 in the stability and transactivation of Nrf2. This finding is important, because a number of studies have suggested that Nrf2 activation could be an effective therapeutic strategy for the chemoprevention of cancer. However, based on some recent studies in the past 2 years, currently, there is a controversy regarding the role of Nrf2 in cancer. Genetic analyses of human tumors have suggested that Nrf2 can have an oncogenic function and may cause resistance to chemotherapy. Therefore, it is somewhat controversial whether the activation, or the inhibition, of Nrf2 is needed for cancer management. Although abundant evidence is available which suggests that Nrf2 activation could be a plausible strategy for cancer chemoprevention, further studies are needed to identify a genetically appropriate population for the use of Nrf2 activators. In addition, further research is needed to ascertain the possibility of using Nrf2 inhibitors for enhancing the effectiveness of chemotherapy in patients with metastatic cancer.

In another original research communications article on pages 102–114 (9), Yum *et al.* have determined the effects of oligonol, a low-molecular-weight polyphenol derived from lychee fruit, on experimentally induced colitis and colonic adenoma formation in mice. This study provided evidence that the observed effects of oligonol may be mediated *via* modulations in important cancer therapeutic targets nuclear transcription factor kappa B (NF-κB), signal transducer and activator of transcription-3, cyclooxygenase-2, inducible nitric oxide synthase, and cyclin D1. Oligonol was also shown to significantly reduce oxidative stress in colon tissues. Overall, this study is important, because ulcerative colitis, in some cases, can be a risk factor for colon cancer. However, it is not clear whether the observed effects of oligonol are specific to this particular polyphenol or a common feature of other flavonoids, especially as the affected molecular targets are common to several other flavonoids.

In a review on pages 163–180 of this Forum (1), Bode and Dong have discussed signal transduction and molecular targets of selected flavonoids. For several years, the authors of this review have focused their efforts on identifying the specific molecular targets of cancer chemopreventive flavonoid compounds with the help of supercomputer technology combined with protein crystallography, molecular biology, and experimental laboratory verification. Based on studies from the laboratory of authors and from other laboratories worldwide, it is clear that flavonoids and other dietary and natural compounds target multiple signaling pathways. This may be a favorable property, because targeting multiple pathways could have a better response than selective targeting by agent(s). The flavonoid supplement market has been exponentially growing in the recent past. However, research on the effectiveness of supplementation is still in its infancy, and clinical studies are lacking regarding the effects of long-term use of supplements in humans. Further, emerging scientific evidence is suggesting that the "whole foods" concept may be better for cancer prevention than any single nutrient or specific combinations. It is being increasingly appreciated that the flavonoids and phytochemicals present in fruits and vegetables can enhance the bioavailability of different nutrients and can target multiple molecular pathways to yield a better response.

On pages 192–208 of this Forum, in another review article by Wilking *et al.* (8), a strong case is presented which promotes the idea that redox regulation and circadian rhythms may be connected processes within a living system and may be playing a critical role in a number of diseases, including cancer. The authors provide evidence regarding existing interactions between the redox signaling and circadian rhythms regulation. A case is made that for the management of cancer and other diseases, which have both circadian rhythm and oxidative stress components in their pathogenesis, it may be useful to target both components. The pineal hormone melatonin is intimately involved in the regulation of circadian rhythms on one hand and also functions as an antioxidant on the other hand. Interestingly, a number of studies have connected low levels of melatonin with certain cancers. Similarly, a number of studies have shown the cancer chemopreventive potential of melatonin in multiple models. Recent studies are providing evidence that several redox-sensitive genes, proteins, and transcription factors, which play critical role in the pathogenesis of diseases including cancer, have strong circadian rhythmicity. Thus, it is reasonable to think that for a more efficacious management of diseases, targeting the two systems in tandem could be more useful. However, a cause-and-effect association between circadian rhythms and oxidative stress is not clear at present, and further studies and collaborative efforts of scientists from diverse disciplines are needed to explore the possibilities presented by the authors.

The review by Khan and colleagues, on pages 151–162 of this Forum (2), has highlighted the health beneficial effects of the flavonoid antioxidant fisetin, which is found in a wide range of fruits and vegetables, such as strawberry, apple, persimmon, grape, onion, and cucumber. Several studies have suggested the beneficial effects of fisetin against numerous diseases in which oxidative stress is important. Based on the available literature, it appears that fisetin possesses potential as a cancer chemopreventive agent, especially due to its wide dietary availability. Although the cancer chemopreventive effects of fisetin are emerging in a variety of models, as discussed by Khan *et al.* (2), further in-depth studies are needed to ascertain the *in vivo* chemopreventive efficacy of this excellent agent as well as its molecular targets. In another review on pages 139–150 of this Forum (4), Li and colleagues have discussed the cancer chemopreventive potential of the isoflavone indole-3-carbinol (I3C) and its *in vivo* dimeric compound 3,3′-diindolylmethane (DIM), which have been shown to inhibit the NF-κB pathway as well as other pathways that are related to oxidative stress. It appears that although the molecular targets of these agents have been extensively studied, clinical studies that evaluate the effects of I3C and DIM for cancer prevention and treatment are lacking. Thus, future pre-clinical studies in specific *in vivo* cancer

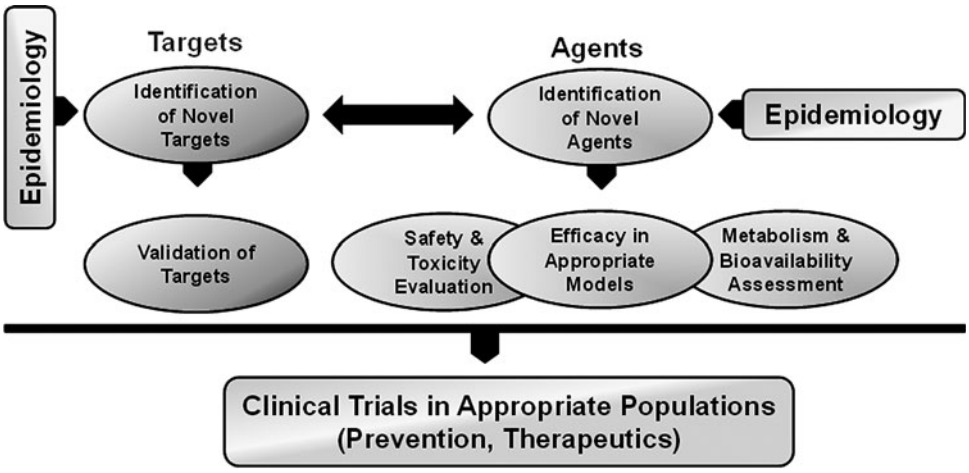

FIG. 1. Antioxidants in cancer management: important issues for future research.

models and clinical trials are needed to further assess the usefulness of I3C and DIM against cancer.

While clinical trials are lacking for most of the cancer chemopreventive agents, the Selenium and Vitamin E Cancer Prevention Trial (SELECT) using oral selenium (Se) and vitamin E supplementation in disease-free volunteers, designed to test a prostate cancer chemoprevention hypothesis, was terminated early because of negative data and safety concerns. Interestingly, the cell culture and animal studies have provided strong evidence regarding the anti-proliferative effects of various Se-compounds. On pages 181–191 in this Forum (7), Steinbrenner *et al.* have discussed a number of important issues and possibilities to understand the success and failures of Se in cancer prevention. As pointed out by the authors, the effectiveness of dietary Se supplementation depends on a number of factors, including baseline Se status, genetic background of an individual, individual variants in selenoprotein, age, gender, type of cancer, time point of intervention, as well as metabolic conversion and dose of Se compounds used. The failure of SELECT teaches us that it is important to consider a variety of issues and seek answers in carefully designed preclinical and small clinical studies before moving to big efforts. Even before SELECT started, several investigators voiced significant concerns regarding the rationale/design of this trial. The major concerns included (i) whether researchers are acting too quickly or whether there are sufficient data on these supplements to justify this trial; and (ii) whether reasons are available for how researchers became so interested in these two supplements exclusively. It was also argued that the formulations and doses of vitamin E and selenium were not optimal in SELECT. These issues need to be avoided in future clinical trials in the case of any chemopreventive agent.

Finally, on pages 115–138 of this Forum (5), Park and Pezzuto have discussed the potential of antioxidant marine agents in cancer management. Although seaweeds are widely consumed in coastal areas for a variety of reasons, the cancer chemopreventive potential of marine botanicals is an underexplored area of research. The authors have discussed the possible antioxidant and chemopreventive potential of a variety of seaweed agents as well as some marine fungi and bacteria. However, a lot of research needs to be done to assess the properties, chemopreventive effects, safety and toxicity, and mechanism of action of marine components. Sine oceans may have significant pollutants, such as heavy metals; one issue that needs to be handled carefully regarding the use of seaweed is to ensure that the seaweed supplements are free from harmful toxic substances.

In conclusion, extensive preclinical research has been done in the area of cancer prevention and therapeutics by antioxidants, especially using naturally occurring and diet based agents. However, a lot needs to be done in order to take some of the most promising antioxidant agent into the clinics. As depicted in Figure 1, the research in this direction revolves around two major themes "Targets" and "Agents." It is important to continue to focus our efforts toward defining and validating novel targets. Similarly, it is equally important to identify novel antioxidant agents followed by extensive efforts to assess their (i) efficacy, (ii) safety and toxicity, and (iii) metabolism and bioavailability, in appropriate model systems. Robust epidemiological studies can feed into and are important to the identification of both novel targets and novel agents. Similarly, the discovery of new targets can feed into the identification of new agents or *vice versa*. Finally, carefully planned clinical trials in well-identified and best-suited populations are needed to ascertain the efficacy of antioxidant agents in cancer prevention as well as cancer therapeutic settings. When designing clinical trials, it will be important to try to eliminate the agents with little development potential, possibly *via* quicker phase 0 trials (6). For example, an agent for cancer prevention needs to have adequate bioavailability and ability to be administered by non-invasive means such as *via* oral or topical administration (6). Further, the agents for prevention of cancer need to be cost affordable because of a need of long-term chronic administration. Thus, multidisciplinary efforts of scientists from diverse scientific disciplines are needed to be able to take the most promising antioxidant agents from *the bench to the bedside*.

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Abbreviations Used

DIM = 3,3′-diindolylmethane
HO-1 = heme oxygenase-1
I3C = indole-3-carbinol
IQGAP1 = IQ motif containing GTPase activating protein 1
NF-κB = nuclear transcription factor kappa B
Nrf2 = NFE2-related factor 2
Se = selenium
SELECT = Selenium and Vitamin E Cancer Prevention Trial