



COMMENTARY

Pro-oxidant activity of dietary chemopreventive agents: an under-appreciated anti-cancer property [v1; ref status: indexed, <http://f1000r.es/15s>]

Asfar S Azmi¹, Fazlul H Sarkar^{1,2}, SM Hadi³¹Department of Pathology, Wayne State University School of Medicine, Detroit MI, 48201, USA²Department of Pathology and Oncology, Karmanos Cancer Institute, Wayne State University School of Medicine, Detroit MI, 48201, USA³Department of Biochemistry, Aligarh Muslim University, Aligarh, 202002, India

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Abstract

"Let food be thy medicine and medicine be thy food" was quoted by Hippocrates more than two thousand years ago and since ancient times the health benefits of different natural agents have been exploited. In modern research, the disease preventive benefits of many such natural agents, particularly dietary compounds and their derivatives, has been attributed to their well recognized activity as the regulators of redox state of the cell. Nevertheless, most of these studies have focused on their antioxidant activity. A large body of evidence indicates that a major fraction of these agents can elicit pro-oxidant (radical generating) behavior which has been linked to their anti-cancer effects. This editorial provides an overview of the under-appreciated pro-oxidant activity of natural products, with a special focus on their ability to generate reactive oxygen species in the presence of transition metal ions, and discusses their possible use as cancer chemotherapeutic agents.

Article Status Summary

Referee Responses

Referees	1	2
v1 published 04 Jun 2013	 report	 report
1 Hasan Mukhtar , School of Medicine and Public Health, University of Wisconsin USA		
2 Surinder Batra , University of Nebraska USA		

Latest Comments

No Comments Yet

Corresponding author: Asfar S Azmi (azmia@karmanos.org)

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Introduction

Plant derived agents (fruit and vegetable derived agents and their derivatives) have been recognized to provide a number of disease preventive properties¹. It is now more than 20 years since the anti-cancer properties of some of the major natural agents such as resveratrol, tea catechins (particularly epigallocatechin (EGCG)) and the turmeric compound curcumin were recognized². Their anti-cancer activity has been primarily attributed to their antioxidant behavior and the popular notion of these agents as antioxidants has driven increased consumption in developed countries³. Further work in independent laboratories has shown that most of these agents can induce cell death through apoptosis, suppress anti-apoptotic pathways, block cell proliferation and modulate a number of important proteins implicated in sustaining the growth of cancer cells⁴. Nevertheless, there is no single unifying mechanism supporting their anti-cancer effects *in vitro* and *in vivo*. Recently, researchers have utilized systems and computational techniques to pinpoint the exact pathways modulated by some of these agents⁵. The results point to a highly promiscuous or having multiple mechanisms of action that involves modulation of multiple signaling in cancer cells⁶. Most importantly, researchers are yet to identify the main mechanism underlying the preferential cancer selectivity elicited by these agents, which spare normal cells. These are some of the major reasons for the skepticism surrounding their health benefits, which has become a hurdle in their clinical application. In order to solidify their clinical utility, researchers have to convincingly chalk out the primary mechanism of these promising agents.

Pro-oxidant activity of natural agents: an underappreciated activity

While the antioxidant action of dietary plant derived agents is unquestionable, over the last decade a parallel field has evolved that has convincingly presented evidence in support of their pro-oxidant (oxygen radical generating) behavior⁷, which may be context dependent. Supporting the significance of pro-oxidant drug action, Jim Watson recently presented a strong case that most of the anti-cancer regimens (ionizing radiation, chemotherapeutics and targeted therapeutics) work either directly or indirectly by generating reactive oxygen species (ROS)⁸.

This concept is not new. For example a PubMed search on May 21, 2013 using the keywords ‘*natural anti-cancer agents*’ and ‘*prooxidants*’ returns 1159 publications, while the keywords ‘*prooxidant*’ and ‘*anticancer agents*’ retrieve more than 11,200 papers. Various plant extract and agents such as resveratrol, catechins, EGCG, quercetin, gossypol, curcumin and caffeic acid have routinely been shown to induce damage to isolated plasmid DNA, albeit under certain conditions (e.g. in the presence of transition metal ions, especially copper⁹). Copper is an essential metal found in chromatin, and observed to be elevated in a number of malignancies¹⁰. In the late 1980s, our group was the first to describe quercetin-induced DNA strand scission in the presence of copper¹¹. Following our line of research, Fukuhara and Miyata were the first to demonstrate that resveratrol can induce oxidative DNA damage in the presence of copper ions¹². Such activity is dependent on a Fenton type reaction in which resveratrol oxidation results in the generation of superoxide radicals that re-oxidize to produce hydroxyl radicals in the presence of Cu(II) (elaborated in Zheng’s article¹³). Our laboratory

showed similar behavior for a number of natural agents, which led us to propose the hypothesis that one of the major anti-cancer mechanisms of plant polyphenolic compounds involves their pro-oxidant behavior in the presence of transition metal ions¹⁴. Other groups have also shown similar oxidant behavior in the presence of iron¹⁵.

Using an alkaline gel single cell electrophoresis/Comet assay, our group was among the first to demonstrate such pro-oxidant behavior in a cellular/biological system either alone or in the presence of copper¹⁶. Since then a number of laboratories have confirmed these results^{17,18}, which resulted in the increasing acceptance of the pro-oxidant behavior of a number of natural agents. Recently, we also showed the pro-oxidant DNA damaging effects of resveratrol in a mice model with supra-copper concentrations¹⁹. These and other studies²⁰ unequivocally prove that, in addition to their antioxidant behavior, natural dietary and diet-derived agents can elicit pro-oxidant behavior and this property cannot be ignored if drawing up a design for a clinical trial investigating the anti-cancer activity of natural agents.

It has been recognized that additional work is needed to further elucidate the mechanism supporting such pro-oxidant behavior of natural agents and the reasons for their cancer cell selectivity. Key questions remain such as:

- (a) Is pro-oxidant action the primary mechanism of these agents since ROS generation is a very rapid event that occurs within nanoseconds?
- (b) What are the metabolic differences in tumors that switch the plant derived agents’ antioxidant behavior to pro-oxidant?
- (c) Apart from DNA damage, what is the effect of such pro-oxidant behavior on other cellular components since it is well known that proteins and lipids are also prone to oxidative damage?
- (d) Is there any preferential selectivity of natural agents towards copper to generate reactive oxygen species over other metal ions found in the cellular milieu?
- (e) Are supra concentrations of copper or other transitional metals required to achieve such pro-oxidant behavior and does this have any value in actual patients?

Some of the answers came from very recent studies of ours in which we showed that agents such as resveratrol can take advantage of the metabolic differences in solid tumors leading to selective cancer cell killing²¹. Our findings have demonstrated that in solid tumors, the preferential dependence of cancer cells on glycolysis (Warburg effect) leads to a lowering of tumor cell pH, which loosens the DNA structure thereby leading to exposure of chromatin-bound copper²². Such labile copper is more prone to attack by resveratrol, leading to oxidative DNA damage. In non-cancerous cells, the primary source of ATP generation is through the citric acid cycle, and therefore these cells maintain physiological pH and so have normal DNA integrity. This observation is just one small step in explaining the cancer cell selectivity of natural pro-oxidant agents.

The cell culture artifact conundrum

As convincing as it sounds, the pro-oxidant activities of natural agents come with caveats. For example, Barry Halliwell and colleagues have suggested that the pro-oxidant behavior is nothing but an artifact of cell culture conditions²³. They propose that most natural agents get oxidized in the presence of the cell culture media component phenol red, which can generate oxygen radicals leading to lipid peroxidation. However, these assumptions were proved to be wrong when different dietary agents were tested in phenol-free media and their pro-oxidant behavior was retained²⁴. Such experiment put to rest the controversy involving ROS generation in experimental conditions. In another study, Burkitt and colleagues showed that under normal physiological conditions, i.e. in the presence of either ascorbic acid or glutathione, resveratrol loses its pro-oxidant property and behaves as an antioxidant²⁵. In the ascorbate system, resveratrol had no effect on the rate of hydroxyl radical formation, but protected DNA from damage by acting as a radical-scavenging antioxidant. Through these studies the authors have concluded that the DNA-damaging properties of resveratrol, as identified by Fukuhara and Miyata, will be of no significance under standard physiological conditions. Nevertheless, as described above, we have shown both in cellular and animal models that their pro-oxidant behavior in tumors remains relevant^{15,16}.

Conclusion and future direction

As much as the anti-cancer activity of natural agents has intrigued researchers, it has also generated a lot of skepticism and controversy. Even though their promiscuous behavior is gaining some acceptance, the lack of a unifying mechanism supporting their activity

has kept them at the periphery of clinical application. These agents are multi-mechanistic in nature and merely focusing on their antioxidant behavior will not do justice to their multifarious health benefits. Similarly, honing in on their effect on only a specific set of cancer hallmarks will not allow a proper understanding of the anti-cancer activity of these agents. Researchers need to pay more attention to the dogma-challenging yet under-appreciated pro-oxidant behavior of dietary agents. This exercise is expected to highlight unanswered questions and may lead to the definition of a single unifying mechanism of action related to their unquestionable anti-cancer effects. This in turn will help in the rapid clinical application of agents or their derivatives from nature's bounty for the treatment of human malignancies.

Author contributions

Asfar S Azmi developed the hypothesis, wrote and revised the article. Fazlul H Sarkar wrote and revised the article. SM Hadi conceived the hypothesis and revised the manuscript.

Competing interests

No competing interests were disclosed.

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Current Referee Status:



Referee Responses for Version 1



Surinder Batra

University of Nebraska, Lincoln NE, USA

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Referee Report: 04 September 2013

This is well-written manuscript.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Competing Interests: No competing interests were disclosed.



Hasan Mukhtar

Department of Dermatology, School of Medicine and Public Health, University of Wisconsin, Madison, WI, USA

Approved: 05 June 2013

Referee Report: 05 June 2013

Very well written. Was a joy to read.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Competing Interests: No competing interests were disclosed.
