Free Radical Biology and Medicine xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Free Radical Biology and Medicine

journal homepage: www.elsevier.com/locate/freeradbiomed

Review Article

Redox regulation of protein kinase C by selenometabolites and selenoprotein thioredoxin reductase limits cancer prevention by selenium

Rayudu Gopalakrishna[a,*], Usha Gundimeda[a], Sarah Zhou[a], Helena Bui[a], Arne Holmgren[b]

[a] Department of Integrative Anatomical Sciences, Keck School of Medicine, University of Southern California, Los Angeles, CA 90089, USA
[b] Department of Medical Biochemistry and Biophysics, Karolinska Institute, 171 77 Stockholm, Sweden

ARTICLE INFO

Keywords:
Protein kinase C
Thioredoxin reductase
Methylselenol
Methylseleninic acid
Prostate cancer prevention
Selenoproteins

ABSTRACT

The cancer-preventive mechanism of selenium should address the way low concentrations of selenometabolites react with cellular targets without being diffused from the sites of generation, the way selenium selectively kills tumor cells, and the intriguing U-shaped curve that is seen with dietary supplementation of selenium and cancer prevention. Protein kinase C (PKC), a receptor for tumor promoters, is well suited for this mechanism. Due to the catalytic redox cycle, low concentrations of methylselenol, a postulated active metabolite of selenium, react with the tumor-promoting lipid hydroperoxide bound to PKC to form methylseleninic acid (MSA), which selectively reacts with thiol residues present within the vicinity of the PKC catalytic domain to inactivate it. Given that lipid hydroperoxide levels are high in promoting cells, PKC inactivation selectively leads to death in these cells. A biphasic effect of MSA in inducing cell death was observed in certain prostate cancer cell lines; lower concentrations of MSA induced cell death, while higher concentrations failed to do so. Lower concentrations of selenium inactivate more sensitive antiapoptotic isoenzymes of PKC (ε and α), sparing less sensitive proapoptotic isoenzymes (PKCδ and PKCζ). Higher concentrations of selenium also inactivate proapoptotic isoenzymes and consequently make tumor cells resistant to apoptosis. Due to a high-affinity binding of thioredoxin to the PKC catalytic domain, this thiol oxidation is explicitly reversed by thioredoxin reductase (TXNRD), a selenoprotein. Therefore, overexpression of TXNRD in advanced tumor cells could make them resistant to selenium-induced death. Conceivably, this mechanism, at least in part, explains why selenium prevents cancer only in certain cases.

1. Introduction

Both epidemiologic and experimental data generated over nearly half a century, support the fact that supplementation with dietary selenium above the nutritionally required level may decrease the incidence of cancer in some cases but fail to do so in others [1,2]. Depending on the form of selenium taken, higher amounts seem to cause toxicity [3]. Greater interest in selenium in cancer prevention in humans was generated when the landmark Nutritional Prevention of Cancer trial, conducted by Clark and his associates, suggested that supplemental selenium in the form of selenized yeast may reduce the incidence and mortality of cancers of the prostate, lung, and colon-rectum [4]. This led to the large-scale clinical intervention Selenium and Vitamin E Cancer Prevention Trial (SELECT), which employed selenomethionine to prevent prostate cancer in men [5]. The results of SELECT did not show any efficacy of selenium in preventing prostate cancer [6]. Some have suggested that the failure of this intervention trial was due to the use of a less efficient form of selenium [7–9], while others have suggested that the failure was due to the use of higher doses of selenium than the optimal dose for cancer prevention [10,11]. This generated a greater interest in understanding the cancer-preventive mechanism of selenium to determine why it prevents cancer in some cases and either fails to dos so or causes toxicity in others.

The redox chemistry of selenium plays an important role in its actions as a nutrient, cancer-preventive agent, and toxicant [2,12]. Selenium exerts its antioxidant actions primarily through its role as selenocysteine in selenoproteins [13–16]. Selenoprotein biosynthesis, structure, and regulation are well established in many cases [17–20]. The requirement of dietary selenium for the synthesis of many of these selenoproteins is low and within the levels of nutritionally required selenium (0.1 ppm). However, the amount of selenium required for cancer prevention (1–3 ppm) is well above the levels of selenium

Abbreviations: PKC, protein kinase C; TXN, thioredoxin; TXNRD, thioredoxin reductase; SELECT, Selenium and Vitamin E Cancer Prevention Trial; NPC, Nutrition Prevention of Cancer; MSA, methylseleninic acid

* Correspondence to: Department of Integrative Anatomical Sciences, Keck School of Medicine of USC, 1333 San Pablo Street, BMT-306, Los Angeles, CA 90089, USA.
E-mail address: rgopalak@usc.edu (R. Gopalakrishna).

https://doi.org/10.1016/j.freeradbiomed.2018.05.062
Received 10 March 2018; Received in revised form 10 May 2018; Accepted 13 May 2018

needed for optimal synthesis of selenoproteins [21]. This prompted the notion that cancer-preventive actions may be caused by low-molecular-weight selenometabolites [22]. Although some limited concentrations of selenium exist in low-molecular-weight forms, their concentrations are extremely low and these compounds are not well characterized [21]. Others suggested that the cancer-preventive actions of selenium might be mediated by methylated metabolites, particularly by methylselenol [23]. However, the way methylated metabolites, which are present in very low concentrations in the cells and are also volatile, mediate the cancer-preventive action of selenium is not well established. Furthermore, it is not known whether there is any relationship between selenoproteins and selenometabolites in the cancer-preventive actions of selenium.

The ability of redox-active selenocompounds to induce protein thiol oxidation and the formation of disulfides was demonstrated using reduced ribonuclease [24]. Protein thiol redox modifications are important mechanisms in regulating key intracellular enzymes such as protein kinases, protein tyrosine phosphatases, and various transcriptional factors [25–29]. As these players are essential in tumor promotion and cell survival, these redox regulatory mechanisms have an important role in tumorigenesis as well as in cancer prevention [30].

In this review, we discuss the oxidation of critical protein thiols in PKC isoenzymes by selenometabolites and its reversal by selenoprotein thioredoxin reductase (TXNRD), as well as its significance in tumor cell susceptibility to selenium and how selenometabolites, at small concentrations, can induce these thiol modifications and selectively kill precancer cells.

2. Selenium in the promotional stage of carcinogenesis

Selenium inhibits various stages of carcinogenesis [31–33]. However, the promotional stage has a longer duration which is well-suited for cancer prevention by selenium [32,34]. For example, decades of prolonged preneoplasia during the development of prostate cancer makes it suitable for cancer prevention [35,36]. Apoptosis-inducing mechanisms of selenium may be important in the promotional stage of carcinogenesis [37]. There is limited evidence that dietary selenium at a level nontoxic to the host can prevent the growth of tumors in experimental animals [38].

Protein kinase C (PKC) isoenzymes may play key roles in tumor promotion and cancer metastasis [39,40]. PKC represents a family of more than 11 isoenzymes [39,41]. These isoenzymes are divided into three categories based on the cofactors needed for their activation. Conventional PKC isoenzymes α, β, and γ are calcium-dependent and are activated by diacylglycerol. Novel PKC isoenzymes δ, ε, θ, and η are calcium-independent but are also activated by diacylglycerol. Atypical PKCs ζ and ι/λ require neither calcium nor diacylglycerol for optimal activity. PKC isoenzymes respond differently to the stimuli that cause tumor promotion, cell growth, and cell death and in some cases, even play opposite roles from one another [42]. PKCε is a promitogenic and prosurvival enzyme and has oncogenic potential [42]. The expression of PKCε significantly increases in prostate cancer in a manner correlating with the aggressiveness of the disease [43]. In contrast, PKCδ is a proapoptotic and antitumorigenic isoenzyme [42]. By limited proteolysis, caspase-3 converts this kinase into a cofactor-independent form which plays an important role in executing apoptosis [42]. PKCζ, which is activated by ceramide, also plays a key role in inducing apoptosis [44]. Therefore, it is imperative to know how selenium inactivates antiapoptotic and proapoptotic PKC isoenzymes to variable extents, which results in either apoptosis or tumor cell survival.

PKC isoenzymes are well known for their role as receptors for phorbol esters, which are experimental tumor promoters. Diacylglycerol, the second messenger that activates PKC, is generated to levels that are four-fold higher in prostate cancer tissue in comparison to levels in benign tissue, suggesting a role for PKC in prostate cancer development [45]. PKC is also directly activated by a variety of

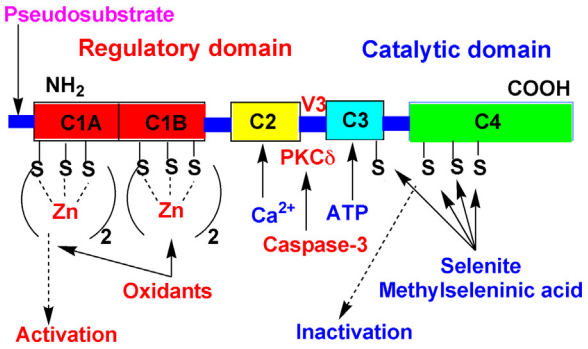

Fig. 1. Oxidation-susceptible sites in PKC isoenzymes. C1: cysteine-rich constant region present in various PKC isoenzymes; C1A and C1B domains: together have four zinc thiolates; C2: Ca^{2+}-binding domain present only in conventional PKC isoenzymes (α, β, and γ), but absent in novel PKC isoenzymes (δ, ε, η and θ) and atypical PKC isoenzymes (ζ, ι, and λ); C3: ATP-binding region in the catalytic domain; C4: protein substrate-binding region in the catalytic domain; V3: proteolysis susceptible variable region present in various PKC isoenzymes, which also has a caspase-3 sensitive region in PKCδ; pseudosubstrate: autoinhibitory region in the regulatory domain prevents the binding of protein substrate to the catalytic domain.

oxidants, either from environmental exposure or generated through inflammatory processes, which play a crucial role in carcinogenesis [30,46]. PKC has unique structural features that make it respond to oxidant tumor promoters such as H_2O_2, periodate, organic peroxides such as benzoyl peroxide, and tobacco tumor promoters in a bidirectional manner [30,47]. Oxidants at lower concentrations activate this kinase while at higher concentrations inactivate the enzyme. Such bimodal regulation of PKC by oxidants may have a role in tumor promotion.

PKC has cysteine-rich regions in both its regulatory and catalytic domains (Fig. 1). The cysteine-rich region present in the regulatory domain coordinates the binding of four zinc atoms [48]. Zinc-thiolate structures in the regulatory domain are more sensitive to oxidative modification than the free thiolates in the catalytic domain. Therefore, oxidants at low concentrations selectively oxidize thiolates coordinating zinc in the regulatory domain, causing the collapse of the autoinhibitory region leading to cofactor-independent activation of PKC [26,27,30,49–52]. Thus, oxidant tumor promoters mimic phorbol ester tumor promoters in activating PKC; phorbol esters bind to a structure supported by zinc-thiolates to relieve the inhibition of the regulatory domain over the catalytic site, and oxidants collapse the regulatory domain to produce the same effect. On the contrary, alkylating agents and oxidants at high concentrations modify the conserved cysteine residues present in the catalytic domain of the enzyme, instead leading to the inactivation of PKC [53]. Since a variety of cancer-preventive agents form oxidation products, this enzyme is inactivated by these agents [30]. Thus, PKC is not only a receptor for tumor promoters but also a molecular target for cancer-preventive agents that may effectively inhibit tumor promotion [30].

3. Reversible inactivation of PKC isoenzymes by selenocompounds

Since protein thiols are very sensitive targets for redox modifications, enzymes such as PKC that have thiol-rich regions are relevant targets for selenometabolites. Inhibition of PKC activity by selenocompounds was previously reported [54]. Nevertheless, it was not known in such studies whether selencompounds bound to and inhibited the enzyme or induced modification of the enzyme. Later studies addressed this issue by utilizing various redox-active, low-molecular-weight selenocompounds to determine whether they modify critical sulfhydryls in PKC to cause a decrease in its kinase activity and phorbol