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REVIEWS: CURRENT TOPICS

Zinc deficiency, DNA damage and cancer risk

Emily Ho*

Department of Nutrition and Food Management and Linus Pauling Institute, Oregon State University, Corvallis, OR 97331, USA

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Abstract

A large body of evidence suggests that a significant percentage of deaths resulting from cancer in the United States could be avoided through greater attention to proper and adequate nutrition. Although many dietary compounds have been suggested to contribute to the prevention of cancer, there is strong evidence to support the fact that zinc, a key constituent or cofactor of over 300 mammalian proteins, may be of particular importance in host defense against the initiation and progression of cancer. Remarkably, 10% of the U.S. population consumes less than half the recommended dietary allowance for zinc and are at increased risk for zinc deficiency. Zinc is known to be an essential component of DNA-binding proteins with zinc fingers, as well as copper/zinc superoxide dismutase and several proteins involved in DNA repair. Thus, zinc plays an important role in transcription factor function, antioxidant defense and DNA repair. Dietary deficiencies in zinc can contribute to single- and double-strand DNA breaks and oxidative modifications to DNA that increase risk for cancer development. This review will focus on potential mechanisms by which zinc deficiency impairs host protective mechanisms designed to protect against DNA damage, enhances susceptibility to DNA-damaging agents and ultimately increases risk for cancer.

Keywords: Zinc deficiency; DNA damage; Cancer; DNA repair; Oxidative stress

1. Introduction

The role of zinc in a wide range of cellular processes, including cell proliferation, reproduction, immune function and defense against free radicals, has been well established [1,2]. Zinc is considered the most abundant trace intracellular element, and there exists increasing evidence that zinc plays an important role in both genetic stability and function [3]. About 25% of the total zinc present in rat liver is found within the nucleus [4]. In vitro, significant amounts of zinc are incorporated in the nuclei [4]. It is clear that, mechanistically, zinc has a significant impact on DNA as a component of chromatin structure, DNA replication and transcription and DNA repair [5]. Zinc is a component of more than 3000 zinc-associated transcription factors, including DNA-binding proteins with zinc fingers, and more than 300 enzymes, including copper/zinc superoxide dismutase (CuZnSOD) and several proteins involved in DNA repair [6–8]. Thus, zinc plays an important role in protecting cellular components from oxidation and damage to DNA. This review will examine the links among zinc deficiency, oxidative stress, DNA damage and repair and risk for cancer.

2. Zinc deficiency and oxidative stress

A significant portion of the North American population does not consume adequate zinc; 10% of the U.S. population consumes less than half the recommended level for zinc [9]. Importantly, zinc deficiency results in an increased sensitivity to oxidative stress [10] and may, in part, account for the mechanism by which zinc deficiency increases the risk for cancer development. There is now increasing evidence that oxidative stress is an important contributing factor in several chronic degenerative diseases, such as cancer [11].

2.1. Potential mechanisms of zinc antioxidant function

Zinc's antioxidant function may be related to several factors. First, zinc is an essential component of CuZnSOD, one of the cell's first lines of defense against reactive

* Tel.: +1 541 737 9559; fax: +1 541 737 6914.
E-mail address: emily.ho@oregonstate.edu.

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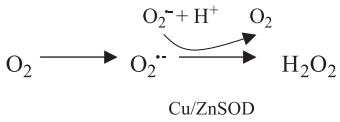
oxygen species (ROS), which functions to remove the superoxide anion ($O_2^{\bullet -}$).



The second potential mechanism for zinc's antioxidant effects is the antagonism of redox-active transition metals, such as iron or copper, and the prevention of oxidation of sulfhydryl groups within proteins. In chemically defined systems, zinc can prevent hydroxyl radical formation by transition metals. Most biological molecules cannot be damaged at a significant rate by direct reactions with oxygen, superoxide or hydrogen peroxide. However, they can be oxidized by hydroxyl radicals (HO•). This species is formed when a single electron is transferred to H_2O_2.

$$e^- + H_2O_2 \rightarrow HO\bullet + OH^-$$

Transition metals, such as iron and copper, can donate electrons to hydrogen peroxide via Fenton reactions.

$$Fe^{2+} + H_2O_2 \rightarrow HO\bullet + OH^- + Fe^{3+}$$

$$Cu^{1+} + H2O_2 \rightarrow HO\bullet + OH^- + Cu^{2+}$$

By competing for binding sites for pro-oxidant transition metals, such as iron and copper, zinc can decrease their ability to transfer electrons and their availability Fenton-like reactions [12–17].

Zinc may also act as an antioxidant by protecting protein sulfhydryl groups against oxidation. This stabilizing effect of zinc on sulfhydryls has been extensively studied on the enzyme δ-aminolevulinate dehydratase. Zinc protects this enzyme from oxygen inactivation, preventing enzyme thiol oxidation and disulfide formation. Removal of zinc results in enzyme inactivation. Several other sulfhydryl-containing proteins, such as dihydroorotase, tubulin, zinc finger proteins, alanyl tRNA synthetase and protein farnesyltransferase, appear to be protected by zinc (for a review, see Ref. [2]). However, zinc does not protect against oxidation in all sulfhydryl-containing proteins. For example, the zinc in zinc metallothionein does offer protection against oxidation; reaction with OH· leads to disulfide formation and a loss of zinc atoms [1].

A third mechanism by which zinc acts as an antioxidant is through its regulation of metallothionein metabolism. Metallothionein, a small-molecular-weight protein, is important in zinc homeostasis and has potent antioxidant activity. Zinc can induce the expression of the cysteine-rich metallothionein protein via activation of the metal transcription factor 1 (MTF-1). Zinc can directly bind and activate MTF-1, which then binds to metal-responsive elements to induce gene expression of proteins, such as metallothionein. Metallothionein itself acts as a potent scavenger of hydroxyl radical. Thus, loss of this protein can impair cellular antioxidant defenses and contribute to the cell's sensitivity to oxidative stress.

2.2. Low zinc and increases in oxidative susceptibility

Although the precise mechanism by which zinc acts as an antioxidant is unclear, compromised zinc status clearly has a significant impact on the antioxidant capacity of the cell. Several laboratories, including our own, have shown increased oxidative stress with low cellular zinc. Increased oxidant production has been shown in cell cultures grown in zinc-deficient media [18]. In vivo, increases in oxidative protein and DNA damage has been shown in zinc-deficient rats [10,19–22]. Zinc deficiency also increases the susceptibility to oxidative injury. Several researchers have shown increased free radical production or increased injury with zinc deficiency both in vitro and in vivo. For example, increases in hyperoxic lung damage, carbon tetrachloride toxicity and lipid peroxidation have been found in zinc-deficient rats compared with their zinc adequate and pair-fed controls [10,20,23,24]. These studies clearly show that zinc deficiency renders animals more sensitive to oxidative insults. On the other hand, zinc supplementation strategies have also been shown to be beneficial against oxidant damage and the progression of ROS-induced diseases. For example, zinc supplementation has shown protection against chemically induced models of Type I diabetes [25,26], and addition of zinc protects cells from UV-induced DNA damage and apotosis [27,28]. In humans, zinc supplementation appears to help patients by slowing the progression of age-related macular degeneration [29,30]. All of these disorders can be linked to increased free radical production. Thus, the antioxidant role of zinc could be an important mechanism in maintaining DNA integrity in the cell by preventing oxidative DNA damage in the cell.

ROS are commonly produced during normal cellular metabolism. However, under certain conditions of stress, such as poor nutrition, an increase in the production of ROS may overwhelm host antioxidant defenses, resulting in oxidative damage. There is increasing evidence that the pathology and disease development associated with oxidative stress may not be due simply to increases in oxidative damage [31]. Rather, ROS may also act as signaling molecules that trigger distinct pathways to induce pathology. Thus, the role of zinc deficiency in the development of chronic diseases, such as diabetes and cancer, may be far more complex than simply causing oxidative damage. Zinc status may also affect redox-sensitive signals and ultimately may alter signal pathways involved in stress response, DNA repair and apoptosis.

3. Zinc and DNA repair

The effects of zinc on DNA integrity may not be limited to zinc's antioxidant properties. Zinc plays a critical role in the regulation of transcription and replication of DNA

through zinc finger proteins. Additionally, many DNA repair mechanisms involve zinc. Many proteins involved in both base and nucleotide excision repairs are zinc finger or zinc-associated proteins. For example, the tumor suppressor protein p53 plays an important role in coordinating events leading to appropriate DNA repair. p53 plays a role in modulating cell cycle progression, apoptosis, DNA repair and cell proliferation/differentiation [32]. p53 is well-known for its ability to induce G_1 arrest in the cell cycle, allowing the cell to induce adequate repair of DNA before cellular division [33]. Notably, the prevalence of p53 mutations in tumors is very high. In fact, over 50% of human malignancies contain a mutation in p53 [34]. The majority of these mutations are found in the region of the gene that encodes for the DNA-binding region of p53 [35,36]. This binding region also contains the zinc-binding domain. Several lines of evidence suggest that p53 acts, in part, as a DNA-binding protein [37]. Thus, to coordinate the events related to DNA repair, p53 must be able to bind to specific DNA-binding domains to transcriptionally activate downstream targets involved in DNA repair. In our laboratory, we have found an increase in p53 protein expression with low intracellular zinc in several different cell lines [38,39]. An up-regulation in p53 expression is seen both when using the intracellular zinc chelator TPEN and by feeding cells zinc-depleted media. This up-regulation of p53 expression is most likely in response to DNA damage induced by zinc deficiency. Although there is an increase in p53 expression with low intracellular zinc, there is evidence that p53 may be dysfunctional, and hence, DNA repair would be severely compromised. A marked decrease in the ability of p53 to bind to downstream DNA targets has been found with zinc deficiency [18]. The p53 protein is a transcription factor; hence, the ability of p53 to bind to DNA promoter regions is critical for coordinating the events that control DNA repair and apoptosis. Thus, despite increases in p53 protein level in zinc-deficient cells, this p53 is likely dysfunctional and activation of gene transcription of specific genes needed for DNA repair could be compromised.

The DNA-binding activity of p53 is largely mediated by a conformation-sensitive structure in the central portion of the protein (residues 102–292) [36]. Mutations in this region cause an “unfolding” of this structure and a loss of binding activity. Other researchers have also found that the removal of zinc, either by chemical chelation or by adding zinc-deficient media, alters the expression of p53 [40,41]. Direct chemical chelation also appears to reversibly alter p53 conformation, with the loss of DNA-binding activity [42]. Thus, zinc deficiency may render the p53 protein to adopt a “mutant-like” conformation that will alter the cell’s ability to appropriately respond to DNA damage.

We have also investigated the effect of low intracellular zinc on the expression of apyrimidic endonuclease (APE), an important endonuclease in base excision repair [43]. DNA base excision repair is a major pathway responsible for the repair of both cellular alkylation and oxidative DNA damage. A critical step in this pathway involves the cleavage of damaged sites in DNA by APE. APE (which is also known as Ref-1) is a multifunctional protein that not only repairs activator protein (AP) sites but also controls DNA-binding activity, via redox mechanisms, of numerous transcription factors that are involved in cancer promotion and progression, such as AP-1, nuclear factor kappaB (NFκB) and p53 [44]. In addition, APE levels appear to be elevated in a number of cancers [45–47]. Low cellular zinc increases the expression of APE, most likely in response to DNA damage induced by low zinc [18].

4. Zinc deficiency, cell signaling and apoptosis

Redox-sensitive transcription factors, such as NFκB and AP-1, play important roles in controlling oxidative stress responses and help control cell proliferation and apoptosis [48,49]. We have also found a marked decrease in binding of AP-1 and NFκB with zinc deficiency [18]. Other investigators have also detected alterations in AP-1 and NFκB signaling with low cellular zinc [50]. Mechanistically, it is unclear how zinc deficiencies may alter transcription factor binding and cell signaling. As discussed previously, this could be related to protective effects of zinc on sulfhydryl groups in zinc fingers or other transcription factors or due to increases in ROS signals. More work needs to be done to identify transcription factors that are sensitive to zinc deficiency; it is unlikely that all zinc-containing transcription factors are sensitive. It is possible that transcription factors, such as AP-1, NFκB and p53, that show alterations with low cellular zinc are more susceptible because they are also redox-sensitive. Regardless, perturbations in their binding and signal pathways could significantly impair the cell’s ability to handle oxidative stresses and alter apoptosis mechanisms.

Therefore, the impact of zinc status on DNA integrity is most likely a multilayered process involving both increases

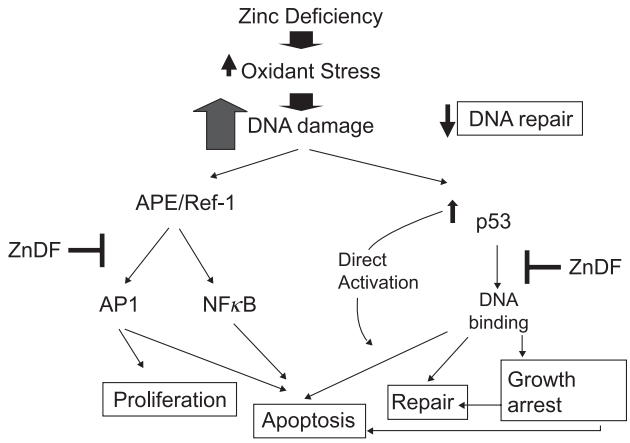


Fig. 1. Summary of the potential effects of zinc deficiency on DNA integrity. The effects of zinc deficiency on DNA damage are multifold. Zinc deficiency can enhance oxidative stress and oxidative DNA damage and, at the same time, can block critical cellular signals that normally lead to repair mechanisms. ZnDF=zinc deficiency.

in oxidative stress and combined perturbations in DNA repair and other oxidant-sensitive signal pathways. Fig. 1 summarizes a putative schematic of how zinc deficiency may induce oxidative stress, DNA damage and impair the cellular signals that aid in repairing this damage, thus, severely compromising DNA integrity and the health of the cell. Thus, in the absence of zinc, an environment with increased oxidative stress and DNA damage along with an inability to adequately signal DNA repair mechanisms, will provide an optimal environment for increases in steady-state DNA damage, which could ultimately lead to increases in cancer development.

Zinc also plays an important role in the regulation of apoptosis. Apoptosis is a mechanism for the cell to undergo "programmed cell death" and is characterized by distinct morphological and biochemical events that include cell shrinkage, membrane blebbing and DNA fragmentation (i.e., DNA damage). Several groups have shown links between zinc deficiency and increased apoptosis in various cells and tissues. One example is in the immune system. One hallmark of human zinc deficiency is an increased susceptibility to infection [51]. Zinc deficiency induces markedly reduced lymphocyte numbers in both the thymus and marrow of zinc-deficient mice. Alterations in apoptosis are critical in the changes in lymphopoiesis and myelopoiesis in T and B cells with zinc deficiency [52,53]. Zinc depletion has also been associated with caspase activation and increases in apoptosis in airway epithelial cells, lung and hepatocytes [54–56]. The induction of apoptosis is an important mechanism for the cell to "commit suicide" in response to DNA damage. Regulation of apoptosis is a complex process, which has multiple known triggers. The direct mechanistic effects of zinc deficiency on apoptosis are still unclear. The possibilities include a direct effect loss of zinc in critical proteins involved in the apoptotic cascade or indirect effects due to increased oxidative stress or a response to DNA damage. ROS are also known inducers of apoptosis. Oxidation of critical protein involved in the apoptotic cascade may be present with zinc deficiency. More study needs to be done to clearly identify targets and determine levels of zinc needed to cause DNA damage or apoptosis. It is clear that zinc deficiency causes oxidative DNA damage, DNA strand breaks and DNA fragmentation, but their interrelationship between zinc-deficiency related DNA damage and programmed cell death is not clear. However, this reinforces the concept that the effect of zinc deficiency on DNA integrity is a multilayered process that integrates oxidative stress, signaling pathways and DNA repair/apoptotic mechanisms.

5. Zinc deficiency and its relevance to cancer

Zinc deficiency affects many cellular systems in the host because of zinc's essential role in many aspects of cellular metabolism. Zinc deficiency can occur in populations with low dietary zinc intake and high concentration of phytate, a powerful chelator of divalent metals. The pathological signs of zinc deficiency include stunted growth, impaired parturition (dystocia), neuropathy, decreased food intake, diarrhea, dermatitis, hair loss, bleeding tendency, hypotension and hypothermia [57]. Although severe zinc deficiency is rare, mild deficiency is highly prevalent even in developed countries. Populations that are at high risk include individuals at early stages of the life cycle (i.e., infancy and childhood) when requirements for zinc are high. In addition, the elderly have an increased risk of zinc depletion because zinc absorption may be impaired and they tend to consume low-zinc diets [58]. Foods rich in zinc include red meat and seafood. Several plant sources, such as whole grains and legumes, also contain zinc, but the zinc in these sources are much less bioavailable. Thus, vegetarians may also be at risk for zinc deficiency.

The role of zinc in cancer has received increasing attention. The link between zinc deficiency and cancer has been established in human, animal and cell culture studies. Zinc status is compromised in cancer patients compared with healthy controls [59,60]. Zinc deficiency causes oxidative DNA damage [22], and chromosome breaks have been reported in animals fed a zinc-deficient diet [61]. In rats, dietary zinc deficiency causes an increased susceptibility to tumor development when exposed to carcinogenic compounds [62–66]. Zinc deficiency has also been suggested to be a contributor to the development of esophageal tumors in rats [64,65,67]. In vitro, cell culture studies have also shown that zinc deficiency can lead to increased oxidative damage to testicular cell DNA [20]. Zinc also appears to play an important role in maintaining prostate health, but the precise role of zinc in the prostate is unknown. Normal human prostate accumulates the highest levels of zinc of any soft tissue in the body [68,69]. However, a marked decrease in zinc content is associated with prostate cancer [70–72]. Zinc deficiency also compromises hormonal function in men [73–76]. Several studies have implicated changes in zinc accumulation in the development and progression of prostate malignancy (for reviews, see Refs. [77,78]). There also exists some evidence that increased dietary zinc is associated with a decrease in the incidence of prostate cancer [79]. However, high-dose supplementation of zinc may increase prostate cancer risk. Currently, it is unknown why the prostate accumulates high zinc concentrations. However, this phenomenon may render the prostate sensitive to changes in zinc intake. We have shown in other various cell types that changes in intracellular zinc has a dramatic effect on DNA damage and repair and hence risk for cancer [18]. It is possible that dietary zinc deficiency will increase an individual's risk for oxidative DNA damage in the prostate and prostate cancer. In addition, there appears to be a loss of zinc during prostate cancer. Thus, zinc need may be enhanced in prostate cancer patients. Zinc supplementation strategies may not only aid in the prevention of cancer, but could also play an important role in limiting its malignancy.

Zinc plays an important role in protecting DNA from damage as an antioxidant and a component of many DNA repair proteins. Zinc is also unique in that it bears antioxidant [1], anti-inflammatory [80] and proapoptotic activity [55,81]. Thus, zinc supplementation has the potential to target multiple points of the carcinogenesis cascade. There is some controversy toward the efficacy of zinc supplements to prevent prostate cancer. Several studies have shown that high cellular zinc levels inhibit prostate cancer cell growth [77,82,83]. However, a recent epidemiologic study has shown an increase in risk for prostate cancer with high zinc supplement use [84]. Increased risk was seen in subjects with very high dose or very long term zinc supplement use (over 100 mg/day zinc supplement use or >10 years). The current upper level of intake for zinc is 40 mg/day. Thus, it is possible that these subjects could have been in the "toxic" range for zinc intake. As with most therapeutics, higher doses do not always equate with an increase in efficacy.

The exact molecular mechanisms by which zinc deficiency impacts DNA integrity and cancer risk remain unclear. It is likely that a complex multilayered process that involves oxidative stress and alterations in cellular signal processes controls DNA repair and apoptosis. Understanding the basic molecular mechanisms behind human nutrient metabolism will help us better understand the role of proper nutrition in the prevention of many chronic disease states, such as cancer.

6. Conclusions and future directions

While there is compelling evidence to support the conclusion that zinc deficiency can lead to DNA damage, the concept that zinc deficiency can directly increase the susceptibility to DNA damage, as well as adversely alter the host response to DNA-damaging agents, has not been extensively explored. Further, while it is known that there exist marked differences in individual responses to DNA-damaging agents, the source of this variation is not clear, although nutritional factors are rarely considered. Approximately 40 micronutrients are required in the human diet. There exists increasing evidence that deficiency of certain micronutrients, such as vitamins B12, folic acid, B6, niacin, C or E, iron or zinc, causes single- and double-strand breaks, oxidative lesions or both [85]. Common micronutrient deficiencies damage DNA by the same mechanism as other known DNA-damaging agents, such as radiation and many chemicals. In some cases, micronutrient (such as folate) deficiency can be more damaging than DNA-damaging agents (such as low-dose ionizing radiation) [86]. It appears clear that zinc deficiency has a significant impact on DNA integrity, and zinc deficiency by itself is damaging to DNA.

Given the fact that zinc is a required cofactor for antioxidant defense proteins and DNA repair enzymes, it is important to determine the extent to which zinc deficiency enhances susceptibility to DNA-damaging agents and impairs host protective mechanisms designed to protect against DNA damage in the development of cancer. It appears that the effects of zinc deficiency on DNA integrity are a multilayered effect involving both oxidative stress and signaling pathways. An important future direction of research is to characterize the impact of zinc nutrition on stress response and cancer-susceptibility genes. A significant portion of genetic mutations that increase risk for cancer are those involved in maintaining DNA integrity and DNA repair. However, the presence of a mutation does not always mean the individual will get cancer. A key factor that pushes the balance to a cancerous phenotype may be inadequate intake of nutrients, such as zinc. If zinc plays a critical role in antioxidant defense and maintenance of DNA integrity, it is likely that inadequate zinc will be highly detrimental to these susceptible individuals. Characterization of the mechanisms by which zinc affects DNA integrity will aid in understanding both nutrient–gene and nutrient–environment interactions and will result in a basis for nutrition-based cancer prevention strategies.

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