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Chromium and Genomic Stability

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Abstract

Many metals serve as micronutrients which protect against genomic instability. Chromium is most abundant in its trivalent and hexavalent forms. Trivalent chromium has historically been considered an essential element, though recent data indicate that while it can have pharmacological effects and value, it is not essential. There are no data indicating that trivalent chromium promotes genomic stability and, instead may promote genomic instability. Hexavalent chromium is widely accepted as highly toxic and carcinogenic with no nutritional value. Recent data indicate that it causes genomic instability and also has no role in promoting genomic stability.

Keywords

chromium; chromate; trivalent chromium; hexavalent chromium; genomic instability

Introduction

Genome instability is a key characteristic of most forms of cancer [1]. In the majority of cancers, the integrity of chromosome structure and number is compromised [2]. Genomic instability refers to a large range of genomic changes; numerical and structural chromosome abnormalities are generally referred to as chromosome instability (CIN) and events causing replication slippage or impaired mismatch repair which lead to microsatellite instability (MIN). While the link between genomic instability and cancer is well established, the molecular events leading to genomic instability are not.

In recent years, trace elements have become of significant interest in the prevention of cancer and are thought to have a significant role in the maintenance of the genome [3–6]. Trace elements, those in which less than 100 mg is required daily [6], can have major impacts on the genome when present in excess or when deficient. Of particular concern are metals. Excess metal ions can cause oxidative stress and contribute to the presence of free

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radicals; however, many of them are required for the normal function and stability of the cellular environment including the detoxification of radicals. Thus, for most metals, it is important to establish a balance for optimum function to maintain cellular and genomic stability.

Metals such as copper, magnesium and zinc are essential to genome stability and play roles as antioxidant and free radical scavenging agents as well as aiding in DNA synthesis and repair processes [4–6]. Copper serves as a co-factor in sodium oxide dismutatse (SOD), thionein and ceruloplasmin, important antioxidants and radical scavengers [5]. Magnesium is involved in almost all DNA processing events including several forms of DNA repair [4]. Levels of zinc have been shown to be related to such things as redox activity, DNA repair activity and chemically-induced carcinogenesis [6].

Chromium is an element which has been a significant concern in the last few decades given its carcinogenicity and its common occurrence. It is a naturally occurring transition metal most commonly found in the oxidation states Cr(III) and Cr(VI) [7]. The biochemistry of chromium is very complex and contributes to its reactivity and availability in biological systems. In biological systems, Cr(VI) is found as the anion, chromate, which is similar to sulfate and phosphate and is readily transported across the cell membrane [8]. Alternatively, trivalent chromium (Cr(III)) forms large bulky molecules such as ternary complexes with amino acids and proteins in biological systems which are poorly absorbed by cells [9]. Cr(VI) is considered the most potent form of chromium with regards to toxicity and carcinogenicity. Cr(VI) does not directly interact with DNA, rather it is the intracellular reduction of Cr(VI) to Cr(III) which ultimately interacts with and causes the DNA damaging events leading to neoplastic transformation of cells and ultimately cancer [10].

Chromium (III)

Trivalent chromium has historically been considered an essential trace element. Initial studies, performed in the 1950’s suggested a glucose tolerance factor (GTF) containing chromium as the active component, however, subsequent studies have failed to fully isolate and characterize a GTF containing chromium and multiple studies have failed to reproduce the original study [11,12]. A handful of studies have been reported on patients receiving total parenteral nutrition (TPN) and lead to the adoption of chromium as an essential nutrient. To summarize, patients on TPN developed symptoms of glucose intolerance which when given Cr supplementation, were reversed. However, flaws in these studies including Cr contamination in TPN solution, lack of data regarding serum levels of Cr before or after supplementation, inconsistent times on non-supplemented TPN between patients, and large variation in onset of symptoms, in addition to small sample size of unhealthy patients, probably lead to an over interpretation of the contribution of Cr [12,13]. In addition, prior to 1980, accurate measurements of chromium in biological material were not valid due to poor detection methods [11]. To date there is no clinically described chromium deficient disease state for humans and research in the area of chromium essentiality is still widely debated [13,14].

Although the essentiality of Cr as a nutrient is now in doubt, evidence does show that Cr can have a therapeutic or pharmacologic effect in certain clinical populations. A meta-analysis of 41 Cr supplementation studies suggested that supplementation specifically benefitted patients with diabetes [15]. One study observed that Cr supplementation in individuals with type 2 diabetes had inconsistent effects across phenotypes but showed significant effects specifically in patients classified as insulin resistant [16]. However, additional studies [17,18] reported no beneficial effect of Cr supplementation for patients with metabolic syndrome, impaired glucose tolerance, or type 2 diabetes. Thus, the studies investigating the beneficial effects of chromium supplementation are still controversial and observed are restricted to very specific health compromised individuals. No beneficial pharmacologic effects have been documented in normal healthy individuals.

The basic biochemistry of Cr(III) has been well described. Studies in cell free systems [19,20] characterized Cr(III)'s role in showing that it binds to DNA leading to a decrease in the fidelity and an increase in the processivity of DNA polymerases which may ultimately lead to increased mutations. A review of Cr(III) effects in cell-free systems shows positive results for a variety genotoxic outcomes including increased mutations [14]. Although Cr(III) is likely the ultimate reactant with DNA, the absorption of Cr(III) across the cell membrane is poor, and thus, studies in cell culture are difficult. Most of these studies have been conducted with less active inorganic salts which are poorly absorbed and require non-relevant treatment concentrations. In addition, studies considering the effect of inorganic Cr(III) *in vivo* are largely negative with regard to genotoxic outcomes [14]. Studies of tannery workers with long term exposures to inorganic chromium do show evidence of genotoxic effects including chromosomal aberrations, micronuclei formation and DNA breaks [21–23]. Additionally, epidemiology studies have shown that workers exposed to inorganic Cr(III) have an increased potential risk for developing cancer [24]. These studies suggest that long term exposure is key in the contribution of Cr(III)-induced genotoxicity. In a more recent study, the long term effects of physiologically relevant concentrations of Cr(III) exposure in patients with CoCr hip implants were considered [25]. In this study, fibroblast cells were treated with levels of chromium equivalent to those measured in patients with both well functioning and worn implants and measured over time for chromosome stability. Cr(III) treated cells exhibited both numerical and structural chromosome instability. Indeed numerical and structural chromosome instability has recently been proposed as a novel mechanism for metal-induced carcinogenesis [26,27].

With the development of more bioavailable forms of Cr(III), such as chromium picolinate and chromium nicotinate, widely used in nutritional and pharmacological applications, the potential for toxicity has needed to be revisited. Cell culture studies have shown that Cr picolinate is able to damage DNA [28, 29], cause chromosomal aberrations [30], and induce mutations [31,32] at physiologically relevant doses. One study was negative for induction of chromosome damage by Cr picolinate, however, treatment times were limited to only 4 hours [33]. A study investigating the metabolic fate of Cr(III) organic complexes suggests that high levels of intracellular Cr(III) can accumulate [34] leading to the formation of Cr-DNA adducts and potentially causing genotoxic effects. In addition, recent developments in the ability to detect chemical speciation in biological media have revealed that many Cr(III)

compounds can be oxidized in extracellular fluids leading to more efficient cellular uptake and DNA damage [35]. Animal studies provide further insight into the potential damage that Cr(III) may cause to the genome. The NTP (National Toxicology Program) recently reported equivocal findings of carcinogenicity in a 2 year rat and mice study of Cr picolinate, with adrenal cancers developing in male rats [36]. One study reported no chromosome damage found in bone marrow of rats treated with chromium picolinate; however, this was in a single 24 h oral exposure tested after 18 and 42 h [37]. There are no long term studies of organic Cr(III) that are comparable to the worker studies of inorganic Cr(III); the human studies of Cr(III) supplementation are focused on effects of glucose metabolism and do not measure genotoxicity. Overall the more bioavailable forms of Cr(III) have the potential to produce reactive oxygen species which can have profound consequences on its ability to react with DNA causing mutations and chromosome breakage leading to genomic instability and potentially carcinogenic effects [35,38].

While the field of Cr(III) research is wrought with controversy with respect to both its essentiality and its toxicity, there is no evidence of a role for Cr(III) with respect to maintaining genomic stability. Though all of the Cr(III) studies are heavily criticized by opposing viewpoints, from the studies investigated to date, Cr(III) does not play a role in DNA repair or its synthesis and it does not aid in free radical scavenging similar to other trace metals, such as zinc and copper.

Chromium (VI)

Hexavalent chromium has long been known to be a human respiratory carcinogen. The mechanisms for how chromium induces cancer are still being elucidated. The traditional view is that Cr(VI) acts by inducing mutations in the DNA sequence. This view is largely based on mammalian cell culture studies. For example, numerous studies used a shuttle-vector mutagenesis system [39–42]. This system involved treating an SV-40 based plasmid in a cell-free system and then transfecting the damaged plasmid into cells. These studies report an increase in mutations after exposure to 10–200 uM soluble Cr(VI).

Other cell culture studies considered mutagenesis with all components inside cells including systems with reporter genes (e.g. the bacterial *gpt* reporter gene) or an endogenous locus (e.g. the hprt locus) [43–45]. These studies also found an increase in mutagenesis though the toxicity of the doses used were high and the mutation frequency was low (i.e. 0–3.5%). The one exception was a study that preloaded cells with 1.4 mM ascorbate and found a 19.2-fold increase after exposure to 40 uM Cr(VI) and no mutations without ascorbate. These data suggest that ascorbate levels may be an important factor for Cr(VI)-induced mutagenesis.

The Cr(VI)-ascorbate studies argue that physiologically relevant levels of ascorbate are typically greater than 1 mM and, thus, one needs to supplement with ascorbate to address this factor [41,42,44]. However, while mM ascorbate levels are consistent with levels found in freshly purified human lymphocytes [44], they are dramatically higher than those seen in human lung tissue. Specifically, two studies show that in adults, lung tissue ascorbate levels range from 0.045 – 0.065 mg/g which is approximately 256–369 uM ascorbate [46]. A second study reported a range of 2.91–62.35 mg/100 g [47]. This measure can be converted

to a range of 165 uM to 3.5 mM. Recent data show that the methods used by these authors tend to overestimate ascorbate levels by a factor of 3 so that these ranges are more accurately 55 uM -1.2 mM. Thus, it would appear that 1.4 mM is above the normal range making it unclear how much of an issue ascorbate is. It is also notable that the study of ascorbate found no mutations at the hprt locus in hamster cells when ascorbate was absent, while another study did find mutations in the absence of ascorbate in this system indicating significant interlaboratory variation in results.

One study considered Cr(VI) mutagenesis in the Big Blue transgenic mouse [48]. Mice were exposed via intratracheal instillation and showed an increased mutation frequency in the lung. The challenge in interpreting these data are that no measures of inflammation in these animals were taken and Cr(VI) is a known irritant. Thus, it is unclear if the mutations are due to Cr(VI) directly or secondary to Cr(VI)-induced inflammation

Considered together, one could argue that Cr(VI) induces mutations. On the other hand, the data also suggest that such events occur only under conditions of high dose, high toxicity or experimentally contrived systems such as the shuttle vector approach. Interestingly, the data for Cr(VI)-induced tumors in workers show few mutations further supporting a conclusion that Cr(VI) is only weakly or indirectly mutagenic.

A newer mechanism that has been proposed lies in the ability of chromium to destabilize the genome [49]. Major causes of genomic instability include the induction of microsatellite instability, defective DNA repair, and the induction of both structural and numerical chromosome defects. Here, we discuss the mechanism of genomic instability with respect to chromium in order to emphasize the fact that chromium promotes genomic instability as opposed to providing protection.

Genomic instability is a common event in lung cancers with both microsatellite instability (MIN) and chromosome instability (CIN) occurring simultaneously. As reviewed by Holmes et al [49], Cr(VI)-induced tumors are no exception and are characterized by both MIN and CIN. MIN and reduced expression of both hMLH1 and hMLH2, key mismatch repair (MMR) genes, were reported in Cr(VI)-induced tumors [50]. However, MIN is considered to occur in cells when they are deficient in MMR. Thus, MIN may play a role in the development of tumors but, only after MMR deficiency has developed. Thus, it is currently unclear if MIN is a driving factor or a consequence of other changes in the genome.

CIN includes both structural and numerical chromosome abnormalities. CIN has been reported in Cr(VI)-induced tumors evidenced by an increase in the loss of heterozygosity consistent with cell culture studies that show a profound and consistent effect on chromosome structure in cultured cells treated with Cr(VI) [49]. Numerical abnormalities, have not been assessed in Cr(VI)-induced tumors, but, multiple studies show that Cr(VI) dramatically alters chromosome number in cultured cells treated with Cr(VI) [26].

Epidemiology, animal and cell culture studies show that the particulate form of chromate is most potent with regards to carcinogenicity [51,52]. A mechanism for particulate Cr(VI)-induced CIN has been proposed and supported [49]. Once the particle dissolves, the ability of Cr(VI) to cross the cell membrane using channel proteins aids in its rapid transport into

the cell [52–55]. Once Cr(VI) enters the cell, it is rapidly reduced to Cr(III) which forms stable complexes with intracellular structures and accumulates within the cell [8]. It is able to form DNA adduct-based lesions, which lead to stalled replication forks and ultimately double strand breaks [39–42,44,45]. These DSBs overwhelm the repair systems of the cells which then go unrepaired or are mis-repaired leading to translocations and other structural aberrations [56,57]. The accumulation of DSBs also causes a G2 arrest leading to centrosome amplification and spindle assembly checkpoint bypass which lead to aneuploidy [58,59].

In addition to the occupational studies of chromate workers, recent studies of patients with cobalt-chromium metal on metal hip implants have shown increases in CIN measured in their peripheral blood but the consequences of this are unknown [60]. To better understand the impact that chromium may have on these results, Figgett et al [25] investigated the effects of physiologically relevant metal levels on fibroblast cells. They found that cells treated with these physiological concentrations of Cr(VI) induced both structural and numerical chromosome aberrations, including complex aneuploidy.

NTP studies of rats and mice have revealed that long term oral exposure to Cr(VI) leads to higher accumulation in multiple tissues [61]. These long term exposures of Cr(VI) lead to the development of intestinal tumors in mice and in the oral mucosa of rats [62,63]. In addition, a recent epidemiologic study implicated Cr(VI) in drinking water as the cause of liver, lung and urologic cancers [64]. However, the mode of action is unclear for these novel Cr(VI)-induced cancers and more research needs to be done in these specific tissue and cell types [65]. There have also been no investigations performed to determine direct effects on DNA or chromosome status in these tumors.

Cr(VI)-induced DNA damage is repaired by several mechanism depending on the type of damage incurred. Base excision repair, single strand break repair, nucleotide excision repair, mismatch repair, homologous recombination, and crosslink repair have all been studied in the repair of Cr(VI)-induced DNA damage as reviewed in Wise et al [7]. All of the lesions, if unrepaired or mis-repaired, can directly impact chromosome structure and overall genomic integrity leading to carcinogenesis.

Conclusion

As outlined above, metals can have crucial roles in the maintenance of the genome. These roles include antioxidant and free radical scavenging agents, DNA synthesis and repair processes. None of these roles have been elucidated with respect to chromium. In fact, as reviewed here, both Cr(III) and Cr(VI) have shown evidence for quite the opposite. Both Cr(III) and Cr(VI) have been shown to damage DNA and break chromosomes which can lead to genome instability and cancer.

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