Cancer: Cell Structures, Carcinogens and Genomic Instability
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Metal ions and carcinogenesis

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Abstract. Metals are essential for the normal functioning of living organisms. Their uses in biological systems are varied, but are frequently associated with sites of critical protein function, such as zinc finger motifs and electron or oxygen carriers. These functions only require essential metals in minute amounts, hence they are termed trace metals. Other metals are, however, less beneficial, owing to their ability to promote a wide variety of deleterious health effects, including cancer. Metals such as arsenic, for example, can produce a variety of diseases ranging from keratosis of the palms and feet to cancers in multiple target organs. The nature and type of metal-induced pathologies appear to be dependent on the concentration, speciation, and length of exposure. Unfortunately, human contact with metals is an inescapable consequence of human life, with exposures occurring from both occupational and environmental sources. A uniform mechanism of action for all harmful metals is unlikely, if not implausible, given the diverse chemical properties of each metal. In this chapter we will review the mechanisms of carcinogenesis of arsenic, cadmium, chromium, and nickel, the four known carcinogenic metals that are best understood. The key areas of speciation, bioavailability, and mechanisms of action are discussed with particular reference to the role of metals in alteration of gene expression and maintenance of genomic integrity.

Key words: Arsenic, cadmium, carcinogenesis, chromium, nickel, oxidative stress.

Introduction

The association of metal exposure with cancer is a well-documented phenomenon. Metals such as arsenic (As), cadmium (Cd), chromium (Cr), and nickel (Ni) are part of an ever growing list of environmental agents that have been formally classified by the International Agency for Cancer Research (IARC) as being known carcinogens [1–4]. For other metals such as iron, copper, beryllium, lead, and mercury there exists an ever increasing body of evidence to support their inclusion in the IARC listings [5–8]. Iron [8] and copper [7], in particular, are carcinogenic in excess, but are highly regulated and generally only produce cancer in animal models or in people with genetic diseases that prevent appropriate metabolic regulation. There is even less information on beryllium carcinogenesis, and no definitive studies that indicate the species, conditions or length of exposure by which lead and mercury metals cause cancer in humans. For these reasons, this review will focus on the known carcinogenic metals: As, Cd, Cr and Ni.

Despite increasing numbers of researchers in the field and the expanding role of metals in environmental health issues, the nature of cancer induction by

metals remains a complex and poorly understood process. However, what is known is that metals can promote change in normal cellular functions, leading to aberrant cell growth and development [6]. All metals are now thought to promote cancer by a number of common mechanisms. These include the formation of free radicals, either actively as key players in redox reactions, or through less direct means such as biomethylation [5, 6, 9, 10]. Similarly, many metals can also influence cell control by altering gene regulation [7, 11–14]. In terms of direct damage to DNA, most metals are only weakly mutagenic; however, many are strong co-carcinogens, promoting a synergistic effect in the presence of other cancer-causing agents [5, 6, 15]. Hence, the ability of metals to promote cellular alterations may be far more dynamic than has been classically assumed. Thus, it is the purpose of this review to evaluate mechanisms that are central to the role of metals as carcinogenic agents. This review outlines current evidence related to the mechanisms of genotoxicity and gene expression, as well as other mechanisms unique to specific metals. The principle focus is on those metals for which the IARC has deemed there to be sufficient evidence to classify as carcinogens, and that there is the most information regarding genotoxic mechanisms. Because of the great amount of data now available on this topic, this chapter does not claim to be exhaustive, but will hopefully provide a useful survey of the field, with selected references focusing heavily on recent reviews.

Mechanisms of metal carcinogenesis: an overview

The process of carcinogenesis has classically been described as occurring in four stages: initiation, promotion, progression, and metastasis. Initiation is generally thought to be the result of genotoxicity leading to DNA mutation. Cancer initiation by metals most often involves the production of free radicals which can potentially damage DNA [5, 6, 16, 17]. This process can occur by multiple mechanisms, such as redox cycling, metabolism, and the induction of genes producing reactive species. The products of oxidative damage in DNA are frequently single base lesions, most notably the 7,8-dihydro-8-oxo-2'-deoxyguanosine base modification [18, 19]. Of all the metals, chromate salts produce the greatest genotoxic response in the shortest period of time [5, 13, 20, 21]. Chromium salts also exhibit the ability to form mutation-inducing crosslinks between DNA and protein [22]. Iron similarly is highly reactive and readily able to donate or accept electrons from a variety of sources. It is, however, highly regulated, such that it is unlikely that genotoxic affects or adduct formation would occur, except in circumstances where iron overload occurs [8].

Initiation of cancer, however, is not solely the result of point mutations. It may also be caused by DNA strand breaks, which can result in chromosomal rearrangements, or through alterations in DNA repair that reduce the capacity for repair of lesions not associated with the metal. Metal-dependent

changes in transcriptional control of specific genes may also play a role in cancer initiation.

Altered gene expression through the induction of specific signal cascades is most often associated with cancer promotion. Metal salts can alter gene regulation through a number of mechanisms, most frequently by activation of transcription factors or through changes to gene methylation patterns [23–25]. Similarly, signal cascades may be modified by the interaction of metals with any of the steps in the pathway, often through direct binding to receptors or intermediate proteins [10, 13, 14].

Progression of tumors is akin to the point where aberrant control mechanisms in the cell begin to predominate, and is characterized by changes to the cell phenotype and metabolic processes. Metals have strong affects on cells, particularly with regard to redox status. Both chromium and arsenic have been shown to alter redox potential in mammalian cells *in vitro* [26–28]. Changes of this nature can facilitate an increased competitiveness of these types of cells as against their non-tumorigenic counterparts, with greater growth potential and rate.

The final phase of malignant tumorigenesis involves the migration of cancerous cells to other regions of the body. This process normally sees the formation of secondary sites of colonization by cells that have altered cell signaling cascades, phenotypic characteristics and proliferative capacity. Of the metals presented here, cadmium is the only one that has been shown to affect the extracellular matrix of cells [29] by interfering with cadherins, which link cells together, preventing their formation and subsequently enabling cells to move to other sites.

Speciation, uptake and health effects of specific metals

Arsenic

Arsenic has had a long and somewhat chequered history. Unlike many of the other carcinogenic metals, it has been used as a prophylactic agent for around 2500 years. It has been said that Hippocrates used arsenic sulfides to treat ulcers and that arsenic may have been used to treat the plague in the Middle Ages [30]. In the 1800s, arsenic formed the basis of the common medication, Fowler's reagent. This reagent was used for the better part of 200 years to treat anything from the common cold, to asthma and psoriasis [30]. Despite its wide medical use, arsenic was also a highly successful murder agent due to its relatively high toxicity at low doses. Clear, odorless and almost tasteless by nature, arsenic was easy to conceal in food or drink. It has been suggested that Napoleon was killed by an overdose of arsenic [31]. More recently, arsenic has been found to be an effective herbicide, as well as being useful in reducing discoloration in glass and as a preservative of wood. Medically, it has again found favor as an agent in the fight against acute promyelocytic leukemia in individ-

uals who have become resistant to normal therapies [32]. Occupational exposure to arsenic is greatest in mining and metal smelting industries; however, it can also occur through glass manufacturing and as the result of coal burning for power production [33]. But the greatest extent of exposure is from arsenic-contaminated water sources.

Arsenic is normally found in close association with heavy metals such as gold, copper and silver. Mining of these heavy metals brings arsenic to the surface where it is concentrated through the refining processes [34]. Arsenic can also be brought to the surface when it is leached from the rock surrounding underground aquifers. It is in this circumstance that arsenic has had its most profound effects on human health. Since the 1980s countries such as Bangladesh, India, and China, where surface water is frequently contaminated with microbial pathogens, have invested heavily in alternate water sources that are now known to be heavily contaminated with arsenic [34, 35]. Concentrations of arsenic in these water sources vary wildly; however, in many regions they exceed by 10 to 15 times the current World Health Organization's (WHO) recommended level of 10 ppm [36]. Even at these levels, arsenic is not acutely toxic. However, as early as 1968, similar high levels of arsenic in the artesian well water in regions of southern Taiwan were recognized as a likely cause of carcinogenesis [37]. Increased cancer rates associated with arsenic-contaminated drinking water have now been recorded in many countries, including Taiwan, Argentina, Chile, and Mexico. The arsenic-associated cancer incidence in Bangladesh and West Bengal, India is expected to reach catastrophic levels over the next several decades [38, 39].

Arsenic in the environment can take a range of forms, both organic and inorganic. Inorganic arsenic has two possible valencies, arsenite, or As(III) and arsenate, As(V). Arsenite is the more toxic of the two species with cell viability assays indicating that concentrations anywhere from 1 to 10 μM and upwards are able to promote toxicity [5]. Arsenate is approximately three to fivefold less toxic than arsenite, presumably because As(V) requires reduction to As(III) to exert its toxicity. Organoarsenic species can also be formed by biometabolism. Many organoarsenic species are significantly less toxic than inorganic As(III). However, methyl As(III) species can be significantly more toxic than inorganic As(III) [40] and may contribute to arsenic carcinogenesis. The relative toxicity of the different forms of arsenic is predominantly the result of their different chemical properties, but may also relate to the relative efficiency of their uptake [41, 42], the duration of the exposure, and the time when the toxicity assay is performed [42, 43]. Arsenic excretion rates vary, but it is generally accepted that arsenic, unlike other carcinogenic metals, is rapidly excreted by the body, to the extent that more than 50% is removed within 2 days in acute poisoning cases [33].

Organic metabolites of arsenic that are of most interest are the monomethyl and dimethyl species of both arsenite and arsenate. These species are generated through biomethylation of inorganic arsenic, followed by reduction and subsequent methylation of monomethyl As(III) to produce dimethyl As(V)

(DMA). The intermediate methylated As(III) species are thought to be considerably more toxic than either methyl As(V) species or even inorganic As(III) species [40, 44]. However, the levels of available organic As(III) species in human tissues relative to other arsenic species are still largely undetermined.

Other organoarsenic species such as arsenobetaine and arsenosugars are commonly found in marine species. Although arsenobetaine is often found in high concentration in marine animals, it is largely excreted unmetabolized, and has very low toxicity [45]. Arsenosugars are frequently found in seaweed and in crustaceans [46]. Recent evidence suggests that these compounds may be metabolized to DMA, which can then be further metabolized to more toxic species [46]. This raises the possibility that consumption of seafoods can be a source of considerable arsenic intake, some of which may result in the retention of some relatively toxic arsenic species. This may have implications for Asian populations, particularly those which live on high fish diets, such as the Japanese [46].

Arsenic pathology is complex. When ingested at very high doses, in excess of 200 mg, it produces acute toxicity characterized by nausea, vomiting, sloughing off of epithelial tissues, internal bleeding, changes to blood pressure, and atrial fibrillation [33]. This can lead to heart attack, coma, and death. At sublethal doses arsenic ingestion can be treated with a range of metal chelators, which reduce its effects; however, few if any other treatments for arsenic ingestion exist. At very low doses, arsenic appears to have minimal short-term effect however, over longer periods a range of pathologies are seen [2]. Chronic low-dose exposure initially produces blotching of the skin, followed by hyperkeratosis of the palms and soles of the feet. If exposure continues, alterations to peripheral vasculature are seen along with the formation of skin lesions, which left untreated, can become cancerous [47, 48]. Arsenic is also associated with an increased risk for cancer of the lungs, liver and bladder [47].

Induction of cancer by arsenic is not thought to originate from a single exposure, but rather is the result of gradual changes to a variety of processes within the cell. Different arsenic species enter cells by different mechanisms. Arsenate is able to mimic phosphate, and hence is able to enter cells using phosphate transport proteins. Arsenite, however, is thought to enter through aquaglyceroporins [49]. Once in the blood stream, arsenic is taken to the liver where biometabolism occurs. This process involves the progressive methylation of arsenic, with As(III) converted to the less toxic methyl As(V) species. The ingested arsenic is excreted predominantly in the urine as inorganic As(III) and As(V), methyl As(V), and dimethyl As(V), with the proportions of these being variable and related to arsenic dose [50–52]. Some intermediate trivalent arsenic metabolites are also produced, and can be found in the urine [53]. Despite their greater toxicity, relative to either inorganic As or organic As(V) species, it is yet to be determined whether these methyl As(III) and dimethyl As(III) species play a significant role in carcinogenesis.

Cadmium

Unlike many other metals, cadmium is found in only one valence state, that of Cd(II). Exposure to cadmium has also been far less common than other carcinogenic metals. Of greatest note was the historical use of cadmium as a paint additive giving rise to the bright yellows seen in many paintings, such as those of Claude Monet [54]. Industrial use of cadmium is only a recent phenomenon, beginning in the 1940s. Cadmium is now most commonly encountered in cadmium-nickel battery production [10], although it continues to be used in paints, as well as in plastic production where it is an effective stabilizing agent. Like arsenic, occupational exposure to cadmium can occur through metal refining processes, where cadmium is often associated with copper and can be released into the atmosphere during heating [55]. The greatest exposure to cadmium, however, comes from cigarette smoke [10]. Particulate cadmium in cigarette smoke collects in the lungs where it can be transported into the bloodstream across the alveoli. Unlike arsenic, cadmium has a long biological half life, considered to be somewhere between 15 and 25 years [4, 56]. This means cadmium can accumulate to levels many times greater than an individual would be subjected to in a single exposure. Cadmium is only a weak mutagen, but is a strong co-mutagen [4, 57, 58]. This is of particular concern for cigarette smokers who simultaneously inhale cadmium and benzo[a]pyrene, as well as a range of other chemicals, including arsenic and other metals.

Health effects of cadmium are quite dissimilar to other metals. Non-toxic doses of cadmium produce a wide variety of effects, many of which are related to bone development and maintenance. Individuals exposed to cadmium can develop osteoporosis, anemia, eosinophilia, emphysema, and renal tubular damage [59]. Long-term cadmium toxicity can produce Itai-Itai disease, in which individuals suffer from bone fractures, severe pain, proteinuria and severe osteomalacia [59]. Acute high-level exposure to cadmium is also able to produce severe lung damage. However, like other metals, prolonged repeated exposures are required to induce carcinogenesis. Target organs for cadmium are varied however, lung cancers predominate [4]. Other tissues subject to malignant transformation by cadmium include the prostate, pancreas and kidney. The testes are also thought to be a site of cadmium carcinogenesis; however, this has only been shown in animal models. Like arsenic, cadmium is only a weak mutagen. This suggests that tumors result from either epigenetic or co-carcinogenic effects, particularly in cases of smoking-induced lung cancer [10].

Chromium

Chromium is widely available, complex in action, and used industrially in a myriad of applications including, pigment production, chrome plating, welding, production of ferrochrome metals, leather tanning and as a dietary sup-

plement [3, 60]. Dietary supplementation is of particular interest because of the critical nature of Cr(III) for optimum insulin binding [61]. Occupational exposure to Cr(VI) is a well-established source of human carcinogenesis; however, occupational health initiatives have had a considerable impact in reducing incidence levels. Non-occupational sources of exposure are thought to originate from engine emissions, atmospheric particles released from smelting and refining industries, as well as through cigarette smoke [13]. Chromium speciation is complex, and chromium is often found in compounds with other metals. Environmental chromium is generally found in two principle valency states, the more toxic and carcinogenic Cr(VI) [60] and the essential Cr(III). Cr(VI) species are readily taken up into cells by phosphate/sulfate anion channels [62–64]. Cr(III), however, cannot move into cells by the same mechanism, and is required at considerably higher concentrations to produce toxicity in cells. It must be noted, however, that not all Cr(VI) species are of equal carcinogenic risk. Animal models have shown that the largely insoluble chromium compounds are far more carcinogenic than their soluble counterparts [3, 65]. It appears that particulate matter containing insoluble chromium is deposited on the epithelial surface of the lung where it accumulates to levels high enough to produce cancer [66].

The mechanism of chromium carcinogenesis is unclear; however, the complex intracellular redox cycling of chromium is thought to produce a range of reactive species as well as producing DNA-protein crosslinks. Generally, Cr(VI) on entry into cells is rapidly reduced by interaction with any of a number of low molecular weight thiols, from glutathione (GSH) to cysteine, as well as a range of other reductants such as ascorbic acid, hydrogen peroxide, cytochrome P450 reductase and NADPH [13]. Of these, GSH, ascorbic acid and cysteine residues appear to be the most critical. The reduction process itself is thought to occur either by sequential single electron transfers, progressively reducing Cr(VI) to Cr(V) and then Cr(III), or by a two electron transfer to Cr(IV) then by single electron transfer to Cr(III) [13, 16, 67]. These reactions can produce a variety of other reactive intermediates and provide the mechanism for crosslinking of DNA to proteins by means of a bifunctional Cr(III) intermediate. Both the oxidative DNA damage caused by redox reactive intermediates and, more importantly, the Cr(III)-mediated DNA-protein crosslinks [22] can cause mutations, thereby initiating the process of carcinogenesis. Similarly, it is possible that the interaction of reactive species may also alter cell signaling pathways causing alterations in gene regulation [13].

Although Cr(III)-DNA adducts generated by reduction of Cr(VI) are known to be mutagenic, it is often believed that Cr(III) compounds are non-toxic and, in fact, Cr(III) is promoted as a highly beneficial dietary additive. However, some forms of Cr(III) are known to be capable of producing DNA damage *in vitro* [68] and the possibility that excess Cr(III) supplementation might eventually lead to increased cancer risk is seldom acknowledged [69].

Unlike arsenic and cadmium, chromium is an essential trace element in its trivalent form. That said, Cr(VI) species can be highly toxic to humans [13].

Inhalation of particulate Cr(VI) can cause irritation to the nasal tissue, leading to nose bleeds, ulceration and formation of lesions in the nasal passage [60]. Damage to lung tissue is also not uncommon [70, 71]. Ingestion of Cr(VI) can cause nausea, vomiting, ulceration of the stomach, damage to the liver and kidney, and finally death [60]. Both species of chromium can cause contact hypersensitivity, leading to rashes, swelling and ulcerations. Cr(VI) is the most carcinogenic form of chromium, with insoluble particulate chromium compounds being the most persistent [66] and the most hazardous [72].

Nickel

Nickel has many common industrial uses, thanks largely to its unique chemical properties. Industrially, it is used in electroplating, electroforming, in circuitry, and in nickel-cadmium batteries. Nickel alloys, including stainless steel, are used in a wide variety of objects, from kitchen knives to building tools [73]. Nickel is also used in jewelry and medical implements. Metallic nickel is non-carcinogenic to humans; however, all other nickel compounds, such as nickel sulfides, oxides, and silicates, and other soluble salts, are known carcinogens [12]. Carcinogenic nickel exposure is greatest through the inhalation of nickel-containing particulates. The burning of fossil fuels, as well as the refining of metals such as copper, introduces considerable amounts of nickel into the atmosphere [12]. Like arsenic, nickel can also be leached from soils and rock, thereby contaminating water supplies. In lower organisms such as bacteria, nickel is an essential trace element found in up to seven different enzymes [74]. Higher organisms, however, have failed to show any definitive role for nickel in normal cellular function. That said, studies in the 1970s and 80s showed that the removal of nickel from the diet of rats had significant effects both physically and mentally, which, with continued exclusion of nickel from the diet, were more profound in the subsequent generations [75]. It may be that nickel is not required for normal cellular function in humans, but rather is essential for our intestinal microflora. Like both arsenic and chromium, nickel occurs in different oxidation states, ranging from I to IV, with Ni(II) being most common in biological systems.

As with chromium, particulate nickel is most harmful to humans, especially in the lung where crystalline nickel becomes lodged in the mucous prior to being phagocytized by both epithelial cells and macrophages [76]. Once inside the cells, the nickel compounds are gradually broken down releasing reactive nickel ions. The phagocytic nature of nickel uptake means considerable amounts of nickel are able to accumulate over time, damaging lung tissue and frequently causing latent effects in individuals who may have been exposed to nickel many years earlier [76].

Nickel is not overly toxic to individuals at low doses; however, nickel-containing jewelry can produce contact hypersensitivity in many people [73]. This normally results in rashes and inflammation of the region of contact. However,

in more extreme reactions, individuals can suffer from asthma attacks. Individuals who inhale nickel fumes for prolonged periods of time frequently develop bronchitis and chronic lung infections. While ingestion of large quantities of nickel is not normally fatal, it can produce stomach aches, kidney pain and blood in the urine [73]. Nickel carcinogenesis is generally limited to the lung, because phagocytosis is necessary to bring the nickel ions to the DNA in the target tissue [12, 77].

Metals and oxidative stress

Most, if not all, of the carcinogenic metals, have the capacity to produce a variety of radical species that can damage cells. Arsenic, chromium, copper, iron, nickel and, to a lesser extent, cadmium, have all been shown to be able to participate in reactions resulting in the formation of reactive oxygen, sulfur or nitrogen species (for reviews see [6, 7, 11, 14, 27, 76–79]). In most cases these metals produce either radicals based on oxygen species or those based on nitrogen species; however, the formation of oxygen species appears to predominate. The formation of radical species can originate from a variety of sources, from redox cycling, through Fenton/Haber-Weiss chemistry, as products of biometabolism, as messengers in signal cascades, and as normal products of cellular metabolism [6, 80, 81]. Essential transition metals, such as iron and copper, are most likely to participate in redox cycling and Fenton/Haber-Weiss chemistry; however, these metals are highly regulated and are of less concern with regard to carcinogenesis. Nevertheless, other carcinogenic metals may also react in similar fashion and thereby produce reactive species that can cause DNA damage and mutations. Some of the key reactions responsible for the metal-related formation of reactive oxygen species (ROS) are described briefly below.

The production of reactive oxygen species

Superoxide ($O_2^{\cdot -}$) was first shown to be produced in phagocytic cells by membrane-bound NADPH oxidase [82, 83]. More recently, it has been observed that epithelial and endothelial cells also express NADPH oxidase [84, 85]. Phagocytic cells produce large concentrations of $O_2^{\cdot -}$ as a killing agent. However, in most non-phagocytic cells, superoxide is primarily formed as a byproduct of mitochondrial metabolism [86], although it is also used as an intracellular messenger in signal cascades [87, 88]. Recent data suggest that at least some of the ROS induced by both arsenic [85, 89, 90] and chromium [91] at low doses is due to activation of NADPH oxidase. This is in direct contrast to earlier theories that assumed that the majority of metal-induced ROS were the result of direct metal-catalyzed redox reactions. Because of its reactivity, the level of $O_2^{\cdot -}$ in cells is normally tightly regulated by superoxide dismutase

(SOD), thereby producing the less reactive, but more mobile, H_2O_2 [92]. Like $O_2^{\cdot-}$, H_2O_2, is also tightly regulated by a multiplicity of catalase and peroxidase enzymes.

Production of ROS by arsenic

The formation of ROS by arsenic is considered one of the most probable mechanisms of arsenic carcinogenesis [79, 93]. However, unlike iron and copper, arsenic does not actively participate in the generation of ROS by conventional processes, such as the Fenton reaction [94]. Importantly, methyl metabolites of arsenic can be more reactive and capable of producing ROS than inorganic arsenic [44, 95]. DMA(V), for example, can be reduced to form either the very reactive DMA(III) [96] or the highly toxic and reactive dimethylarsine gas [95]. Dimethylarsine can react with molecular oxygen to produce both superoxide and dimethylarsenic radicals, which in turn can interact with free transition metals, producing the highly damaging hydroxyl radical [95]. Because DMA(V) appears to specifically target the lung [95], the formation of oxidative DNA damage through the intermediary of DMA(V) and its metabolites may well correlate with the high incidence of lung cancers seen in chronically exposed individuals. The presence of methylated As(III) metabolites in the urine also correlates with increased levels of bladder cancer [96, 97].

Arsenite, through the upregulation of hepatic and renal heme oxygenase, has been shown to release free iron, carbon monoxide and biliverdin from heme, making them available for free radical-generating reactions [93, 98, 99]. The release of bound iron by this mechanism is dependent on the arsenic species, with dimethylarsenite, DMA(III), being the most effective [100]. Thus, the methylated metabolites of arsenic, which are produced almost exclusively in the liver [96, 101], are most capable of producing ROS, such as superoxide, hydroxyl radicals, singlet oxygen and H_2O_2. Although the skin, lungs, and bladder seem to be the primary targets for arsenic carcinogenesis, increased levels of liver cancer have been reported in chronically exposed populations, as well as in experimental animal models [25, 35, 102].

Inorganic arsenic species can also produce ROS in non-hepatic mammalian cells. A number of groups have reported the production of ROS using the DCF fluorescence assay [103–105]. Similarly, Shi et al. [106] have shown that arsenite is able to produce superoxide species in keratinocyte cells; however, significant amounts of superoxide were only detected at concentrations that promote apoptosis. Indirect evidence to support the formation of ROS has also been reported. Various studies have shown that arsenic promotes the upregulation of GSH and antioxidants [107–110]. Similarly, depletion of GSH results in an increase in the toxic and clastogenic effects of arsenic [111]. Biomarkers for oxidative stress, such as 8-oxo-dG, have also been shown to be increased after exposure to arsenic in mammalian cell culture and human tissues [44,

103, 109, 112, 113]. In myeloid leukemia (NB4) and epithelial cells, arsenic treatment at low doses has been shown to induce NADPH oxidase [85, 90]. Recent data show that arsenic can also activate NADPH oxidase in endothelial cells [85, 89].

In addition to ROS, nitrogen-based radicals, such as nitric oxide and peroxynitrite, have also been implicated in oxidative damage by arsenic. The formation of micronuclei and induction of poly(ADP-ribosylation) in Chinese hamster ovary (CHO) cells and bovine endothelial cells and the formation of oxidative DNA damage [measured by cleavage with formamidopyrimidine-DNA glycosylase (Fpg) enzyme] have all been shown to be effectively blocked by the addition of inhibitors of nitric oxide synthase, suggesting that these radicals may account for some of the damage seen in cells [114, 115]. In all, the formation of radical species by arsenic appears to be an important mechanism by which arsenic may promote its carcinogenic effects.

Chromium

Chromium, like arsenic, has been shown to produce oxidative stress in cells by multiple mechanisms; however, the extent to which these are able to produce cancer is still subject to debate. As mentioned above, Cr(VI) can undergo a series of reductions leading to the formation of Cr(III). Chromium(VI) is a strong oxidizing agent and, like copper and iron, can produce ROS directly through Fenton type chemistry, whereby Cr(VI), or one of its metabolites, is able to interact with H_2O_2 in the presence of a reductant to produce both superoxide and hydroxyl radicals [116–119]. However, it is not only the ROS produced by the reduction of chromium species that can produce oxidative damage in cells, there is a growing body of evidence to suggest that the genotoxicity of chromium can be caused in part by the reactive chromium species themselves, such as Cr(V) [120]. O'Brien et al. [13] have raised the possibility that these species may in fact be the direct cause of the oxidative stress response measured by DCFH and rhodamine 123. Even the use of ROS scavengers is not sufficient to rule out this possibility, since these scavanges can also react directly with Cr(V) to prevent DNA damage [121, 122]. It must be noted, however, that the formation of radicals by this mechanism has only been shown to occur when both chromium and H_2O_2 were present at concentrations that are unlikely to be physiologically achievable within cells.

Like most metals that have the capacity to undergo redox reactions, chromium has been shown to deplete intracellular GSH and alter the regulation of the redox enzymes such as catalase and SOD [123–125]. Glutathione has shown to be a critical factor in the reduction of Cr(VI) to Cr(III). The relationship between chromium-induced oxidative stress, DNA damage and repair processes, and apoptotic cell death are complex [13, 22]. Moreover, the relationship between these processes and the induction of cancer is far from well understood.

Cadmium

In contrast to chromium, cadmium has been shown not to have any capacity to produce free radical species by Fenton type chemistry [10, 126]. However, cadmium is able to promote oxidative stress in a variety of model systems via the formation of superoxide and H_2O_2 radicals [127–129]. Indirect evidence in support of free radical generation in cells is also abundant. Studies of cell culture, rat and mouse models all show a general downregulation of GSH and thioredoxin reductase, as well as expression changes in radical converting enzymes such as SOD [10, 130, 131]. This suggests that cadmium may not produce significant free radical species by itself, but rather prevents the normal regulation of radicals produced by other agents and metabolic processes of the cell [132]. Similarly, it appears that cadmium may be able to induce the release of iron from its bound state in proteins and biological membranes [133, 134]. The release of iron would then provide a catalyst for ROS production through Fenton/Haber Weiss chemistry.

Nickel

Unlike either arsenic or chromium, nickel is not readily metabolized by cells and, therefore, does not have the capacity to produce radicals by this mechanism. However, nickel is able to produce ROS by redox cycling and other less direct mechanisms. Soluble nickel particles exist in cells in two states, either as Ni(II) or Ni(III). Nickel has the capacity to bind to amino acid residues and can subsequently undergo redox cycling reactions between these two states in the presence of molecular oxygen and H_2O_2. These processes produce a variety of radicals including $OH^{\bullet}$, carbon- and sulfur-centered radicals, as well as nickel-based radicals [6, 12, 135, 136]. Direct evidence for the formation of radical species by nickel in CHO, lymphoblast and A549 cells has been shown by a number of groups [24, 137–139]. Likewise, fumes from nickel welding processes have been shown to promote the formation of both radical species and lipid peroxidation of cell membranes [140]. Similarly, 8-oxo-dG and other oxidative base modifications have been generated in DNA through interaction of nickel and H_2O_2, suggesting a capacity for nickel to generate damage by Fenton type reactions [12, 141]. Thus, phagocytosis of particulate nickel compounds such as nickel sulfide and nickel subsulfide and subsequent release of Ni(II) can produce oxidative stress in the lungs and other tissues [12, 24, 142]. Moreover, dissolution of nickel by these processes can occur over extended periods of time, leading to continuous production of radicals within the cell [12], thereby initiating and actively promoting the development of tumors [143]. Nickel has indirectly been shown to effect GSH levels and the levels of key enzymes such as SOD and glutathione peroxidase in both cell and animal models [140, 144–146]. The potential for nickel to generate radical species and oxidative stress by these

mechanisms, forms a likely means to both induce and promote alteration and disregulation in cells.

Mechanisms of metal induced alterations in DNA repair

DNA is a dynamic molecule, constantly under assault from both endogenous and exogenous agents, which can often facilitate mutational changes to its sequence. DNA replication also causes changes in genetic material through the infidelity of replication enzymes, most notably during bypass of DNA lesions. The error rate of replication and repair of endogenous base damage has been shown to lead to the formation of lesions with a frequency of one in every 10^4–10^9 bases per cell per day [147]. To combat this, cells have developed a variety of DNA repair mechanisms. In mammalian cells these repair processes fall within several distinct pathways: mismatch repair (MMR), homologous and non-homologous rejoining, nucleotide excision repair, base excision repair, and direct reversion of damage. Alterations in the regulation and activity of repair processes have been shown to occur through interactions of cells with a variety of agents, including many metals. Interference by metal ions with DNA repair has the capacity to increase the potential for mutations, which then persist in the genome. A major consequence of this is the initiation of carcinogenesis. The following paragraphs outline the repair processes that have been shown to be affected by As, Cr, Cd, and Ni.

Mismatch repair

Spontaneous alteration of DNA bases and mistakes by DNA polymerases are commonly recognized and repaired by the MMR system [148]. The principle role of MMR is to remove nucleotides that have been inadvertently incorporated opposite non-pairing partner bases and to correct the insertion/deletion of bases. These errors normally occur as a byproduct of DNA replication and, if not corrected, can result in either base substitution or frameshift errors [148, 149]. In *E. coli,* the MMR system consists of a number of key proteins, including: MutS, MutL, MutH, DNA polymerases, single-stranded binding proteins, and DNA ligase [150]. Eukaryotes, however, have evolved a more complex system whereby many of these proteins have been duplicated, and now have specific roles in certain parts of the cell, or work only under certain circumstances. The specificity and efficiency of MMR means that defects in these proteins can lead to an accumulation of errors in the genome, producing cancers such as hereditary nonpolyposis colon cancer (HNPCC) [151].

Although MMR plays a significant role in the repair of oxidative DNA damage [152], interactions between carcinogenic metals and the MMR pathway appear to be limited. Currently, cadmium is the only carcinogenic metal shown to interfere with MMR [153]. Physiologically relevant concentrations of cad-

mium, on the order of 5 μM, can inhibit MMR in yeast and extracts from human cells by between 20% and 50% [54, 153]. Inhibition of MMR to this extent can have significant implications for the accumulation of errors in the genome generated by endogenous processes [154].

Nucleotide excision repair

Nucleotide excision repair (NER) is principally concerned with the removal of larger lesions and adducts produced from exogenous sources such as UV light [150, 155, 156]. NER is able to correct a variety of lesion types, including 6–4 photoproducts, cyclobutane pyrimidine dimers (CPDs) and large chemical adducts such as benzo[a]pyrene diolepoxides. Recently, NER has been shown to repair adducts formed by chromium species [157]. The process of NER is complex. At present approximately 30 proteins are known to be involved in NER, with several others thought to be necessary for the repair process [156, 158]. A variety of UV sensitivity disorders, such as xeroderma pigmentosum and Cockayne's syndrome, are associated with defects in the NER pathway, highlighting its importance in genome maintenance [150]. The pathway is divided into two distinct processes: global genomic repair (GGR) and transcription-coupled repair (TCR) [156]. The GGR pathway is mostly concerned with the repair of adducts in non-coding regions and on the non-transcribed strand of the genome, while the TCR pathway deals with damage that inhibits RNA transcription. In addition to the formation of oxidative DNA damage, NER is probably one of the most important cellular targets for carcinogenic metals. Changes to the functioning of the NER pathway have been shown to occur after exposure to As, Cr, and Ni.

Arsenic(III) has been shown to reduce the capacity of a variety of cells to repair UV-induced damage such as thymine dimers [159]. Hartwig et al. [160] have shown that arsenic has an inhibitory effect on both the GGR and TCR pathways, primarily by inhibiting damage recognition, with subsequent inhibition of ligation at higher concentrations. Other studies have reported that the ligation step of NER is specifically affected by arsenic treatment [110, 161–164]. Nickel has also been shown to have inhibitory effects on both the incision and the ligation step of UV-induced DNA damage repair [165, 166].

Although ligation appears to be uniquely sensitive to arsenic, other steps in the NER pathway can also be affected by carcinogenic metals. Hartwig et al. [167, 168] have shown that both cadmium and nickel are able to reduce recognition of UV-induced lesions by the xeroderma pigmentosum group A (XPA) protein. Interestingly, the inhibition of XPA binding by nickel or cadmium can be partly reversed by the addition of zinc, suggesting that nickel and cadmium can substitute for zinc in the DNA-binding domain of the protein [168, 169]. Presumably as a consequence of this inhibition of damage recognition, both nickel oxide and nickel chloride are capable of impairing the repair of benzo[a]pyrene adducts in lung cells [170]. Similar results have been shown in

NER proficient human cells in which nickel treatment reduced repair and increased mutagenesis of benzo[a]pyrene adducts [171].

Base excision repair

Base excision repair (BER), a simpler process than NER, is the primary mechanism for the repair of endogenous damage produced by ROS and small adducts, such as methyl groups. As a consequence, this pathway is critically important with regard to maintaining genome integrity, especially with regard to metal carcinogenesis. In the BER pathway, damage recognition begins with a series of damage-specific glycosylases, each of which recognizes and excises a single class of damaged or modified bases, such as oxidized purines (OGG1 or Fapy glycosylases) or pyrimidines (e.g., NEI-l and -2 glycosylases [172]) producing either an apurinic/apyrimidinic (AP) site or an abasic site plus a single strand break (having associated lyase activity) [156, 173–175]. AP endonuclease is responsible for the cleavage of the backbone for those glycosylases that do not have intrinsic lyase activity. From the point of nucleotide insertion, the BER pathway is divided into two different sub-pathways depending on the original damage type. After cleavage leaving a free 3'-OH, DNA polymerase β excises the abasic sugar on the 5'-side of the break and inserts a single correct nucleotide [149, 176].

In cases where the AP site is unsuitable for a single nucleotide replacement, polymerase β dissociates from the damage site and a PCNA-dependant long-patch repair complex takes over [177]. In this instance, up to 10 nucleotides adjacent to the site of damage are removed and replaced. Closure of the phosphodiester backbone then occurs via either DNA ligase I or a ligase III/XRCC1 complex [178, 179].

Despite the importance of this pathway with regard to repair of oxidative damage, studies into the effects of metals on BER are limited. Of the carcinogenic metals, it is arsenic that appears to have the greatest effect on this pathway. BER activity has long been known to be inhibited by arsenic. It was first noted by Li and Rossman [161] and later Lynn et al. [163], who showed that CHO cells exposed to 5 μM or more As(III) exhibited a reduced capacity to repair methyl methane-sulfonate (MMS)-induced damage, and that this reduced activity could be attributed to a decrease in ligase activity. However, in contrast to the inhibition of DNA damage recognition by nickel and cadmium, the inhibition of DNA repair by arsenic is not due to direct inhibition of the repair proteins [164]. Asmuss et al. [169] have also shown that the activity of the bacterial formamidopyrimidine-DNA glycosylase is unaffected by less than 1 mM As(III) and Ni(II). However, the trivalent methylated metabolites of arsenic do appear to have a dose dependant inhibitory effect on this enzyme [180].

Most evidence to date suggests that inhibition of BER by arsenic is primarily due to downregulation of the repair genes [110, 181, 182]. More recently, it has been discovered that at lower doses (below 1 μM) of arsenic can also

promote a protective (hormetic) effect by upregulating BER genes such as AP endonuclease and polymerase β. However, above 1 μM As(III) these proteins also exhibit downregulation. This is reflected in both mRNA and protein levels in a number of cell types exposed to short-term arsenic treatments [110, 181]. DNA ligase activity and protein levels exhibit a very similar dose response (Sykora and Snow, unpublished). Interestingly, this dose-response pattern has also been observed with telomerase, another enzyme involved in the maintenance of genomic integrity [183, 184]. This pattern of altered gene regulation is not uniform across all cell types, and it is unknown to what extent it occurs *in vivo*, or after periods of chronic exposure.

The effects of cadmium on BER appear to be varied, with studies showing that it can affect a number of major proteins in the pathway [185, 186]. For example, cadmium can inhibit the activities of two critical DNA glycosylases, 8-oxoguanine DNA glycosylase and endonuclease III [186, 187]. Exposure of rats to aerosolized cadmium showed a time- and dose-dependent downregulation of 8-oxoguanine DNA glycosylase mRNA and protein levels in the lung epithelium [188]. *In vitro* studies of AP endonuclease have also shown inhibition by cadmium; however, this occurs at concentrations that are largely cytotoxic to cells [189]. Like arsenic, cadmium appears to be able to inhibit both the insertion of new nucleotides and strand ligation. Evidence for this has been shown in cadmium-treated cells, in which oxidative damage accumulates to a greater extent, and is repaired more slowly than in untreated controls [185]. Reduced rates of repair of oxidative DNA lesions may have long-term mutagenic consequences for metal exposed cells.

The effect of Cr(VI) on the BER pathway appears to be more limited than that of either arsenic or cadmium. Although chromium can inhibit the expression of 8-oxoguanine DNA glycosylase in human cells [190], it has little or no effect on AP endonuclease activity. Nickel has no known role in regulating the BER pathway, and appears to exert its effects exclusively on NER.

Given the critical nature of the BER pathway for the repair of DNA damage created by oxidative stress, it is probable that any downregulation of proteins in the pathway would have serious effects on the cell and could be very important for the ability of metals to produce cancer.

Direct repair

In contrast to other pathways mentioned previously, direct repair is by far the most simple, generally consisting of a single protein which produces chemical reversion of nucleotide damage. The best known of these reactions in mammalian cells is O^6-methylguanine-DNA methyltransferase (MGMT) [150, 156]. Left unrepaired, O^6-methylguanine lesions in DNA can produce large numbers of GC → AT transition mutations [156]. Importantly, arsenic can alter methylation of the promoter region of this gene, downregulating protein expression [101, 191]. Cadmium and nickel have also been shown to alter the

activity of MGMT. Cadmium appears to directly interfere with the MGMT protein itself [192, 193]. Nickel, however, inhibits the pathway indirectly at concentrations above 50 μM [194], possibly by also causing methylation changes in the promoter. Other metals have been shown not to interact with the pathway either directly or indirectly [192].

DNA and protein interactions

The formation of metal complexes with amino acids, proteins and DNA is common in cells. Interactions of this nature have been speculated to have a wide range of consequences, including initiation of signal cascades, constitutive activation or inactivation of enzymes, as well as inhibition of both DNA repair and replication. Arsenic, chromium and nickel all exhibit the capacity to create or become part of a variety of complexes in cells. Cadmium and other metals may also form protein complexes, although the role of these complexes in carcinogenesis is less well understood.

Arsenic

Trivalent arsenic species are well known to bind to protein thiols [195], particularly when the cysteine residues are in close proximity within the protein. Binding of As(III) to critical cysteine residues has been demonstrated to inactivate both the glucocorticoid receptor [196, 197] and the glucose transporter, GLUT4 [195, 198], as well as prevent the activation of NF-κB [199]. Phenylarsine oxide has also been shown to bind a range of proteins including NADPH oxidase, both stimulating and inhibiting ROS production dependant on dose [90, 200].

Cadmium

Beyond the more obvious mechanisms of carcinogenesis, such as increased ROS and altered gene expression, cadmium can also facilitate malignant transformation by altering cell-cell adhesion. Both vascular endothelial cells and transport epithelia rely on cell adhesion complexes to control intercellular transport. A number of key proteins have been identified in these adhesion complexes, including the catenins, connexins, cadherins, and integrins [201–203]. Of particular interest are the cadherins, which appear to be most affected by cadmium [10, 204, 205]. Cadherins are unique cell-cell adhesion proteins that require calcium to facilitate binding. They are coupled to catenins, which in turn link them to actin polymers within cells [201, 206]. It is the E-cadherins, which link epithelial cells that are thought to be the most susceptible to cadmium [205]. E-cadherin is important to cell development and has also been shown

to suppress tumor formation in a range of tissues [204, 206]. The effect of cadmium on cell adhesion was first characterized by a significant loss of tissue integrity that was not initially due to apoptosis [207–209]. Later studies, especially those of Prozialeck et al. [210] showed that cell adhesion and, in particular, the integrity of E-cadherin was an early target of cadmium toxicity. It was also shown that cadmium was able to exert its greatest effects on E-cadherins when calcium levels were low, suggesting that cadmium competes for calcium binding sites [211, 212] The loss of E-cadherins are thought to enhance tumor metastasis, promote toxicity, and promote changes to gene expression profiles through altered β-catenin signaling [204, 206].

Chromium

Complexes formed by chromium are considerably more varied than those of other metals discussed here. The binding of chromium to DNA does not occur with Cr(VI); however, the reduced metabolites of chromium, Cr(III, IV, and V) have all been shown to be reactive towards DNA [213, 214]. Although the structure and efficiency of formation of these chromium-DNA complexes is strongly affected by the reductant involved, such as GSH, ascorbate, or cysteine, most of the resulting adducts seem to be both genotoxic and mutagenic [22]. Binding of chromium species to DNA appears to be preferential for guanine nucleotides, and occurs largely with phosphates in the backbone [22, 215]. The formation of chromium-DNA adducts has a twofold effect, they both inhibit DNA replication and prevent DNA repair, thereby promoting mutagenesis.

Nickel

Nickel shows a strong affinity for histidines and, to a lesser extent, cysteines, and is able to form complexes with a wide variety of proteins [216, 217]. As a result, nickel is frequently used to extract and purify proteins that have been histidine tagged [218, 219]. Proteins that have been shown to bind nickel include: serum albumins, the neuroblastoma-associated tumor suppressor (DAN), and histones [220–222]. Like other metals that form protein complexes, it is thought that nickel interacts with proteins, altering their conformation in such a way that they are no longer able to perform normal cellular functions. Nickel has also been shown to crosslink DNA as the result of oxidation of DNA-associated proteins [12, 223].

Effects on gene regulation: direct and epigenetic changes

Metals have been shown to alter the expression of a great number of genes, too many to cover in detail here. These changes in gene expression are generally

transient, and can be produced or caused by a multitude of different factors. Accordingly, this section looks at a limited number of genes that best illustrate the effects of carcinogenic metals on gene expression. For more detailed information on gene expression, the following recent reviews cover each metal in detail [10, 12–14]. Changes in gene expression are often thought to be the indirect result of signal cascades, DNA methylation changes and ROS; however, metals may also be directly responsible for changes in transcription factor activity.

Epigenetic mechanisms are heritable changes that can impart effects on the regulation of genes without altering the genomic sequence itself. Hypermethylation generally causes genes to be downregulated or effectively switched off, while hypomethylation often results in increased levels of gene expression. A number of agents that induce carcinogenesis, such as X-rays, have been shown to affect cells in this manner [224]. Similarly, nickel, arsenic, and, to a lesser extent, cadmium and chromium, are able to produce extensive alterations in genomic methylation [10, 23, 25, 97, 225–228].

Arsenic

Arsenic can both induce and suppress gene expression, depending on its concentration and the length of exposure. Microarray analyses of gene expression in arsenic-treated cells have identified hundreds of genes, most of which fall within several categories: cellular stress response, cell cycle control, redox regulation, and DNA repair [25, 99, 229, 230]. Different cell types and different treatment conditions can produce different effects on gene expression. In some cases, for example, such as arsenic-induced Bowen's disease, p53 expression is upregulated compared to non-arsenic-related disease controls [231]. Low-dose arsenic also promotes upregulation of p53 in cultured fibroblasts after both acute and longer treatments [232, 233]. In contrast, microarray analysis of normal human keratinocytes exposed to between 0.005 and 5 μM As(III), showed a generalized downregulation of p53 [234]. Transcription factors such as AP-1 and NF-κB are also regulated by arsenic, presumably the result of the activation of signal transduction pathways and the formation of ROS [235–237]. Hu et al. [237] showed that acute low-dose treatments of human fibroblasts with arsenite produced upregulation of both AP-1 and NF-κB expression, while chronic exposures lead to a downregulation of AP-1 and NF-κB. The AP-1 transcription factor is important for the regulation of DNA repair, inflammatory responses and cell growth [238, 239]. The activation of NF-κB can increase the expression of cytokines and growth factors, which may be responsible for tumor promotion [240, 241]. In aortic endothelial cells, it has been shown that acute low-dose arsenic treatments promote nuclear accumulation of NF-κB, similar to results seen in rat lung slices [242, 243]. These changes in transcription factor expression and activation are likely to lead to the observed changes in gene expression, and, more importantly perhaps, the observed inhibition of BER by arsenic.

It has only recently been discovered that arsenic can also modify DNA methylation patterns [244]. Dose-dependent hypermethylation of gene promoters was first noticed in regions of the p53 gene following exposure of cultured cells to either As(III) or As(V) [245]. Similarly, the p53 promoter region was shown to be hypermethylated in basal cell carcinomas (BCCs) from arsenic-exposed individuals relative to BCCs from non exposed patients [246]. In contrast, Zhao et al. [244] showed that chronic treatment of rat liver cells with arsenic caused global hypomethylation of promoters and malignant transformation. This hypomethylation was thought to occur as the result of depletion of *S*-adenosyl-methionine [244]. Changes to methylation patterns induced by arsenic are persistent, destabilizing [247], and have the potential to promote aberrant expression of genes involved in cell development and regulation, leading to cancer induction [25, 97, 226].

Cadmium

Like most metals, cadmium is responsible for alterations in the expression of many genes, including the immediate early response genes, *c-fos, c-jun* and *c-myc*; stress response proteins, such as metallothionein and heat shock proteins; and transcription factors, such as NF-κB [248–251]. Zheng et al. [252] have also shown that the livers of mice treated with 10 μmol/kg $CdCl_2$ exhibit increased expression of c-jun and p53. All of these proteins are believed to be involved in tumor promotion. Immediate early response genes (IEGs) induce mitogenic growth signals causing increased proliferation, particularly of cells that already possess mutations in critical regulatory genes.

Activation of stress response genes in response to changes in the extracellular environment enables cells to both protect themselves against oxidative stress and maintain normal cellular function. Cadmium activates a variety of these genes, the most notable of which is metallothionein. Metallothionein is a cysteine-rich low molecular weight protein, which binds excess heavy metal ions preventing their toxic effects [253]. Differential tissue expression of metallothioneins is thought to be a major reason for the tissue specificity of cadmium carcinogenesis [10, 254]. Cadmium is readily able to induce metallothionein expression in the liver and kidneys, but not in the testes or prostate [253, 255, 256]. Reduced expression of metallothionein in the testes and prostate relative to the liver of rats, correlates with increased levels of tumors and toxicity in these tissues [253]. Similarly, the use of transgenic mice has demonstrated that metallothionein reduces cadmium-induced ROS formation and activation of other genes that protect against oxidative stress [257]. Several key antioxidant genes in cells, most notably SOD and catalase, show reduced levels of expression in response to cadmium treatment [10, 130, 131, 258]. Depression of these enzymes can facilitate an increased build up of ROS, which can cause significant damage to cells.

Cadmium-induced methylation changes are less well characterized than those produced by other metals. However, the effects of DNA methylation changes on cadmium toxicity have long been of interest, since it was shown that methylation of the metallothionein promoter results in decreased expression and increased cadmium toxicity [259]. Subsequently, it has been shown that demethylation of the silenced metallothionein promoter with 5-azacytidine (5-aza-CR) is able to induce cadmium resistance in cells that were previously cadmium sensitive [260]. More recently, it has been shown that cadmium, itself, can induce alterations in DNA methylation patterns [228, 261]. However, unlike arsenic, cadmium appears to interfere with methylation by direct interaction with the DNA binding domains of the DNA methyltransferases [228].

Chromium

Chromium can also induce changes in gene expression due to its ability to produce radical species and oxidative stress. For example, as with arsenic and cadmium, both NF-κB and AP-1 are modulated by chromium exposure, with NF-κB being up regulated, which in turn activates *c-myc* [16, 262, 263]. Microarray studies in various cell cultures and *in vitro* models exposed to low to medium doses of chromium show an increase in a variety of genes, including those of the oxidative stress response, particularly those involved in redox regulation [70, 262, 264, 265]. Chromium species, like nickel species, have also been shown to affect the expression of hypoxia-inducible factor-1 (HIF-1) proteins [266]. Unlike the other metals described here, there is very little evidence to suggest that chromium also produces epigenetic changes, with the exception of a report by Cheng et al. [267] showing transgenerational changes in hormonal control in mice fed a diet supplemented with high levels of Cr(III).

Nickel

Nickel, like the other metals is able to alter the regulation of a variety of genes, including NF-κB [12]. Nickel has also been shown to promote the induction of hypoxia through activation of the transcription factor HIF-1 [268]. Increased levels of HIF-1 correlate with angiogenesis of new vasculature in tumors [269]. Other microarray studies have shown that nickel acetate exposure induces large-scale alterations of gene expression in human lung epithelial cells [270]. Some of the genes most strikingly affected include metallothionein and the heat shock proteins. Similarly, nickel sulfate-induced lung injury in mice showed gene expression patterns representative of both hypoxic and oxidative stress responses [271].

In contrast to the other metals presented in this section, the principle carcinogenic mechanism of nickel appears to be epigenetic in nature (reviewed in [12, 227]). The effects of nickel on DNA methylation were first suggested when it was noted that nickel-immortalized cells could be induced to senesce by demethylation with 5-azacytidine [272]. Since then it has been shown that nickel treatment alters methylation-dependent chromatin condensation [224], causes gene silencing [273], and modifies the activity of DNA methyltransferases [274]. Additionally, when mice are injected with nickel sulfide, the resultant tumors all exhibit hypermethylation of the p16 gene, an important regulator of cell cycle control [275].

More recently, it has been shown that nickel can induce epigenetic changes by both hypoacetylation and localized hypermethylation [276, 277] and that chemical demethylation and deacetylation can reverse gene silencing [278, 279].

Summary

Agents responsible for human carcinogenesis are grossly varied in their properties, and metals are no exception. However, it seems likely that metals share several common means by which to induce cancer. Critically, the most important of these appears to be the generation of oxidative stress and deregulation of key maintenance genes within cells. That said, the nature of the dose of each of these metals, as well as confounding variables required to produce a carcinogenesis, remain at best an unresolved issue. However, with time, and as research progresses, it is likely that a more complete picture will emerge on metal-induced carcinogenesis.

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