

# 3 Metals, Metalloids, and Cancer

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## 3.1 INTRODUCTION: METALS AND METALLOIDS

Mankind has sought out and worked with metals for millennia. The earliest known metalwork dates to approximately 5000 B.C.<sup>1</sup> Metals are ubiquitous in modern society. They form the structures of our buildings and our automobiles, and they are vital components of our computers and appliances. Many chemical reactions, including those used to manufacture products ranging from foods to drugs, are catalyzed by metals. Metals, including iron, chromium, and cobalt, are essential components of our diet.<sup>2</sup> Not all exposures to metal are benign, however. For example, various forms of the metalloid arsenic have been used for centuries as a poison.<sup>3</sup> Exposure to specific forms of metal, especially in the workplace, where workers may be exposed to high concentrations for many hours per day, has been linked to cancers of the lung, skin, and other organs. Because metals do not biodegrade, the level of environmental metal exposure tends to increase over time in areas where they are utilized. Archaeological data indicate that humans today possess levels of skeletal lead and cadmium many times higher than those found in ancient humans.<sup>4</sup> The purpose of this chapter is to discuss metals and metalloids that have been identified as carcinogens in humans via epidemiological studies. Special emphasis will be given to the free-radical and molecular mechanisms by which metals may induce or promote cancer.

Metals are defined as elements that have an unoccupied space in their outer valence shell, possess a positive charge in solution, and are good conductors of electricity. Metalloids are defined less stringently than metals. They are semiconductor elements that may or may not display metal



characteristics, depending on chemical conditions.<sup>5</sup> The metalloid of greatest interest to cancer research is arsenic. The majority of elements in the periodic chart (96 of 113) can be classified as either metals or metalloids. Of these, only seven have been positively associated with human cancer.

The complex chemistry of metals defies simple categorization with respect to carcinogenesis. Even metals recognized as carcinogens vary greatly with respect to carcinogenic potential, which is often a function of oxidation state. For example, exposure to chromium (VI) increases cancer risk, whereas chromium (III) exposure does not.<sup>6</sup> It is, therefore, more correct to refer to specific metal forms (metal salts versus pure metals, for example) or oxidation states than to categorize any single metal as carcinogenic.

### 3.2 EPIDEMIOLOGY: THE CASE FOR METALS AND CANCER

Numerous epidemiological studies have examined the effects of metals on human cancer. The first epidemiological studies linking metals and human cancer date from the early 1900s.<sup>7</sup> Based on available epidemiological data, a number of government agencies — including the U.S. Environmental Protection Agency, the Occupational Safety and Health Administration, the National Institute for Occupational Safety and Health, and the National Toxicology Program, as well as nongovernmental advisory organizations such as the American Conference of Governmental Industrial Hygienists and the International Agency for Research on Cancer — have classified metals with regard to their carcinogenicity in humans. Although there is some disagreement among these organizations as to the carcinogenic potential of specific metal compounds, there is general agreement among them that a given metal (chromium, for example) may occur in carcinogenic forms. Table 3.1 lists the metals and metalloids that have been positively identified as human carcinogens by one or more of these organizations, as well as the metallic form involved, the organ sites they affect, and the relative risk of lung cancer (the most common form of metal-induced cancer) associated with each metal/metalloid.

Relative risk is an epidemiological estimate of the fold increase in risk for a particular disease compared with the general population. Relative risk also serves as a means of comparison among factors associated with a particular disease. Based on current data, the relative carcinogenic potential of the seven metals implicated as human lung carcinogens is as follows: arsenic > chromium > nickel > beryllium and cadmium > lead > cobalt.

One of the greatest difficulties for the epidemiologist studying metal-related cancer is the determination of the risk associated with a single metal or metal compound. Multiple types of metals, or multiple forms of a single metal, are often present in an industrial work environment. For example, welders may be exposed to fumes containing mixtures of iron, chromium, nickel, and manganese, whereas workers in copper or lead smelting plants may also be exposed to significant quantities of arsenic. In addition, persons who work with metals may be exposed to other cancer-causing agents, such as asbestos and cigarette smoke.<sup>13</sup> These factors, especially cigarette smoking, may play an additive or synergistic role in cancer causation. It is interesting to note that cigarettes, the major cause of human lung cancer, contain a number of carcinogenic metals, including cadmium, arsenic, nickel, and chromium, and are associated with a five-fold increase in the risk of lung cancer, higher than any single metal.<sup>15</sup> The result of these multicarcinogen exposures is that it is generally possible to identify specific metals and metal compounds as human carcinogens, but more difficult to quantify the level of risk associated with a given metal species.

Animal studies have been used to determine the relative carcinogenic potential of various metal species. Because traditional *in vitro* methods of determining mutagenic or carcinogenic potential, such as Ames testing, do not consistently identify metals as mutagens, animal studies are especially important to determine the relative carcinogenic potentials of metals. An advantage of animal studies is that they allow for the use of single metals or mixtures of known composition and concentration. The disadvantage of animal studies of metal-induced cancer is that they do not always reflect what has been observed in human epidemiological studies. This is especially true for arsenic, which has been associated with human carcinogenesis for more than a century, but which, in animals, does not produce the same lesions

TABLE 3.1

## Metal and Metalloid Forms Positively Identified as Human Carcinogens

| Metals/Metalloids | Forms Considered Human Carcinogens   | Target Organs  | Relative Lung Cancer Risk <sup>a</sup> |
|-------------------|--|--|--|
| Arsenic           | elemental arsenic; inorganic arsenic compounds   | lung, skin, kidney, bladder, liver, lymphoma, leukemia | 3.69                                   |
| Beryllium         | pure metal; alloys   | lung   | 1.49                                   |
| Cadmium           | dust and fumes; carbonate, chloride, fluoroborate, nitrate, oxide, sulfate, and sulfite forms                                      | lung, prostate   | 1.49                                   |
| Chromium          | chromium metal; chromite (mineral form); chromic acid; hexavalent (chromate) compounds; carbamate, phosphate, and triacetate forms | lung, nasal passages                                   | 2.78                                   |
| Cobalt            | chromium/cobalt alloys   | lung   | 1.20                                   |
| Lead              | chromate and phosphate compounds   | respiratory tract, kidney                              | 1.33                                   |
| Nickel            | nickel metal; inorganic nickel; carbonyl and sulfide forms; soluble and insoluble forms  | lung, nasal passages                                   | 1.56                                   |

<sup>a</sup> Relative risk is an epidemiological estimate of the fold increase in risk for a particular disease compared with the general population.

Sources: EPA, Health Assessment Document for Cadmium, U.S. Environmental Protection Agency, Washington, DC, 1981; EPA, Evaluation of the Potential Carcinogenicity of Lead and Lead Compounds, U.S. Environmental Protection Agency, Washington, DC, 1989a; IARC, Monograph on the Evaluation of Carcinogenicity: an Update of IARC Monographs, Vol. 1-42, Suppl. 7, World Health Organization, International Agency for Research on Cancer, Lyon, France, 1987; NTP, National Toxicology Program Report on Carcinogens, Public Health Service, U.S. Department of Health and Human Services, Washington, DC, 2002; Fu, H. and Boffetta, P., *Occup. Environ. Med.*, 52, 73, 1995; Steenland, K. et al., *Am. J. Ind. Med.*, 29, 474, 1996; Tuchsén, F. et al., *Scand. J. Work Environ. Health*, 22, 444, 1996.

or multi-organ carcinogenesis observed in humans.<sup>16</sup> Despite their limitations, animal studies have proven invaluable for the study of the mechanisms of a number of metal-related cancers.

### 3.3 CARCINOGENIC METALS

As Table 3.1 indicates, at least seven metals have been identified as human carcinogens, primarily of the lung. This may lead to the conclusion that these metals are extremely dangerous, and that any contact may result in cancer. On the contrary, based on available epidemiological evidence, it appears that the majority of metal-associated cancers are the result of chronic overexposure over a period of years or decades. With the exception of arsenic and lead, metal-induced cancers are largely preventable through the use of proper environmental controls or respiratory protective equipment. Surprisingly, two of the seven metals listed in Table 3.1, chromium and cobalt, are required in minute quantities as essential nutrients. Three others (arsenic, cadmium, and lead) may also be essential dietary nutrients.<sup>2</sup> As Paracelsus indicated, it is not the substance that makes the poison, but the dose.



### 3.3.1 ARSENIC

Arsenic is a metalloid that occurs naturally in both organic and inorganic forms as either arsenic (III) or arsenic (V). Arsenic (III), or arsenite, is considered to be the most toxic and is also the form most closely associated with cancer, both as a carcinogen and as a chemotherapy. Arsenic exposure may be occupational or may result from drinking arsenate-contaminated water. Workers in pesticide manufacturing plants, smelting operations, and the art glass industry have the greatest potential for exposure. Persons living in countries including Bangladesh, India, Taiwan, Argentina, and Chile are at risk for overexposure due to high arsenic levels in ground water.<sup>17,18</sup> Shellfish consumption is a third source of arsenic exposure, although the primarily methylated forms contained in these foods are rapidly excreted in urine and do not appear to increase cancer risk.

Arsenic is the most potent of the metals, both as a carcinogen and as a chemotherapy. Chronic exposure to arsenic has been associated with cancers of the lung, skin, and other organs. Lung cancers are most common in arsenic smelter workers and are typically adenocarcinomas.<sup>19</sup> One of the unique characteristics of arsenic carcinogenesis relative to other carcinogenic metals is that either respiration or ingestion can result in lung cancer. Skin cancers resulting from arsenic exposure may be of either basal- or squamous-cell origin and are unrelated to sun exposure. Other cancers, including lymphoma, leukemia, kidney, bladder, and liver cancer, have also been linked to arsenic exposure. Prior to the advent of radiation and other forms of chemotherapy, arsenic was used as a cancer treatment. Studies in China during the 1970s renewed interest in the use of arsenic to treat specific forms of cancer. Today, arsenic trioxide (III) is considered one of the most effective treatments for promyelocytic leukemia.<sup>20</sup>

### 3.3.2 BERYLLIUM

Beryllium is a light, heat-resistant metal that is often alloyed with other metals to increase their strength. Beryllium is used in nuclear reactors, spacecraft, rocket fuel, and aircraft. There is some disagreement concerning the carcinogenicity of beryllium in humans. Groups including the National Institute for Occupational Safety and Health and the National Toxicology Program consider beryllium a confirmed human carcinogen. Other organizations, such as the U.S. Environmental Protection Agency, list it as a probable carcinogen, and the International Agency for Research on Cancer "reasonably anticipates" that beryllium is a carcinogen.<sup>11,13,21,22</sup> The greatest anthropogenic source of beryllium results from the burning of fossil fuels, particularly coal. Beryllium exposure can result from respiration of dust or ingestion in food or water. Occupational exposure has been reported to increase lung cancer risk.<sup>23,24</sup> Environmental exposure does not appear to significantly increase cancer risk, however.<sup>25</sup>

### 3.3.3 CADMIUM

Relative to other metals described in this chapter, cadmium has come into use only recently, during the second half of the 20th century. It is used in galvanizing applications (because of its resistance to corrosion) and forms the positive pole of nickel/cadmium and zinc/cadmium batteries. Cadmium (II) is produced as a by-product of zinc and lead refining. In areas where soil is contaminated with cadmium, plant foods such as rice are a major source of exposure. Divalent cadmium is also present in seafood and in cigarettes.<sup>16</sup> At normal exposure levels, cadmium is rendered harmless by binding to metallothionein, although it may be stored in the body for more than two decades. Excessive exposure to cadmium overwhelms the ability of metallothionein to bind cadmium, and organ damage occurs, mainly in the kidneys and liver.<sup>26</sup> Curiously, the organ sites most commonly associated with cadmium-induced cancer are the lung and prostate. Workers in nickel/cadmium battery factories were found to have an increased risk for both types of cancer.<sup>27</sup> Some authors have suggested that cadmium-associated cancers do not result from cadmium exposure alone but are, instead, related to coexposure to nickel or lead.<sup>28</sup> Unlike carcinogenic metal forms, such as

chromium (IV) and cobalt (II), cadmium (II) does not directly participate in the formation of reactive oxygen species. Instead, it indirectly enhances free-radical formation through the depletion of glutathione and other cellular reducing agents.<sup>29</sup>

### 3.3.4 CHROMIUM

Although chromium can exist in at least four oxidation states, trivalent and hexavalent chromium are the forms of greatest interest to human health. Chromium compounds are used in steel production, in the construction of furnaces, in leather tanning, in pigments, and in welding. Chromium (III) is the form most commonly encountered in nature. Unlike chromium (VI), ingested or respired chromium (III) is neither toxic nor carcinogenic. Instead, chromium (III) is an essential dietary nutrient with a recommended daily intake of 50 to 200  $\mu\text{g}$  for adults. A component of the "glucose tolerance factor," chromium is necessary for the proper function of insulin.<sup>2</sup>

Hexavalent forms of chromium are the most important for industrial applications. Chromium (VI) is associated with an increased risk of lung cancer. It is primarily utilized by the steel industry for the production of ferrochrome, which is, in turn, used for the production of stainless steel. Unlike trivalent chromium, hexavalent chromium easily crosses cellular and nuclear membranes. Once inside the cell, it is converted by glutathione reductase, ascorbic acid, or any of a number of other intracellular reducing agents to chromium (V), chromium (IV), or chromium (III). It is the formation of reactive oxygen species during reduction of chromium and the binding of chromium (III) and chromium (V) to DNA that are considered to be the major causes of chromium-induced carcinogenesis.<sup>30</sup> Hexavalent chromium differs from other metal carcinogens in that it is also a potent mutagen.

### 3.3.5 COBALT

Cobalt is a by-product of copper refining. Its industrial applications include uses in alloys, magnets, and pigments. Radioactive forms of cobalt, including cobalt-57 and cobalt-60, have been used for over 30 years as chemotherapy agents. Cobalt (II) is associated with human lung cancer. According to the most recent epidemiological data, it is the weakest of the metal carcinogens.<sup>14</sup> Like trivalent chromium, cobalt is an essential nutrient. Indeed, it forms the center of the vitamin B-12 molecule.<sup>2</sup>

### 3.3.6 LEAD

Lead is the most common of the carcinogenic metals in the environment. In the past, it has been used as an additive in gasoline and in paint. Industrial uses of lead today include lead/acid batteries, alloys, and the use of the pure metal to form nonreactive surfaces. At least two lead-containing compounds (lead phosphate and lead chromate) are associated with cancer.<sup>31,32</sup> Lead workers are at increased risk for cancers of the respiratory tract, the digestive tract, and to a lesser extent, the kidney. Recent data indicate that some of the cancers attributed to lead may have been the result of arsenic contamination.<sup>33</sup> Like cadmium, lead compounds do not participate in redox cycling, but they do indirectly increase the formation of free radicals via the depletion of glutathione and other cellular reducing agents.<sup>34</sup>

### 3.3.7 NICKEL

Nickel is used in stainless steel alloys, for metal plating, and in batteries. Sources of nickel exposure most closely associated with cancer include metal refining operations, oil fly ash, and cigarette smoke. Several forms of nickel, both soluble and insoluble, have been identified as human carcinogens. Nickel (II) chloride is a soluble form of nickel that has been identified as a weak lung carcinogen. Nickel subsulfide ( $\text{Ni}_3\text{S}_2$ ) and nickel oxide (NiO) are sparingly soluble forms of nickel that are potent lung and nasal carcinogens. Nickel sulfides are more carcinogenic than other forms



of nickel.<sup>35</sup> It has been theorized that this is due to the formation of radicals by both the metal and the sulfur components. Insoluble metallic nickel is also a lung and nasal carcinogen. One explanation for the greater carcinogenic potential of less soluble nickel compounds is that they are more persistent in the respiratory tract and are phagocytized. Inside phagocytes, in the low-pH environment of lysosomes, nickel species that are insoluble or sparingly soluble at neutral pH can be solubilized and released into the cytoplasm at high concentrations.<sup>36</sup>

### 3.3.8 IRON AND COPPER

Although not considered to be carcinogenic metals per se, iron and copper are considered by some authors to be the ultimate carcinogens in metal-induced cancer. The introduction of carcinogenic metals to the body and the resulting damage they induce may cause the release of iron and copper from the sequesterant proteins to which they are normally bound tightly. The resulting free-radical reactions and DNA damage may lead to an initiation of cancer.<sup>37</sup> In support of this hypothesis, experimental data indicate that iron-overload diseases increase the risk for liver cancer (where iron is concentrated) and other cancers as well.<sup>38</sup>

## 3.4 MECHANISMS OF CANCER CAUSATION BY METALS

In this section, we discuss the possible mechanisms by which metals may induce cancer. A review of the current literature reveals several common themes with regard to the effects of carcinogenic metals on the cellular level. These include DNA damage, the inhibition of DNA repair, generation of reactive oxygen species, and effects on apoptosis and signal transduction. These are not discrete phenomena but, rather, are interrelated in the pathology of metal-induced cancer.

### 3.4.1 DNA DAMAGE

DNA damage is considered to be the initiating step in cancer causation. If left unrepaired, this damage can result in cell death or a permanent mutation. Accumulated mutations in oncogenes or tumor suppressor genes may then result in cancer. Metals are capable of inducing a wide range of DNA damage, including strand breaks, depurinations, base modifications, and DNA cross-links. The effect of metals on DNA depends on the type of metal in question. Nickel (II) is capable of inducing DNA strand breaks at cytosine, thymine, and guanine residues, whereas chromium (VI) appears to target guanine selectively.<sup>39,40</sup> Strand breaks may also be caused by the attack of a DNA sugar by the hydroxyl radical. Chromium (VI) and nickel (II) can cause depurination at guanine or adenine residues. Metals are capable of inducing two base modifications in DNA that are associated with cancer, including 8-hydroxy-2'-deoxyguanosine and O-6-methylguanine. The formation of cancer-associated DNA-base alterations as a result of metal-induced free-radical reactions is, perhaps, the strongest link between metal-induced cancer and reactive oxygen species. Cross-links are the most common form of DNA damage induced by metals. Cross-links can result from noncovalent binding of DNA complexes or the formation of covalent bonds between strands.<sup>37</sup>

In addition to effects on DNA, metals can also affect protein expression, structure, and function. Damage to protein is generally not considered as serious as DNA mutations because protein is a "renewable resource." Protein alterations can have pronounced effects on essential cell functions, however, including the inhibition of DNA repair. Metals can oxidize sulfhydryl groups to disulfides, as well as bind to cysteine, histidine, arginine, lysine, or proline. Conformational and functional changes result from these interactions. Protein-protein and protein-DNA cross-links can also occur. In addition, binding of metals to protein may enhance their ability to form free radicals, thus causing further damage to the protein. Metals can also bind to the chromatin protein associated with DNA or to the DNA itself. Chromatin cross-links have been identified in workers exposed to nickel and chromium.<sup>41</sup>

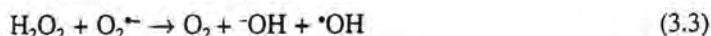
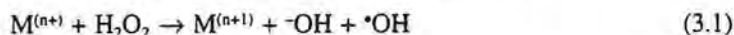
### 3.4.2 INHIBITION OF DNA REPAIR

Arsenic, nickel, lead, cadmium, and cobalt, are weak mutagens. Since mutagenicity is related to carcinogenic potential, they might be expected to lack carcinogenic activity. In fact, they are classified as human carcinogens. This apparent paradox is partly explained by the fact that these metals also inhibit DNA repair. Metals can inhibit DNA repair in several ways: by interfering with mismatch repair, by base excision, or by nucleotide excision. Many DNA repair proteins contain a zinc-finger motif. DNA repair can be inhibited either through oxidative inactivation of these proteins or by the exchange of zinc for another metal.<sup>42</sup> Arsenic (III) inhibits DNA repair through oxidative mechanisms. Nickel (II) chloride inhibits the removal of oxidatively damaged DNA bases and inhibits the ligation of broken DNA strands. Both nickel (II) chloride and nickel oxide enhance the mutagenic activity of the carcinogen benzo(a)pyrene by inhibiting DNA repair. Other carcinogenic metals, including arsenic, cadmium, and cobalt, share this ability to enhance the mutagenic activity of directly acting (DNA-damaging) carcinogens.<sup>38</sup> In addition to the inhibition of DNA repair itself, metals including cadmium, nickel, and cobalt are capable of inactivating zinc-finger proteins that are involved in the detection of DNA repair. In this way, metals are capable of compromising not only DNA repair itself, but the ability to detect DNA damage as well. The mechanism by which these repair proteins are inactivated involves the replacement of metals essential to DNA-repair protein function (zinc and magnesium) with other metals. Cadmium (II), nickel (II) and cobalt (II) are all capable of this activity.<sup>43</sup>

### 3.4.3 GENERATION OF REACTIVE OXYGEN SPECIES

The formation of reactive oxygen species (ROS) is an inevitable consequence of oxidative metabolism. In the body, the levels of these potentially damaging chemicals are kept in check by a complex system of reducing agents and enzymes that serve to convert ROS into less harmful species. These systems include glutathione, antioxidant vitamins and their precursors (vitamins C and E, beta-carotene), and natural chelators and sequestrants, such as metallothionein. Not all ROS generation is undesirable, as some ROS serve to relay intracellular signals. Metals can upset this delicate balance between formation and disappearance of ROS, tipping the balance in favor of oxidation, a state known as oxidative stress.

Major forms of oxygen-centered ROS include hydrogen peroxide ( $\text{H}_2\text{O}_2$ ), superoxide radical ( $\text{O}_2^{\bullet-}$ ), and the hydroxyl radical ( $\bullet\text{OH}$ ). The hydroxyl radical is particularly toxic because it reacts with any biological molecule in its vicinity, including DNA, RNA, or protein.



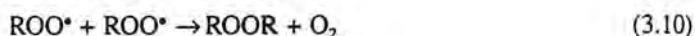
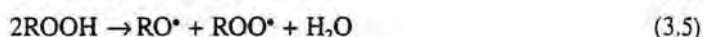
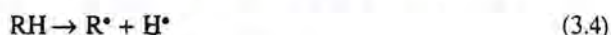
Equation 3.1 through Equation 3.3 demonstrate the effects of metals that possess multiple oxidation states (transition metals) on the formation of these radicals. In Fenton-type reactions (Equation 3.1), metal oxidation ( $\text{M}^{(n+)} \rightarrow \text{M}^{(n+1)}$ ) is coupled to  $\text{H}_2\text{O}_2$  disproportionation to form the hydroxyl ion and the hydroxyl radical. Equation 3.2 demonstrates the ability of superoxide to reduce an oxidized metal through the donation of a single electron. It is this reduction that can, in turn, catalyze Haber-Weiss type reactions (Equation 3.3), in which hydrogen peroxide reacts with the superoxide radical to form molecular oxygen and the hydroxyl radical. In fact, the reaction in Equation 3.3 will not proceed unless it is coupled to metal reduction.<sup>44</sup> The reactions described in Equation 3.1 through Equation 3.3 can function in a continuous cycle wherein metals are repeatedly oxidized and reduced (thus the term *redox cycling*). This oxidation-reduction cycle results in the formation of increasingly greater quantities of the highly toxic hydroxyl radical, as both the Fenton and Haber-Weiss reactions produce this radical. This continual supply of  $\bullet\text{OH}$  mimics the effects of one of the most potent known



carcinogens, ionizing radiation.<sup>45</sup> Chromium (VI), nickel (II), and cobalt (II) are capable of producing hydroxyl radicals and inducing DNA damage via Fenton and Haber–Weiss reactions.

Metal chelation may enhance or inhibit the Fenton reaction, depending on the metal and the chelator in question. Chelation of iron (II) by EDTA enhances the formation of hydroxyl radical, while deferoxamine, another chelator, reduces its formation. This is significant because peptides or proteins can chelate metals in the body, thus influencing the resulting degree of damage. The formation of hydroxyl radicals by nickel (II) and cobalt (II) is enhanced by this type of chelation. In addition to the Fenton and Haber–Weiss reactions, metals can also catalyze the formation of the hydroxyl radical via reaction with hypochlorite (HOCl), which is produced by neutrophils.<sup>37</sup>

In addition to the hydroxyl radical, metals catalyze the formation of a wide variety of radical species through numerous types of reactions with lipids. These processes are collectively known as lipid peroxidation or autoxidation. Autoxidation occurs in three basic stages: initiation, propagation, and termination (Equation 3.4 through Equation 3.10). RH represents a lipid molecule; ROOH represents a lipid peroxide. Initiation reactions (Equation 3.4 and Equation 3.5) are typically slow, but metals can dramatically increase reaction rates by lowering the energies of activation and by the donation of electrons. In Equation 3.4, a lipid radical (R•) is formed. In Equation 3.5, the cleavage of two lipid molecules results in the formation of an alkoxy (RO•) and a peroxy radical (ROO•). Equation 3.6 and Equation 3.7 illustrate propagation reactions. First, a lipid radical reacts with molecular oxygen to form a peroxy radical. The peroxy radical can then react with other lipids to re-form a lipid peroxide and to form a lipid radical (Equation 3.7), thus “propagating” free-radical reactions. In much the same way as the Fenton and Haber–Weiss reactions, the initiation and propagation reactions of autoxidation can form a cycle, resulting in the formation of ever greater quantities of lipid radicals. The final stage of autoxidation is termination (Equation 3.8 through Equation 3.10), in which lipid and peroxy radicals react with one another to form nonradical compounds.<sup>46</sup>



Autoxidation is theorized to be important to the pathology of cancer because of its ability to change the permeability of cell membranes and to form organic peroxides. Changes in membrane permeability affect sodium channels and proteins involved in signal transduction. Changes in mitochondrial membrane permeability can result in increased formation of ROS and/or apoptosis.<sup>47</sup> Organic peroxides formed during autoxidation reactions can form hydroxyl radicals via Fenton and Haber–Weiss types of reactions. Theories concerning the importance of autoxidation in cancer are supported by the increases in measures of oxidative stress and cell permeability in human cancer patients.<sup>48</sup>

#### 3.4.4 EFFECTS ON SIGNAL TRANSDUCTION AND APOPTOSIS

The presence of metals can affect signal transduction, especially for genes known to be responsive to the oxidative status of the cell, such as p53. This will, in turn, affect the actions of the cell controlled by these genes. One of the effects most commonly associated with cancer-causing metals is apoptosis. Metals can cause apoptosis via extrinsically (cell receptor) or intrinsically (mitochondrial or DNA damage) mediated mechanisms. Apoptosis can be induced through p53-dependent or p53-independent



pathways.<sup>49</sup> Chromium (VI) can indirectly induce p53-mediated apoptosis in at least three ways: through direct DNA damage, through activation of mitogen-activated (MAP) kinases upstream of p53, and through the oxidative activation of p53 itself. Reduced forms of chromium or the reactive oxygen species produced by them both have the capacity to damage DNA. Damage, such as DNA strand breaks, activates upstream kinases, such as DNA protein kinase, *Ataxia telangiectasia* mutated protein kinase, and the *Ataxia telangiectasia* and rad3<sup>+</sup>-related protein kinase, all of which are capable of activating p53.<sup>50</sup> The binding of chromium (III) and chromium (V) to DNA can result in the activation of MAP kinases, such as c-Jun N-terminal kinase and p38, which, in turn, can activate p53 without DNA damage. Due to the presence of cysteine residues within the protein, p53 is sensitive to and can be activated by changes in oxidative conditions within the cell.<sup>51</sup>

Arsenic-induced apoptosis is independent of p53 and is mediated through direct mitochondrial damage and MAP kinase activation. Arsenic can cause M-phase cell-cycle arrest and apoptosis in p53-negative cells. Arsenic damages mitochondria via free-radical mechanisms. Activation of either p38 or c-Jun N-terminal kinase subsequently activates growth arrest and DNA damage (GADD) genes. Activation of GADD45 causes arsenic-induced cell-cycle arrest.<sup>52</sup>

Paradoxically, both apoptosis and the inhibition of apoptosis can be carcinogenic. Generalized apoptosis of a cell population may select for apoptosis-resistant cells. The inhibition of apoptosis may allow for the growth and replication of cells that contain substantial DNA damage, thus perpetuating potentially carcinogenic cell traits. Chromium (VI) appears to do both of these, as it initially activates and later inactivates p53.

Although many of the effects of metals appear to be mediated through free-radical production, this does not explain all of the effects of metals. For example, chromium (VI) is a stronger pro-oxidant and mutagen than arsenic (III); however, arsenic (III) is a more potent carcinogen. One explanation for this is the differing ways in which metals affect the signal transduction pathways of cancer-related genes. The nuclear transcription factor kappa-B (NFκB) is known to be up-regulated in many cancers. Arsenic (III) and Cr (VI), both human carcinogens, have been shown to both activate and inactivate NFκB.<sup>53</sup> The extent to which activation or inactivation occurs appears to be dependent both on metal concentration and cell type. At levels that are physiologically achievable *in vivo* (in the low micromolar range), arsenic (III) appears to induce NFκB.

A number of authors have proposed that this activation is due to the production of free radicals. However, three experimental findings indicate that the mechanism may also be independent of oxidation. First of all, the binding of the NFκB protein to DNA, which is necessary for its activity, is reduced when the NFκB protein itself is oxidized. In other words, the NFκB protein is more effective as a transcription factor when it has not been oxidized. Second, chromium (VI) generates more radicals than arsenic (III) but induces NFκB to a lesser extent. Finally, the signal transduction pathways known to control NFκB activation (LPS, IL-1, Toll, CD28) are not activated by oxidative stress. Together, these findings indicate that carcinogenic metals may have mechanisms that are independent of or in addition to free-radical formation, at least with regard to NFκB.<sup>54</sup>

### 3.5 CONCLUSIONS

Mankind has used metals for millennia, but the relation between metals and cancer has only been known for over a century. Only in the past three decades have the tools been available to analyze the molecular and cellular effects of metals on cancer, and only very recently has it been possible to examine the role of free radicals in normal and disease states. Because of their ability to produce free radicals, transition metals provide a unique means by which to study not only metal-related diseases, but the effects of free radicals on DNA damage, intracellular signaling, and cell-to-cell communication. With the completion of the human genome project and the use of novel technologies such as genomics and proteomics, it will soon be possible to examine the global effects of free radicals on genes and their expression.

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