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Conference overview: Molecular mechanisms of metal toxicity and carcinogenesis

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Abstract

Chronic exposure to many heavy metals and metal-derivatives is associated with an increased risk of cancer, although the mechanisms of tumorigenesis are largely unknown. Approximately 125 scientists attended the 3rd Conference on Molecular Mechanisms of Metal Toxicity and Carcinogenesis and presented the latest research concerning these mechanisms. Major areas of focus included exposure assessment and biomarker identification, roles of ROS and antioxidants in carcinogenesis, mechanisms of metal-induced DNA damage, metal signalling, and the development of animal models for use in metal toxicology studies. Here we highlight some of the research presented, and summarize the conference proceedings. (Mol Cell Biochem **279:** 3–15, 2005)

Key words: antioxidants, carcinogenesis, DNA damage, metals, reactive oxygen species

Introduction

The 3rd Conference on Molecular Mechanisms of Metal Toxicity and Carcinogenesis was held at the National Institute for Occupational Safety and Health (NIOSH) in Morgantown, West Virginia. Over 125 participants from 13 different countries gathered at NIOSH to present their research on September 12–15, 2004. This conference was the third meeting of metal researchers in the past 5 years, with previous conferences convening in 2000 and 2002. Major areas of research emphasized at the conference included metal epidemiology, identification of biomarkers, reactive oxygen species (ROS) production, antioxidant effects, induction of DNA damage/lesions, metal-induced signalling pathways, and animal exposure studies. Here, we summarize the conference proceedings and examine the future directions of research concerning the molecular mechanisms that facilitate metal-induced carcinogenesis.

Epidemiological implications of metal exposure and the search for reliable biomarkers

Many metals are considered essential trace elements and must be present in low concentrations in the human body in order for normal cellular function. However, altered concentrations or transition states of metals in the body are thought to lead to a wide range of deleterious conditions, especially an increase in cancer incidence. Increased metal exposure in humans can occur via ingestion, inhalation, dermal contact, and occupational exposure [1–4]. Although correlations between metal exposure and cancer are well documented, more research is still needed in order to determine the exact mechanisms of metal-induced carcinogenesis. Much of the metal toxicology and carcinogenicity data available are acquired in animal model systems, with few studies representing the

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effects of metals in humans. Therefore, several groups at the conference focused their research on the link between the level of metals in the human body and carcinogenesis.

Puneet and colleagues presented work examining the role of metals in gallbladder carcinoma. Using atomic absorption spectrometry, they showed that the bile of gallbladder carcinoma patients contained higher concentrations of cadmium (Cd), chromium (Cr), and lead (Pb) than patients with other non-cancerous gallbladder diseases, such as gallstones. Additionally, Puneet's group observed an increase in the expression of the metal-exposure protection protein metallothionein (MT) in 70% of the gallbladder carcinoma patients. These results suggest that the body may concentrate these metals in the hepatobiliary system and lead to gallbladder carcinoma. This group also examined the lipid peroxidation profiles of gallbladder carcinoma patients in order to determine if a free radical-induced mechanism might be involved. They found a significant increase in the amount of a major lipid peroxidation product, 4-hydroxynonenal (4-HNE), in gallbladder carcinoma patients compared to patients with other non-cancerous gallbladder diseases. These results suggest that the formation of lipid peroxidation products in the gallbladder, induced by metal exposure and free radical generation, may be an underlying cause of tumorigenesis.

Many metals must be controlled by a tightly regulated homeostasis. Two such examples are copper (Cu) and iron (Fe), the accumulation of which results in oxidative stress-induced cellular damage [5, 6]. Wilson's disease (WD) and primary hemochromatosis (PH) are metal storage diseases which are associated with DNA damage in the liver. In WD, a defect in a Cu-transporting ATPase results in the intracellular accumulation of Cu [7]. In PH, Fe accumulates in the liver because of a mutation in the *hfe* gene, which is responsible for mediating the effects of the transferrin receptor [8]. Patients with both WD and PH are shown to exhibit increased oxidative stress-induced etheno-DNA adducts [9]. Vinyl chloride, urethane, and 4-HNE produce etheno-DNA adducts as well [10–12]. These adducts can subsequently lead to the formation of strong miscoding lesions and cause mutations and/or genomic instability in patients suffering from WD and PH. Nair and Bartsch further examined the correlation between the formation of 4-HNE-induced DNA adducts and susceptibility to the development of liver cancer. They found that both WD and PH patients exhibiting high 4-HNE-induced DNA adducts were the most susceptible to the development of liver cancer, particularly those patients suffering from PH.

Conversely, the dietary deficiency of many micronutrients, such as certain trace metals, has also been associated with an increased risk of cancer [13–16]. Ansari *et al*. presented data suggesting that significantly lower levels of selenium (Se) and zinc (Zn) were found in the serum, biliary fluid, and gallbladder tissue of gallbladder carcinoma patients when compared to healthy individuals. Serum manganese (Mn) was also reduced in gallbladder carcinoma patients, leading Ansari's group to conclude that a deficiency in these trace elements is also associated with gallbladder carcinoma.

Since animal studies assessing the effects of metal exposure can often be difficult to interpret and inconclusive due to the role of some metals as co-carcinogens, reliable biomarkers are needed in order to assess metal exposure in humans. A good candidate for a human biomarker must be specific to the effects of the causal agent and easily quantitated and/or assayed for its presence in a noninvasive manner. Several studies presented at the conference examined the reliability of biomarkers for metal exposure. Using atomic absorption spectrometry, Rahnama *et al*. analysed the Zn, Cu, Mn, and Fe content of saliva and serum from patients with squamous cell carcinoma (SCC) of the oral cavity. SCC patients exhibited increased concentrations of Cu, Mn, and Fe in their blood and increased concentrations of all four metals examined in saliva when compared to healthy individuals. These results suggest that analysis of saliva may be an easy and effective way to detect SCC of the oral cavity.

In order to identify specific biomarkers of mercury (Hg) poisoning Song *et al*. examined cases of peripheral neuropathy in Hg-exposed patients. Levels of selected urinary proteins were measured in patients with acute or chronic mercury poisoning and healthy control individuals. Elevated levels of the α1-m, β2-m, TRF, IgG, and NAG proteins were observed in both acute and chronic Hg-exposed patients, suggesting that urine samples may be a simple and inexpensive way to test for Hg exposure.

In addition to studying the adverse effects of metal exposure, it is important to try to develop methods of exposure prevention. For example, Huang's group attempted to develop a correlation model between Fe exposure in coal mines and Coal Workers' Pneumoconiosis (CWP). Fe is an essential trace metal that is stored in Fe-containing proteins, such as ferritin and transferrin, where it is tightly bound and unavailable to cause oxidative damage [17–19]. Bio-available iron (BAI), such as Fe associated with citrate and ATP, has been shown to produce oxidants in a pH-dependent manner [20]. Some coal dusts contain BAI, and have been shown to activate the stress-inducible AP-1 and NFAT transcription factors, while coal dust lacking BAI had no effect on either transcription factor [21]. Previously, BAI content in Pennsylvanian coal was shown to be much higher than the BAI content in coal dust originating from Utah [22]. Huang posited that BAI content could be used as a predictor of coal toxicity. The BAI content of coal from Utah and Pennsylvania was calculated and compared to epidemiological studies reporting the incidence of CWP. A significant correlation was found between BAI levels and CWP incidence, suggesting that BAI levels may be used to predict the toxicity of coal before mining begins.

A second example of an *in vitro* predictive model for metal-induced disease was introduced by Hockertz *et al.* Primary mast cells from human lung tissue and foreskin specimens were exposed to wear particle antigens including chromium–cobalt–molybdenum alloy (CrCoMo), a titanium alloy (TiAL6V4), or ultra-high molecular weight polyethylene (UMHWPE), all of which can be released into the human body by the degradation of implanted osseoprostheses [23]. Histamine release, indicative of an allergic reaction, was observed when the antigens were added to serum-containing media and applied to primary mast cells. If a patient's IgE was present, histamine release increased, particularly for the CrCoMo antigen. This *in vitro* model could be useful in preventing potentially dangerous allergic reactions in patients with osseoprostheses and reduce the need for the administration of immunosuppressants.

In some cases, a single biomarker candidate may be difficult to obtain and a profiling-based approach may be useful in identifying a molecular fingerprint for toxicant exposure. Boyd *et al*. presented an example of a high throughput strategy utilizing an automated robotic workstation (COPAS BIOSORT) to dispense and measure the length of *Caenorhabditis elegans* worms. Toxicological endpoints were examined using a fluorescent dye that only penetrates deceased worms. An imaging workstation was used to track the motion of the live worms and acquire multidimensional image analysis data. This type of high throughput method can be used to examine the properties of potential neurological and developmental toxicants and detect subtle changes in gene or protein expression that may be undetectable by conventional methods.

Balancing act: The role of reactive oxygen species (ROS) and antioxidants

Many transition metals have been shown to produce elevated amounts of damaging ROS [24–28]. Disturbing the redox balance of a system leads to an increase in DNA damage, DNA-protein crosslink formation, lipid peroxidation, apoptosis, cellular toxicity, and/or the inappropriate activation of cellular signalling pathways [29–35]. Since the mechanisms of ROS action are not well understood, many conference participants focused their studies on the elucidation of these mechanisms. For example, Cr(VI) is a transition metal that is known for its ability to produce increased quantities of ROS in cellular systems, particularly $^{\bullet}OH$ [36, 37]. Previous studies have focused on the effects of soluble Cr(VI), since solubility is thought to be an important factor in its ability to contribute to carcinogenesis [38]. However, water insoluble (or particulate) Cr(VI) has also been shown to be genotoxic and cytotoxic [24, 39–41]. Leonard *et al*. examined the capability of insoluble Cr(VI) in the form of $PbCrO_4$ to produce ROS using electron spin resonance (ESR) spectroscopy and the comet assay. Their results showed that insoluble Cr(VI) was capable of producing oxidative stress in a similar pattern to that of soluble Cr(VI).

Additional studies examining the effects of $PbCrO_4$ in the human lung cell line WHTBF-6, were presented by Wise *et al.* $PbCrO_4$ was shown to enter the lung cells and exert clastogenic effects in a dose-dependent manner, although dicentric chromosome formation was not observed. In order to exclude Pb ions as the culprits of clastogenicity, WHTBF-6 cells were also exposed to Pb-glutamate, which yielded no clastogenesis. However, Na_2CrO_4 treatment also proved to be clastogenic in the lung cells, suggesting that the CrO_4^{2-} ions were the cause of clastogenicity. In addition, the antioxidant vitamin C prevented the uptake of CrO_4^{2-} ions and exhibited only a slight effect on Pb levels. The cytotoxicity profiles of WHTBF-6 cells exposed to CrO_4^{2-} ions and $PbCrO_4$ were comparable. Both $PbCrO_4$ and Na_2CrO_4 induced cell cycle arrest and inhibited growth, whereas Pb ions alone induced only mitotic arrest.

ROS production often leads to cell death through cytotoxicity and/or apoptosis, prompting several groups to examine the benefits of inducing ROS in a cellular system through metal treatment. Farmer *et al*. used synthetic melanins derived from dihydroxy indole (DHI) to study the speciation, metal-binding affinity, and redox reactivity of those synthetic melanins. Several metals bound the DHI-melanin, increasing redox cycling and the production of $^{\bullet}OH$ and $O_2^{\bullet-}$ surprisingly. Indium(III) ions alone and lipophillic metallodithiocarbamate exhibited an unusual amount of toxicity towards melanoma cells in culture.

The dithiocarbamate disulfide disulfiram (DSF), which is traditionally prescribed as an alcohol-abuse deterrent, also showed strong Cu-dependent activity towards melanoma cells. Cen's group confirmed that DSF exhibited a Cu-dependent toxicity towards melanoma cells, whereas non-transformed melanocytes were only slightly affected. Upon DSF treatment, melanoma cell survival decreased to less than 10% as the intracellular Cu concentrations increased. The addition of Cu chelators blocked the DSF-induced cell toxicity and Cu uptake by melanoma cells. Formation of a $Cu(deDTC)_2$ complex was proposed to be the likely active agent. This response is unique to Cu(II) and was not observed with Fe(II), Mn(III), or Zn(II), suggesting that local administration of Cu and DSF or DHI may be useful in the treatment of melanoma.

Exposure to metal-containing particulates can also increase ROS production in cellular systems. For example, diesel exhaust particles (DEP) have been shown to induce DNA adducts and increase inflammation in the respiratory tract [42–46]. DEP contents include polycyclic-aromatic hydrocarbons (PAHs), redox active semi-quinones, and trace heavy metals [47, 48]. Park *et al*. compared ROS production

of the DEP standard reference material 2975 (SRM 2975) and DEP collected from the air in Korea as measured by thiobarbituric acid – reactive substances of deoxyribose (TBARS). Differences in transition metal composition were observed between samples; DEP from Korea contained more Fe, less Cu, and less Zn than SRM 2975. TBARS measurements of the water soluble fraction of SRM 2975 showed a broad absorption in the visible range, which was absent in the DEP from Korea. Although SRM 2975 increased the amount of TBARS fluorescence, H_2O_2 generated more TBARS in the DEP from Korea, probably due to the increase in Fe composition. These results support a role for Fe in DEP-induced H_2O_2 toxicity, which may be the underlying cause of the adverse health effects of DEP.

Some substances seem to exert both pro-oxidant and antioxidant effects. Recently, many epidemiological and experimental studies have suggested that drinking green or black tea may protect against cancer [49, 50]. Sinha *et al*. presented evidence suggesting that tea extracts, in particular the polyphenolic components, exert their chemopreventative effects by acting as antioxidants. They proposed that tea extracts can prevent DNA damage caused by sodium arsenite in V-79 Chinese hamster lung fibroblasts. However, Azmi *et al*. presented evidence that the presence of at least one of those polyphenolic components, (−) epigallocatechin-3-gallate (EGCG), can induce $^{\bullet}OH/O_2^{\bullet-}$ formation and increase the rate of DNA oxidation in the presence of Cu ions. The results of Azmi's group suggest that EGCG's apoptosis-inducing pro-oxidant properties may be more important than its preventative antioxidant properties. These studies are also supported by the findings of Qanungo *et al*. and Zykova *et al*. which suggest that tea polyphenols may serve as either an antioxidant or an oxidant, depending on the physiological environment [51–53].

Ascorbate/ascorbic acid/vitamin C is another compound that has been shown to exhibit both pro-oxidant and antioxidant effects [54–58]. Huq and Hussain presented evidence suggesting that exposure to Cd acetates increases DNA damage in the presence of ascorbate. The mechanism occurs via the formation of a 1:1 covalent Cd(II)/ascorbate adduct, causing a molecular activation of the transition metal. This group proposed that the deprotonated ascorbate ions bind to $^3O_2$ producing the volatile ROS species, singlet molecular oxygen ($^1O_2$).

Ironically, some transition metals may serve as antioxidants and prevent oxidative cellular damage. Aravind and Prasad presented evidence that Zn may be able to prevent Cd toxicity. *Ceratophyllum demersum* L. plants were treated with different concentrations of $CdCl_2$ and/or $ZnCl_2$. The production of $^{\bullet}OH$, an oxidative shift in GSH levels, loss of carbonic anhydrase conformation, and DNA fragmentation were observed with $CdCl_2$ treatment. Upon treatment with both $CdCl_2$ and $ZnCl_2$, the presence of Zn inhibited all of the aforementioned markers of oxidative damage, suggesting that Zn may be able to prevent the cytotoxic effects induced by Cd.

Rhenium is a second transition metal that exhibits low ROS-induced toxicity. Thus, cluster rhenium compounds (CRCs) are thought to be a potential drug delivery agent. CRCs possess lower toxic effects than other metals currently used as anti-tumour agents, including palladium, platinum, and cisplatin [59]. Shtemenko and Shtemenko examined the mechanisms of lipid-CRC interactions in solution and during the formation of liposomes. They had previously shown that CRCs bind to carboxylic acids, amino acids, or adamantanic acids, and subsequently interact with the surface of red blood cells to stabilize the membrane in response to free radicals [60]. Administration of CRC1-dichlorotetra-μ-(i-butirato) dirhenium(III) or CRC2-tetrachlorodi-μ-(γ-aminobutirato) dirhenium(III) chloride in models of hemolytic anaemia led to an increase in hemoglobin and resistance of erythrocytes to oxidative stress, extending the life of hemolytic animals. In addition, CRC1 or CRC2 administration increased reduced glutathione pools, glutathione reductase expression, and glutathione peroxidase (GPx) expression in the tissues of animals with hemolytic anaemia. Thus, CRCs demonstrate antioxidant and antiradical properties *in vivo* and may be useful in the treatment of hemolytic anaemia.

Cellular systems have developed a wide range of antioxidant mechanimsms to combat the dangerous accumulation of ROS. Examples include the increased expression of ROS detoxification enzymes, such as catalase and superoxide dismutase (SOD), and the maintenance of pools of small molecular ROS scavengers, such as N-acetyl-cysteine (NAC) and reduced glutathione (GSH). These ROS scavenging molecules react with excess ROS, thus minimizing cellular damage. Glutathione (GSH), although widely known for its antioxidant activity, is also involved in Se metabolism and bioactivity [61]. Shen *et al*. presented evidence that Se caused a dose-dependent onset of mitochondrial permeability transition (MPT) in the presence of GSH, which could be inhibited by the MPT inhibitor cyclosporin A. An SOD mimic, Mn[III]tetrakis [4-benzoic acid] porphyrin, has been previously shown to prevent MPT caused by xanthine (X)–xanthine oxidase (XO) [62]. Surprisingly, SOD had no effect on MPT and was unable to prevent the reduction of cytochrome *c* induced by selenite-GSH (Se-GSH) formation. Although others have observed an increase in $O_2^{\bullet-}$ formation during Se-GSH generation, Shen's group measured $O_2^{\bullet-}$ via the lucigenin assay and found no significant increase in $O_2^{\bullet-}$ when compared to $O_2^{\bullet-}$ production by the X–XO pathway [63]. These results suggest that $O_2^{\bullet-}$ is not responsible for Se-GSH-induced MPT in this system.

Resveratrol is a polyphenolic antioxidant that is synthesized by many plant species and has been shown to inhibit the growth of cultured cancer cells [64]. Xia *et al*. presented evidence that ROS-induced activation of the PI3K/Akt survival

pathway can be inhibited by the presence of resveratrol in prostate cancer cells. Resveratrol also inhibited the expression of proteins involved in angiogenesis, such as HIF-1α and VEGF, in a dose-dependent manner. Xia's results suggest that resveratrol may be able to inhibit prostate cancer progression and angiogenesis through the inhibition of these pathways.

DNA damage/lesions and mitotic effects

In addition to producing ROS, many metals have been shown to directly modify and/or damage DNA by forming DNA adducts, facilitating DNA protein crosslink formation, or inducing chromosomal breaks [33, 44, 65, 66]. Many researchers at the conference presented work reflecting efforts to identify the mechanisms of metal-induced DNA damage. For example, O'Brien *et al*. examined the role of O_2 in Cr(VI)-induced DNA lesions and found that hypoxic conditions did not alter Cr(VI) reduction by ascorbic acid. However, both Cr(VI)-DNA binding and the occurrence of polymerase arresting lesions (PALs) decreased under hypoxic conditions by 70% and 50–90%, respectively. These results suggest that O_2 can help facilitate the formation of Cr(VI)-DNA complexes.

A common oxidative DNA modification, the 7,8-dihydro-8-oxo-2′-deoxyguanosine (8-oxo-dG) lesion, can lead to the misincorporation of extraneous nucleotides during DNA replication [67, 68]. The 8-oxo-dG lesions are prone to further oxidation by many transition metals, including Cr(V) and Iridium (IV). Further oxidation of 8-oxo-dG leads to the formation of guanidinohydantoin (Gh) and spiroiminodihydantoin (Sp) lesions, subsequently causing G $\rightarrow$ T and G $\rightarrow$ C transversions [69, 70]. Gh and Sp lesions are thought to enhance base misincorporation and the polymerase blocking effects of the 8-oxo-dG lesion [71, 72]. Certain base excision repair (BER) enzymes recognize 8-oxo-dG lesions, but not Gh or Sp lesions [73, 74]. Hailer *et al*. showed that the mammalian BER glycosylases NEIL1 and NEIL2 both recognize and cleave Gh and Sp lesions in single-stranded DNA [75]. These results demonstrate that an alternative pathway is activated in response to the formation of Gh and Sp DNA lesions.

The 8-oxo-dG lesion also affects transcription factor binding [76, 77]. Previous studies by Sugden's group reported that binding activity of the NF-κB p50 subunit increases if the G_1 nucleotide of the IκB consensus binding sequence (5′-dAGTTGA $G_1G_2G_3G_4$ACTTTCCCAGCC-3′) is replaced with an 8-oxo-dG modified base [77]. In contrast, replacing the G_3 nucleotide with an 8-oxo-dG decreases p50 binding activity. Sugden *et al*. found that p50 binding activity to the modified IκB consensus sequences correlated with p50 *in vitro* transcription activity, providing a direct link between the regulation of gene transcription and ROS formation.

In addition to inducing DNA lesions, metals can interfere with cellular processes such as mitosis. Kligerman *et al*. presented evidence that arsenicals induced c-type anaphases consisting of small, condensed chromosomes – an effect usually seen with spindle poisons [78]. They proceeded to investigate the effects of several different modified arsenicals on mitosis and found that monomethylarsenous acid (MMA^{III}) exhibited the most potent mitotic interference. When tubulin was directly exposed to MMA^{III}, an increase in the number of mitotic indices (MIs) was observed. None of the arsenicals with a valence state of V showed an obvious effect on tubulin polymerization, whereas all arsenicals with a valance state of III inhibited tubulin polymerization. Taken together, these results indicate that As(III) and its metabolites can interfere with cell division.

Activation of DNA repair enzymes is often observed in response to metal-induced DNA damage [79–81]. Lee *et al*. examined the effects of DNA repair gene polymorphisms on the number of Cr(VI)-induced strand breaks in human white blood cells. Several DNA repair genes with known genetic polymorphisms were examined, including glutathione S transferase M1 (GSTM1), glutathione S transferase T1 (GSTT1), NADPH quinine oxidoreductase 1 (NQO1), X-ray repair cross complementation factor 1 (XRCC1), and the 8-oxo-7,8-dihydroguanine (8-oxo-G) DNA glycosylase (OGG1). White blood cells homozygous for the OGG1 Cys^{326} polymorphism exhibited an increased number of DNA strand breaks when compared to the OGG1 Ser^{326}/Ser^{326} and OGG1 Ser^{326}/Cys^{326} polymorphisms. White blood cells homozygous for the OGG1 Cys^{326} variation also exhibited an increase in the ratio of oxidative DNA damage to plasma antioxidant capacity. These results suggest that OGG1 Cys^{326} homozygous individuals may be defective in the repair of DNA adducts during oxidative stress conditions, possibly due to the oxidation of these residues.

Metal-induced cellular signalling

Although each metal activates its own unique set of signalling events, many metals also activate general ROS-mediated stress response pathways. DNA microarray technology has recently been used to examine the global effects of metals in several model systems. For example, Jin *et al*. examined the global transcriptional response of the yeast *Sacchromyces cerevisiae* to Zn, Cd, Hg, Cu, silver (Ag), Cr, or arsenic (As) exposure. Approximately 25% of the yeast genes were affected by at least one metal (798 upregulated genes and 774 downregulated genes). Most of the metals upregulated genes involved in the oxidative damage process, sulfur assimilation, sulfur metabolism, and GSH biosynthesis. Genes encoding biomolecular transporters of sugar and lipid metabolism were downregulated. Approximately 10% of the total number of

S. cerevisiae genes were affected by As treatment – a 2–5-fold increase in the number of genes affected by any other metal examined. Additional classes of genes induced by As treatment included double-stranded break (DSB) repair and DNA replication genes.

Other examples of the use of microarray analysis in toxicology studies included Song and Freedman's examination of the global transcriptional effects of Cu treatment on a human hepatoma cell line, Hep2G. After 4 h of Cu exposure, 42 genes were upregulated and 24 were downregulated. The upregulated genes included metallothionein IIA (MT2A), myosin heavy polypeptide (MYH8), CYP1A1, IL-1 receptor alpha (IL11RA), heat shock protein A1A (HSPA1A), and heme oxygenase 1 (HO-1). Downregulated genes included the HT017 protein, dynamin 1-like (DNM1L), DEAD box polypeptide 18 (DDX18), and GST theta 2 (GSTT2). RT-PCR confirmed the upregulation of HSPA1A, CYP1A1, and HO-1 with peak induction levels at 12, 24, and 8 h respectively.

Many of the global effects of metals on transcription are thought to be controlled by the interactions between metal response elements (MREs) and MRE binding transcription factors such as MTF-1 [82]. Since PKC inhibitors can abrogate MT induction, it is thought that some metals induce signalling cascades through PKC activation, subsequent alteration of MTF-1 phosphorylation, and activation of MT transcription [83]. Craft and Freedman examined the role of p53 activation in MT induction by Hg and Cd and found MT transcription was reduced in p53 $-/-$ cells when compared to wild-type p53 cells. These results suggest that p53 may be part of a general mechanism in the cellular response to both Hg and Cd exposure.

Arsenic signalling

After a metal ion is transported across the cytoplasmic membrane, its transition state is often altered by cellular reducing agents. Reduction of some metals to a lower transition state, such as As(V) $\rightarrow$ As(III), often results in an increase in toxicity; thus it is beneficial to examine the metal's cellular point of entry. The Ars ATPase is a catalytic subunit of the *Escherichia coli* ArsAB pump that translocates As(III) across the cellular membrane, conferring resistance to arsenicals and antimonials [84, 85]. There are two halves of the Ars ATPase, A1 and A2, each consisting of a nucleotide binding domain (NBD) and a metal binding domain (MBD). The NBD and MBD are connected by two amino acid linkers which serve as a signal transduction domain [85, 86]. Using site-directed mutagenesis, Bhattacharjee and Rosen altered residues of the signal transduction domain and found that the D142A and P145A mutations caused a loss of As(III) resistance. These results indicate that this domain is important in the signalling events between the catalytic and allosteric sites of the Ars ATPase.

The aquaglyceroporin (AQP) family is responsible for the uptake of As(III) and Sb(III) in humans [87, 88]. Mukhopadhyay *et al*. reported that overexpression of human AQP9 in the chronic myelogenous leukemia cell line K562 increased uptake of the As(III)-based drug Trisenox [89]. Since AQP9 overexpression in K562 cells exhibited hypersensitivity to Trisenox, it is possible that pharmacological alteration of AQP9 expression could boost Trisenox effectiveness in cancer patients.

As(III) induces oxidative stress and delays cell cycle reentry into the G1 phase by an unknown signalling mechanism [90, 91]. Chen *et al*. presented evidence that As(III) induces ubiquitination and subsequent degradation of the Cdc25C protein, which is thought to cause the exit of cells from the G_2/M phase [92]. These effects were not abrogated by treatment with antioxidants. Furthermore, immunoprecipitation showed that Cdc25C associates with the Fzr/Cdh1 recognition subunit of the mitotic APC ubiquitin ligase complex. siRNA silencing of Fzr/Cdh1 expression protected Cdc25C from degradation and decreased ubiquitin conjugating activity. Chen's group concluded that As(III) induces the redox-independent ubiquitination and degradation of Cdc25C through its association with Fzr/Cdh1.

As(III) exposure also induces remodelling of the cytoskeleton [93]. Qian *et al*. demonstrated that As(III) activated Cdc42 and NADPH oxidase. Activated NADPH oxidase produced $O_2^{\bullet -}$ and induced actin filament reorganization in SVEC4-10 cells [94]. Overexpression of a dominant negative Cdc42 or treatment with the actin filament stabilization agent jasplakinolide inhibited actin filament reorganization and prevented NADPH oxidase activation. Qian's group concluded that As(III) activates NADPH oxidase through Cdc42 and leads to actin filament reorganization and the formation of lamellipodia and filopodia. These effects could possibly lead to increased cell migration and/or metastasis of As(III)-induced tumours.

Paradoxically, As also exhibits apoptotic-inducing effects. Ivanov and Hei presented evidence that human melanoma cells exposed to combinations of low concentrations of As (2–10 μM) and EGFR, PI3K/Akt, or MEK/Erk inhibitors effectively induced apoptosis via the TRAIL and TNFα pathways in spite of low Fas levels [95]. The combination of As and synthetic small molecule inhibitors could potentially be used to sensitize melanoma cells to cytotoxic drugs, enabling the development of more effective chemotherapy regimens.

Lemarie *et al*. further examined the apoptotic pathway induced by As and found that As_2O_3 prevented monocyte adhesion and expression of the macrophage phenotypical markers transferrin receptor CD71 and integrin CDIIc. As_2O_3 induced monocyte apoptosis, increased caspase 8 and caspase 3 expression, decreased cFLIP and XIAP expression, decreased NFκB binding activity, and exhibited a loss of mitochondrial

membrane potential. Lemarie's group suggested that As_2O_3 may induce apoptosis by inhibiting NFκB activity and subsequently decreasing the expression of the cFLIP and XIAP pro-survival proteins, both of which are transcriptionally controlled by NFκB. Taken together, these studies may provide insight into the signalling pathways responsible for As's ability to induce apoptotic effects as well as carcinogenic effects.

Chromium signalling

Cr(VI) has been known to produce ROS, induce DNA damage, and subsequently activate the ATM, p53, and Chk2 proteins [96, 97]. It is known that ATM is activated specifically in response to DSBs, however no Cr-induced DSBs have been reported [98–101]. Ceryak *et al*. exposed normal human dermal fibroblasts to Cr(VI) or the radiomimetic agent neocarzinostatin (NCS) and analysed the occurrence of DSBs, the phosphorylation status of histone H2A.X (γ-H2AX), and the formation of γ-H2AX nuclear foci. Their evidence suggested that DSBs were formed in response to Cr(VI) exposure in an S phase-dependent manner, while NCS exposure exhibited an equal distribution of DSB formation throughout all phases of the cell cycle. Additionally, Cr(VI) exposure showed γ-H2AX foci formation in PCNA positive cells; an effect that was absent in the NCS-exposed control cells and decreased in ATM$-/-$ cells.

Xie *et al*. independently showed that $PbCrO_4$ induced DSBs in a dose-dependent manner, resulting in the activation of ATM in human lung cells. γ-H2AX is rapidly phosphorylated on Ser^{139} when DSBs are induced by ionizing radiation [102]. Xie's group also showed that $PbCrO_4$ induced a concentration-dependent phosphorylation of γ-H2AX and subsequent foci formation. Taken together, these results demonstrate a general mechanism for Cr(VI)-induced carcinogenesis involving the induction of S phase-dependent DSBs and marked by γ-H2AX foci formation.

Xu presented additional evidence that Cr(VI) exposure activates ATM/ATR, phosphorylates the structural maintenance chromosome 1 (SMC1) protein, and activates caffeine-sensitive S phase cell cycle arrest in a dose-dependent manner. Surprisingly, Cr(VI)-induced S phase cell cycle arrest was shown to be independent of ATM at high concentrations. Xu proceeded to examine alternate pathways responsible for Cr(VI)-induced S phase cell cycle arrest and found that Rad17, which is required for the release of active ATR, bound the site of Cr(VI)-induced DNA damage. A non-functional Rad17 showed impaired S phase arrest in response to Cr(VI) exposure, suggesting that a low dose of Cr(VI) activates an ATM-dependent pathway, whereas a high dose of Cr(VI) activates an ATR-dependent pathway.

Cadmium signalling

The activation of transcription factors by metals is one of the most commonly studied effects of metal-induced cellular signalling. However, recent interest has been expressed in alternative targets of metal signalling, such as the translational machinery pathways. Aberrant regulation of the expression of the translational control proteins eIF3 and eEF1δ has been shown to be responsible for Cd(II)-induced transformation and tumorigenesis [103, 104]. Orthumpangat *et al*. presented evidence that a third translational control protein may also affect the cellular response to metals. Cell lines exposed to Cd(II) exhibited a decrease in eIF4E protein expression. siRNA silencing of eIF4E induced cell death, whereas eIF4E overexpression resulted in cell survival after exposure to an otherwise lethal dose of Cd(II). Cd(II) exposure also activated the ubiquitin pathway, resulting in the degradation of eIF4E and a subsequent decrease in cyclin D1 expression. The expression of other members of the translational machinery family were altered upon exposure to Cd(II). One such protein, eEF1A2 is a cellular proto-oncogene which is overexpressed in many cancer cell lines and tumours. Orthumpangat's group reported that eEF1A2 expression was increased in response to Cd(II), while overexpression of eEF1A2 conferred resistance to Cd(II)-induced apoptosis. These results confirm that the translational machinery is a potential mechanism for Cd(II)-induced carcinogenesis.

Hep3B human hepatocarcinoma cells have been shown to undergo Cd-induced apoptosis in a caspase-independent manner [105]. The mechanism is thought to involve the nuclear translocation of endonuclease G (endoG) and the apoptosis-inducing factor (AIF), both of which are mitochondrial apoptogenic proteins. Lemarie *et al*. showed that the release of endoG and AIF in response to Cd exposure was preceded by an increase in cytoplasmic Ca^{2+} and a loss of mitochondrial membrane potential. Bapta-AM, a Ca^{2+} chelator, blocked these events and prevented apoptosis. Inhibition of ROS production by the mitochondrial inhibitors ruthenium red, rotenone, and diphenyleneiodonium prevented the loss of mitochondrial membrane potential. Bapta-AM and diphenyleneiodonium also blocked expression of the NFκB-regulated anti-apoptotic protein bcl-x(L) in Cd exposed cells. Lemarie's group concluded that Cd induces apoptosis in Hep3B cells through Ca^{2+} release and ROS-induced impairment of the mitochondria. These results are in agreement with the hypothesis that Cd-induced apoptosis occurs through the release of endoG and AIF.

Cobalt and copper signalling

Exposure to the hard metal dust tungsten carbide–cobalt mixture (WC–Co) directly correlates with an increased risk of

cancer in exposed workers [106]. Lombaert *et al*. demonstrated that both cobalt (Co) and WC–Co induced apoptosis of peripheral blood mononucleated cells (PBMCs). WC–Co apoptosis occurred at a higher rate than Co-induced apoptosis and was dependent on caspase 9 activation, whereas Co-induced apoptosis depended on the activation of both caspases 8 and 9. These results demonstrate that although WC–Co and Co can produce similar cellular effects, the signalling mechanisms may be different.

Although Cu is a trace element necessary for the function of enzymes such as SOD, it can also produce ROS, DNA damage, and other carcinogenic effects when present in excess. Cherian and Ostrakhovitch examined the effects of Cu exposure on the p53 tumour suppressor signalling pathways in the breast cancer epithelial cell lines MDA-MB-231 and MCF7. p53 mutant MDA-MB-231 cells are resistant to metal toxicity, while p53 wild-type MCF7 cells are sensitive to metal-induced apoptosis [107, 108]. ROS production was not observed in MDA-MB-231 cells exposed to Cu; however, an increase in Akt phosphorylation, Akt nuclear translocation, Cyclin D1 expression, and cell cycle progression were still apparent. In contrast, ROS production in MCF7 cells increased in response to Cu exposure. Increases in p53 expression, p21 expression, G1 phase arrest, and apoptosis in MCF7 cells were also observed. Additionally, Cu-exposed MCF7 cells exhibited a loss of mitochondrial membrane potential and lacked Akt activation and translocation. Suppression of p53 in MCF7 cells with pifithrin or E6 protein decreased p53 phosphorylation and increased Akt phosphorylation, suggesting that p53 is involved in the response to Cu-induced ROS formation [109].

Nickel signalling

Insoluble nickel (Ni) compounds, such as Ni_3S_2, green NiO, black NiO, and Ni refinery dust, have been shown to exert carcinogenic effects in a variety of animal experiments [110]. Landolph *et al*. showed that Ni_3S_2, green NiO, and black NiO are phagocytosed by C3H/10T1/2 mouse embryo cells. Cytotoxicity, chromosomal aberrations, and morphological transformation were observed upon Ni exposure. The extent of phagocytosis was shown to correlate with each Ni compound's ability to induce transformation. RAP-PCR differential display experiments showed 130 genes that were differentially expressed between the Ni-transformed and non-transformed cell lines. Increased expression of the *ect-2*, calnexin, and *wdr1* genes in transformed cells were confirmed by Western blot. Respectively, these genes encode a Rho GDP/GTP exchange factor that modulates microtubule assembly, a molecular chaperone, and a stress-inducible protein. Decreased expression of the vitamin D receptor interacting protein/thyroid hormone activating protein 80 (DRIP/TRAP-80) and two other novel genes was also observed in transformed cells. Landolph's group concluded that Ni ions generate ROS, mutate and activate proto-oncogenes and/or tumour suppressor genes, and also induce chromosomal aberrations.

Paradoxically, Ni can upregulate the expression of some genes while downregulating others, possibly through the post-translational modification of histones. Ni suppresses the acetylation of core histones H3 and H4 and induces *de novo* DNA methylation, leading to gene silencing [34, 111]. Costa's group found that water-soluble Ni salts could inhibit the acetylation of the core histones H2A, H2B, H3, and H4. Ni exposure also induced methylation of Lys 9 in histone H3 and increased the ubiquitination of histones H2A and H2B. Golebiowski and Kasprzak also examined the acetylation status of the N-terminal tails of the core histones in Ni-exposed human and rodent cells exposed to Ni(II). Histone H2B exhibited the least acetylation, followed by histones H4, H3, and H2A in human airway epithelial cells and normal rat kidney cells. However, these effects were not seen in Chinese hamster ovary (CHO) cells, suggesting that the decrease in acetylation may be cell-type dependent.

Ni(II) has been shown to cleave the –SHHKAKGK motif of the C-terminal tail of histone H2A *in vitro* and in cell culture [112, 113]. Karaczyn *et al*. examined the effects of Ni(II) on other histones and showed that histone H2B abundance increases with Ni(II) treatment over time. Mass spectrometry and amino acid sequencing analysis confirmed that the H2B immunoreactive bands were variants of histone H2B. The larger band represented a truncated histone H2B lacking 16 amino acids from the N-terminal tail. The smaller band represented a histone H2B variant lacking both the 16 N-terminal amino acids and an additional nine amino acids from the C-terminal tail. Post-translational modifications of the smaller band included two acetylated Lys residues. Similar, but weaker effects on histone H2B were seen with Co(II), but no effects were seen with Cu(II), Cd(II), or Zn(II). Since the terminal tails of histones are thought to play key roles in structuring chromatin and regulating gene expression, the loss of these regions may be involved in the mechanisms relating to Ni(II)-induced carcinogenesis. Taken together, these results suggest that histone modification may play a major role in the mechanism of Ni-induced gene activation and/or silencing.

In addition to modifying core histones, Ni-exposure also activates the HIF-1α transcription factor via the depletion of intracellular Fe and the subsequent inhibition of the Fe-dependent enzyme proline hydroxylase, which leads to the stabilization of HIF-1α [114]. Huang *et al*. examined the effects of Ni_3S_2 or $NiCl_2$ exposure on other transcription factors and found that Ni ions increased ROS production and activated NFAT. NFAT activation was inhibited by catalase, NAC, and desferoxamine (DFO), but not by SOD or sodium

formate. Ni activation of the HIF-1α, PI3K, Akt, and p70^{S6K} was abrogated by PI3K inhibitors. Overexpression of Akt and PI3K dominant negative (DN) mutants also inhibited HIF-1α activation and Cap43 expression, while exposure to rapamycin, a p70^{S6K} inhibitor, had no effect. In conclusion, Huang's group suggested that Ni generates H_2O_2 which in turn activates NFAT. HIF-1α and Cap43 are also activated by Ni via a PI3K/Akt-dependent pathway, independently of p70^{S6K} activity.

Animal studies

Although animal studies provide valuable information concerning the toxic effects and signalling pathways of environmental contaminants, adequate animal models do not exist for some metals due in part to their roles as co-carcinogens. Thus, it is difficult to assess the effects of the metal itself because a known carcinogenic compound must be administered simultaneously in order for a tumour to develop. For example, Burns *et al*. presented evidence that hairless mice (Skh1 strain) exposed to ultra-violet radiation (UVR) and Cr- or As-contaminated drinking water elevated the number of tumours produced by UVR to 8.3- and 4.6-fold, respectively. These data confirm that metals can act as hazardous co-carcinogens even at low levels of exposure.

Most metal toxicity studies have been conducted on adult animals, yet few have focused on the effects of metal-exposure during the pubescent period. Chatterjee *et al*. examined Sprague–Dawley rats exposed to intravenous-infused $CdCl_2$ 40–100 days after birth. Histopathological examination of the mammary tissue showed that Cd(II) exposure slightly suppressed apoptosis while DNA strand breaks increased by 61%. At low doses, no change in histopathology of the mammary tissue was observed. However, at high doses, exposed rats exhibited intraductal proliferations. In addition, MT expression was elevated 60–86% in Cd(II)-treated animals in a dose-dependent manner. These results indicate that Cd(II) exposure may lead to an increased incidence of cancer in pubescent animals.

Additionally, Chatterjee *et al*. examined the chemopreventive effects of vanadium (V) supplementation. Rats were exposed to diethlnitrosamine (DEN) and phenobarbitol to induce hepatocarcinogenesis, and subsequently fed V-supplemented chow for 20 weeks. V supplementation reduced the nodular incidence, total number of tumours, and multiplicity of tumours, and decreased metallothionein expression, BrdU labelling index (a marker of cell proliferation), and iNOS expression. In addition, V supplementation increased p53 immunoreactivity and the apoptotic labelling index. These results suggest that V may play a significant role in controlling cellular activities during chemical-induced hepatocarcinogenesis.

Depleted uranium (DU) is a radioactive heavy metal used to manufacture military munitions. United States military personnel wounded by DU shrapnel may have a higher risk of cancer incidence, due to DU's genotoxic and mutagenic effects [115, 116]. DU can also cause radiation-specific cellular damage and transformation of human osteoblast cells [117, 118]. In order to simulate the effects of a DU-containing shrapnel wound, Miller *et al*. implanted non-tumourigenic immortalized cells into syngenic DBA/2 mice with or without DU implantation pellets. Mice implanted with DU pellets showed an 80% incidence of tumorigenesis, whereas control mice exhibited a 10% incidence of leukemia, suggesting that DU can significantly enhance tumour incidence.

Chromium(III) Picolinate ($Cr(Pic)_3$) is a dietary supplement associated with oxidative damage, DNA damage, antioxidant enzyme depletion, and renal/liver dysfunction in rats [119, 120]. In cell culture, $Cr(Pic)_3$ has been shown to cause mutations and DNA fragmentation [121]. Rasco *et al*. examined the prenatal effects of dietary supplementation of $Cr(Pic)_3$, $CrCl_3$, or picolinic acid in pregnant female mice. The fetuses birthed from the $Cr(Pic)_3$-fed females showed an increase in bifurcated cervical arches, delays in righting reflex, delays in hind limb grasp, and deficiencies in motor skills. These results demonstrate that dietary supplementation of $Cr(Pic)_3$ exerts negative effects on the developing nervous system of mammalian fetuses.

Barchowsky *et al*. developed an animal model to examine the effects of As(III) on angiogenesis and the expression of tissue remodelling genes in cardiac tissue. Male mice were exposed to As(III)-contaminated drinking water at low to moderately high concentrations for up to 20 weeks. Enhanced vascularization of Matrigel implants was observed after 5 weeks of As(III) exposure. RT-PCR showed a dose- and time-dependent induction of VEGF, VEGF receptors, plasminogen activator inhibitor-1, endothelin-1, and matrix metalloproteinase-9 in cardiac tissue. Barchowsky's group concluded that the effects of chronic As(III) exposure on the cardiovascular tissue varies with dose and length of exposure.

Summary

The research presented at the 3rd Conference on Molecular Mechanisms of Metal Toxicity and Carcinogenesis provided new insights into the mechanisms of ROS production, the mechanisms of metal signalling, and the development of animal exposure models. However, the following areas of metal research still need further exploration. First, there is a need for the development of new human biomarkers for metal exposure. Without definitive and specific biomarkers, it is very difficult to assess the extent of metal exposure in humans, and thus, difficult to treat the resulting ailments. Second, since most metals cause some form of ROS-induced cellular

stress, the elucidation of the mechanisms of ROS production may provide information concerning the general mechanisms of metal-induced carcinogenesis. Third, defining the mechanisms of metal-induced DNA damage may provide new insights into the signalling events involved in DNA damage repair pathways. Fourth, a better understanding of the complex signalling networks that lead to the adverse effects of metals could lead to the identification of superior and more specific novel therapeutic agents. Finally, better animal models for metal exposure are necessary in order to thoroughly evaluate the toxic and carcinogenic effects of metals in a model system. Through the use of current technology and the implementation of new experimental strategies, metal researchers can address these concerns.

References

1. Churg A, Brauer M, Carmen Avila-Casado M, Fortoul TI, Wright JL: Chronic exposure to high levels of particulate air pollution and small airway remodeling. Environ Health Perspect 111: 714–718, 2003
2. Gambelunghe A, Piccinini R, Ambrogi M, Villarini M, Moretti M, Marchetti C, Abbritti G, Muzi G: Primary DNA damage in chrome-plating workers. Toxicology 188: 187–195, 2003
3. Hunder G, Javdani J, Elsenhans B, Schumann K: 109Cd accumulation in the calcified parts of rat bones. Toxicology 159: 1–10, 2001
4. Tsai SM, Wang TN, Ko YC: Mortality for certain diseases in areas with high levels of arsenic in drinking water. Arch Environ Health 54: 186–193, 1999
5. Gaetke LM, Chow CK: Copper toxicity, oxidative stress, and antioxidant nutrients. Toxicology 189: 147–163, 2003
6. Papanikolaou G, Pantopoulos K: Iron metabolism and toxicity. Toxicol Appl Pharmacol 202: 199–211, 2005
7. Tanzi RE, Petrukhin K, Chernov I, Pellequer JL, Wasco W, Ross B, Romano DM, Parano E, Pavone L, Brzustowicz LM: The Wilson disease gene is a copper transporting ATPase with homology to the Menkes disease gene. Nat Genet 5: 344–350, 1993
8. Parkkila S, Waheed A, Britton RS, Bacon BR, Zhou XY, Tomatsu S, Fleming RE, Sly WS: Association of the transferrin receptor in human placenta with HFE, the protein defective in hereditary hemochromatosis. Proc Natl Acad Sci USA 94: 13198–13202, 1997
9. Nair J, Carmichael PL, Fernando RC, Phillips DH, Strain AJ, Bartsch H: Lipid peroxidation-induced etheno-DNA adducts in the liver of patients with the genetic metal storage disorders Wilson's disease and primary hemochromatosis. Cancer Epidemiol Biomarkers Prev 7: 435–440, 1998
10. Feng Z, Hu W, Amin S, Tang MS: Mutational spectrum and genotoxicity of the major lipid peroxidation product, trans-4-hydroxy-2-nonenal, induced DNA adducts in nucleotide excision repair-proficient and -deficient human cells. Biochemistry 42: 7848–7854, 2003
11. Benedetti A, Pompella A, Fulceri R, Romani A, Comporti M: 4-Hydroxynonenal and other aldehydes produced in the liver in vivo after bromobenzene intoxication. Toxicol Pathol 14: 457–461, 1986
12. Bartsch H, Nair J, Velic I: Etheno-DNA base adducts as tools in human cancer aetiology and chemoprevention. Eur J Cancer Prev 6: 529–534, 1997
13. McCullough ML, Giovannucci EL: Diet and cancer prevention. Oncogene 23: 6349–6364, 2004
14. Rohrmann S, Smit E, Giovannucci E, Platz EA: Association between serum concentrations of micronutrients and lower urinary tract symptoms in older men in the Third National Health and Nutrition Examination Survey. Urology 64: 504–509, 2004
15. Karunasinghe N, Ryan J, Tuckey J, Masters J, Jamieson M, Clarke LC, Marshall JR, Ferguson LR: DNA stability and serum selenium levels in a high-risk group for prostate cancer. Cancer Epidemiol Biomarkers Prev 13: 391–397, 2004
16. Ames BN: DNA damage from micronutrient deficiencies is likely to be a major cause of cancer. Mutat Res 475: 7–20, 2001
17. Goddard JG, Gower JD, Green CJ: A chelator is required for microsomal lipid peroxidation following reductive ferritin-iron mobilisation. Free Radic Res Commun 17: 177–185, 1992
18. Reif DW, Simmons RD: Nitric oxide mediates iron release from ferritin. Arch Biochem Biophys 283: 537–541, 1990
19. Winston GW, Feierman DE, Cederbaum AI: The role of iron chelates in hydroxyl radical production by rat liver microsomes, NADPH-cytochrome P-450 reductase and xanthine oxidase. Arch Biochem Biophys 232: 378–390, 1984
20. Morgan EH: Studies on the mechanism of iron release from transferrin. Biochim Biophys Acta 580: 312–326, 1979
21. Huang C, Li J, Zhang Q, Huang X: Role of bioavailable iron in coal dust-induced activation of activator protein-1 and nuclear factor of activated T cells: difference between Pennsylvania and Utah coal dusts. Am J Respir Cell Mol Biol 27: 568–574, 2002
22. Zhang Q, Dai J, Ali A, Chen L, Huang X: Roles of bioavailable iron and calcium in coal dust-induced oxidative stress: Possible implications in coal workers' lung disease. Free Radic Res 36: 285–294, 2002
23. Katzer A, Hockertz S, Buchhorn GH, Loehr JF: *In vitro* toxicity and mutagenicity of CoCrMo and Ti6Al wear particles. Toxicology 190: 145–154, 2003
24. Leonard SS, Roberts JR, Antonini JM, Castranova V, Shi X: PbCrO4 mediates cellular responses via reactive oxygen species. Mol Cell Biochem 255: 171–179, 2004
25. Shi X, Flynn DC, Porter DW, Leonard SS, Vallyathan V, Castranova V: Efficacy of taurine based compounds as hydroxyl radical scavengers in silica induced peroxidation. Ann Clin Lab Sci 27: 365–374, 1997
26. Wang Y, Fang J, Leonard SS, Rao KM: Cadmium inhibits the electron transfer chain and induces reactive oxygen species. Free Radic Biol Med 36: 1434–1443, 2004
27. Leonard S, Gannett PM, Rojanasakul Y, Schwegler-Berry D, Castranova V, Vallyathan V, Shi X: Cobalt-mediated generation of reactive oxygen species and its possible mechanism. J Inorg Biochem 70: 239–244, 1998
28. Hei TK, Filipic M: Role of oxidative damage in the genotoxicity of arsenic. Free Radic Biol Med 37: 574–581, 2004
29. Lefebvre Y, Pezerat H: Reactive oxygen species produced from chromate pigments and ascorbate. Environ Health Perspect 102(Suppl 3): 243–245, 1994
30. Wang S, Leonard SS, Ye J, Ding M, Shi X: The role of hydroxyl radical as a messenger in Cr(VI)-induced p53 activation. Am J Physiol Cell Physiol 279: C868–C875, 2000
31. Balamurugan K, Rajaram R, Ramasami T, Narayanan S: Chromium(III)-induced apoptosis of lymphocytes: Death decision by ROS and Src-family tyrosine kinases. Free Radic Biol Med 33: 1622–1640, 2002
32. Petit A, Mwale F, Tkaczyk C, Antoniou J, Zukor DJ, Huk OL: Induction of protein oxidation by cobalt and chromium ions in human U937 macrophages. Biomaterials 26: 4416–4422, 2005
33. Chakrabarti SK, Bai C, Subramanian KS: DNA-protein crosslinks induced by nickel compounds in isolated rat lymphocytes: role of reactive oxygen species and specific amino acids. Toxicol Appl Pharmacol 170: 153–165, 2001

34. Kang J, Zhang Y, Chen J, Chen H, Lin C, Wang Q, Ou Y: Nickel-induced histone hypoacetylation: the role of reactive oxygen species. Toxicol Sci 74: 279–286, 2003
35. Lin C, Kang J, Zheng R: Oxidative stress is involved in inhibition of copper on histone acetylation in cells. Chem Biol Interact 151: 167–176, 2005
36. Bagchi D, Bagchi M, Stohs SJ: Chromium (VI)-induced oxidative stress, apoptotic cell death and modulation of p53 tumor suppressor gene. Mol Cell Biochem 222: 149–158, 2001
37. Wang S, Leonard SS, Ye J, Gao N, Wang L, Shi X: Role of reactive oxygen species and Cr(VI) in Ras-mediated signal transduction. Mol Cell Biochem 255: 119–127, 2004
38. Newbold RF, Amos J, Connell JR: The cytotoxic, mutagenic and clastogenic effects of chromium-containing compounds on mammalian cells in culture. Mutat Res 67: 55–63, 1979
39. Wise JP, Leonard JC, Patierno SR: Clastogenicity of lead chromate particles in hamster and human cells. Mutat Res 278: 69–79, 1992
40. Wise JP, Sr., Wise SS, Little JE: The cytotoxicity and genotoxicity of particulate and soluble hexavalent chromium in human lung cells. Mutat Res 517: 221–229, 2002
41. Leonard SS, Vallyathan V, Castranova V, Shi X: Generation of reactive oxygen species in the enzymatic reduction of PbCrO4 and related DNA damage. Mol Cell Biochem 234–235: 309–315, 2002
42. Han JY, Takeshita K, Utsumi H: Noninvasive detection of hydroxyl radical generation in lung by diesel exhaust particles. Free Radic Biol Med 30: 516–525, 2001
43. Casillas AM, Hiura T, Li N, Nel AE: Enhancement of allergic inflammation by diesel exhaust particles: Permissive role of reactive oxygen species. Ann Allergy Asthma Immunol 83: 624–629, 1999
44. Tsurudome Y, Hirano T, Yamato H, Tanaka I, Sagai M, Hirano H, Nagata N, Itoh H, Kasai H: Changes in levels of 8-hydroxyguanine in DNA, its repair and OGG1 mRNA in rat lungs after intratracheal administration of diesel exhaust particles. Carcinogenesis 20: 1573–1576, 1999
45. Baulig A, Garlatti M, Bonvallot V, Marchand A, Barouki R, Marano F, Baeza-Squiban A: Involvement of reactive oxygen species in the metabolic pathways triggered by diesel exhaust particles in human airway epithelial cells. Am J Physiol Lung Cell Mol Physiol 285: L671–L679, 2003
46. Siegel PD, Saxena RK, Saxena QB, Ma JK, Ma JY, Yin XJ, Castranova V, Al Humadi N, Lewis DM: Effect of diesel exhaust particulate (DEP) on immune responses: Contributions of particulate versus organic soluble components. J Toxicol Environ Health A 67: 221–231, 2004
47. Schuetzle D: Sampling of vehicle emissions for chemical analysis and biological testing. Environ Health Perspect 47: 65–80, 1983
48. Ichinose T, Furuyama A, Sagai M: Biological effects of diesel exhaust particles (DEP). II. Acute toxicity of DEP introduced into lung by intratracheal instillation. Toxicology 99: 153–167, 1995
49. Rah DK, Han DW, Baek HS, Hyon SH, Park JC: Prevention of reactive oxygen species-induced oxidative stress in human microvascular endothelial cells by green tea polyphenol. Toxicol Lett 155: 269–275, 2005
50. Erba D, Riso P, Bordoni A, Foti P, Biagi PL, Testolin G: Effectiveness of moderate green tea consumption on antioxidative status and plasma lipid profile in humans. J Nutr Biochem 16: 144–149, 2005
51. Qanungo S, Das M, Haldar S, Basu A: Epigallocatechin-3-gallate induces mitochondrial membrane depolarization and caspase-dependent apoptosis in pancreatic cancer cells. Carcinogenesis, 2005
52. Zykova TA, Zhang Y, Zhu F, Bode AM, Dong Z: The signal transduction networks required for phosphorylation of STAT1 at Ser727 in mouse epidermal JB6 cells in the UVB response and inhibitory mechanisms of tea polyphenols. Carcinogenesis 26: 331–342, 2005
53. Elbling L, Weiss RM, Teufelhofer O, Uhl M, Knasmueller S, Schulte-Hermann R, Berger W, Micksche M: Green tea extract and (−)-epigallocatechin-3-gallate, the major tea catechin, exert oxidant but lack antioxidant activities. FASEB J 2005
54. McCord JM: The evolution of free radicals and oxidative stress. Am J Med 108: 652–659, 2000
55. Stahl W, Sies H: Antioxidant defense: Vitamins E and C and carotenoids. Diabetes 46(Suppl 2): S14–S18, 1997
56. Podmore ID, Griffiths HR, Herbert KE, Mistry N, Mistry P, Lunec J: Vitamin C exhibits pro-oxidant properties. Nature 392: 559, 1998
57. Herbert V, Shaw S, Jayatilleke E: Vitamin C-driven free radical generation from iron. J Nutr 126: 1213S–1220S, 1996
58. Leonard SS, Cutler D, Ding M, Vallyathan V, Castranova V, Shi X: Antioxidant properties of fruit and vegetable juices: More to the story than ascorbic acid. Ann Clin Lab Sci 32: 193–200, 2002
59. Pirozhkova-Patalah IV, Shtemenko NI: Influence of cis-[Re2GABA2Cl4]Cl2 on the antioxidant defense system parameters of normal human blood. Biochemistry (Mosc) 66: 721–724, 2001
60. Hrynevych I, Oliinyk SA, Shtemenko NI, Shtemenko OV: Antioxidant properties of rhenium cluster complexes with butyric acid derivatives in blood plasma and erythrocytes. Ukr Biokhim Zh 75: 65–71, 2003
61. Turner RJ, Weiner JH, Taylor DE: Selenium metabolism in *Escherichia coli*. Biometals 11: 223–227, 1998
62. Madesh M, Hajnoczky G: VDAC-dependent permeabilization of the outer mitochondrial membrane by superoxide induces rapid and massive cytochrome c release. J Cell Biol 155: 1003–1015, 2001
63. Yan L, Spallholz JE: Generation of reactive oxygen species from the reaction of selenium compounds with thiols and mammary tumor cells. Biochem Pharmacol 45: 429–437, 1993
64. ElAttar TM, Virji AS: Modulating effect of resveratrol and quercetin on oral cancer cell growth and proliferation. Anticancer Drugs 10: 187–193, 1999
65. Bau DT, Wang TS, Chung CH, Wang AS, Wang AS, Jan KY: Oxidative DNA adducts and DNA-protein cross-links are the major DNA lesions induced by arsenite. Environ Health Perspect 110(Suppl 5): 753–756, 2002
66. Matsui M, Nishigori C, Toyokuni S, Takada J, Akaboshi M, Ishikawa M, Imamura S, Miyachi Y: The role of oxidative DNA damage in human arsenic carcinogenesis: detection of 8-hydroxy-2′-deoxyguanosine in arsenic-related Bowen's disease. J Invest Dermatol 113: 26–31, 1999
67. Shibutani S, Takeshita M, Grollman AP: Insertion of specific bases during DNA synthesis past the oxidation-damaged base 8-oxodG. Nature 349: 431–434, 1991
68. Grollman AP, Moriya M: Mutagenesis by 8-oxoguanine: An enemy within. Trends Genet 9: 246–249, 1993
69. Henderson PT, Delaney JC, Muller JG, Neeley WL, Tannenbaum SR, Burrows CJ, Essigmann JM: The hydantoin lesions formed from oxidation of 7,8-dihydro-8-oxoguanine are potent sources of replication errors in vivo. Biochemistry 42: 9257–9262, 2003
70. Leipold MD, Muller JG, Burrows CJ, David SS: Removal of hydantoin products of 8-oxoguanine oxidation by the *Escherichia coli* DNA repair enzyme, FPG. Biochemistry 39: 14984–14992, 2000
71. Henderson PT, Delaney JC, Gu F, Tannenbaum SR, Essigmann JM: Oxidation of 7,8-dihydro-8-oxoguanine affords lesions that are potent sources of replication errors *in vivo*. Biochemistry 41: 914–921, 2002
72. Muller JG, Duarte V, Hickerson RP, Burrows CJ: Gel electrophoretic detection of 7,8-dihydro-8-oxoguanine and 7,8-dihydro-8-oxoadenine via oxidation by Ir (IV). Nucleic Acids Res 26: 2247–2249, 1998
73. Michaels ML, Tchou J, Grollman AP, Miller JH: A repair system for 8-oxo-7,8-dihydrodeoxyguanine. Biochemistry 31: 10964–10968, 1992
74. Michaels ML, Cruz C, Grollman AP, Miller JH: Evidence that MutY and MutM combine to prevent mutations by an oxidatively damaged

form of guanine in DNA. Proc Natl Acad Sci USA 89: 7022–7025, 1992
75. Hailer MK, Slade PG, Martin BD, Rosenquist TA, Sugden KD: Recognition of the oxidized lesions spiroiminodihydantoin and guanidinohydantoin in DNA by the mammalian base excision repair glycosylases NEIL1 and NEIL2. DNA Repair (Amst) 4: 41–50, 2005
76. Ramon O, Sauvaigo S, Gasparutto D, Faure P, Favier A, Cadet J: Effects of 8-oxo-7,8-dihydro-2′-deoxyguanosine on the binding of the transcription factor Sp1 to its cognate target DNA sequence (GC box). Free Radic Res 31: 217–229, 1999
77. Hailer-Morrison MK, Kotler JM, Martin BD, Sugden KD: Oxidized guanine lesions as modulators of gene transcription. Altered p50 binding affinity and repair shielding by 7,8-dihydro-8-oxo-2′-deoxyguanosine lesions in the NF-kappaB promoter element. Biochemistry 42: 9761–9770, 2003
78. Miller BM, Adler ID: Suspect spindle poisons: Analysis of c-mitotic effects in mouse bone marrow cells. Mutagenesis 4: 208–215, 1989
79. Mateuca R, Aka PV, De Boeck M, Hauspie R, Kirsch-Volders M, Lison D: Influence of hOGG1, XRCC1 and XRCC3 genotypes on biomarkers of genotoxicity in workers exposed to cobalt or hard metal dusts. Toxicol Lett 156: 277–288, 2005
80. Kim YD, An SC, Oyama T, Kawamoto T, Kim H: Oxidative stress, hogg1 expression and NF-kappaB activity in cells exposed to low level chromium. J Occup Health 45: 271–277, 2003
81. Zharkov DO, Rosenquist TA: Inactivation of mammalian 8-oxoguanine-DNA glycosylase by cadmium(II): Implications for cadmium genotoxicity. DNA Repair (Amst) 1: 661–670, 2002
82. Palmiter RD: Regulation of metallothionein genes by heavy metals appears to be mediated by a zinc-sensitive inhibitor that interacts with a constitutively active transcription factor, MTF-1. Proc Natl Acad Sci USA 91: 1219–1223, 1994
83. Saydam N, Adams TK, Steiner F, Schaffner W, Freedman JH: Regulation of metallothionein transcription by the metal-responsive transcription factor MTF-1: Identification of signal transduction cascades that control metal-inducible transcription. J Biol Chem 277: 20438–20445, 2002
84. Rosen BP: Transport and detoxification systems for transition metals, heavy metals and metalloids in eukaryotic and prokaryotic microbes. Comp Biochem Physiol A Mol Integr Physiol 133: 689–693, 2002
85. Rosen BP: Biochemistry of arsenic detoxification. FEBS Lett 529: 86–92, 2002
86. Rosen BP, Hsu CM, Karkaria CE, Owolabi JB, Tisa LS: Molecular analysis of an ATP-dependent anion pump. Philos Trans R Soc Lond B Biol Sci 326: 455–463, 1990
87. Liu Z, Boles E, Rosen BP: Arsenic trioxide uptake by hexose permeases in Saccharomyces cerevisiae. J Biol Chem 279: 17312–17318, 2004
88. Liu Z, Carbrey JM, Agre P, Rosen BP: Arsenic trioxide uptake by human and rat aquaglyceroporins. Biochem Biophys Res Commun 316: 1178–1185, 2004
89. Bhattacharjee H, Carbrey J, Rosen BP, Mukhopadhyay R: Drug uptake and pharmacological modulation of drug sensitivity in leukemia by AQP9. Biochem Biophys Res Commun 322: 836–841, 2004
90. Lee TC, Ho IC: Modulation of cellular antioxidant defense activities by sodium arsenite in human fibroblasts. Arch Toxicol 69: 498–504, 1995
91. Seol JG, Park WH, Kim ES, Jung CW, Hyun JM, Kim BK, Lee YY: Effect of arsenic trioxide on cell cycle arrest in head and neck cancer cell line PCI-1. Biochem Biophys Res Commun 265: 400–404, 1999
92. Millar JB, Russell P: The cdc25 M-phase inducer: An unconventional protein phosphatase. Cell 68: 407–410, 1992
93. Chou IN: Distinct cytoskeletal injuries induced by As, Cd, Co, Cr, and Ni compounds. Biomed Environ Sci 2: 358–365, 1989
94. Qian Y, Liu KJ, Chen Y, Flynn DC, Castranova V, Shi X: Cdc42 regulates arsenic-induced NADPH oxidase activation and cell migration through actin filament reorganization. J Biol Chem 280: 3875–3884, 2005
95. Ivanov VN, Hei TK: Arsenite sensitizes human melanomas to apoptosis via tumor necrosis factor alpha-mediated pathway. J Biol Chem 279: 22747–22758, 2004
96. Wang S, Shi X: Mechanisms of Cr(VI)-induced p53 activation: The role of phosphorylation, mdm2 and ERK. Carcinogenesis 22: 757–762, 2001
97. Ha L, Ceryak S, Patierno SR: Chromium (VI) activates ataxia telangiectasia mutated (ATM) protein. Requirement of ATM for both apoptosis and recovery from terminal growth arrest. J Biol Chem 278: 17885–17894, 2003
98. Buscemi G, Perego P, Carenini N, Nakanishi M, Chessa L, Chen J, Khanna K, Delia D: Activation of ATM and Chk2 kinases in relation to the amount of DNA strand breaks. Oncogene 23: 7691–7700, 2004
99. McKinnon PJ: ATM and ataxia telangiectasia. EMBO Rep 5: 772–776, 2004
100. Falck J, Coates J, Jackson SP: Conserved modes of recruitment of ATM, ATR and DNA-PKcs to sites of DNA damage. Nature 434: 605–611, 2005
101. Lee JH, Paull TT: ATM activation by DNA double-strand breaks through the Mre11-Rad50-Nbs1 complex. Science 308: 551–554, 2005
102. Rogakou EP, Pilch DR, Orr AH, Ivanova VS, Bonner WM: DNA double-stranded breaks induce histone H2AX phosphorylation on serine 139. J Biol Chem 273: 5858–5868, 1998
103. Joseph P, Lei YX, Whong WZ, Ong TM: Molecular cloning and functional analysis of a novel cadmium-responsive proto-oncogene. Cancer Res 62: 703–707, 2002
104. Joseph P, Lei YX, Whong WZ, Ong TM: Oncogenic potential of mouse translation elongation factor-1 delta, a novel cadmium-responsive proto-oncogene. J Biol Chem 277: 6131–6136, 2002
105. Lemarie A, Lagadic-Gossmann D, Morzadec C, Allain N, Fardel O, Vernhet L: Cadmium induces caspase-independent apoptosis in liver Hep3B cells: role for calcium in signaling oxidative stress-related impairment of mitochondria and relocation of endonuclease G and apoptosis-inducing factor. Free Radic Biol Med 36: 1517–1531, 2004
106. Moulin JJ, Wild P, Romazini S, Lasfargues G, Peltier A, Bozec C, Deguerry P, Pellet F, Perdrix A: Lung cancer risk in hard-metal workers. Am J Epidemiol 148: 241–248, 1998
107. Fan LZ, Cherian MG: Potential role of p53 on metallothionein induction in human epithelial breast cancer cells. Br J Cancer 87: 1019–1026, 2002
108. Ostrakhovitch EA, Cherian MG: Differential regulation of signal transduction pathways in wild type and mutated p53 breast cancer epithelial cells by copper and zinc. Arch Biochem Biophys 423: 351–361, 2004
109. Ostrakhovitch EA, Cherian MG: Role of p53 and reactive oxygen species in apoptotic response to copper and zinc in epithelial breast cancer cells. Apoptosis 10: 111–121, 2005
110. Nickel and Nickel Compounds. IARC Monographs 49: 257–445, 1990
111. Zhao J, Yan Y, Salnikow K, Kluz T, Costa M: Nickel-induced down-regulation of serpin by hypoxic signaling. Toxicol Appl Pharmacol 194: 60–68, 2004
112. Bal W, Karantza V, Moudrianakis EN, Kasprzak KS: Interaction of Nickel(II) with histones: *In vitro* binding of nickel(II) to the core histone tetramer. Arch Biochem Biophys 364: 161–166, 1999
113. Bal W, Liang R, Lukszo J, Lee SH, Dizdaroglu M, Kasprzak KS: Ni(II) specifically cleaves the C-terminal tail of the major variant of histone H2A and forms an oxidative damage-mediating complex with the cleaved-off octapeptide. Chem Res Toxicol 13: 616–624, 2000

114. Davidson T, Salnikow K, Costa M: Hypoxia inducible factor-1 alpha-independent suppression of aryl hydrocarbon receptor-regulated genes by nickel. Mol Pharmacol 64: 1485–1493, 2003
115. Miller AC, Blakely WF, Livengood D, Whittaker T, Xu J, Ejnik JW, Hamilton MM, Parlette E, John TS, Gerstenberg HM, Hsu H: Transformation of human osteoblast cells to the tumorigenic phenotype by depleted uranium-uranyl chloride. Environ Health Perspect 106: 465–471, 1998
116. Miller AC, Fuciarelli AF, Jackson WE, Ejnik EJ, Emond C, Strocko S, Hogan J, Page N, Pellmar T: Urinary and serum mutagenicity studies with rats implanted with depleted uranium or tantalum pellets. Mutagenesis 13: 643–648, 1998
117. Yazzie M, Gamble SL, Civitello ER, Stearns DM: Uranyl acetate causes DNA single strand breaks *in vitro* in the presence of ascorbate (vitamin C). Chem Res Toxicol 16: 524–530, 2003
118. Miller AC, Brooks K, Stewart M, Anderson B, Shi L, McClain D, Page N: Genomic instability in human osteoblast cells after exposure to depleted uranium: Delayed lethality and micronuclei formation. J Environ Radioact 64: 247–259, 2003
119. Stearns DM, Wise JP, Sr., Patierno SR, Wetterhahn KE: Chromium(III) picolinate produces chromosome damage in Chinese hamster ovary cells. FASEB J 9: 1643–1648, 1995
120. Bagchi D, Bagchi M, Balmoori J, Ye X, Stohs SJ: Comparative induction of oxidative stress in cultured J774A.1 macrophage cells by chromium picolinate and chromium nicotinate. Res Commun Mol Pathol Pharmacol 97: 335–346, 1997
121. Stearns DM, Silveira SM, Wolf KK, Luke AM: Chromium(III) tris(picolinate) is mutagenic at the hypoxanthine (guanine) phosphoribosyltransferase locus in Chinese hamster ovary cells. Mutat Res 513: 135–142, 2002