Molecular and Cellular Biochemistry **222:** 3–9, 2001.

Molecular mechanisms of metal toxicity and carcinogenesis

Suwei Wang and Xianglin Shi

Pathology and Physiology Research Branch, Health Effects Laboratory Division, National Institute for Occupational Safety and Health, Morgantown, WV; Department of Basic Pharmaceutical Sciences, West Virginia University, Morgantown, WV, USA

Abstract

Many metals and metal-containing compounds have been identified to be potent mutagens and carcinogens. Recently, a new sub-discipline of molecular toxicology and carcinogenesis has been developed. The combination of newly developed molecular techniques and free radical approach makes it possible to insightfully examine metal-induced carcinogenesis in precise molecular terms so that intricate biological interrelationships can be elucidated. In consideration of the increased amount of new findings deciphered by utilizing these new methods, the 1st Conference on Molecular Mechanisms of Metal Toxicity and Carcinogenesis was held. In this conference, more than 50 scientists from nine countries presented their novel discoveries concerning metal-induced carcinogenesis, delineated molecular mechanism of metal carcinogenesis, and proposed novel therapeutic intervention and prevention strategies. This article reviews some of the state-of-the-art information presented at the meeting regarding the molecular mechanisms of metal cytotoxicity and carcinogenesis. (Mol Cell Biochem **222**: 3–9, 2001)

Key words: ROS, cell growth regulation, transcription factor, signal transduction, cytotoxicity and carcinogenesis

Introduction

Both environmental and occupational exposures to chromium (Cr), nickel (Ni), and arsenic (As) compounds have been reported to cause human tumors in various systems [1, 2]. Although a variety of metals, including Cr, Ni, As, vanadium (V), cadmium (Cd), and cobalt (Co), have been reported to induce carcinogenesis both in animals and in humans, the underlying mechanisms are not well understood. Since carcinogenesis induced by metals involves a variety of facets, it is difficult to identify one common mechanism. It is very likely that each metal has its own unique molecular mechanisms which contribute to the cancer development.

During the last two decades, chemical and cellular studies have provided a variety of contributions to extend our understanding for metal-induced carcinogenesis. Generally, carcinogenesis is considered to have the following four stages: initiation, promotion, progression, and metastasis. Although mutations on genome DNA which are capable of activating oncogenes or inactivating tumor suppressors, are traditionally considered to be the crucial factor for cancer initiation, other events, such as transcription activation, recombination, and oncogene amplification, which may or may not require DNA damage, also contribute to the tumor initiation process [3–7]. Altered gene expression and cell signaling are considered to be related to tumor promotion and progression [8, 9]. The mechanisms of metal-induced carcinogenesis are believed to be involved in all stages of cancer development.

Metal compounds are found to have effects on the cellular organelles and components, such as cell membrane, mitochondrial, lysosome, endoplasmic reticulum, nuclei, and some enzymes involved in metabolism, detoxification, and damage repair [10]. All these systems are considered to influence metal-induced cellular responses. Metal ions have been found to directly bind to some cell components such as DNA and nuclear proteins through ionic and coordination bonds, causing

Address for offprints: X. Shi, Pathology and Physiology Research Branch, National Institute for Occupational Safety and Health, Morgantown, WV 26505, USA

DNA damage and conformation changes [11, 12]. Since the binding of metal ions to DNA and proteins are reversible, it appears that this direct binding can not be fully responsible for carcinogenic metal-induced DNA lesions, including DNA strand breaks and base modifications, which result from the breakage or formation of covalent bonds [13–16]. Based on these observations, in addition to direct binding, other indirect effects of metals must be involved in metal-induced cell responses.

Accumulating evidence suggests that reactive oxygen species (ROS) play a major role in mediating metal-induced cellular responses and carcinogenesis [16–19]. ROS mainly include superoxide radical ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), hypochloride (HOCl), hydroxyl radical (·OH), peroxyl radical (ROO·), alkoxyl radical (RO·), thiyl radical (RS·), and nitric oxide (NO). Several groups have reported that carcinogenic metal-induced chromatin damage can be mimicked by ROS, suggesting the important role of ROS in these processes [16–19]. In addition, various metal ions, such as Cr (VI), V (V), and Co (II), have been reported to produce ROS in cellular systems through a Fenton-like reaction and Haber–Weiss cycle [20–23]. The generated ROS are believed to mediate metal-induced cell injuries.

Recently, a new sub-discipline of molecular toxicology and carcinogenesis has been developed. The combination of newly developed molecular techniques and free radical approach makes it possible to insightfully examine metal-induced carcinogenesis in precise molecular terms so that intricate biological interrelationships can be elucidated. In view of the increased amount of new findings deciphered by utilizing these new methods, the 1st Conference on Molecular Mechanisms of Metal Toxicity and Carcinogenesis was held at National Institute for Occupational Safety and Health (NIOSH), Morgantown, WV, in September, 2000. In this conference, more than 50 scientists from nine countries presented their novel discoveries concerning metal-induced carcinogenesis, delineated molecular mechanism of metal carcinogenesis, and proposed novel therapeutic intervention and prevention strategies. The following categories have been emphasized during the meeting: (a) effects of metal on apoptosis and cell growth regulation; (b) mechanism of metal-induced regulation of nuclear transcription factors, such as NF-κB, AP-1, p53, and NFAT; (c) activation of signal transduction pathway, MAP kinase pathway; (d) effects of metals on gene expression; (e) mechanism of metal-induced cytotoxicity, cellular responses, mutagenesis, and carcinogenesis; (f) detection and mechanism of metal-induced free radical generation.

Effects of metal on apoptosis and cell growth regulation

Apoptosis, or programmed cell death, is a genetically controlled response for cells to commit suicide. It provides a major form of cell death in normal development of vertebrate embryos, appropriate functions of the immune system, and maintenance of tissue homeostasis [24–29]. Apoptosis can be triggered by a variety of extrinsic and intrinsic signals, including UV and ion radiations, viral infection, bacterial toxins, nutrient deprivation, oncogene expressions, oxidants, etc. [30–32]. Apoptosis that results from these stimuli regulates the elimination of excess, genetically damaged, and improperly developed cells from tissues. Various toxic metals, including Cr, V, Cd, Ni, and As, have been reported to be able to induce apoptosis [33–37]. However, the mechanisms and the effects of apoptosis are still not clear.

In this conference, Ye *et al.* presented the evidence indicating that both ROS and p53 play very important roles in Cr (VI)-induced apoptosis. ROS were generated within a few minutes after Cr (VI) treatment, while p53 induction took at least 5 h. The observations that p53 positive cells have a higher apoptosis level compared to p53 negative cells only at later stage (3–24 h) but not at early stage (0–3 h) indicate that ROS generation through Cr (VI) reduction was responsible for the early stage of apoptosis, whereas p53 contributed to the later enhanced apoptosis induced by Cr (VI). Meanwhile, Patierno *et al.* and Bagchi *et al.* also reported that Cr (VI) was able to induce apoptosis in several different cell lines at different levels in a dose and time dependent manner both *in vitro* and *in vivo*. Induction of p53, disruption of the mitochondrial membrane permeability transition (MMPT), release of mitochondrial cytochrome C into the cytoplasm, and activation of caspase 3 have been implicated in the process of apoptosis induced by Cr (VI). More interestingly, they found that Cr-induced DNA–DNA crosslinks were crucial for apoptosis resulting from Cr exposure. These findings indicate that both ROS and Cr metabolites from Cr (VI) reduction play important roles in the mechanism of Cr (VI)-induced apoptosis.

Ong *et al.* investigated the role of calcium (Ca^{2+}) in Cd-induced apoptosis in primary cultured mouse thymocytes. Exposure of thymocytes to Cd resulted in a rapid and sustained intracellular Ca^{2+} elevation, followed by caspase-3 activation, PARP cleavage, and DNA fragmentation. They believe that intracellular Ca^{2+} elevation may trigger caspase-3 activation, contributing to Cd-induced apoptosis in thymocytes. This study has provided new information for a better understanding of the immunotoxic and immunomodulatory effects of Cd.

Under normal conditions, cell cycle proceeds from G_1 phase to S phase, then to G_2 and M phase, without interruption. These processes are monitored at several checkpoints for the proper DNA duplication, regulated by complexes of cyclins and cyclin-dependent kinases. Two important checkpoints are in charge of G_1/S and G_2/M transitions. When damage occurs to the cells, most of them are capable of arresting the cell cycle, repairing the damage, and resuming the proliferation when the damages are fixed. By this way, the cells take con-

trol of the precision of DNA replication and maintain the integrity of the genome [38–41]. However, for the damage that cannot be repaired by arresting the cell cycle, the cells would undergo apoptosis. Therefore, cell cycle arrest is a passive defense mechanism of cells in response to external toxicants, and improper cell growth regulation may result in cancer development.

At present, there are only a few reports available on the effects of metal on cell growth regulation. In this meeting, two articles from Dr. Xianglin Shi's laboratory presented their novel findings on mechanisms of metal-induced cell growth arrest. Zhang *et al.* found that V (V) was able to induce G_2/M phase arrest in A549 cells. During this process, several regulatory pathways are involved, including activation of p53 and p21 and inactivation of cdc2/cyclin B complex resulting from both increased chk1 expression and inhibition of cdc25C. ROS generated in V (V)-stimulated cells, especially H_2O_2, are involved in the regulatory mechanisms. In another study, Chen *et al.* utilized Cr (VI) and As (III), focusing on GADD45, a cell cycle checkpoint protein whose activation arrests cells at G_2/M phase. Inhibition of NF-κB activation caused an increased and prolonged induction of GADD45, resulting in G_2/M arrest induced by As (III). On the other hand, abrogation of c-*jun* N-terminal kinase (JNK) activation decreased GADD45 induction by As (III), suggesting that JNK and NF-κB may have distinct contribution to cell growth regulation through their opposite effects on GADD45 in response to toxic metals. The results indicate that toxic metals indeed can cause cell cycle arrest, and several different pathways and regulatory proteins are involved in these cell growth arrest process. The investigations on metal-induced cell cycle arrest opened a new window to study the mechanisms of metal-induced cancer development.

Mechanism of metal-induced regulation of nuclear transcription factors

One of the characteristics of nuclear transcription factors is that they are able to recognize and bind to specific sequences of DNA, regulating the expression of their target genes. Due to this unique property, the activation of transcription factors act as a key switch in mediating cell responses upon stimulation. Accumulating evidence indicate that some metals such as Cr, V, and lead (Pb), are able to activate various transcription factors including NF-κB, AP-1, and p53 protein [42–52]. While it has been suggested that ROS may be involved in the process of transcription factors activation, the underlying mechanisms are still need to be investigated.

In this conference, Wang *et al.* provided direct evidence, indicating that ROS do play an important role in mediating p53 activation induced by Cr (VI). Among all the ROS generated during the Cr (VI) reduction, ·OH radical is the one that is responsible for Cr (VI)-induced p53 activation. Thorough investigation on transcription factor activation in response to vanadate exposure has been carried out by Dr. Xianglin Shi's group. In addition to the findings that vanadate is able to induce the activation of NF-κB, AP-1, and p53, they performed studies to uncover the mechanisms of those activation from different angles. Huang *et al.* reported an increase in p53 transactivation induced by vanadate. Furthermore, they pointed out that this activation of p53 was mainly through H_2O_2, which is generated through vanadate reduction.

Chen *et al.* presented evidence that vanadate also was able to induce the activation of both NF-κB and JNK in macrophages. In their study, they also investigated the possible cross talk between MAP kinase pathway and NF-κB upstream signal transduction. Blockage of JNK activation resulted in partial inhibition of vanadate-induced IκBα degradation, but inactivation of IKKβ, which results in decreased IκBα degradation, did not have any effect on the activation of JNK induced by vanadate. These results suggest that the involvement of JNK in regulating the upstream signal transduction of NF-κB activation. Furthermore, they found that both vanadate-induced IκBα degradation and JNK activation could be inhibited by antioxidant, N-acetylcysteine (NAC), indicating that regulatory role of ROS in the upstream of NF-κB activation. Transcription factor AP-1, which is important in mediating cell proliferation, also could be induced by vanadate. Ding *et al.* observed AP-1 activation induced by vanadate both in JB6 cells and rat lung epithelia cells, and the activation was mediated by $O_2^{\cdot-}$ and H_2O_2, but not by ·OH radical.

NFAT (nuclear factor of activated T cells) family proteins, which are expressed in most immune-system cells, play a pivotal role in the transcription of cytokine genes and other genes critical for the immune response. Huang *et al.* reported that NFAT could be activated upon vanadate exposure. Blockage of both calcium and calcineurin inhibited the activation of NFAT by vanadate. Similar inhibition was observed when specific antioxidants for H_2O_2 was added, suggesting the potential role of H_2O_2 in mediating calcium/calcineurin pathway-dependent activation of NFAT induced by vanadate. In addition, another heavy metal, nickel was also found to be able to induce NFAT activation. The activation of NFAT by nickel is involved in regulating the expression of Cap43 gene, a novel gene induced by nickel and hypoxia [53, 54]. These findings provide evidence for the activation of NFAT by metal for the first time, and help to understand the signal transduction pathways involved in carcinogenic effects of nickel compounds, as well as other transition metals.

Effects of metals on signal transduction pathway

Since metals have been found to activate transcription factor AP-1, it is not surprising to find them affecting the AP-1

upstream regulatory signal transduction pathway, namely the MAP kinase pathway. Different effects of Cr (VI) and Cd on the activation of three major MAP kinases, ERK, JNK, and p38 kinase were presented by Dr. Jia-LingYang's group. Cr (VI) is able to activate all of the three MAP kinases in dose- and time-dependent manner. In contrast, Cd decreased the ERK activity with a concomitant enhancement in JNK at low cytotoxic dosages, but persistently activated the three MAP kinases at high cytotoxic dosages. Cross talk of the MAP kinase signals was observed in cells exposed to Cr (VI) but not to Cd. Increased JNK and p38 kinase activity, as well as reduced ERK activity, contribute to Cd-induced apoptosis. In contrast, the activation of these three MAP kinases was not involved in apoptosis induced by Cr (VI). These results imply that although ROS are considered to play important roles in the cell responses induced by both Cd and Cr (VI), these two metals may induce MAP kinase activation and regulate cell growth arrest and apoptosis through different mechanisms.

Another interesting and novel finding presented in this conference is that ROS were reported to induce the phosphorylation of tyrosine kinase. Cr (VI) was utilized as a source for whole spectrum of ROS generation. A dose- and time-dependent increase of tyrosine phosphorylation was observed in human lung epithelial cells after Cr (VI) exposure. Among all the ROS generated by Cr (VI) reduction, H_2O_2 and ·OH radical are the major species to mediate the increased tyrosine phosphorylation. This study provides direct evidence demonstrating the involvement of ROS, especially H_2O_2 and ·OH radical, in tyrosine phosphorylation for the first time, providing a new direction for the further mechanism studies on the effects of ROS on regulating signal transduction pathway.

Effects of metals on gene expression

Most of the toxic heavy metals are also reported to be carcinogens and mutagens due to their ability to cause gene mutation. Water-insoluble nickel has been reported by Costa *et al.* to silence genes in mammalian cells [55]. In this meeting, Dr. Costa presented the results of their recent investigations on the molecular mechanisms of nickel-induced gene silencing. The inhibition of the acetylation of histone H4 at specific N-terminal lysine sites by nickel was observed to contribute to nickel induced gene silencing. Trichostatin A, which is an inhibitor of histone deacetylase, is able to inhibit the ability of nickel to silence genes by reducing the inhibition of histone H4 acetylation. The results indicated that as a major component of the transcriptional machinery, the acetylation of histone plays an important role in nickel-induced silencing, which is presumed to be a crucial step in nickel-induced carcinogenesis.

In addition to inducing gene silencing, insoluble nickel compounds can also cause morphological transformation by positively altering gene expression as presented by Landolph *et al.* By using RAP-PCR mRNA Differential Display to examine cells transformed by carcinogenic insoluble nickel compounds, they found that 130 genes were differentially expressed compared to nontransformed cells. These results indicate that the global gene expression in the transformed cells was substantially modified. Among these altered 130 genes, 10 genes which are involved in various functions have been identified. Further work to determine how these alterations contribute to nickel-induced transformation will help in understanding the tumor development caused by nickel.

Similarly, by using microarray technique, Ye *et al.* found that Cr (VI) was able to alter the expression of about 220 genes, of which 150 genes was up-regulated and 70 were down-regulated by Cr (VI). Functional analysis indicates that these genes are involved in several categories, including redox stress, calcium mobilization, fat degradation, protein metabolism, cell cycle arrest, and carcinogenesis. The results provide critical information and directions for understanding the molecular mechanisms involved in Cr (VI)-induced carcinogenesis.

To further understand the precise mechanisms of As and Cr induced toxicity and carcinogenesis, Hamilton *et al.* examined the effects of these two metals on gene expression. Their previous studies indicate that both As (III) and Cr (VI) have profound but preferential effects on expression of several inducible genes, including the hormone-regulated phosphoenolpyruvate carboxykinase (PEPCK) gene, whose expression is associated with the glucocorticoid receptor (GR)-mediated regulatory pathway [56, 57]. Their novel study presented at this conference indicated that As (III) significantly suppressed both basal and inducible expression of PEPCK through specific suppression of GR as a transcription factor within the nucleus following hormone binding and nuclear translocation. In contrast, Cr (VI) enhanced both basal and inducible gene expression of PEPCK via Cr (III)-involved cAMP signaling pathway.

Metallothionein (MT) is a low-molecular-weight protein with approximately one-third of its amino acids being cysteine residues. This protein is inducible by various metals, exerting its role in essential trace metal homeostasis and heavy metal detoxification. MTF-1, a protein which specific binds to metal response element (MRE), plays a major role in regulating metal-inducible MT transcription [58]. At present, there are two models that describe MTF-1-mediated regulation of MT transcription. However, neither of them is able to explain how metals other than zinc can activate MT transcription. Freedman *et al.* presented a novel mechanism for the activation of MT induced by metal: MTF-1 phosphorylation. They found that MTF-1 was phosphorylated following zinc exposure but not by Cd treatment. The results confirmed that MTF-1 can be phosphorylated *in vivo*, and this phosphor-

ylation might be the mechanism for metal-induced MT transcription. Although both zinc and Cd are able to up-regulate MT transcription, the mechanisms for their effects might be different.

Mechanism of metal-induced cytotoxicity, cellular responses, mutagenesis, and carcinogenesis

Cr (VI)-containing compounds are well established mutagens and carcinogens. Although the mechanisms for their effects are not clear yet, it is generally believed that Cr (VI)-induced cellular responses are mediated by the reactive intermediates generated directly from Cr (VI) reduction, including ROS, Cr (V), Cr (IV), and Cr (III). Previous studies and a variety investigations presented in this meeting have shown that ROS generated from Cr (VI) reduction play crucial roles in DNA damage, lipid peroxidation, and transcription factor activation [42–44, 46]. At the meeting, Pritchard reported his recent finding, implicating nitrogen species in the mechanisms of Cr (VI)-induced endothelial cell inflammation. Cr (VI) was observed to be capable of inducing inflammation in human umbilical vein endothelial cells (HUVEC). During this process, ICAM-1 gene expression was increased at mRNA level, suggesting the possible involvement of this gene in the inflammation. Cr (VI) increased the generation of $O_2^{\cdot-}$ and decreased the intracellular NO without affecting the NO generation. At the same time, an increased formation of peroxynitrite was observed, indicating that generated $O_2^{\cdot-}$ scavenged NO to produce peroxynitrite. Through this mechanism, decreased NO activity and increased formation of peroxynitrite appear to play a critical role in enhancing the expression of ICAM-1, which is crucial in Cr (VI)-induced endothelial cell injury. The other project carried out by the same group using bovine coronary endothelial cells (BCEC) investigated the mechanisms of effects of Cr (VI) on eNOS (endothelial NO synthase) activity. Even at very low concentration, Cr (VI) perturbed eNOS function in BCEC cells. This perturbation is possibly due to the disruption of the interaction between eNOS and heat shock protein 90 (Hsp90), whose association to eNOS ensures the proper functions of this synthase. This disruption may be an important step in the mechanisms by which endothelial cells can be induced to generate $O_2^{\cdot-}$ in an eNOS-dependent manner.

In addition to ROS and reactive nitrogen species, the importance of chromates in Cr (VI)-induced cellular responses have been re-emphasized during this meeting. Several groups presented their recent findings on Cr (VI)-induced DNA damage. Cystine may act as a reductant when mixing with Cr (VI), and reduce Cr (VI) to Cr (III). The generated Cr (III) is able to form ternary Cr (III)-DNA adducts, which contribute to a significant increase in mutation frequencies. The study by Zhitkovich *et al.* provided strong evidence for the importance of Cr (III)-dependent pathway in the genotoxicity of Cr (VI) compounds. In addition, Krepkiy *et al.* reported that Cr (III)-Cys might be the intermediate for the formation of Cr (III)-DNA complex. Cr (V), another reactive metabolite of Cr (VI), is also reported to cause DNA damage [59]. To investigate the oxidative DNA damage by Cr (V), Sugden *et al.* established two model Cr (V) complexes: Cr (V)-ehba and Cr (V)-salen, which have similar oxidative mechanisms in terms of their abilities to serve as direct-acting oxidants but oxidizing different sites on DNA. Cr (V)-ehba complex has a preference for oxidation at the deoxyribose sugar moiety resulting in sequence-neutral, frank- and alkaline-labile strand breaks, while Cr (V)-salen complex with has shown a preference for oxidation at the nucleic acid base guanine to yield a piperidine-labile cleavage site. The use of these tow model Cr (V) complexes has shown an excellent correlation with the DNA damage associated with cellular exposure to chromate, facilitating further understanding for the mechanisms of Cr (VI)-induced DNA damage and other responses.

Cr (VI) has been shown to be mutagenic in a variety of assay systems. Using Big Blue transgenic mouse, Dixon *et al.* reported that manipulating glutathione (GSH) level could alter the mutant frequency of Cr (VI): reduced tissue GSH levels resulted in a lower mutant frequency, suggesting the important role of GSH in regulating Cr (VI) mutagenesis. Most interestingly, As, a co-mutagen, was found to suppress the Cr (VI) mutagenesis in this system. The possible explanation is that As reduced the availability of free GSH required for Cr reduction and mutagenesis.

Although As has a much lower potency to induce mutagenesis compared to Cr (VI), it is able to induce significant cytotoxicity and carcinogenesis. Increasing evidence indicates that epigenetic mechanisms, including modulation of cell growth signaling, may play a role in As carcinogenesis. Simeonova *et al.* have demonstrated that As exposure activates cell proliferation of urinary bladder epithelium by using both *in vitro* and *in vivo* models. A persistent activation of AP-1 transcription factor, as well as the altered expression of a variety of genes associated with cell growth and cell arrest were observed following exposure of the cells to As. These molecular alterations might be involved in As-induced carcinogenesis. Moreover, the toxicity and carcinogenesis induced by As can be significantly enhanced by atrazine.

Detection and mechanism of free radical generation

It is generally believed that Cr (VI) compounds exert their functions via their reduction metabolites. However, there is no systematic investigation on how Cr (VI) compounds are converted to the metabolites. To solve this question, Leonard *et al.* thoroughly investigated the mechanisms of the genera-

tion of ROS and chromate metabolites. They have shown that Cr (VI) can be reduced to Cr (V) by glutathione reductase. During this process, $O_2^{\cdot -}$ radical is generated from molecular oxygen, followed by the generation of H_2O_2 via $O_2^{\cdot -}$ dismutation catalyzed by superoxide dismutase (SOD). Furthermore, H_2O_2 reacts with Cr (V) through a Fenton-like reaction to produce ·OH radical and regenerate Cr (VI). ·OH radicals generated by the enzymatic Cr (VI) reduction are capable of causing DNA strand breaks. This study provides a clear picture of ROS and reactive Cr intermediates generated from Cr (VI), facilitating further biological studies on Cr (VI)-induced carcinogenesis. The other study carried out by Liu *et al.* confirmed the generation of Cr (V) in the biological system. By using a newly developed *in vivo* electron paramagnetic resonance (EPR) spectrometer, they observed the formation of Cr (V) directly from intact mice after intravenous injection of Cr (VI). The intensity of Cr (V) signals decreased when the animal was pretreated with antioxidants and metal chelators. The study verified the importance of Cr (V) in mediating Cr (VI)-induced responses, and illustrated the possibility of using EPR to study paramagnetic metal ions in intact animals.

Discussion

During the meeting, a number of groups presented their state-of-the-art information in their research with metals. The presentations cover a wide range, including metal-induced cytotoxicity, cellular responses, mutagenesis, apoptosis, cell growth regulation, regulation of nuclear transcription factors, activation of signal transduction pathway, and gene expression. It should be noted that impressive progress has been achieved concerning the molecular mechanisms of metal-induced toxicity and carcinogenesis. However, there are still a lot of questions to be solved. A great number of investigations have been conducted concerning the roles of metals in regulating cell signaling. However, some of the findings are contradictory, and a link is needed to connect all the observations and findings. The question of how ROS and metal metabolites affect the signal transduction still remains to be answered. Metal-induced cell growth control has got more attention during this meeting. Three presentations from Dr. Xianglin Shi's group reported the effects of V on cell growth regulation. These findings provide a new direction for the study of mechanisms of metal-induced carcinogenesis. Furthermore, some new emerging techniques provide more convenient and powerful tools for the investigation of metal-induced carcinogenesis. For example, the microarray technique makes it possible to screen a large amount of genes which are possibly induced by certain metals, providing the hints and directions necessary for more insightful studies.

Acknowledgements

The authors thank Drs Vince Castranova, Murali Rao, and Val Vallyathan for a critical reading of the manuscript. Research funded under Interagency Agreement number 98-18-00m2 between the Occupational Safety and Health Administration (OSHA) and the National Institute for Occupational Safety and Health (NIOSH). The views expressed in the paper are those of the authors and do not necessarily reflect the official position of OSHA.

References

1. IARC: Chromium, nickel, and welding. In: Monographs on the Evaluation of the Carcinogenic Risks to Humans, Vol. 49. International Agency for Research on Cancer, Lyon, France, 1990, pp 49–256
2. NRC: National Research Council Report: Arsenic in the Drinking Water. National Academy Press, Washington, DC, 1999
3. Solomon E, Borrow J, Goddard AD: Chromosome aberrations and cancer. Science 254: 1153–1160, 1991
4. Bos JL: Ras oncogenes in human cancer: A review. Cancer Res 49: 4682–4689, 1989
5. Hollstein M, Sidransky D, Vogelstein B, Harris CC: p53 mutations in human cancers. Science 253: 49–53, 1991
6. Koberle B, Speit G: Molecular characterization of mutations at the hprt locus in V79 Chinese hamster cells induced by bleomycin in the presence of inhibitors of DNA repair. Mutat Res 249: 161–167, 1991
7. Schwab M: Oncogene amplification in neoplastic development and progression of human cancers. Crit Rev Oncog 2: 35–51, 1990
8. Flamigni F, Marmiroli S, Caldarera CM, Guarnieri C: Effect of sodium arsenite on the induction and turnover of ornithine decarboxylase activity in erythroleukemia cells. Cell Biochem Funct 7: 213–217, 1989
9. Mikalsen SO: Effects of heavy metal ions on intercellular communication in Syrian hamster embryo cells. Carcinogenesis 11: 1621–1626, 1990
10. Squibb KS, Fowler BA: Relationship between metal toxicity to subcellular systems and the carcinogenic response. Environ Health Perspect 40: 181–188, 1981
11. Berg JM: Potential metal-binding domains in nucleic acid binding proteins. Science 232: 485–487, 1986
12. Leonard A: Chromosome damage in individuals exposed to heavy metals. In: H. Sigel (ed). Metal Ions in Biological Systems, Vol. 20. Marcel Dekker, New York, 1986, pp 229–258
13. Hamilton-Koch W, Snyder RD, Lavelle JM: Metal-induced DNA damage and repair in human diploid fibroblasts and Chinese hamster ovary cells. Chem Biol Interact 59: 17–28, 1986
14. Oleinick NL, Chiu SM, Ramakrishnan N, Xue LY: The formation, identification, and significance of DNA-protein cross-links in mammalian cells. Br J Cancer (suppl 8): 135–140, 1987
15. Kasprzak KS, Diwan BA, Rice JM, Misra M, Riggs CW, Olinski R, Dizdaroglu M: Nickel (II)-mediated oxidative DNA base damage in renal and hepatic chromatin of pregnant rats and their fetuses. Possible relevance to carcinogenesis. Chem Res Toxicol 5: 809–815, 1992
16. Kasprzak KS: Possible role of oxidative damage in metal-induced carcinogenesis. Cancer Invest 13: 411–430, 1995
17. Breimer LH: Molecular mechanisms of oxygen radical carcinogenesis and mutagenesis: The role of DNA base damage. Mol Carcinog 3: 188–197, 1990
18. Dizdaroglu M: Chemical determination of free radical-induced damage to DNA. Free Radic Biol Med 10: 225–242, 1991

19. Vallyathan V, Shi X, Castranova V: Reactive oxygen species: Their relation to pneumoconiosis and carcinogenesis. Environ Health Perspect 106 (suppl 5): 1151–1155, 1998
20. Shi X, Dalal NS: Evidence for a Fenton-type mechanism for the generation of .OH radicals in the reduction of Cr (VI) in cellular media. Arch Biochem Biophys 281: 90–95, 1990
21. Shi X, Dalal NS: Superoxide-independent reduction of vanadate by rat liver microsomes/NAD(P)H: Vanadate reductase activity. Arch Biochem Biophys 295: 70–75,1992
22. Wang G, Hazra TK, Mitra S, Lee HM, Englander EW: Mitochondrial DNA damage and a hypoxic response are induced by CoCl(2) in rat neuronal PC12 cells. Nucleic Acids Res 28: 2135–2140, 2000
23. Vallyathan V, Shi X: The role of oxygen free radicals in occupational and environmental lung diseases. Environ Health Perspect 105 (suppl 1): 165–177, 1997
24. Kerr JFR, Harmon B, Searle J: An electron-microscope study of cell deletion in the anuran tadpole tail during spontaneous metamorphosis with special reference to apoptosis of striated muscle fibers. J Cell Sci 14: 571–585, 1974
25. Kerr JFR, Serale J, Harmon BV, Bishop CJ: Apoptosis. In: C.S. Potten (ed). Perspectives on Mammalian Cell Death. Oxford University Press, Oxford, 1987, pp 93–128
26. Cohen JJ: Programmed cell death in the immune system. Adv Immunol 50: 55–85, 1991
27. Golstein P, Ojcius DM, Young JD-E: Cell death mechanisms and the immune system. Immunol Rev 121: 29–65, 1991
28. Savill JS, Wyllie AH, Henson JE, Walport MJ, Henson PM, Haslett C: Macrophage phagocytosis of aging neutrophils in inflammation: Programmed cell death in the neutrophil leads to its recognition by macrophages. J Clin Invest 83: 865–875, 1989
29. Schwartzman RA, Cidlowski JA: Apoptosis: The biochemistry and molecular biology of programmed cell death. Endocr Rev 14: 133–150, 1993
30. Sachs L, Lotem J: Control of programmed cell death in normal and leukemia cells: New implications for therapy. Blood 82: 15–21, 1993
31. Reed JC: Bcl-2 and the regulation of programmed cell death. J Cell Biol 124: 1–6, 1994
32. Vaux DL, Weissman IL, Kim SK: Prevention of programmed cell death in *Caenorhabditis elegans* by human bcl-2. Science 258: 1955–1957, 1992
33. Blankenship LJ, Manning FC, Orenstein JM, Patierno SR: Apoptosis is the mode of cell death caused by carcinogenic chromium. Toxicol Appl Pharmacol 126: 75–83, 1994
34. Schieven GL, Wahl AF, Myrdal S, Grosmaire L, Ledbetter JA: Lineage-specific induction of B cell apoptosis and altered signal transduction by the phosphotyrosine phosphatase inhibitor bis(maltolato)oxovanadium (IV). J Biol Chem 270: 20824–20831, 1995
35. el Azzouzi B, Tsangaris GT, Pellegrini O, Manuel Y, Benveniste J, Thomas Y: Cadmium induces apoptosis in a human T cell line. Toxicology 88: 127–139, 1994
36. Shiao YH, Lee SH, Kasprzak KS: Cell cycle arrest, apoptosis and p53 expression in nickel (II) acetate-treated Chinese hamster ovary cells. Carcinogenesis 19: 1203–1207, 1998
37. Ochi T, Nakajima F, Sakurai T, Kaise T, Oya-Ohta Y: Dimethylarsinic acid causes apoptosis in HL-60 cells via interaction with glutathione. Arch Toxicol 70: 815–821, 1996
38. Shackelford RE, Kaufmann WK, Paules RS: Cell cycle control, checkpoint mechanisms, and genotoxic stress. Environ Health Perspect 107 (suppl 1): 5–24, 1999
39. Huang LC, Clarkin KC, Wahl GM: Sensitivity and selectivity of the DNA damage sensor responsible for activating p53-dependent G1 arrest. Proc Natl Acad Sci USA 93: 4827–4832, 1996
40. Hartwell LH, Kastan MB: Cell cycle control and cancer. Science 266: 1821–1828, 1994
41. Mercer WE: Checking on the cell cycle. J Cell Biochem Suppl 30–31: 50–54, 1998
42. Ye J, Zhang X, Young HA, Mao Y, Shi X: Chromium (VI)-induced nuclear factor-kappa B activation in intact cells via free radical reactions. Carcinogenesis 16: 2401–2405, 1995
43. Shi X, Ding M, Ye J, Wang S, Leonard SS, Zang L, Castranova V, Vallyathan V, Chiu A, Dalal N, Liu K: Cr (IV) causes activation of nuclear transcription factor-kappa B, DNA strand breaks and dG hydroxylation via free radical reactions. J Inorg Biochem 75: 37–44, 1999
44. Shi X, Chiu A, Chen CT, Halliwell B, Castranova V, Vallyathan V: Reduction of chromium (VI) and its relationship to carcinogenesis. J Toxicol Environ Health B Crit Rev 2: 87–104, 1999
45. Chen F, Ding M, Lu Y, Leonard SS, Vallyathan V, Castranova V, Shi X: Participation of MAP kinase p38 and IkappaB kinase in chromium (VI)-induced NF-kappaB and AP-1 activation. J Environ Pathol Toxicol Oncol 19: 231–238, 2000
46. 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: C868–875, 2000
47. Huang C, Chen N, Ma WY, Dong Z: Vanadium induces AP-1- and NFkappB-dependent transcription activity. Int J Oncol 13: 711–715, 1998
48. Ding M, Li JJ, Leonard SS, Ye JP, Shi X, Colburn NH, Castranova V, Vallyathan V: Vanadate-induced activation of activator protein-1: Role of reactive oxygen species. Carcinogenesis 20: 663–638, 1999
49. Hart BA, Lee CH, Shukla GS, Shukla A, Osier M, Eneman JD, Chiu JF: Characterization of cadmium-induced apoptosis in rat lung epithelial cells: Evidence for the participation of oxidant stress. Toxicology 133: 43–58, 1999
50. Barchowsky A, Dudek EJ, Treadwell MD, Wetterhahn KE: Arsenic induces oxidant stress and NF-kappa B activation in cultured aortic endothelial cells. Free Radic Biol Med 21: 783–790, 1996
51. Cavigelli M, Li WW, Lin A, Su B, Yoshioka K, Karin M: The tumor promoter arsenite stimulates AP-1 activity by inhibiting a JNK phosphatase. EMBO J 15: 6269–6279, 1996
52. Ramesh GT, Manna SK, Aggarwal BB, Jadhav AL: Lead activates nuclear transcription factor-kappaB, activator protein-1, and amino-terminal c-Jun kinase in pheochromocytoma cells. Toxicol Appl Pharmacol 155: 280–286, 1999
53. Zhou D, Salnikow K, Costa M: Cap43, a novel gene specifically induced by Ni^{2+} compounds. Cancer Res 58: 2182–2189, 1998
54. Salnikow K, Blagosklonny MV, Ryan H, Johnson R, Costa M: Carcinogenic nickel induces genes involved with hypoxic stress. Cancer Res 60: 38–41, 2000
55. Broday L, Cai J, Costa M: Nickel enhances telomeric silencing in *Saccharomyces cerevisiae*. Mutat Res 440: 121–130, 1999
56. McCaffrey J, Wolf CM, Hamilton JW: Effects of the genotoxic carcinogen chromium (VI) on basal and hormone-inducible phosphoenolpyruvate carboxykinase gene expression *in vivo*: Correlation with glucocorticoid- and developmentally regulated expression. Mol Carcinog 10: 189–198, 1994
57. Hamilton JW, Kaltreider RC, Bajenova OV, Ihnat MA, McCaffrey J, Turpie BW, Rowell EE, Oh J, Nemeth MJ, Pesce CA, Lariviere JP: Molecular basis for effects of carcinogenic heavy metals on inducible gene expression. Environ Health Perspect 106 Suppl 4: 1005–1015, 1998
58. Murphy BJ, Andrews GK, Bittel D, Discher DJ, McCue J, Green CJ, Yanovsky M, Giaccia A, Sutherland RM, Laderoute KR, Webster KA: Activation of metallothionein gene expression by hypoxia involves metal response elements and metal transcription factor-1. Cancer Res 59: 1315–1322, 1999
59. Bose RN, Fonkeng BS, Moghaddas S, Stroup D: Mechanisms of DNA damage by chromium (V) carcinogens. Nucleic Acids Res 26: 1588–1596, 1998