19

Vanadium- and Chromium-Induced Cell Signal Transduction

YONG QIAN

National Institute for Occupational Safety and Health
Morgantown, West Virginia, U.S.A.

XIANGLIN SHI

National Institute for Occupational Safety and Health
and West Virginia University
Morgantown, West Virginia, U.S.A

I. Introduction

Both vanadium and chromium are two common toxic metals. Accumulating evidence indicates that exposure of humans to either metal induces a wide spectrum of adverse effects, including carcinogencity, neurotoxicity, and immunotoxicity (1). Among these adverse effects, carcinogencity is the major geological consequence. The underlying molecular mechanisms have not been fully identified yet. For the past several years, much attention has been focused on how these two metals alter cellular signal transduction to lead to carcinogenesis. This chapter will serve as a summary of recent advances in the knowledge base for cellular signal transduction, including induction of tyrosine phosphorylation, phosphatidylinositol 3-kinase (PI3 kinase) signaling, nuclear factor-κB (NF-κB) activation, cell cycle arrest, and apoptosis, in response to vanadium or chrominum.

II. Vanadium

Vanadium is an essential metal for many species and is widespread in the environment (2). Accumulating evidence indicates that administration of vanadium is toxic to humans. An epidemiological study showed that exposure to vanadium was closely related to the incidence of lung cancers in humans (3). Vanadium mainly exists in two different forms, tetravalent (IV)

vanadyl (oxovanadate) and pentavalent (V) vanadate, dependent on the oxidation state. The pentavalent vanadate is stable at the physiological pH and is mainly located in the extracellular area (4). Upon administration into the body, it is reduced to tetravalent vanadyl by glutathione and the respiratory NADPH oxidase in association with the formation of reactive oxygen species (ROS) (2). Inside cells, vanadium mainly exists as a tetravalent oxidate form (2). Pervanadate is a widely used form of vanadate that is produced by the reaction of H_2O_2 and orthovanadate(IV). It is more toxic than orthovanadate (5). Among all forms of vanadates, peroxovanadate(IV) has the most potent toxic effects on cells (6). The vanadium reduction generates several kinds of ROS, including superoxide anion radical $O_2^{\bullet -}$, hydrogen peroxide (H_2O_2), and hydroxyl radical ($^{\bullet}OH$) (7,8). Increasing evidence indicates that ROS play a central role in vanadium-induced cell signal transduction (7,9–13).

A. Tyrosine Phosphorylation

Tyrosine phosphorylation is an important cellular process to control cell proliferation, differentiation, cell cycle, apoptosis, and cell signal transduction (14). The induction of tyrosine phosphorylation is tightly controlled by protein tyrosine kinases (PTKs) and protein tyrosine phosphotases (PTPs). PTKs are divided into two categories: receptor tyrosine kinases and nonreceptor tyrosine kinases. Most cell growth receptors belong to the receptor tyrosine kinases. They normally have a transmembrane-spanning receptor and intrinsic protein tyrosine kinase activity. Upon stimulation by either ligand binding or receptor cross-linking, the receptor tyrosine kinases undergo autotyrosine phosphorylation on the tyrosine residues located in their own carboxy terminus and induce conformational changes, which lead to the full activation of kinase activities and the creation of binding sites for the cellular substrates. The nonreceptor tyrosine kinases are among these cellular substrates. They are activated upon either binding to the receptor tyrosine kinases or dephosphorylation by protein tyrosine phosphatases. Followed the activation, the nonreceptor tyrosine kinases are able to phosphorylate a vast variety of cellular proteins to affect numerous cellular functions (15). Src protein tyrosine kinase family is an important nonreceptor tyrosine kinase family.

Vanadium is a potent PTP inhibitor, and the mechanisms of inhibition have not been elucidated yet. It is a phosphate analogue, and was proposed to inhibit PTPs via binding to an essential cysteine residue at the catalytic site of PTPs (5), which can be mimicked by H_2O_2 (16). It has been demonstrated that exposure of the cells to vanadium inhibits numerous PTP activities, which result in an increase in the protein tyrosine

phosphorylation in human airway epithelial cells (HAECs) (17). Earlier studies indicate that vanadium-induced inhibition of PTPs mimicks the action of insulin (18). However, further studies showed that insulin and vanadium activated an overlapping set of signaling proteins through stimulating two distinct protein tyrosine phosphorylation profiles (19). Indeed, vanadium was found to inhibit basal and insulin-stimulated system A amino acid uptake and cell proliferation in addition to its action of insulin-like stimulation through the inhibition of PTPs (20), indicating that vanadium-induced signaling involves some unique cell signal pathways besides insulin-mediated signaling transduction.

It is controversial whether vanadium-induced signaling involves the activation of insulin receptor tyrosine kinase and the induction of tyrosine phosphorylation of insulin receptor substrate 1 (IRS-1). Chronic treatment of diabetic rats with sodium orthovanadate increased the tyrosine phosphorylation and the activity of the insulin receptor in association with the normalization of plasma glucose levels. In vitro studies found that vanadium prevented the dephosphorylation of the insulin receptor and increased its kinase activities, indicating that vanadium had an ability to induce the activation of insulin receptor tyrosine kinase (21). Furthermore, it has been found that vanadium-activated insulin receptor tyrosine kinase induces the tyrosine phosphorylation of IRS-1 in numerous cell types, which subsequently activates PI3 kinase, Akt, and MAPKs (22,23).

However, other groups found that vanadium-induced tyrosine phosphorylation signaling was independent of the activation of insulin receptor tyrosine kinase and the tyrosine phosphorylation of IRS-1 (24). Inhibition of insulin receptor kinase using the specific inhibitor quercetin was unable to block vanadium-induced signal transduction (25). Their studies further demonstrated that vanadium induces cellular signal transduction through both a cytosolic and a membranous nonreceptor tyrosine kinases, instead of insulin receptor tyrosine kinase and IRS-1 in rat adipocytes. The cytosolic nonreceptor tyrosine kinase has a molecular mass of 53 kDa and is activated upon vanadium treatment. Inhibition of its kinase activities blocks vanadium-induced insulin-like actions on glucose utilization (26). The 55- to 60-kDa membranous nonreceptor tyrosine kinase was found to be related to vanadium-induced insulin-like actions of hexose uptake, antilipolytic activity, and PI3 kinase activation in rat adipocytes (24).

The induction of tyrosine phosphorylation plays a central role in cell signal transduction in response to vanadium stimulation, such as vanadium-induced activation of phosphoinositide-dependent protein kinase-1 (PDK1), MAPK, and NF-κB. It has been found that peroxovanadate(IV) treatment induces the tyrosine phosphorylation of PDK1, concomitant with the translocation of PDK1 to the membrane and the activation of its kinase in

adipocytes. The activation of PDK1 by vanadium is abolished upon inhibition of the tyrosine phosphorylation of PDK1 by either preincubation with Src kinase inhibitor or mutation of tyrosine 373 of PDK1, indicating an essential role of tyrosine phosphorylation in vanadium-induced activation of PDK1 (27). It was found that vanadium-induced MAPK activation is mediated by the induction of epidermal growth factor receptor (EGFR) tyrosine phosphorylation, which involves the activation of Ras and Raf in human airway epithelial cell lines (28,29). Recently, several reports have demonstrated that tyrosine phosphorylation is involved in vanadium-induced NF-κB activation. Wu's group found that vanadium treatment induces the tyrosine phosphorylation of EGFR and the subsequent activation of Ras, which results in serine phosphorylation and degradation of IκB, as well as the subsequent activation of NF-κB (29). Other groups found that vanadium treatment induced the activation of NF-κB through the tyrosine phosphorylation of IκB at Tyr42 and subsequent IκB degradation in several different cell types. They further demonstrated that IκB was tyrosine phosphorylated by p56LCK and ZAP-70 tyrosine kinases. Either deletion or inhibition of these two tyrosine kinases abolishes vanadium-induced activation of NF-κB (30,31).

B. Phosphatidylinositol 3-Kinase

PI3 kinase is a key enzyme that is involved in cell growth, proliferation, differentiation, and survival. PDK1 and PKB/Akt are its two major downstream signal protein kinases. Vanadium has been demonstrated to activate PI3 kinase through either tyrosine phosphorylation or a member of ROS, H_2O_2, in numerous cell types (24,32). Activation of PI3 kinase has a major role in a wide variety of signal transduction pathways in response to vanadium stimulation, including the activation of PDK1 (27,32), Akt (23), $p70^{s6k}$ (33), p38 (23), ERK (34), and Ras/Raf (34). Much attention has been focused on dissecting the cross-talk among these downstream signaling proteins in mediating the effects of vanadium. Recently, our group found that PI3 kinase was essential for vanadium-induced expressions of hypoxia-inducible factor-1α (HIF-1α) and vascular endothelial growth factor (VEGF) (35). HIF-1 is a transcription factor composed of HIF-1α and HIF-1β. It overexpresses in many human cancers, and its activation is closely related to tumorigenicity and angiogenesis. We demonstrated that vanadium treatment induces HIF-1 expression through the activation of PI3 kinase in both a dose-dependent and a time-dependent manner in DU145 human prostate carcinoma cells. Further studies showed that PI3 kinase regulates vanadium-induced HIF-1α expression via the activation of AKT and mTOR/FRAP. HIF-1 regulates many gene transcriptions,

including VEGF. VEGF is a major player in tumor progression and angiogenesis, and inhibition of VEGF expression decreases tumor growth, metastasis, and invasion. We have demonstrated that vanadium-induced HIF-1α activation is responsible for VEGF expression through a PI3 kinase/Akt/mTOR/FRAP signaling pathway. Furthermore, we found that ROS plays a central role in vanadium-induced HIF-1α, and VEGF expression. Removal of ROS abolishs vanadium-induced activation of PI3 kinase, HIF-1α, and VEGF. Therefore, our studies defined a unique pathway of vanadium-induced carcinogenic signaling, which may be through ROS → PI3 kinase → Akt → mTOR/FRAP → HIF-1α → VEGF (35).

C. Nuclear Factor-κB (NF-κB)

NF-κB is a key transcriptional factor that has a major role in cell signal transduction. It is a hetrodimer composed of p50 and p65 (RelA). NF-κB is inactive in the cytoplasm and is associated with one of its inhibitors, IκB, or p105, or p100. Upon stimulation, IκB undergoes either tyrosine phosphorylation or serine phosphorylation, followed by degradation and dissociation from NF-κB. IκB is mainly regulated by protein kinase IKK that is further controlled by MEKK1, Akt, NAK, and PKC. After the dissociation from its inhibitor IκB, NF-κB can translocate to nucleus to activate transcription. Numerous stimuli can activate NF-κB, including cytokines, mitogens, environmental and occupational particles, toxic metals, intracellular stresses, and ultraviolet light (36).

Vanadium has been demonstrated to activate NF-κB in virtually all kinds of cells. However, there is controversy about the underlying molecular mechanisms. As discussed earlier, some studies demonstrate that vanadium activates NF-κB through tyrosine phosphorylation and the degradation of IκB (30,31). Two tyrosine kinases, p56LCK and ZAP-70, were reported to be involved in the tyrosine phosphorylation of IκB in T cells (31). Furthermore, it was found that the degradation of tyrosine phosphorylated IκB is independent of the induction of ubiquitination upon vanadium treatment (30). However, various studies pointed out that vanadium activates NF-κB through the serine phosphorylation and subsequent degradation of IκB, which involves ubiquitination. The different results could be due to (a) the different forms of vanadium used in stimulation of the cells; and (b) the different cell types and different dosage of vanadium used in the experiments (37).

Oxidative stress and MAPKs have been found to participate in vanadium-induced NF-κB activation. Our group demonstrated that vanadium treatment induces the activation of both NF-κB and JNK partially via ROS-mediated reactions. Inhibition of IKKβ, an upstream kinase of IκB, blocks

vanadium-induced activation of NF-κB but is unable to affect vanadium-induced JNK activation. However, inhibition of vanadate-induced JNK activation partially decreases vanadium-induced IκB degradation and NF-κB activation. Furthermore, pretreatment of the cells with the antioxidants inhibits the activation of both NF-κB and JNK in response to vanadium stimulation (13). Our results indicate that both the cellular redox states and the activation of MAPK play an important role in vanadium-induced NF-κB activation. Interestingly, Jaspers' group found that ROS mediated vanadium-induced NF-κB activation through two distinct pathways: NF-κB nuclear translocation and NF-κB-dependent transcription, which involves p38MAPK (38). They found that NF-κB nuclear translocation is dependent on vanadium-induced formation of ROS, whereas NF-κB-dependent transcription is dependent on both vanadium-induced ROS formation and p38 activation.

D. Cell Cycle

Cell cycle is the cellular process by which cells reproduce themselves by duplicating their own contents and subsequently dividing into two. Normally, the processes of the cell cycle are divided into four phases: S, G_2, M, and G_1 phases. Cells replicate their DNA in the S phase and separate into two daughter cells in the M phase. The interval period between the completion of M phase and the beginning of S phase is called G_1 phase, and the G_2 phase is the interval period between the end of S phase and the initiation of M phase. The cell cycle is mainly controlled by a set of regulatory proteins called cyclins and cyclin-dependent kinases (CDKs), which are further regulated by a series of kinases and phosphatases. The control of cell cycle is mainly executed at three checkpoints, namely, G_1, G_2 and M phase. The G1 checkpoint is mainly regulated by a tumor suppressor gene p53, which involves p21, cyclin E/CDK2, and cyclin D/CDK4/6. The G2 checkpoint is predominately regulated by ATM-mediated cell signal transduction, which involves GADD45, CDC25, and CDC2/cyclin B. The M phase checkpoint is regulated by microtubules. Cells arrest their growth at these three checkpoints to correct damage and ultimately reenter the cell cycle. If cells are unable to correct the damage during the cell cycle arrest, they will go to apoptosis, a programmed cell death, or cell transformation (39).

Given the facts that vanadium and its ROS derivatives can damage DNA and vanadium itself is a potent phosphatase inhibitor, it is expected that vanadium treatment will affect the cell cycle. Indeed, vanadium treatment induces G_2/M cell arrest in association with the inhibition of nuclear translocation of Cdc25B2, a dual specific protein phosphatase and a cell cycle activator (40). Our group also found that vanadium treatment induces

a significant increase in G_2/M phase cell arrest in a human lung epithelial cell line (A549). Examining the changes in the cell cycle regulatory proteins, we found that vanadium treatment increased p21 and Chk1 expression, and decreased Cdc25C expression in a dose- and time-dependent manner. Consistent with the changes in p21, Chk1, and Cdc25C, the activities of Cdc2/cyclin B1 complex were inhibited upon vanadium treatment. Our group also showed that vanadium treatment induces the formation of ROS, $^{\bullet}OH$, H_2O_2, and $O_2^{\bullet-}$. The inhibition of H_2O_2 with catalase abolished vanadium-induced cell cycle arrest, indicating that H_2O_2 has a major role in vanadium-induced G_2/M phase arrest (7). Furthermore, our recent results demonstrate that vanadium treatment induces S-phase arrest in a mouse epidermal cell line (C141) (10). Interestingly, we found that the type of vanadium-induced cell cycle arrest was dependent on the activation of p53. p53 is a tumor suppressor protein and is activated upon DNA damage. We demonstrated that vanadium treatment induced G_2/M phase arrest in p53-deficient C141 cells, whereas it induced S-phase arrest in p53 wild-type C141 cells in association with the activation of p53 (11). Our results indicate that vanadium-induced G_2/M arrest is p53 independent whereas vanadium-induced S arrest is p53 dependent.

It was speculated that vanadium may induce the cell cycle arrest through activation of the MAPK family (2). Yan's group found that vanadium treatment down-regulates cyclin D1 expression and induces S-phase arrest in association with the activation of MAPK and $p70^{S6K}$ in mouse embryonic fibroblast C3H10T1/2 cells (41). Recently, our group found that vanadium treatment induces the activation of p53, the inactivation of p-cdk2 (an important S-phase progression kinase), and S-phase arrest in association with the activation of ERK and p38 in C141 cells in a dose- and time-dependent manner. Inhibition of either ERK or p38 dramatically decreased the activation of p53, restored the activation of p-cdk2, and subsequently abolished vanadium-induced S-phase arrest upon vanadium stimulation, indicating that the MAPK family has an essential role in vanadium-induced S-phase arrest (42).

E. Apoptosis

Apoptosis or a programmed cell death is a physiologically regulated process to kill cells. It is a critical cellular event to maintain normal cell development. The dysregulation of apoptosis is directly related to the occurrence of cancinogenesis. The characteristics of apoptosis are plasma membrane blebbing, cytoplasmic shrinkage, chromosomal DNA condensation and breakdown, and cell death. The processes of apoptosis involve the tumor necrosis factor receptor (TNFR) family, adaptor proteins, the Bcl-2 family,

and the caspase family. The TNFR receptor family, also called death receptors, triggers the processes of apoptosis through binding to death-inducing ligands. The engagement of TNFR and ligands recruits the adaptor proteins, which further activate the caspase family proteins. There are two kinds of caspase proteins: initiator caspases (such as caspase 8 and 9) and effector caspases (such as caspase 3). Upon the activation, the caspase family proteins induce apoptosis through the cleavage of mitochondria and the degradation of numerous cellular proteins. The Bcl-2 family proteins are a group of proteins that are either antiapoptotic (such as Bcl-2 and Bcl-x_L) or proapoptotic (such as bax and Bak), and are regulated by a variety of cellular signaling proteins, including the caspase family (43).

Accumulating evidence indicates that vanadium treatment induced apoptosis in some cell types. It has been shown that vanadium treatment induces apoptosis through the activation of caspases 3, 8, and 9, the induction of mitochondrial permeability transition, the promotion of cytochrome *c* release from mitochondria, and the fragmentation of chromosomal DNA in lymphoid cell lines, which is independent of the activation of tyrosine kinase $p56^{lck}$ or phosphatase CD45 (44). It is controversial whether vanadium-induced apoptosis is related to the inhibition of PTPs (2), although one report showed that vanadium facilitated interferon-α-mediated apoptosis via the inhibition of PTPs (45).

Convincing evidence shows that the generation of H_2O_2 is involved in vanadium-induced apoptosis. Vanadium induces apoptosis, concomitant with an increase in oxygen consumption and H_2O_2 formation in mouse epidermal JB6 cells (12). Inhibition of H_2O_2 formation by pretreatment with catalase abolishes vanadium-induced apoptosis, indicating a regulatory role of H_2O_2 in vanadium-induced apoptosis (12). Further studies found that the generation of H_2O_2 was responsible for the transactivation of p53 upon vanadium stimulation in JB6 cells (9). Recently, our studies demonstrated that vanadium treatment induced apoptosis in cultured cerebellar granule progenitors (CGPs), concomitant with the production of H_2O_2, the activation of ERK and JNK, the induction of c-Jun phosphorylation, the aggregation of Fas (CD95), the increase of Fas-FADD association, and the activation of caspase 8 (46). Interestingly, vanadium-activated CD95-FADD-caspase 8 pathway is dependent on the activation of MAPK but not H_2O_2, whereas vanadium-induced FasL production is dependent on H_2O_2 but not MAPK. Furthermore, vanadium-induced MAPK activation was independent of the production of H_2O_2 in CGPs. These results indicate that vanadium induces apoptosis in CGPs through two distinct pathways: (a) H_2O_2-induced FasL production and (b) MAPK-induced CD95 aggregation, FADD association, and caspase 8 activation (46).

III. Chromium

Chromium is one of the most widely used metals in industry. There are several forms of chromium, e.g., III, IV, V, and VI. The trivalent chromium compounds do not easily enter cells and therefore are neither toxic nor carcinogenic. In contrast, chromium(IV) easily enters cells and has been proven to induce chromosomal aberrations and mutations, DNA damage, and cell transformation (47). Epidemiological investigations found that workers in the chromium chemical production process area with high exposure to chromium have an elevated risk of developing lung cancer (48). Chromium(VI) itself is unable to react with the isolated DNA. Upon reaction by cellular reductants, chromium intermediates are produced and molecular oxygen is consumed. During this process, ROS are generated, which induce DNA damage and mutations (47). Chromium(VI) can be reduced to chromium(IV) and (V) by low molecular weight molecules, such as glutathione, ascorbate, cysteine, lipoic acid, NAD(P)H, ribose, fructose, and arabinose (47). In cells, chromium(VI) can be reduced by a variety of enzymatic and nonenzymatic factors, including microsomes, mitochondria, glutathione reductase (GSSG-R), lipoyl dehydrogenase, and ferredoxin-$NADP^+$ oxidoreductase (47). Compared to other metals, chromium is potent in the production of ROS. It produces a whole spectrum of ROS during the chromium(VI) reduction process, which is important in the mechanism of chromium-induced carcinogenicity (47).

A. Tyrosine Phosphorylation

Earlier studies showed that chromium treatment of rat hepatoma cells induces the tyrosine phosphorylation of three proteins that have molecular masses of 210, 125, and 87 kDa, respectively, in a dose- and time-dependent manner (49). Since the profile of chromium-induced tyrosine phosphorylation has some degrees of similarity with that of vanadium-induced tyrosine phosphorylation, it was speculated that chromium might have the capability to inhibit the PTPs as vanadium does. However, further studies found that chromium treatment of the cells did not facilitate the autophosphorylation of insulin receptor β subunit in contrast with stimulation by vanadium, indicating that chromium and vanadium may induce an increase in the protein tyrosine phosphorylation through the distinct mechanisms. Indeed, there are no data to show that chromium can directly inhibit the PTPs as vanadium does.

Our group recently found that chromium induces protein tyrosine phosphorylation as well as production of H_2O_2 and $^{\bullet}OH$ in human lung epithelial cell lines (A549) (50). Western blot analysis using an

antiphosphotyrosine antibody revealed that numerous proteins were tyrosinephosphorylated ranging from 215 to 30 kDa. Either inhibition of H_2O_2 with catalase or inhibition of $^{\bullet}OH$ with *N*-acetyl-L-cysteine (NAC) abolishes the chromium-induced increase in tyrosine phosphorylation, indicating that chromium may induce tyrosine phosphorylation through ROS-mediated signaling (50). Interestingly, microarray analysis of the gene expression profiles demonstrates that the expressions of two Src-related kinases, cytoplasmic tyrosine kinase (Cyl) and HYL tyrosine kinase, are increased to more than sixfold in response to chromium stimulation (51). Recently, Balamurugan's group found that chromium treatment of human peripheral blood lymphocytes induces the generation of ROS and subsequently activates Src family tyrosine kinases, which results in apoptosis (52). Taken together, these results indicate that chromium may induce tyrosine phosphorylation through the activation of protein tyrosine kinases. Further studies are needed to identify the individual tyrosine kinases in chromium-induced cell signaling.

B. Nuclear Factor-κB (NF-κB)

A relatively low concentration of chromium can induce the activation of NF-κB in a variety of cells, including T cells (53), macrophages (54), bronchial epithelial cells (55), and human breast cancer cells (56). The molecular mechanisms still have not been identified yet. There has been no report showing that chromium can activate the upstream signaling proteins of NF-κB, such as IKK. However, our previous studies demonstrate that the integrity of IKK is essential for chromium-induced activation of NF-κB. We found that chromium stimulation induces the activation of NF-κB in mouse JB6 fibroblast cell lines, and that activation of NF-κB is substantially inhibited upon the transient expression of a dominant negative IKKβ, indicating that IKKβ is required for the activation of NF-κB in response to chromium stimulation (54). Until now there has been no convincing evidence to prove that IκB, an NF-κB inhibitory protein, undergoes phosphorylation at either serines or tyrosines, or degradation upon chromium stimulation. However, several results revealed that chromium stimulation might increase NF-κB activity through altering the nuclear binding ability of NF-κB to its cis-acting elements in several cell lines (56).

It has been speculated that ROS may be an important factor in the activation of NF-κB in response to toxic metal stimulation, including chromium. Chromium is potent in the production of ROS. Our earlier results show that chromium stimulation induces the formation of $^{\bullet}OH$ and H_2O_2 as well as the activation of NF-κB. The removal of ROS inhibits chromium-induced NF-κB activation, indicating that ROS is involved in the

activation of NF-κB in response to chromium stimulation. However, our recent studies generated some controversial results. Compared with arsenic, chromium generates far more ROS in the cells, yet our NF-κB-dependent CAT activity assays show that chromium induces much weaker NF-κB reporter gene activity than arsenic did, indicating that the molecular mechanisms of ROS-mediated NF-κB activation are far more complicated than originally thought (57).

Our recent data indicated that the activation of NF-κB might induce a protective role in chromium-induced cytotoxicity. Stimulation of mouse embryo fibroblast cells with chromium induces the activation of NF-κB. Interestingly, disruption of IKKβ, an upstream kinase of NF-κB, abolishes chromium-induced NF-κB activation and elicits necrotic-like cell death as well. Both the results of Western blot and DNA microarray analysis show that the inhibition of NF-κB by IKKβ disruption decreases the expression of cIAP1, cIAP2, and caspase inhibitors, and subsequently increases the expression of caspase-3 upon chromium stimulation. Furthermore, the expression of antiapoptotic protein, Bcl-xl, is decreased because of the increased cleavage activities of caspase-3. Overexpression of exogenous cIAP1 dramatically reduces the chromium-induced necrotic-like cell death in these NF-κB-suppressed cells. These results indicate that the activities of NF-κB are essential for protecting the cells from chromium-induced cytotoxicity (55).

C. Cell Cycle Arrest

It is not unexpected that stimulation of cells with chromium induces cell cycle arrest, given the facts that (a) chromium(VI) and its reduction forms have an ability to directly damage DNA and (b) chromium-induced ROS can damage DNA (47). Indeed, earlier studies provided a direct linkage between DNA damage and the S-phase cell cycle arrest in response to chromium stimulation (58). These studies demonstrate that the S-phase arrest results from chromium-induced polymerase-arresting lesions and guanine-guanine DNA interstrand cross-links in normal human lung cells. They further found that chromium-induced cycle arrest is likely an initial response to chromium exposure in a low dose range, indicating that chromium-induced cell cycle arrest is tightly dependent on the extent of DNA damage and the ability of cells to regain the replicative potential (59). Our group found that chromium treatment induces the G_2/M-phase arrest as well as the generation of $^{\bullet}OH$ and H_2O_2 in human lung epithelial A549 cells. Inhibition of H_2O_2 production with catalase abolishes chromium-induced cell cycle arrest, indicating that the formation of H_2O_2 is essential for chromium-induced G_2/M-phase arrest in A549 cells (60).

The molecular mechanisms of chromium-induced cell cycle arrest are far from clear. Microarray analysis of the gene expression demonstrate that many cell cycle regulatory genes are affected in response to chromium stimulation, including INK4 p19 and Rbp (51). INK4 p19 is a CDK inhibitor, and Rbp is a suppressor of the transcription factor E2F. Overexpression of INK4 p19 or Rbp may lead to the inhibition of cell growth. Microarray analysis shows that the expression levels of both genes are increased about threefold upon chromium stimulation. Gene expression profiles also show that other cell cycle regulatory genes, CDC25B, CDC2, CDC47, and casein kinase II (CK2), are reduced upon chromium stimulation. CDC47 and CK2 are two kinases that are directly related to DNA replication. Therefore, the results of microarray analysis indicate that chromium may induce the cell cycle arrest through the multiple signaling pathways. However, further studies are needed to confirm the results of microarray analysis. For example, Western blot analysis may be needed to examine the changes in protein expression levels of these genes in response to chromium stimulation.

D. Apoptosis

Exposure of the cells to chromium induces a wide spectrum of cell responses, including cell growth arrest and apoptosis, dependent on the dose and the duration of the stimulation. A high concentration and a long exposure of chromium tend to induce apoptosis (59,61). Either the generation of ROS or the activation of p53 is essential for chromium-induced apoptosis. Our earlier studies show that exposure of human lung epithelial cells to chromium induces apoptosis, as well as the production of ROS and the activation of p53. Removal of ROS inhibits chromium-induced apoptosis, indicting the essential role of ROS in chromium-induced apoptosis. Interestingly, we also found that the generation of ROS occurs within several minutes whereas the activation of p53 takes several hours upon chromium stimulation, indicating that two different mechanisms are involved in chromium-induced apoptosis in different stages. The early stage of apoptosis could be ROS dependent, whereas the later stage of apoptosis could be both ROS and p53 dependent. Indeed, we demonstrated that chromium-induced apoptosis is independent of the activation of p53 for the first 3 h and is related to the activities of p53 at the later stage of response (62).

Three mechanisms may be involved in chromium-induced activation of p53 as previously summarized: (a) chromium-generated ROS induce DNA damage which activates several upstream signaling proteins of p53 and subsequently induces the activation of p53; or (b) chromium-mediated activation of MAPKs induces the activation of p53; or (c) p53 protein has a set of redox-sensitive cysteines that are located within the DNA-binding

domain and are critical for DNA binding with p53 (36). Carlisle's group demonstrated that chromium treatment induced a fourfold to sixfold increase in p53 expression, concomitant with the occurrence of apoptosis in human lung fibroblast cells. Their immunofluorescence data show that chromium stimulation facilitates the nuclear translocation of p53. Furthermore, deletion of p53 abolishes chromium-induced apoptosis. Taken together, these results indicate that p53 is essential in chromium-induced apoptosis (63). Our group found that chromium might induce the activation of p53 through the generation of ·OH and H_2O_2 (64). In a separated study, we found that chromium stimulation induces the phosphorylation and acetylation of p53 at Ser15 and Lys382, respectively, an important step toward the dissociation of the mdm2/p53 complex and the subsequent activation of p53. We also found that ERK acted as an upstream kinase for the phosphorylation of p53 at Ser15 in response to chromium stimulation (65). Taken together, these studies indicate that chromium may induce apoptosis through a unique ROS → MAPK → mdm2 → p53 signaling pathway. It may be noted that there have been no reports to show that chromium stimulation increases the expression of numerous p53-targeted proteins or the expression of p53-dependent proapoptotic regulatory proteins.

Other mechanisms are being investigated for chromium-induced apoptosis. Very recently, Catelas's group found that chromium induced apoptosis in macrophages in a dose- and time-dependent manner, concomitant with a dose- and time-dependent increase in TNF-α secretion, indicating that chromium might induce apoptosis through the induction of TNF-α (66). Src family tyrosine kinases may also have a role in chromium-induced apoptosis. As discussed earlier, Balamurugan's group found that inhibition of Src family kinase activities with a specific inhibitor, PP2, abolishes chromium-induced apoptosis (52).

IV. Conclusions

In this chapter we summarized the recent advances in the knowledge base for both vanadium- and chromium-induced cell signal transduction. It is important to note that we artificially divided the signaling pathways into several separated sections for the convenience of discussion. In reality, there is extensive cross-talk among these signaling pathways. For example, the induction of tyrosine phosphorylation is directly related to vanadium-induced activation of NF-κB, which may be regulated by ROS and is further linked to the outcome of cell cycle arrest and apoptosis. It is also important to note that both vanadium and chromium affect far more cell signaling pathways than could be covered in this chapter.

One of major challenges to dissect metal-induced carcinogenic cell signaling transduction pathways is that many metals are the trace elements that are essential for the human body, and the human body has evolved with the existence of metals. Thus, many metals and their derivates are able to evade the natural protective barriers of the human body and utilize existent human metabolic systems to reach the "designated" tissues or cell constituents, which result in alteration of a broad spectrum of cell signal transduction pathways that lead to carcinogenesis.

References

1. Waalkes MP, Fox DA, States JC, Patierno SR, McCabe MJ, Jr. Metals and disorders of cell accumulation: modulation of apoptosis and cell proliferation. Toxicol Sci 2000; 56:255–261.
2. Evangelou AM. Vanadium in cancer treatment. Crit Rev Oncol Hematol 2002; 42:249–265.
3. Hickey RJ, Schoff EP, Clelland RC. Relationship between air pollution and certain chronic disease death rates. Multivariate statistical studies. Arch Environ Health 1967; 15:728–738.
4. Erdmann E, Werdan K, Krawietz W, Schmitz W, Scholz H. Vanadate and its significance in biochemistry and pharmacology. Biochem Pharmacol 1984; 33:945–950.
5. Huyer G, Liu S, Kelly J, Moffat J, Payette P, Kennedy B, Tsaprailis G, Gresser MJ, Ramachandran C. Mechanism of inhibition of protein-tyrosine phosphatases by vanadate and pervanadate. J Biol Chem 1997; 272:843–851.
6. Bevan AP, Drake PG, Yale JF, Shaver A, Posner BI. Peroxovanadium compounds: biological actions and mechanism of insulin mimesis. Mol Cell Biochem 1995; 153:49–58.
7. Zhang Z, Huang C, Li J, Leonard SS, Lanciotti R, Butterworth L, Shi X. Vanadate-induced cell growth regulation and the role of reactive oxygen species. Arch Biochem Biophys 2001; 392:311–320.
8. Shi X, Dalal NS. Hydroxyl radical generation in the NADH/microsomal reduction of vanadate. Free Radic Res Commun 1992; 17:369–376.
9. Huang C, Zhang Z, Ding M, Li J, Ye J, Leonard SS, Shen HM, Butterworth L, Lu Y, Costa M, et al.: Vanadate induces p53 transactivation through hydrogen peroxide and causes apoptosis. J Biol Chem 2000; 275:32516–32522.
10. Zhang Z, Huang C, Li J, Shi X. Vanadate-induced cell growth arrest is p53-dependent through activation of p21 in C141 cells. J Inorg Biochem 2002; 89:142–148.
11. Zhang Z, Chen F, Huang C, Shi X. Vanadate induces G2/M phase arrest in p53-deficient mouse embryo fibroblasts. J Environ Pathol Toxicol Oncol 2002; 21:223–231.
12. Ye J, Ding M, Leonard SS, Robinson VA, Millecchia L, Zhang X, Castranova V, Vallyathan V, Shi X. Vanadate induces apoptosis in epidermal

JB6 P+ cells via hydrogen peroxide-mediated reactions. Mol Cell Biochem 1999; 202:9–17.
13. Chen F, Demers LM, Vallyathan V, Ding M, Lu Y, Castranova V, Shi X. Vanadate induction of NF-kappaB involves IkappaB kinase beta and SAPK/ERK kinase 1 in macrophages. J Biol Chem 1999; 274:20307–20312.
14. Gozin A, Franzini E, Andrieu V, Da Costa L, Rollet-Labelle E, Pasquier C. Reactive oxygen species activate focal adhesion kinase, paxillin and p130cas tyrosine phosphorylation in endothelial cells. Free Radic Biol Med 1998; 25:1021–1032.
15. Blume-Jensen P, Hunter T. Oncogenic kinase signalling. Nature 2001; 411:355–365.
16. Torres M, Forman HJ. Vanadate inhibition of protein tyrosine phosphatases mimics hydrogen peroxide in the activation of the ERK pathway in alveolar macrophages. Ann N Y Acad Sci 2002; 973:345–348.
17. Samet JM, Silbajoris R, Wu W, Graves LM. Tyrosine phosphatases as targets in metal-induced signaling in human airway epithelial cells. Am J Respir Cell Mol Biol 1999; 21:357–364.
18. Fantus IG, Deragon G, Lai R, Tang S. Modulation of insulin action by vanadate: evidence of a role for phosphotyrosine phosphatase activity to alter cellular signaling. Mol Cell Biochem 1995; 153:103–112.
19. Palmer G, Bonjour JP, Caverzasio J. Stimulation of inorganic phosphate transport by insulin-like growth factor I and vanadate in opossum kidney cells is mediated by distinct protein tyrosine phosphorylation processes. Endocrinology 1996; 137:4699–4705.
20. Fantus IG, Tsiani E. Multifunctional actions of vanadium compounds on insulin signaling pathways: evidence for preferential enhancement of metabolic versus mitogenic effects. Mol Cell Biochem 1998; 182:109–119.
21. Pugazhenthi S, Khandelwal RL. Does the insulin-mimetic action of vanadate involve insulin receptor kinase? Mol Cell Biochem 1993; 127–128:211–218.
22. Wilden PA, Broadway D. Combination of insulinomimetic agents H_2O_2 and vanadate enhances insulin receptor mediated tyrosine phosphorylation of IRS-1 leading to IRS-1 association with the phosphatidylinositol 3-kinase. J Cell Biochem 1995; 58:279–291.
23. Tardif A, Julien N, Chiasson JL, Coderre L. Stimulation of glucose uptake by chronic vanadate pretreatment in cardiomyocytes requires phosphatidylinositol 3-kinase and p38 MAPK activation. Am J Physiol Endocrinol Metab 2003; 4:4.
24. Elberg G, He Z, Li J, Sekar N, Shechter Y. Vanadate activates membranous nonreceptor protein tyrosine kinase in rat adipocytes. Diabetes 1997; 46:1684–1690.
25. Shisheva A, Shechter Y. Quercetin selectively inhibits insulin receptor function in vitro and the bioresponses of insulin and insulinomimetic agents in rat adipocytes. Biochemistry 1992; 31:8059–8063.
26. Shisheva A, Shechter Y. Role of cytosolic tyrosine kinase in mediating insulin-like actions of vanadate in rat adipocytes. J Biol Chem 1993; 268:6463–6469.

27. Grillo S, Gremeaux T, Casamayor A, Alessi DR, Le Marchand-Brustel Y, Tanti JF. Peroxovanadate induces tyrosine phosphorylation of phosphoinositide-dependent protein kinase-1 potential involvement of src kinase. Eur J Biochem 2000; 267:6642–6649.
28. Wang YZ, Bonner JC. Mechanism of extracellular signal-regulated kinase (ERK)-1 and ERK-2 activation by vanadium pentoxide in rat pulmonary myofibroblasts. Am J Respir Cell Mol Biol 2000; 22:590–596.
29. Wu W, Jaspers I, Zhang W, Graves LM, Samet JM. Role of Ras in metal-induced EGF receptor signaling and NF-kappaB activation in human airway epithelial cells. Am J Physiol 2002; 282:L1040–1048.
30. Mukhopadhyay A, Manna SK, Aggarwal BB. Pervanadate-induced nuclear factor-kappaB activation requires tyrosine phosphorylation and degradation of IkappaBalpha. Comparison with tumor necrosis factor-alpha. J Biol Chem 2000; 275:8549–8555.
31. Livolsi A, Busuttil V, Imbert V, Abraham RT, Peyron JF. Tyrosine phosphorylation-dependent activation of NF-kappa B. Requirement for p56 LCK and ZAP-70 protein tyrosine kinases. Eur J Biochem 2001; 268:1508–1515.
32. Prasad N, Topping RS, Zhou D, Decker SJ. Oxidative stress and vanadate induce tyrosine phosphorylation of phosphoinositide-dependent kinase 1 (PDK1). Biochemistry 2000; 39:6929–6935.
33. Hei YJ, Farahbakhshian S, Chen X, Battell ML, McNeill JH. Stimulation of MAP kinase and S6 kinase by vanadium and selenium in rat adipocytes. Mol Cell Biochem 1998; 178:367–375.
34. Pandey SK, Theberge JF, Bernier M, Srivastava AK. Phosphatidylinositol 3-kinase requirement in activation of the ras/C- raf-1/MEK/ERK and p70(s6k) signaling cascade by the insulinomimetic agent vanadyl sulfate. Biochemistry 1999; 38:14667–14675.
35. Gao N, Ding M, Zheng JZ, Zhang Z, Leonard SS, Liu KJ, Shi X, Jiang BH. Vanadate-induced expression of hypoxia-inducible factor 1 alpha and vascular endothelial growth factor through phosphatidylinositol 3-kinase/Akt pathway and reactive oxygen species. J Biol Chem 2002; 277:31963–31971.
36. Chen F, Shi X. Intracellular signal transduction of cells in response to carcinogenic metals. Crit Rev Oncol Hematol 2002; 42:105–121.
37. Chen F, Shi X. Signaling from toxic metals to NF-kappaB and beyond: not just a matter of reactive oxygen species. Environ Health Perspect 2002; 110 Suppl 5:807–811.
38. Jaspers I, Samet JM, Erzurum S, Reed W. Vanadium-induced kappaB-dependent transcription depends upon peroxide-induced activation of the p38 mitogen-activated protein kinase. Am J Respir Cell Mol Biol 2000; 23:95–102.
39. Tannoch VJ, Hinds PW, Tsai LH. Cell cycle control. Adv Exp Med Biol 2000; 465:127–140.
40. Woo ES, Rice RL, Lazo JS. Cell cycle dependent subcellular distribution of Cdc25B subtypes. Oncogene 1999; 18:2770–2776.

41. Yan S, Wenner CE. Modulation of cyclin D1 and its signaling components by the phorbol ester TPA and the tyrosine phosphatase inhibitor vanadate. J Cell Physiol 2001; 186:338–349.
42. Zhang Z, He H, Chen F, Huang C, Shi X. MAPKs mediate S phase arrest induced by vanadate through a p53-dependent pathway in mouse epidermal C141 cells. Chem Res Toxicol 2002; 15:950–956.
43. Strasser A, O'Connor L, Dixit VM. Apoptosis signaling. Annu Rev Biochem 2000; 69:217–245.
44. Hehner SP, Hofmann TG, Droge W, Schmitz ML. Inhibition of tyrosine phosphatases induces apoptosis independent from the CD95 system. Cell Death Differ 1999; 6:833–841.
45. Gamero AM, Larner AC. Vanadate facilitates interferon alpha-mediated apoptosis that is dependent on the Jak/Stat pathway. J Biol Chem 2001; 276:13547–13553.
46. Luo J, Sun Y, Lin H, Qian Y, Li Z, Leonard SS, Huang C, Shi X. Activation of JNK by vanadate induces a Fas-associated death domain (FADD)-dependent death of cerebellar granule progenitors in vitro. J Biol Chem 2003; 278:4542–4551.
47. Ding M, Shi X. Molecular mechanisms of Cr(VI)-induced carcinogenesis. Mol Cell Biochem 2002; 234–235:293–300.
48. Hayes RB, Lilienfeld AM, Snell LM. Mortality in chromium chemical production workers: a prospective study. Int J Epidemiol 1979; 8:365–374.
49. Yurkow EJ, Kim G. Effects of chromium on basal and insulin-induced tyrosine phosphorylation in H4 hepatoma cells: comparison with phorbol-12-myristate-13-acetate and sodium orthovanadate. Mol Pharmacol 1995; 47:686–695.
50. Qian Y, Jiang BH, Flynn DC, Leonard SS, Wang S, Zhang Z, Ye J, Chen F, Wang L, Shi X. Cr (VI) increases tyrosine phosphorylation through reactive oxygen species–mediated reactions. Mol Cell Biochem 2001; 222:199–204.
51. Ye J, Shi X. Gene expression profile in response to chromium-induced cell stress in A549 cells. Mol Cell Biochem 2001; 222:189–197.
52. 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 2002; 33:1622–1640.
53. 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 1995; 16:2401–2405.
54. 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 2000; 19:231–238.
55. Chen F, Bower J, Leonard SS, Ding M, Lu Y, Rojanasakul Y, Kung HF, Vallyathan V, Castranova V, Shi X. Protective roles of NF-kappa B for chromium(VI)-induced cytotoxicity is revealed by expression of Ikappa B kinase-beta mutant. J Biol Chem 2002; 277:3342–3349.

56. Kaltreider RC, Pesce CA, Ihnat MA, Lariviere JP, Hamilton JW. Differential effects of arsenic(III) and chromium(VI) on nuclear transcription factor binding. Mol Carcinog 1999; 25:219–229.
57. Chen F, Ding M, Castranova V, Shi X. Carcinogenic metals and NF-kappaB activation. Mol Cell Biochem 2001; 222:159–171.
58. Xu J, Bubley GJ, Detrick B, Blankenship LJ, Patierno SR. Chromium(VI) treatment of normal human lung cells results in guanine-specific DNA polymerase arrest, DNA-DNA cross-links and S-phase blockade of cell cycle. Carcinogenesis 1996; 17:1511–1517.
59. Pritchard DE, Ceryak S, Ha L, Fornsaglio JL, Hartman SK, O'Brien TJ, Patierno SR. Mechanism of apoptosis and determination of cellular fate in chromium(VI)-exposed populations of telomerase-immortalized human fibroblasts. Cell Growth Differ 2001; 12:487–496.
60. Zhang Z, Leonard SS, Wang S, Vallyathan V, Castranova V, Shi X. Cr(VI) induces cell growth arrest through hydrogen peroxide–mediated reactions. Mol Cell Biochem 2001; 222:77–83.
61. Carlisle DL, Pritchard DE, Singh J, Owens BM, Blankenship LJ, Orenstein JM, Patierno SR. Apoptosis and P53 induction in human lung fibroblasts exposed to chromium (VI): effect of ascorbate and tocopherol. Toxicol Sci 2000; 55:60–68.
62. Ye J, Wang S, Leonard SS, Sun Y, Butterworth L, Antonini J, Ding M, Rojanasakul Y, Vallyathan V, Castranova V, et al. Role of reactive oxygen species and p53 in chromium(VI)-induced apoptosis. J Biol Chem 1999; 274:34974–34980.
63. Carlisle DL, Pritchard DE, Singh J, Patierno SR. Chromium(VI) induces p53-dependent apoptosis in diploid human lung and mouse dermal fibroblasts. Mol Carcinog 2000; 28:111–118.
64. 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 2000; 279:C868–875.
65. Wang S, Shi X. Mechanisms of Cr(VI)-induced p53 activation: the role of phosphorylation, mdm2 and ERK. Carcinogenesis 2001; 22:757–762.
66. Catelas I, Petit A, Zukor DJ, Antoniou J, Huk OL. TNF-alpha secretion and macrophage mortality induced by cobalt and chromium ions in vitro: qualitative analysis of apoptosis. Biomaterials 2003; 24:383–391.

...cialty specifically with lung injury induced by oxygen and nitrogen ...discusses molecular, cellular, and mechanistic events leading to patho... ...ole of oxidant species in the induction of these events; and potential ...ch molecular pathways. Important issues regarding oxidative stress ...d progression of pulmonary diseases—including diseases caused ...pental inhalation of toxicants—are discussed in the context of

...d in lung injury, inflammation, and development of disease triggered ...molecular, cellular, functional, and pathological changes as well as ... for their prevention.

...rs in the field contribute chapters highlighting the significant role of ...cies in the development of pulmonary diseases such as acute respi... ...chronic granulomatous disease...chronic obstructive pulmonary ...silicosis...smoking-induced inflammation, injury, and disease... ...ects...hyperoxic disease...and metal-induced carcinogenesis.

...eader with the Pathology and Physiology Research Branch of the ...vision, National Institute for Occupational Safety and Health, ...e is also Professor of Pathology, Anatomy, and Basic Sciences at ...antown, West Virginia. He is the author, coauthor, editor, or coed... ...pters, and 305 journal articles. An editorial board member of the ...*and Physiology* and *Molecular and Cellular Biochemistry* and the ...research in lung health and occupational safety and health, Dr. ...egree (1963) from The Maharaja Sayajirao University of Baroda,

...of the Pathology and Physiology Research Branch of the Health ...ational Institute for Occupational Safety and Health, Morgantown, ...ease Control Distinguished Consultant and an Adjunct Professor ...y, Morgantown, West Virginia, and the University of Pittsburgh, ...search in pulmonary toxicology has resulted in the publication of ...0 peer-reviewed journal papers and textbook chapters. Dr. Castranova ...4) from West Virginia University, Morgantown, West Virginia.

...er with the Pathology and Physiology Research Branch of the ...onal Safety and Health, Morgantown, West Virginia. He is also ...munology, and Cell Biology and Basic Pharmaceutical Sciences ...gantown, West Virginia. The guest editor of several journal focused ...national conferences, and author of 330 journal articles, Dr. Shi ...wo journals and an associate editor of the *Journal of Environmental ...cology*. He received the Ph.D. degree (1988) from West Virginia ...Virginia.

...*America*

ISBN 0-8247-4874-3

Volume 187

Oxygen/Nitrogen Radicals

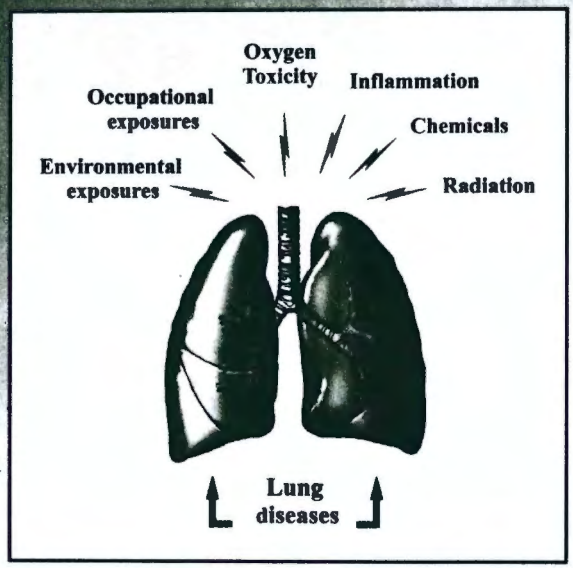
Vallyathan · Castranova · Shi

MARCEL

Volume 187

Oxygen/Nitrogen Radicals

Lung Injury and Disease



edited by

Val Vallyathan
Vincent Castranova
Xianglin Shi