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Toxic Metals and Autophagy

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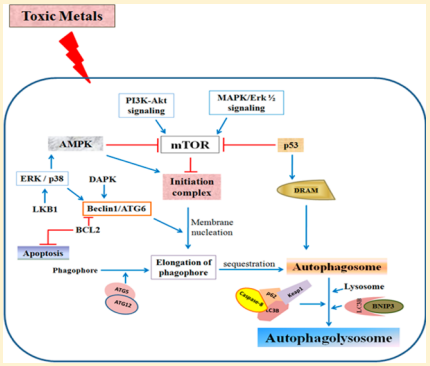
ABSTRACT: The earth's resources are finite, and it can no longer be considered a source of inexhaustible bounty for the human population. However, this realization has not been able to contain the human desire for rapid industrialization. The collateral to overusing environmental resources is the high-level contamination of undesirable toxic metals, leading to bioaccumulation and cellular damage. Cytopathological features of biological systems represent a key variable in several diseases. A review of the literature revealed that autophagy (PCDII), a high-capacity process, may consist of selective elimination of vital organelles and/or proteins that intiate mechanisms of cytoprotection and homeostasis in different biological systems under normal physiological and stress conditions. However, the biological system does survive under various environmental stressors. Currently, there is no consensus that specifies a particular response as being a dependable biomarker of toxicology. Autophagy has been recorded as the initial response of a cell to a toxic metal in a concentration- and time-dependent manner. Various signaling pathways are triggered through cellular proteins and/or protein kinases that can lead to autophagy, apoptosis (or necroptosis), and necrosis. Although the role of autophagy in tumorigenesis is associated with promoting tumor cell survival and/or acting as a tumor suppressive mechanism, PCDII in metal-induced toxicity has not been extensively studied. The aim of this review is to analyze the comparative cytotoxicity of metals/metalloids and nanoparticles (As, Cd, Cr, Hg, Fe, and metal-NP) in cells enduring autophagy. It is noted that metals/metalloids and nanoparticles prefer ATG8/LC3 as a potent inducer of autophagy in several cell lines or animal cells. MAP kinases, death protein kinases, PI3K, AKT, mTOR, and AMP kinase have been found to be the major components of autophagy induction or inhibition in the context of cellular responses to metals/metalloids and nanoparticles.



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INTRODUCTION

Environmental exposure to toxicants or xenobiotics triggers tissue and cell injury by causing regulated and/or unregulated sudden cell death. According to the Committee on Toxicity (COT),[1] >100 000 chemicals are unconfined globally every year as their production, use, and disposal increases. The fate of chemical substances depends on their physicochemical properties and chemical applications. Chemical substances discharged into the environment may be natural or can be anthropogenic. Rapid industrialization and widespread use of compounds containing metals that are nonbiodegradable and have a long residence time in the environment cause serious eco-toxicological problems and are infamous for their tendency to bioaccumulate (Figure 1) and induce pathophysiological vulnerability (Table 1).[2−27] A common characteristic of toxic metals is, therefore, the chronic nature of their toxicity. The toxicological property of metals usually involves an interaction between the free metal ion and the toxicological target.[28]

Toxicant insult can trigger cellular pathways that are broadly classified as death and survival signals. Each organ has its own critical threshold toward a toxicant, regulated by signaling systems. Cells utilize a coordinated, preprogrammed signaling system to maintain the structural and functional homeostasis of the organ not only on exposure to external toxicants but also in their normal life cycle. Depending on the concentration and duration of exposure to toxicants as well as the morphological and molecular definitions of cell death modalities, Galluzzi et al.[29] described programmed cell death, an essential orchestrated process, as being divided into apoptosis (PCDI), autophagy (PCDII), and necroptosis (PCDIII)[30−32] (Figure 2).

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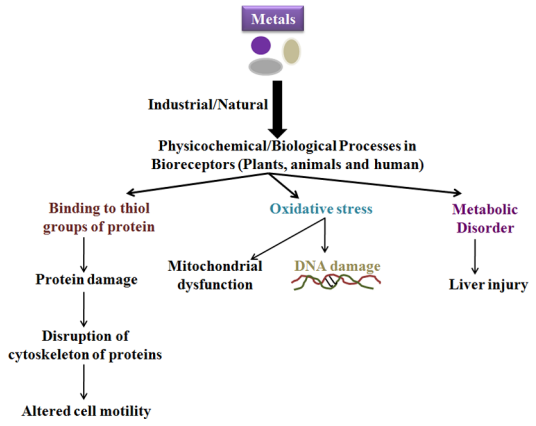

Figure 1. Physicochemical/biological processes in plants, animals, and humans in response to metal exposure.

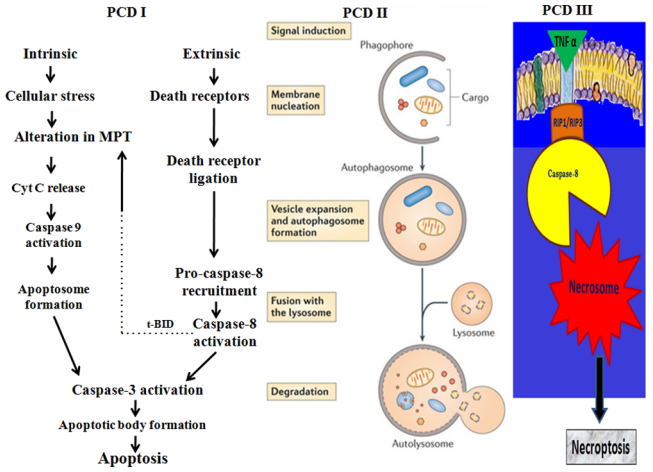

Figure 2. Schematic diagram of programmed cell death. PCDI adapted with permission from refs 30 and 31. Copyright 2006 and 2014, respectively, Nature Publishing Group.

Apoptosis (PCDI) comprises two stages, intrinsic and extrinsic, involving mitochondrial outer membrane permeabilization (MOMP) and death receptor-interacting proteins, respectively.[29,33] Necroptosis (PCDII) is induced by a class of death receptors, mainly TNFR (tumor necrosis factor receptor) and RIPK (receptor-interacting protein kinase).[29,34,35] Investigation into the roles of autophagy has increased and has also been highlighted in recent years, invading the fields of biology and medicine.[28] The process of autophagy occurs constitutively at basal levels and activates a variety of intracellular and extracellular stimuli.[36] Autophagy is an evolutionarily conserved ubiquitous cellular process dominated by dynamic catabolic biochemical mechanisms, generating autophagosomes to engulf intracellular components that ultimately fuse with lysosomes during the maturation step.[36,37] Depending on the different target cargo by which cellular material is transported to lysosomes, there are three categories of autophagy: microphagy, chaperone-mediated autophagy (CMA), and macroautophagy.[36,38] Microautophagy consists of direct lysosomal uptake of cytosol or organelles at the lysosomal surface by protrusion of the lysosomal membrane.[39] CMA selectively degrades proteins with a specific motif, KFERQ, transporting them to the lysosome via lysosomal membrane associated protein 2.[40] Macroautophagy is often referred to as autophagy in general (Figure 3).[41,42] Macroautophagy sets in with the wrapping of the flat membrane around a portion of the cytosol or organelles, forming a closed double membrane vesicle, the autophagosome, which is a hallmark of the process. During the early formation of the autophagosome, the membranes enlarge in magnitude by altering their shape to form a cup-like structure called a phagophore.[43] Phagophores are formed either by isolation of the original membrane, assimilation of additional lipids, or tabulation of the existing compartments. Sequestration of cytosolic content ultimately takes place in these vesicles.[36,37] The segregated cytosol is then delivered to the lysosomal lumen, generating single membrane autophagolysosomes (mature autophagosomes) and degrading their contents by lysosomal hydrolyses.[44−46] The maturation process is connected

Table 1. Cytopathological Features and Clinical Relevance of Metals/Environmental Stressors

	cytopathological features			
metal	acute exposure/deficiency	chronic exposure/deficiency	clinical relevance	refs
arsenic (As)	vomiting, diarrhea, weakness, prostration, and weight loss, cutaneous manifestations, hyperpigmentation, conjunctivitis, photophobia, pharyngitis, or irritating cough, asthma, prolonged QT interval, ↑ BP, neutropenia	Bowen's disease, palmar keratosis, skin cancer, squamous cell carcinoma, acute myeloid leukemia	thiamine deficiency, altered pyruvate dehydrogenase (PDH) complex, ↑ H_2O_2 production, disrupting cellular electrolytic function, cellular PCD	2−5
cadmium (Cd)	disruption of presynaptic function, olfactory dysfunction, nasal epithelial damage, odorant-guided passive avoidance behavior, behavioral change	tubular proteinuria, Alzheimer's disease, Parkinson's disease, lung cancer	blockade of calcium channels, disruption of olfactory epithelium, ↑ intracellular calcium concentrations, overexpression of a proteasomal-resistant of IκB in heme catabolism, autophagy, apoptosis, necroptosis, necrosis	6−11
chromium (Cr)	essential components of "glucose tolerence factor", skin ulcer, nasal membrane inflammation, liver damage, edema	lung function disorder, dermatitis, pharyngitis, lung cancer	hyperglycemia, inhibit phosphotyrosine phosphatase, enhance insulin receptor, cytotoxicity like autophagy, apoptosis	12−15
mercury (Hg)	nausea, dysfunction of GI tract, renal organ system, neurological disruption, facial paresthesias, visual-field constriction, ataxia, dysarthria	hearing loss, blindness, developmental delay, memory loss, hair loss,	disseminated intravascular coagulopathy, cellular stress, oxidative damage, renal cortical necrosis, apoptosis	16−20
iron (Fe)	↑/↓ hepcidin, hypoxia, ↑ erythropoietin production, hemochromatosis,	hypertrophy, dilatation, heart failure, cirrhosis, degenerative arthropathy in thalassemia, sickle cell disease, aplastic anemia,	weight loss, fatigue, bronze/gray skin, polyuria, arthralgias, cachexia, soft, small testes, arthritis, cellular damage	21−24
metal-nanoparticles (NPs)	heat generation, dermaltoxicity, respiratory toxicity	spinal cord injury, gynecological problems	↑ oxidative stress, human alveolar epithelial apoptotic damage, autophagy induction	25−27

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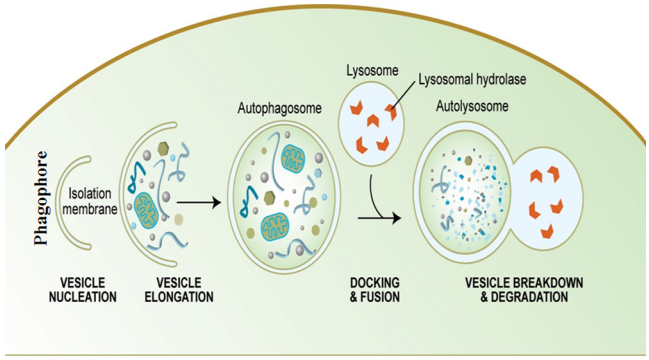

Figure 3. Mechanism of autophagy. Reprinted with permission from ref 42. Copyright 2009 Beth Levine, MD.

with lysosome-associated membrane proteins (LAMPs), maintaining cellular homeostasis by ubiquitination.[47,48] A major microtubule-associated protein-like chain, LC3B (light chain 3B), conjugates to phosphatidylethanolamine by ubiquitination, which functions as an integral membrane protein in the membrane of a nascent autophagosome.[49] Autophagy is upregulated by extra- and intracellular stresses and signals. Several phylogenetically conserved proteins, ATGs (AuTophaGy) and protein kinases, are involved in the formation of the autophagosome and autophagolysosome.[50−54] The interaction of ATG proteins and protein kinases in autophagy are depicted in Figure 4. ATG proteins undergo phosphorylation and

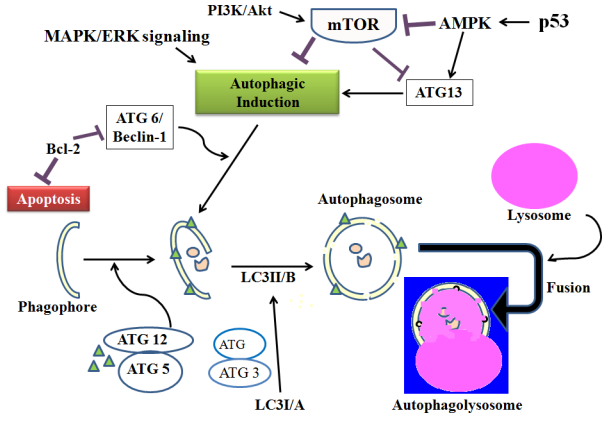

Figure 4. Interactions of ATG proteins in autophagy.

acetylation, which modulates multiple components of the autophagic machinery, as well as post-translational modification of ATG proteins, which may be a crucial factor in regulating autophagy.[55] Crosstalk between signaling pathways ensues to control autophagic pathways. As a central inhibitor of autophagy, serine-threonine protein kinase TOR (target of rapamycin) integrates input information from multiple upstream signal transduction pathways and negatively regulates autophagy via ATG protein suppression.[56]

Autophagy is critical to the processes of embryo development, growth regulation, and maintenance of homeostasis in multicellular organisms.[57] Futhermore, emerging evidence suggests that autophagy can protect the affected cell against toxicant and/or metal-induced toxicity.[38] Degraded products of autophagy may serve as raw materials for cellular metabolism.[58,59] Basal autophagy may remove aged and damaged organelles and proteins under normal circumstances. Sometimes, autophagy in excess may lead to cell death.[60] Morphologically defined autophagic death is the death of cellular organelles; thus, it may provide nutrients to other cells in multicellular organisms from the components of organs or tissues that are removed during PCD that can be reused to survive or maintain cellular homeostasis.[61] Although autophagy is present in dying cells,[62] there is also crosstalk between autophagy and other PCDs. Autophagy can inhibit and/or enhance apoptotic and necrotic cell death in the same cell.[62] The outcome of autophagic cell death, therefore, depends on its crosstalk with the PCDI and PCDIII pathways.[63]

Metals may vary in their oxidation state by losing one or more electrons to form cations. All metals are potentially toxic, yet many metals are essential for life. Nonessential metals follow the same pathways as those of chemically similar essential metals.[64] Hypothetically, the strength of metal toxicity depends principally on absorption, concentration, and persistence of the eventual toxicant at its location of action. Metals, either present in the environment or administered for therapeutic reasons, are prototypical xenobiotics that retard or enhance immune responses. Cells that are involved in the transport of metals, such as, in the gastrointestinal tract, hepatocytes or renal tubular cells, are particularly susceptible to toxicity. A number of biochemical reactions occur (Figure 5) by the

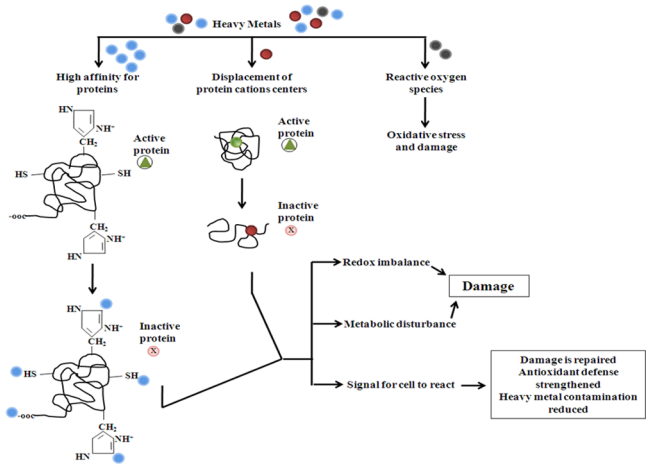

Figure 5. Heavy metals toxicity in cell. Reprinted with permission from ref 65. Copyright 2010 Chilean Society of Soil Science.

displacement of protein cationic centers or the increase of reactive oxygen species when insulted by metal/metalloid.[65] The major hazardous metals of concern in terms of their environmental load and health effects are arsenic (As, slow death mineral), cadmium (Cd, pseudomacho or violent element), chromium (Cr), mercury (Hg, mad hatters mineral), copper (Cu), aluminum (Al), and lead (Pb, horror mineral).[66,67] Some metals, such as Cd and Hg, are generally considered only from a toxicological point of view because they are not essential for the well being of an organism. However, other metals, such as iron (Fe) and Cu, are essential for life but present significant health problems when in excess. Still other metals, such as Pb, Cd, Cr, and Al, may be intimately involved in cellular dysfunction through their presence or absence. Minerals like fluoride and arsenic salts are of natural origin, but human activity can also aggravate the situation. Emissions of heavy metals to the environment occur via a wide range of processes and pathways, including air (during combustion, extraction, and

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processing), surface waters (via runoff and releases from storage and transport), and soil (into ground waters and crops). However, exposure does not result only from the presence of harmful agents in the environment, it also depends on their concentration and contact with the target tissue. Exposure is a function of concentration and time: "an event that occurs when there is contact at a boundary between a human and environment with a contaminant of a specific concentration for an interval of time".[68]

Toxicity is the degree of damage impinged on an organism by a xenobiotic. To know the potential hazard or toxicity of a specific chemical, inputs are essential on the type of effect, dose, duration, physicochemical properties, exposure route, and the susceptibility of the biological system of that chemical. Cells utilize a coordinated, preprogrammed signaling system to maintain the homeostasis of an organ's structure and function. Autophagy is one of the preprogrammed mechanisms (Figure 6)

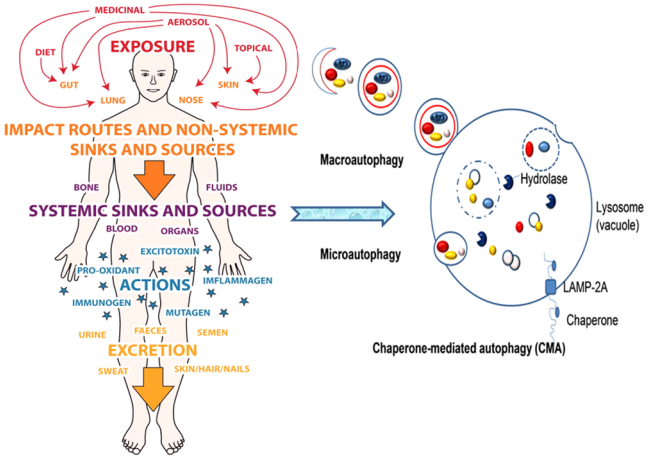

Figure 6. Toxicity and autophagy. Adapted with permission from refs 69 and 70. Copyright 2013 The Royal Society of Chemistry and copyright 2013 Scientific Research Publishing, respectively.

by which a cell can reduce or remove the inserted toxic agents from the body to control homeostasis inside the cell.[69,70] Induction of autophagy, therefore, may provide a novel restorative approach toward toxicology. Considering the dearth of information on the role of autophagy in toxicology, this review concentrates on autophagic pathways caused by different environmental metal contaminants.

■ ARSENIC (As)

Arsenic is a widely distributed metalloid, occurring in rock, soil, water, and air. Inorganic arsenic is present in groundwater used for drinking in several countries, whereas organic arsenic compounds are primarily found in fish.[71] Elevated levels of arsenic are also found in several countries in which it exceeds the World Health Organization (WHO) drinking water guideline (10 μg/L), affecting 100 million people globally. The major incidents of arsenic contamination in groundwater in Asian countries have been recorded in Bangladesh, India, China, Mongolia, Nepal, Cambodia, Myanmar, Afghanistan, Korea, and Pakistan.[72] In India, the major states that are affected by arsenic contamination of water are Assam, Bihar, Chhatishgarh, Uttarpradesh, and West Bengal.[73] In India's neighboring country, Bangladesh, approximately 70 million people are at risk of long-term exposure to high levels of arsenic through groundwater.[74,75] Kurdistan province of Western Iran and Vietnam have a considerable risk of chronic arsenic poisoning. In certain areas of Brazil, Bulgaria, Chile, Canada, Cambodia, Czech Republic, Egypt, Finland, Germany, Ghana, Greece, and Hungary, large amounts of arsenic have been found in drinking water and arsenic-containing air-contaminated food crops.[76−81]

The oxides of arsenic are the most common threat because they are known to be highly toxic to living systems. Inorganic As is more harmful than organic As exposure[82] because it is biotransformed in the liver. The two forms of inorganic As, reduced (trivalent As(III)) and oxidized (pentavalent As(V)) can be absorbed and accumulated in tissues and body fluids,[83] particularly in keratin-rich tissues like hair, nail, and skin. Trivalent As binds to sulphydryl groups with higher affinity, leading to the inhibition of enzymatic systems. In humans, inorganic As is reduced nonenzymatically from a pentoxide to a trioxide state, which increases its bioavailability and toxicity. The remaining unbound As ($\leq$10%) accumulates in cells, which, over time, may lead to skin, bladder, kidney, liver, lung, and prostate cancers.[84] Arsenic disrupts energy transduction reactions, ATP production, and capillary integrity as well as leads to endothelial damage and loss of cellular volume. Arsenic is a well-known carcinogen, and, paradoxically, it is also used as an effective chemotherapeutic agent for acute promyelocytic leukemia.[85,86] Increased As exposure is associated with an enhanced frequency of chromosomal aberrations and sister-chromatid exchanges[87,88] through interaction with zinc finger structures.[89] Monomethylarsenic and dimethylarsenic radicals are able to form reactive oxygen species (ROS) by reaction with molecular oxygen.[90]

Arsenic is a potent inducer of oxidative stress, causing DNA damage and apoptosis.[91] On the basis of the types of exposure, As interrupts the normal control of apoptosis through its influence on signaling pathways. Although much is known about the mechanisms of apoptosis induced by arsenic compounds, there is a dearth of information on the involvement of autophagy as a regulator of As-dependent cell death. Recent studies have revealed that arsenic could cause autophagic cell death in malignant cells, including leukemia and lymphoblastoid and malignant glioma cells (Table 2). Kanzawa et al.[92] reported that Bcl-2/adenovirus E1B 19 kDa-interacting protein 3 (BNIP3) plays a central role in As_2O_3-induced autophagic cell death in malignant glioma cells. BNIP3 is upregulated in As_2O_3-induced autophagic cell death by involvement of the autophagy-specific marker LC3 and disruption of mitochondrial membrane integrity, but not by caspase activation. Four micromolar As_2O_3 promoted downregulation of BAX protein via accumulation of Beclin-1 and triggered autophagic cell death in leukemic cell lines.[93] Immediately after treatment with As_2O_3, the proliferation of HL60 cells was significantly inhibited, and the formation of autophagosomes was increased.[94] However, if As_2O_3 remains in the cell for a longer time, then cell death occurs by apoptosis. Arsenic can induce the ERK1/2 signaling pathway to stimulate autophagy via LC3B and Beclin-1, which are important regulators of autophagosome formation, and DAPK promoter hypermethylation in human uroepithelial SV-HUC-1 cells.[95] Goussetis et al.[96] reported that As_2O_3, a potent inducer of autophagy, appears to require activation of the MEK/ERK pathway but not the AKT/mTOR or JNK pathways in leukemia cells. In human lymphoblastoid cell lines, arsenic insult is strongly associated with autophagy,[97] modulating the regulation of genes encoding autolysosomal constituents[98] and resulting in inhibition of cellular growth.

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Table 2. Arsenic Compounds and Cellular Responses

compd	concentration (μM)	selective marker	effects	ref
As_2O_3	1−4	BNIP3 and LC3	induction of autophagic cell death in malignant glioma cell	92
As_2O_3	4	Beclin-1	downregulation of BAX accumulates Beclin-1 to trigger autophagic cell death in a leukemic cell line	93
As_2O_3	0.625−20	Autophagosome formation	early induction of autophagy as cell survival mechanism in HL60 cells	94
$NaAsO_2$	1−10	ERK1/2, DAPK, LC3B, and Beclin-1	ERK1/2 stimulates Beclin-1 and LC3B and DAPK hypemiethylation to induce autophagy in human uroepithelial SV-HUC-1 cells	95
As_2O_3	2	MEK/ERK Beclin-1, and Atg7	induction of autophagy by MEK/ERK activation to stimulate Beclin-1/ATG7 in leukemia cells	96
$NaAsO_2$	6	p62, LC3B	activation of UPR (unfolded protein response) containing p62 and LC3 induces autophagic puncta formation in human lymphoblastoid cell lines	97, 98
As_4O_6	0.5−3	Beclin-1/ATG6	ROS generation in U-937 human leukemic cells triggers autophagic induction	99

Table 3. Cadmium Compounds and Cellular Responses

compd	concentration (μM)	selective marker	effects	ref
$CdCl_2$	3−24	ERK-LC3	induction of both autophagy and apoptosis in MES-13 cells	106
$Cd(NO_3)_2$	1−10	LC3II/B	accumulation of autolysosomes in HUVECs	108
$Cd(NO_3)_2$	>20			
$CdCl_2$	40 μM in W138 cells and 160 μM in RW138 cells for 24 h	PERK, Atg5, LC3II, p38, Akt, and MRPl	Cd-induced Atg5 and LC3II dephosphorylated p38 and Akt as well as downregulated MRP1 and procaspase-3 to induce autophagy in WI38 human lung epithelial fibroblast cells	109
$CdCl_2$	1−10	LKB1-AMPK and mTOR	ROS generation causes activation of LKB1-AMPK and downregulation of mTOR to induce autophagy	110
$CdCl_2$	1 mM for 18 and 24 h exposures	LC3II/B	cell proliferation and autophagy in rat kidney	107
$CdCl_2$	0.3 mg/kg body mass/1, 3, and 5 days of intoxication	LC3II/B	autophagy as an additional strategy to safeguard the developmental program in sea urchin embryos	111

Han et al.[99] demonstrated that tetraarsenic hexoxide generated ROS production in U-937 human leukemic cells, which triggered both Beclin-1/ATG6-induced autophagic cell death and caspase-dependent apoptosis. Zhang et al.[100] further revealed that the major source of ROS is arsenic-damaged mitochondria, which are catabolically removed by autophagic activation.

CADMIUM (Cd)

Cd occurs naturally in ores together with Zn, Pb, Cr, and Cu. Cd compounds are used as stabilizers, color pigments, rechargeable batteries, and alloys and can be found in some fertilizers. Cd production, consumption, and emissions to the environment have increased worldwide dramatically during the 20th century.[101] Cd-containing products that are rarely recycled are frequently dumped with household waste, thereby contaminating the environment. Cd acts as a catalyst in forming ROS and prefers the +2 oxidation state in most of its compounds. Cd and its congeners are not always considered to be transition metals because they do not have partly filled d or f electron shells in their elemental or common oxidation states.[102] Cd is insoluble in water and is not flammable; however, in its powdered form, it may burn and release toxic fumes.[103] Cadmium acetate ($Cd(CH_3CO_2)_2$) and cadmium chloride ($CdCl_2$) can produce severe respiratory distress[103] from acute exposures of 1−5 mg m^{-3}. Cigarette smoking is the major source of Cd exposure in smokers, whereas food is the principal source of Cd exposure in the general nonsmoking population. Cd toxicity at a low concentration is amplified as a consequence of the long biological half-life of the metal and has been associated with blockage of oxidative phosphorylation, glutathione depletion, inhibition of antioxidant enzymatic activity, production of oxidative stress, DNA damage, reduction of protein synthesis, and cell death.[104] Ingestion of 150 g of cadmium chloride was reported to cause focal hepatic necrosis.[105] The effect of Cd toxicity depends on its concentration and the duration of exposure, which can induce both apoptotic- and autophagic-related pathways.[106,107] Autophagy is implicated in the response of hematopoietic stem/progenitor cells/differentiated cells to toxic concentrations of heavy metal cations.[14] Different Cd concentrations can drive autophagy in various cellular responses, including epidermal, mesangial, and endothelial cells (Table 3). The cytotoxicity of Cd induces both autophagy and apoptosis in MES-13 cells through elevation of cytosolic Cd levels by Ca^{2+}−ERK−LC3 and Ca^{2+}−mitochondrial-caspase signaling pathways.[108] The internalization of Cd^{2+} into human umbilical vein endothelial cells (HUVECs) promoted autophagy at low concentrations (<10 μM) and inhibited apoptosis by deprivation of serum and basic fibroblast growth factor (bFGF).[108] Lim et al.[109] suggest that Cd may protect against autophagy by relieving endoplasmic reticulum stress. Cd-mediated intracellular ROS generation causes induction of autophagy through the activation of LKB1-AMPK (liver kinase B1-adenosine monophosphate kinase) signaling and the downregulation of mTOR in epidermal cells.[110] In the sea urchin embryo exposed to cadmium, Chiarelli et al.[107] showed that autophagy can play a crucial role in stress response of this organism because autophagy can energetically contribute to apoptotic execution through its catabolic role. Chargui et al.[111] reported that in environmental exposures Cd accumulates within lysosomes of proximal convoluted tubule (PCT) cells in rat kidney, triggering cell proliferation and autophagy; persistence of Cd within the cytosol might continuously damage proteins and impair long-term autophagy efficiency. However, the role of Cd in stimulating autophagic cell death warrants further research.

CHROMIUM (Cr)

Water insoluble Cr(III) compounds and Cr metal are not considered to be a health hazard, whereas the toxicity and carcinogenic properties of Cr(VI) have been known for a long time.[112] The World Health Organization[113] indicated that 0.05 mg/L is

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the maximum allowable concentration of Cr(VI) in drinking water.

Several *in vitro* studies indicated that high concentrations of Cr(III) in the cell can lead to DNA damage.[114] Highly reactive hydroxyl radicals and other reactive radicals are byproducts of the reduction of Cr(VI) to Cr(III), which contributes to genotoxicity by binding DNA. Cr(III) can complex with organic compounds inside cells and interfere with metalloenzyme systems at high concentrations.[115,116] Cr is a redox-active soil contaminant with dramatic alterations in its mobility and toxicity with changes in its oxidation state.[117,118] Apoptosis induced by hexavalent Cr exposure is initiated by several pathways, including modulation of the level of micronucleated polychromatic erythrocytes (MN-PCEs) in CD-1 mice, DNA damage,[119] generation of ROS, induction of p53,[120] DNA-dependent protein kinase signaling to p53-dependent intrinsic mitochondrial apoptosis,[15] and oxidative stress, which includes enhanced production of superoxide anion and hydroxyl radicals, increased lipid peroxidation, genomic DNA fragmentation, and activation of protein kinase C[121] to eliminate the damaged cells from the population (CD-1, myelogenous leukemic K562, J774A cells and human lung epithelial A549 cells). Interestingly, hexavalent Cr is also able to induce autophagy in hematopoietic stem/progenitor cell. Gioacchino et al.[14] reported that stem/progenitor cells exposed to subtoxic (0.1 μM) and toxic (10 μM) concentrations of Cr show autophagic morphologies, contributing to the conservation of tissue renewal capacity. Autophagy is an ultrastructural marker of Cr toxicity in human cord blood hematopoietic stem cells.[14] Hence, in the hematopoietic lineage, autophagy and apoptosis are both involved in response to Cr(VI)-induced toxic stress, and the molecular switch between these two pathways could be regulated according to the differentiation of stem/progenitor cells.[28]

MERCURY (Hg)

Hg is an element that occurs naturally in the environment, usually in combination with other elements as Hg compounds or salts. Hg is a ubiquitous environmental toxin that causes a wide range of adverse health effects in humans. According to the position of Hg in the periodic table, it has exceptionally low melting and boiling temperatures for a d-block metal. Since the electron configuration strongly resists removal of an electron, Hg behaves similar to that of noble gas elements, which form weak bonds and become solids that melt easily at relatively low temperatures.[122] The physiological range for these elements (among deficiency, sufficiency, and toxicity) is exceedingly narrow, and there exists a controlled metal homeostasis network to adjust the fluctuation starting from nonavailability to toxicity.[123] Awareness was drawn to Hg poisoning after the occurrence of Minamata disease reported from Minamata Bay, Japan, during 1953−1960.[124] There are three forms of Hg: elemental, inorganic, and organic. Each form has its own profile of toxicity. The most common forms of Hg that are present in the environment are metallic Hg: the inorganic salts, mercuric sulfide, mercuric chloride, and methylmercury. Methylmercury is of particular concern because it bioaccumulates through the food chain.

Within a cell, Hg may bind to a variety of enzymes, producing nonspecific cell injury or cell death. Toxicity of Hg ions is manifested by protein precipitation, enzyme inhibition, and generalized corrosive action. Hg not only binds to SH groups but also to phosphoryl, carboxyl, amide, and amine groups. Readily available proteins (including enzymes) with such groups have great affinity to react with Hg. Bose et al.[125] demonstrated the distribution kinetics of radioactive Hg in different hepatocellular fractions in which Hg treatment increased nuclear and liposomal protein content significantly. Several reports of *in vivo* and *in vitro* Hg toxicity are available regarding its biochemical and cellular toxic effects, which include DNA damage, inhibition of DNA and RNA synthesis, and alteration in protein structure.[126−128] $HgCl_2$ can damage DNA in rat and mouse embryo fibroblasts.[129,130] Nonlethal dose Hg exposure is known to induce oxidative stress-mediated apoptosis in rat liver.[131] A few reports have demonstrated that a lower concentration of Hg causes the induction of cell death in different cell types. Mercuric chloride damages cellular DNA by a nonapoptotic mechanism at a low dose (5 μM) in the U-937 cell line.[132] Chatterjee et al.[133,134] reported that 5 μM Hg toxicity drives autophagy following ATG5-ATG12-LC3B covalent-conjugation pathway modulation by Keap1-p62, ERK-p38, and DRAM-p53 regulator proteins through ubiquitination in rat hepatocytes. In addition, autophagy monitors cell fate in response to Hg, where Fas-associated death domain has been found to recruit one of the most important executioners of programmed cell death, caspase-8, to autophagosomes through interactions with ATG5[134] (Figure 7).

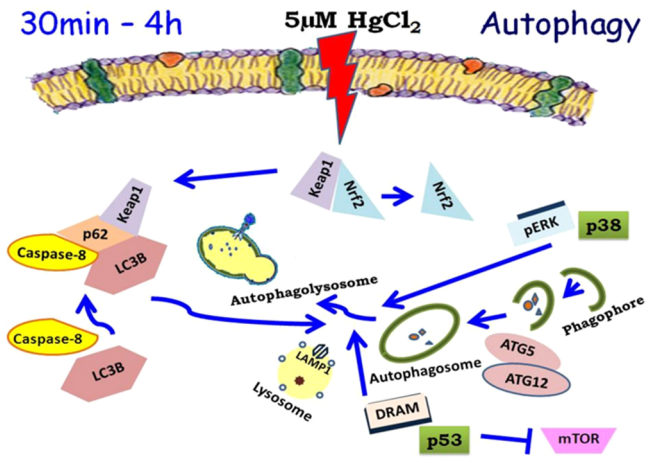

Figure 7. Hg insult results in autophagic cell death in rat hepatocytes.

IRON (Fe)

Fe, an essential nutrient, is vital to the energy generation process of cells. Fe has a knack for switching back and forth between two ionic states: a reduced state as ferrous iron and an oxidized state as ferric iron. Due to these different ionic states, iron can serve as a cofactor in several enzymes involved in oxidation−reduction reactions. Fe is a vital element in nearly all organisms, and iron overload has become one of the key underlying factors in the pathogenesis of neurodegenerative diseases.[135] Accumulation of Fe in the brain is a hallmark of hemorrhagic stroke and several neurodegenerative disorders.[136] Fe overload has been reported to induce brain injury through necrotic and apoptotic mechanisms. Fe^{2+}-mediated toxicity is aggravated by increased oxidative stress and DNA damage. Curiously, Fe^{2+}-mediated cell death does not always appear to involve apoptosis. Instead, the phenomenon seems to occur as a result of excessive autophagic activity.[137]

Autophagy in mammalian cells is one of the cytoprotective measures against various metabolic toxicity or organelle

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damage.[138] Chew et al.[137] revealed that three key players, Fe overload, excessive protein aggregation, and autophagy, are associated in the pathophysiology of programmed cell death. Chen et al.[136] suggest that autophagic cell death may be a mechanism of brain injury in Fe overload disorders. Fe has a crucial role in mitochondrial complexes and in a variety of Fe-containing biomolecules, including enzymes needed for cell proliferation.[139] However, because of its related capacity to induce homolytic cleavage of hydrogen peroxide, forming the aggressive hydroxyl radical (HO^-) or similarly reactive Fe-centered radicals, this transition metal may also be hazardous.[140,141] Thus, cells and organisms need to handle Fe with great care. Most Fe is concealed within biomolecules, where it is not accessible to hydrogen peroxide. Additionally, Fe can be stored for further use in ferritin, a 450 kDa protein that binds up to 4500 atoms of Fe.[142,143] Cells absorb Fe from their environment during proliferation, making tumor cells particularly sensitive to Fe chelators,[144] whereas nondividing cells mainly rely on efficient turnover and reutilization of Fe.[145] Recent data suggest that upregulation of the stress protein ferritin is a rapid adaptive mechanism and that cellular sensitivity to oxidative stress is influenced by ferritin autophagy.[139] As a known cargo receptor, nuclear receptor coactivator 4 (NCOA4) functions with ATG8 protein to recruit a selective cargo−receptor complex into autophagosomes for the autophagic turnover of ferritin (ferritinophagy), which is critical for Fe homeostasis.[146] Cellular iron metabolism is schematically summarized[139] in Figure 8.

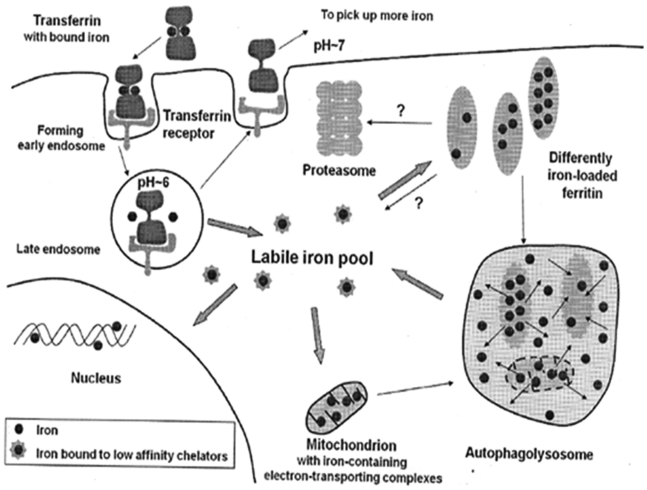

Figure 8. Schematic illustration of cellular iron uptake, intracellular transport, and turnover of iron-containing structures. Reprinted with permission from ref 139. Copyright 2011 Elsevier.

METAL NANOPARTICLES

Nanotechnology, considered one of the key technologies of the 21st century, promises to revolutionize our world.[147] The prefix nano is derived from the Greek word nanos, meaning dwarf. Nanotechnology involves the manipulation and application of engineered particles or systems that have at least one dimension less than 100 nm in length.[148] Objects on the nano scale acquire novel properties and functions because of their much larger surface-to-mass ratio (compared to that of other particles), quantum properties, and ability to absorb and carry other compounds such as probes and proteins. Nanoparticles may be associated with biological molecules such as phospholipids, lipids, lactic acid, dextran, and chitosan or may have more chemical characteristics like those of various polymers, carbon, silica, and metals.[149] Particles generally end intracellularly in endosomes or lysosomes followed by degradation. However, cellular uptake of nanoparticles (~20 nm) can also be possible without the involvement of endocytic mechanisms.[150] Chemical characteristics, such as surface charge, may also determine the fate of nanoparticles in cells. The unique surface properties of nanoparticles in comparison to that of bulk materials impact nanotoxicological studies because their surface is the contact layer with the body. Studies have revealed that the same properties that render the nanoparticles so unique could also be responsible for their potential toxicity.[147] Nanotoxicology[151] encompasses physicochemical determinants, routes of exposure, biodistribution, molecular determinants, genotoxicity, and regulatory aspects (Figure 9).

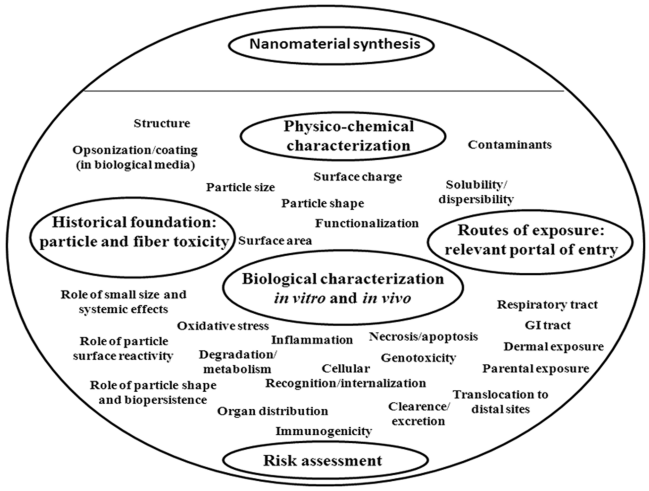

Figure 9. Overview of nanotoxicology. Reprinted with permission from ref 151. Copyright 2010 Elsevier.

Nanomaterials can cross biological membranes and access cells, tissues, and organs that larger-sized particles normally cannot.[152] Nanomaterials can gain access to the bloodstream via inhalation or ingestion.[153,154] At least some nanomaterials can penetrate the skin; even larger microparticles may penetrate skin when it is flexed. Once in the bloodstream, nanomaterials can be transported around the body and be taken up by organs and tissues, including the brain, heart, liver, kidneys, spleen, bone marrow, and nervous system.[153] Nanomaterials are described as triggers of extrinsic and intrinsic apoptotic pathways.[155,156] It has been recently reported that several classes of nanomaterials induce elevated levels of autophagic vacuoles in different cultured animal and human cells as well as in *in vivo* models.[157,158] Such nanomaterials include alumina, europium oxide, gadolinium oxide, gold, iron oxide, manganese, neodymium oxide, palladium, samarium oxide, silica, terbium oxide, titanium dioxide, ytterbium oxide, yttrium oxide nanoparticles, nanoscale carbon black, fullerene, fullerene derivatives, and protein-coated quantum dots.[156] Table 4 depicts the toxicity of nanoparticles, highlighting the area of autophagic cell death. The cytotoxicity of quantum dots (QDs) through activation of the differentiation ability of stem cells may provide size-dependent autophagy signaling[159] and decreases ATP levels by generating ROS to stimulate LC3, a potent marker of autophagy.[160,161] AuNPs (gold nanoparticles) can be taken

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Table 4. Nanoparticles (NPs) and Cellular Responses

compd	selective marker	effects	ref
quantum dots	LC3	size-dependent autophagy signaling in human mesenchymal stem cells	159
Au-NP	P62 and LC3	lysosomal degradation in autophagosome at rat kidney epithelial cells	162
Mn-NP	LC3 and Beclin-1	increased ROS signals autophagy and apoptosis in N27 dopaminergic neuronal cells	163
α alumina-NP	LC3	introduction of antigen to T cells through autophagy in dendritic cells	164
Ag-NW	upregulation of LC3	accumulation of autophagosomes in cell lines of epithelial, endothelial, gastric, and phagocytic origin	26
FeO-NP	LC3, ATG5, ATG12, and AKT signaling	hyperactivation of autophagic cell death in A549 human lung cancer cells	165
ZnO-NP	autophagosome formation	ROS generation activates autophagy in skin cells	166
Cd-quantum dots	LC3	ROS generation decreases ATP levels and increases LC3 to induce autophagy in mouse renal adenocarcinoma cell lines	160
Cd-quantum dots	LC3	combination of Cd ions and CdTe/CdS/ZnS mimics the toxic effect of CdTe, suggesting autophagy in PC12 cells	161
ZnO-NP	MAP-LC3-II, Beclin-1, Akt, PI3K, and mTOR	increased numbers of autophagosomes later supports apoptosis in macrophages	168

into cells through endocytosis in a size-dependent manner. Ma et al.[162] reported that the internalized AuNPs eventually accumulate in lysosomes and cause impairment of lysosome degradation capacity through alkalinization of lysosomal pH, consequently inducing autophagosome accumulation and processing of LC3. Degradation of the autophagy substrate p62 is blocked in AuNP-treated cells, indicating autophagosome accumulation via the blockade of autophagy flux. Exposure to 25−400 μg/mL Mn NPs (Mn nanoparticles) significantly increased ROS in N27 dopaminergic neuronal cells, which resulted in neurotoxic effects by activating apoptotic and autophagy signaling pathways through altering caspase-mediated proteolytic cleavage of proapoptotic protein kinase Cδ (PKCδ) as well as Beclin-1 and LC3, respectively, in a time- and dose-dependent manner.[163] Li et al.[164] demonstrated that α-Al_2O_3 nanoparticles delivered antigens to autophagosomes in dendritic cells, presenting the antigens to T cells through autophagy. A low level of cytotoxicity of AgNW (silver nanowire) was dependent on cell type, nanowire length, dose, and incubation time, which induced autophagosome accumulation together with an upregulation of the autophagy marker protein LC3.[26] Iron oxide NPs selectively induced hyperactivation of autophagic cell death[165] in cancer cells (A549) by generation of ROS through involvement of classical mTOR signaling. Therefore, iron oxide NPs bear potential for applications in biomedicine as a tumor therapy specifically by inducing the autophagy-mediated cell death of cancer cells. On analyzing the data, it is abundantly clear that nanomaterials may induce autophagy via an oxidative stress mechanism, such as accumulation of damaged proteins and subsequent endoplasmic reticulum or mitochondrial stress.[166,167] ZnO-NP (zinc oxide nanoparticles)-induced ROS leads to normal skin cell death through autophagic vacuole accumulation and mitochondrial damage caused by diminished mitochondrial membrane potential and adenosine-5′-triphosphate (ATP) production.[166] Roy et al.[168] recently found that ZnO NPs induced ROS generation by depleting antioxidant enzymes and increasing lipid peroxidation and protein carbonyl content in macrophages. ZnO NPs increased the number of autophagosomes and autophagy marker proteins (microtubule-associated protein 1 light chain 3-isoform II (MAP-LC3-II) and Beclin-1 in macrophages after 30 min to 24 h of treatment in which phosphorylated Akt, PI3K, and mTOR were significantly decreased. In addition, inhibition of LC3-II by siRNA-dependent knockdown attenuated the cleavage of caspase-3, which demonstrated that autophagy supports apoptosis on exposure to ZnO NPs, as addressed by Roy et al.[168] The increase in autophagic vacuoles by nanomaterials may be an adaptive cellular response.[156] In fact, nanoparticles are commonly observed within the autophagosome compartment, suggesting that activation of autophagy is a targeted exertion to sequester and degrade these materials by entering into the cytoplasm.[169] Exposure to airborne pollution has been associated with Alzheimer's and Parkinson's diseases, whereas nanoparticles are the primary particle and surface area components of pollution-derived particulates. Stern and Johnson[158] have recently postulated a relationship between nanoparticle-induced autophagy dysfunction and pollution-associated neurodegradation.

COMBINED STRATEGIES OF CELLS

Interorganelle crosstalk involves several molecular switches within the signaling network[170] (Figure 10). The functional

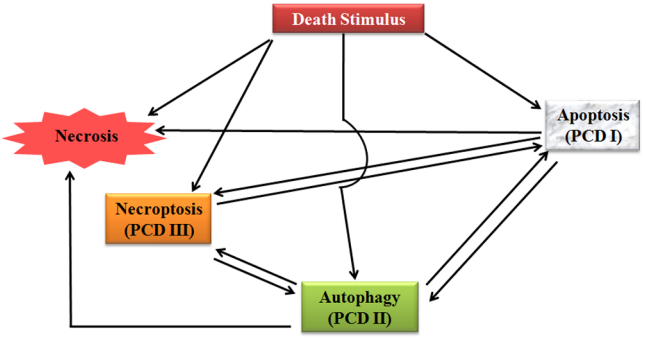

Figure 10. Crosstalk among different forms of cell death.

relationship between apoptosis (self-killing) and autophagy (self-eating) is the switching of cells between these two cytotoxic responses in a mutually exclusive manner, sharing common pathways that either link or polarize cellular responses.[63] Autophagic cell death serves a cytoprotective role in physiologically relevant conditions, which is mediated in many circumstances by negative modulation of apoptosis. On the other hand, apoptotic signaling may inhibit autophagy. The mechanism mediating the complex counter-regulation of apoptosis and autophagy are not yet fully understood, although the crosstalk includes interactions between autophagic marker proteins (Beclin-1, ATG5) and apoptotic factors (Bcl-2/Bcl-xL,

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caspases, calpain).[171] Autophagy and apoptosis, therefore, may be considered as an alternative and/or combined strategy employed by cells exposed to toxic concentrations of metals.[28] Sometimes, low concentrations of a metal preliminarily induces autophagy in a cell, which may then undergo apoptosis after a long duration of metal exposure.[27,172] Nevertheless, apoptosis also comes first as a cellular defense mechanism, which may proceed with autophagy in metal exposure. For example, aluminum (Al) significantly increases rat astrocyte apoptosis and autophagy levels in a dose-dependent manner.[173] At a low dose, Al (400 μM) mediated upregulation of autophagy-related protein is markedly higher, whereas at a high dose of Al (1600 μM), autophagy and apoptosis are both activated simultaneously. Although Zn lethality depends on autophagic proteins, autophagy genes have sometimes been found to participate in Zn-induced necrotic cell death.[174] Moreover, when copper oxide (CuO) coalesces with nanoparticles, it induces autophagy as a survival strategy in MCF7 cells, whereas inhibition of autophagy drives the MCF7 cells toward apoptosis.[27]

CONCLUSIONS

Metals and metalloids represent toxicants that are hazardous to human and environmental health. Different physicochemical parameters of metals may aggravate toxicity in the biota. Although toxicity often deals with cell death by PCDI and necrosis, recent reports suggest that PCDII can also play a critical role as a survival factor and/or death signal when toxicity is exerted by environmental contaminants. PCD II/autophagy has gained the attention of several researchers seeking to analyze the pathogenetic mechanisms of human diseases. Autophagy is effective in limiting inflammation by necrosis, inducing apoptosis, preventing/inducing tumorigenesis, and behaving as a key modulator of cellular senescence. The study of autophagy is, therefore, pivotal in the development of new approaches for toxicological studies. The present review demonstrates that toxic concentrations of metals/metalloids and nanoparticles provoke cell death either by PCDII and PCDI or by a combination of both pathways. ATG8/LC3 plays a vital role in stimulating autophagy in response to metal toxicity. MAPK, p62, p38, AMPK, and DAPK are the inducers of autophagy initiation, whereas PI3K−AKT−mTOR may inhibit autophagy as a component of cellular homeostasis. ATG6/Beclin-1 and BCL-2 are the adapter proteins that modulate PCDII and PCDI in response to metal toxicity. Interestingly, low concentrations of arsenic ($NaAsO_2$) and certain sizes of Au-NPs can stimulate the autophagy−ubiquitination link in different model systems (human lymphoblastoid cell lines and rat kidney epithelial cells). Adaptation to metal stress is therefore much more critical throughout biological evolution. The availability of novel technologies and animal models to study autophagy will progressively reveal the convoluted autophagic pathways in different types of metal-induced cytotoxicity.

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ABBREVIATIONS

COT, Committee on Toxicity; PCD, programmed cell death; MOMP, mitochondrial outer membrane permeabilization; TNFR, tumor necrosis factor receptor; RIPK, receptor-interacting protein kinase; CMA, chaperone mediated autophagy; LAMPs, lysosome-associated membrane proteins; LC3B, light chain 3B; ATG, autophagy; DRAM, damage-regulated autophagy modulator; TOR, target of rapamycin; WHO, World Health Organization; BNIP3, Bcl-2/adenovirus E1B 19 kDa-interacting protein 3; HUVECs, human umbilical vein endothelial cells; bFGF, basic fibroblast growth factor; LKB1-AMPK, liver kinase B1-adenosine monophosphate kinase; PCT, proximal convoluted tubule; MN-PCEs, micronucleated polychromatic erythrocytes; NCOA4, nuclear receptor coactivator 4; QDs, quantum dots; PKCδ, protein kinase Cδ; MAP, microtubule-associated protein; DAPK, death-associated protein kinase

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