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Mini review

Cell apoptosis induced by carcinogenic metals

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Abstract

Well-documented evidence suggests that environmental and occupational exposure of toxic metals or metal-containing compounds can cause a number of human diseases, including inflammation and cancer, through DNA damage, protein modifications, or lipid peroxidation. This mini-review addresses the mechanisms of cell death induced by some carcinogenic metals, including arsenic (III), chromium (VI) and vanadium (V). A possible contribution of reactive oxygen species to metal-induced cell death is also discussed. (Mol Cell Biochem **222**: 183–188, 2001)

Key words: apoptosis, metal, ROS, DNA damage, carcinogenesis

Apoptotic pathways

Programmed cell death or apoptosis is a process in which cell death is initiated and completed in an orderly fashion through the activation of various apoptotic pathways [1, 2]. During the last decade, there has been overwhelming interest in apoptosis and elucidation of mechanisms controlling this process. Apoptosis is an essential process required for development, morphogenesis, immune regulation, tissue remodeling, and some pathological reactions. Most apoptotic cells are characterized by unique morphological features, such as membrane blebbing, cell shrinking, cytosolic and nuclear condensation, and breakdown of chromosomal DNA. Depending on the use of different initiating caspases, signal-induced apoptosis can be roughly divided into two categories: receptor-mediated 'extrinsic' apoptosis and mitochondria-mediated 'intrinsic' apoptosis [1, 2]. Whereas procaspase 8 is activated by receptors for Fas ligand and TNF through the recruitment of intracellular death domain-containing proteins, procaspase 9 is initiated by cytochrome c released from damaged mitochondria (Fig. 1). Both activated caspase 8 and caspase 9 use the same executive caspases, mainly caspase 3, to complete an apoptotic process. All caspases are expressed as inactive precursors and are activated by cleavage at specific peptide bonds. There are a number of cellular proteins which act as roadblocks in the activation cascade of caspases. These proteins act either by counteracting the effect of caspases, such as in the case of cIAP1, cIAP2 and XIAP, or stabilizing the outer membrane of mitochondria, such as in the case of Bcl-x and Bcl-2 [3, 4]. The apoptosis mediated by mitochondria may be more important and relevant in metal-induced cell death.

ROS in apoptosis

Under normal conditions, mitochondria may be the main source for the generation of basic and homeostatic reactive oxygen species (ROS) through the chain reaction of electron transport. Paradoxically, mitochondria are also very vulnerable to the adverse effects of excessive ROS generation. Excessive ROS that can not be neutralized by the limited reducing capacity of the mitochondria and cytosol not only increase mitochondrial membrane permeability, but also diffuse into the mitochondrial matrix to damage the respiratory chain, and consequently amplify the generation of ROS further due to the less efficient transferring of electrons to oxygen (O_2) [5]. Mitochondria with increased membrane permeability release several apoptosis-promoting factors including cytochrome c, apoptosis inducing factor (AIF), and Diablo/Smac [6–9]. Cytochrome c forms a complex with a cytosolic protein, named apoptosis protease-activating factor-1 (Apaf-1), to activate caspase-9. AIF is a flavoprotein with homology to plant ascorbate reductases and bacterial NADH oxidases [9]. After its release from the intermembrane space of damaged

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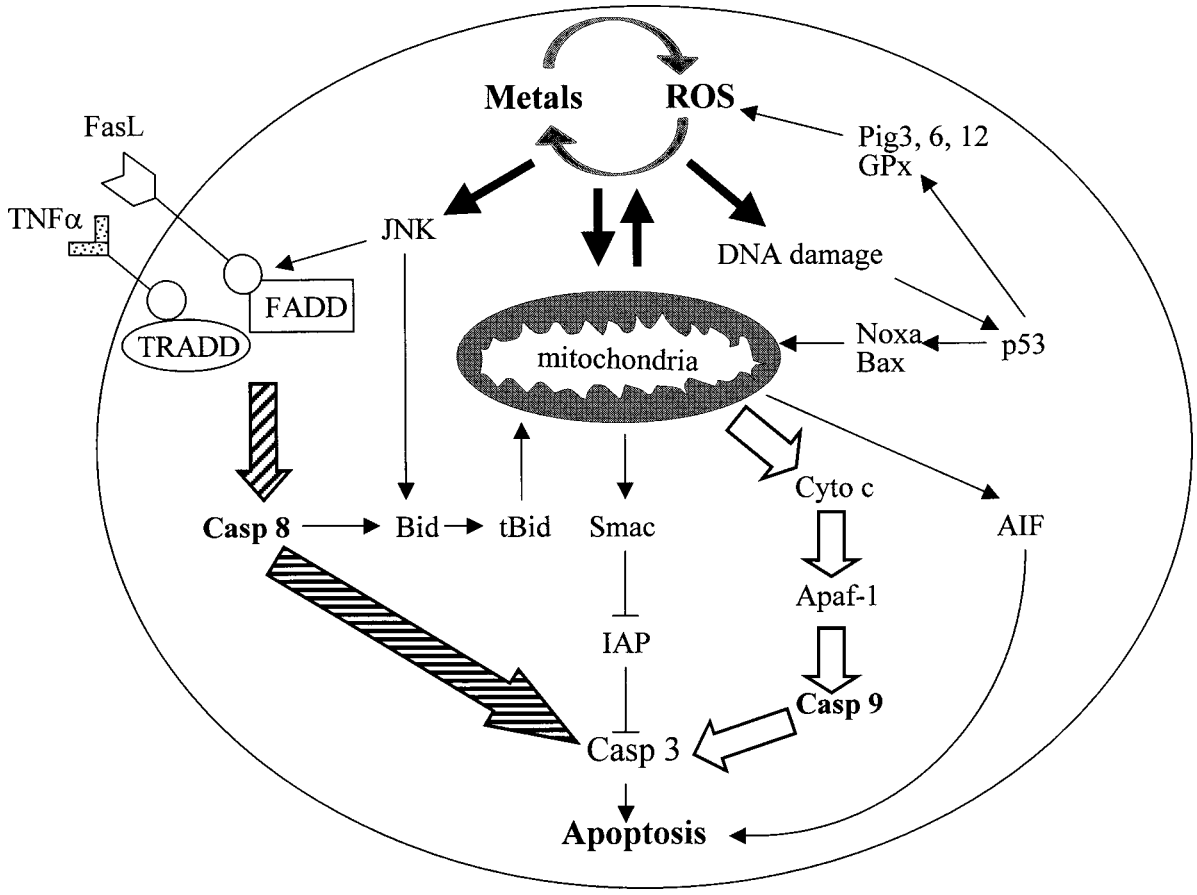

Fig. 1. Apoptosis signaling pathways mediated by metals. Metals or ROS generated by metals directly initiate the intrinsic apoptotic pathway at the mitochondria (unfilled block arrows). Metals or ROS can also indirectly initiate this pathway by activating p53 as a result of metal- or ROS-induced DNA damage. The activation of p53 triggers the expression of Bax and Noxa, two apoptotic proteins which induce an increase in mitochondrial permeability. Cytochrome c is released into the cytosol from damaged mitochondria and binds to Apaf1, resulting in the activation of caspase-9. Damaged mitochondria can also release AIF, which induces apoptosis in a caspase-independent manner, and Smac which antagonizes the antiapoptotic effect of IAP proteins. Metals or ROS may also potentiate the extrinsic apoptotic pathway (shadow block arrows) through MAP kinase-, especially, JNK-mediated induction of FasL or Fas expression. Recruitment of death domain containing proteins, such as FADD and TRADD to these receptor complexes, activates caspase-8. The activation of caspase-8 and caspase-9 leads to the activation of the effector caspases, including caspase-3 and caspase-7.

mitochondria, AIF exhibits a caspase-independent apoptotic function. Recently, another mitochondrial intermembrane protein, Smac/Diablo, has been identified [7, 8]. Once released from the mitochondria to cytoplasm, Smac/Diablo binds to and antagonizes cIAP1 and cIAP2, allowing the activation of caspases.

Mechanisms of metal-induced apoptosis

Cr (VI)

Since apoptosis is an important process influencing the malignant transformation of cells, the regulation of cell apoptosis may be critical in metal-induced carcinogenesis. The earliest report revealing apoptotic induction by chromium (VI) (Cr (VI)) was provided by Blankenship *et al.* [10, 11], who demonstrated that cells treated with Cr (VI) exhibited apoptotic features. Evidence provided by Singh *et al.* [12] indicated that depending on the rate, magnitude and spectrum of genotoxicity and mitochondrial damage, cells exposed to Cr (VI) are fated to undergo either terminal growth arrest or p53-dependent apoptosis. It has been suggested that Cr (VI) was able to damage DNA by forming DNA-Cr-DNA cross links [13]. Cr (VI) itself was found to be unable to react with macromolecules, such as DNA, RNA, proteins and lipids. Instead, Cr (V) and Cr (III), intermediates of Cr (VI) reduction form covalent interactions with DNA and other macromolecules [12], a process which activates DNA-dependent protein kinases (DNA-PK) and induces subsequent p53 activation and cell apoptosis. The DNA damaging effect of Cr (VI) might be also through ROS generated by Cr (VI) reduc-

tion. Supporting this notion is the evidence by our laboratory showing that antioxidants, including pyrroline dithiocarbamate (PDTC) and aspirin, protected DNA from damage and p53 from activation induced by Cr (VI) [14, 15].

Activation of p53 tumor suppressor protein is considered to be one of the critical steps in the induction of apoptosis by Cr (VI) [10, 15, 16], although p53-independent apoptosis induced by Cr (VI) has also been reported [16]. Several mechanisms are involved in the Cr (VI)-induced p53 activation. First, direct DNA damage by Cr (VI) or ROS generated during cellular Cr (VI) reduction activates upstream kinases, including DNA-PK, ATM, ATR and others, for p53 phosphorylation, stabilization/activation [17, 18]. Second, Cr (VI)-induced MAP kinase activation, especially, c-Jun-N-terminal kinase and p38, which is independent of DNA damage, modulates the activation and activity of p53 [19–21]. Third, the p53 protein contains several redox-sensitive cysteines critical for the DNA binding activity of p53 [22, 23]. As a transcription factor, p53 is able to up-regulate the expression of genes involved in either ROS production or metabolism, including quinone oxidoreductase (Pig3), proline oxidase (Pig6) homologues, glutathione transferase (Pig12), and glutathione peroxidase (GPx) [24]. Moreover, p53 also activates the expression of several genes that directly control or regulate the process of apoptosis. These genes include Bax, Fas, Fas ligand, IGF-BP3, PAG608 [25], ei24 (Pig8) [26], and Noxa [27]. The strongest evidence supporting the notion that p53 is involved in Cr (VI)-induced apoptosis was demonstrated through the use of fibroblasts from both wild type mice and p53-deficient mice [16]. Cr (VI) induced a 2- to 3-fold greater increase in apoptosis in wild type fibroblasts than in p53-deficient fibroblasts. Obviously, the activation of p53 by Cr (VI) will lead to the increased expression of p53-targeted apoptotic genes, such as Bax, Fas, Fas ligand, ei24, and Noxa. Several recent studies from our group and others have highlighted the importance of H_2O_2 and hydroxyl radical in Cr (VI)-induced p53 activation and cell death [15, 28–30]. As discussed earlier, excessive ROS are toxic to the mitochondria. Therefore, mitochondrial damage, resulting either from the direct effect of Cr (VI) or indirect effect mediated by Cr (VI)-induced activation of p53, appears to be a critical step in Cr (VI)-induced cell apoptosis. Indeed, studies by Carlisle *et al.* [31] indicated that cyclosporin A, which prevents the pre-apoptotic release of cytochrome c from mitochondria, blocked Cr (VI)-induced apoptosis and increased the survival rate of replication-competent but genetically damaged cells.

As (III)

Chronic exposure to As (III), another important toxic submetal widely found both in the environment and in occupational settings, can cause a number of human diseases, especially cancer [32, 33]. The apoptotic effect of As (III) was originally observed in human acute promyelocytic leukemia cells [34]. Later studies suggested that As (III) might be a direct toxic agent for mitochondria in this type of cell. As (III) (1 μM) induced a condensation of the mitochondrial matrix and caused disruption of the mitochondrial transmembrane potential leading to the release of cytochrome c and AIF [35, 36]. As (III) can also induce apoptosis in other types of cells. Although the mechanism remains unclear, it has been consistently shown that p53 activation may not be involved in As (III)-induced cell apoptosis under many circumstances [34, 37]. Based on studies using several antioxidants or ROS scavengers in Chinese hamster ovary cells, two reports have suggested an oxidative stress model for As (III)-induced apoptosis [38, 39]. In mouse skin epidermal cells, the apoptosis induced by arsenic is possibly through protein kinase C and MAP kinases pathways [40]. MAP kinases, especially c-Jun-N-terminal kinase (JNK) and p38, have been proposed as important mediators in signal-induced apoptosis [40]. Both JNK- and p38-mediated cell death may be due to the rapid induction of FasL as observed in Jurkat cells [41], microglial cells [42] and hepatoma cells [43]. In addition, it has been demonstrated that JNK is also required for UV-induced caspase-independent proteolytic activation of Bid, a pro-apoptotic BH3-only member of the Bcl2 group which translocates to the mitochondria after activation and induces cytochrome c release [44, 45]. Our recent studies have found that JNK activation is required for arsenic-induced gene expression of GADD45, a cell cycle checkpoint protein which arrests cells in G2/M phase transition, and for the possible induction of apoptosis [46].

V (V)

Emerging evidence suggests that various forms of vanadium or vanadium containing particles from environmental and occupational sources are able to trigger or potentiate cell apoptosis. It was believed that among the various oxidation states of vanadium, the pentavalent form, vanadium (V) (V (V)), one of the most common forms of vanadium found in nature and in tissues or cells after exposure, is the most toxic [47]. Short-term exposure to V (V) causes local irritation to the eyes and upper respiratory tract rather than systemic toxicity. There is limited information concerning the possible neoplastic transforming activity of V (V) following long-term exposure [47–49]. V (V) might cause apoptosis in some types of cells, but may be anti-apoptotic in other types of cells. In lymphoid cell lines, V (V) treatment caused the activation of caspases 3, 8 and 9, the induction of mitochondrial permeability transition, the release of cytochrome c and DNA fragmentation. Further studies suggested that there was no involvement of protein tyrosine kinase $p56^{lck}$ or phosphatase CD45 in this

apoptotic effect of V (V) in lymphoid cell lines [50]. V (V)-induced apoptosis in mouse epidermal JB6 cells was thought to involve the generation of ROS and the activation of p53. Pre-treatment of the cells with antioxidants, including NAC and catalase, blunted the apoptotic effects of V (V) on these cells [51]. In other types of cells, V (V) might elicit an anti-apoptotic effect through the activation of Akt/PKB or the inactivation of tyrosine phosphatase. Support for this comes from the work of Chin *et al.* [52], who showed that V (V) protected malignant glioma cells from apoptosis. The addition of wortmannin, a phosphatidylinositol 3-kinase (PI3K) inhibitor, abolished the protective effect of V (V) on these cells, indicating that PI3K and Atk/PKB may be responsible for the V (V)-mediated protective effect. In intestinal epithelial cells, studies by Scheving *et al.* [53] demonstrated a correlation between a decline in protein phosphatase activity and inhibition of cell apoptosis in V (V)-treated cells. It is not clear why V (V) is anti-apoptotic in some types of cells, but is pro-apoptotic in other types of cells. The explanation for this may be the use of different forms of V (V). It was noted that some studies used sodium vanadate, while others used peroxovanadate or pervanadate. The later form is a reactive product of V (V) in the presence of H_2O_2. Many reports indicated that sodium vanadate and peroxovanade exhibit different effects on the induction of cell apoptosis, inactivation of protein phosphatase and generation of ROS. An additional explanation for the contradictory effects of V (V) on cell apoptosis may be due to the use of different doses of V (V). It has been frequently observed that lower concentrations of V (V) protect cells from signal-induced apoptosis, whereas higher concentrations of V (V) are either cytotoxic or pro-apoptotic. The final explanation for the varying effects of V (V) on cell apoptosis is the types of cells used in each experiment. It is well-known that cells, originating from different tissues, exhibit different capacities for ROS generation and respond differently to metal or exogenous ROS stimulation.

Summary

Evidence indicates that many toxic metals, such as Cr (VI), As (III) and V (V), are carcinogenic. It is difficult to reconcile the pro-apoptotic effects of metals with the observed carcinogenic potential of metals in humans. Many studies addressed the pro-apoptotic effect of metals on selected cell types, but failed to delineate how this process would contribute to cell transformation and carcinogenesis. Cell apoptosis was originally viewed as a normal physiologic process by which correct functional cellular population dynamics are maintained through the apoptotic loss of cell populations carrying abnormal genetic information [4]. It is known that metals under certain circumstances are apoptotic, but it is not known whether this apoptotic process induced by metals is a perfect or an imperfect process. An imperfect apoptotic process might result in the escape of cells that would be potentially carcinogenic. Thus, increased apoptosis under the conditions of chronic metal exposure could possibly coincide with an increase in the number of cells carrying damaged but replication-competent genetic information. Conversely, chronic and lower dose exposure of cells or tissues to metals may perturb or even inhibit appropriate apoptosis, leading to the accumulation of cells with carcinogenic potential. Among metals discussed above, it is likely that Cr (VI) is not only the most toxic metal, but also the most carcinogenic metal toward cells [13, 21]. The genotoxicity of Cr (VI), resulting from either direct DNA binding of Cr (VI) derivatives or generation of ROS during cellular Cr (VI) reduction, may create a cell sub-population that may be predisposed to mutagenesis and capable of averting apoptosis. Considering the fact that Cr (VI)-induced apoptosis is p53 dependent under many circumstances, mutations in the p53 gene would undoubtedly facilitate the development of apoptosis resistant and potentially carcinogenic cells. Moreover, although Cr (VI) itself and ROS generated from Cr (VI) reduction intracellularly are able to activate upstream signals leading to the accumulation and activation of p53 in the early phase of cells in response to Cr (VI), neither Cr (VI) nor ROS can persistently activate the function of p53 protein in the later phase. Instead, due to the oxidation of cysteine residues in the DNA binding domain of p53 protein [22, 23], Cr (VI) may actually inactivate the tumor suppressing function of p53 by impairing the DNA binding activity of p53.

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