Calcium Homeostasis Disruption – a Bridge Connecting Cadmium-Induced Apoptosis, Autophagy and Tumorigenesis

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Summary
Calcium and cadmium are divalent metals and have similar chemical properties. Both can enter cells through, albeit different, channels, or through protein-dependent permeation. However, cadmium disturbs the calcium homeostasis by inhibiting calcium channels and/or related proteins. Cadmium can also alter membrane phospholipid concentrations, and so induce a calcium homeostasis disorder. The altered calcium homeostasis induced by cadmium results in cell apoptosis, autophagy or tumorigenesis. In this review, calcium homeostasis disruption is summarized as a bridge connecting cadmium-induced apoptosis, autophagy, and tumorigenesis.

Introduction
Cadmium is a ubiquitous environmental pollutant and presents everywhere: in air, water, soil and foods. Cadmium enters the human body mainly through consumption of contaminated food, smoking and inhalation by workers in metal industries [1]. Cadmium is a cumulative toxicant that the body has difficulty in eliminating. In 1992, the provisional tolerable weekly intake (PTWI) of cadmium was taken as 7 μg/kg body weight. Using the benchmark dose-derived urinary cadmium threshold, which is accepted as a measure of the body burden and the cumulative amount in the kidneys, the TWI for cadmium was 2.5 μg/kg body weight [2]. It has been reported that the normal cadmium level in blood is less than 5.0 ng/ml, and that acute toxicity will occur when the blood cadmium level exceeds 50 ng/ml. Since the 1950s, along with an understanding of development, the questions of cadmium pollution, prevention and control have become urgent issues in various countries. Because of the serious influence of cadmium pollution on human bodily health, the study of cadmium toxicity has attracted much interest [3–5]. A growing amount of evidence suggests that cadmium may cause cell damage by interfering with the homeostasis of essential elements such as zinc, copper, magnesium, iron and calcium. Here we review progress in studies on cadmium-induced calcium homeostasis disruption.

Mechanism of Cadmium Transportation
Experimental results indicate that cadmium is transported across the membrane system either through channels or by protein transporters used by some other essential metals. Cadmium may enter the apical membrane of intestinal epithelial cells through the divalent metal transporter 1 (DMT1), also known as divalent cation transporter 1 (DCT1). Some zinc transporters such as ZIP1, ZIP2, ZIP8, and ZIP14 can also transport cadmium into cells [6, 7]. In addition, cadmium forms cadmium-protein complexes with several proteins, such as multidrug resistant protein 1 (MRP1), cystic fibrosis transmembrane conductance regulator (CFTR), metallothionein (MT), megalin, and cubulin. These complexes enter cells via receptor-mediated endocytosis. In electrogenic cells, cadmium permeates into cells through L- or N-type voltage-dependent calcium channels (VDCCs) [8]. In non-electrogenic cells, cadmium permeates into cells through the store-operated Ca2+ channels (SOCs) [9].
Cadmium can also permeate the cells through the transient receptor potential channel vanilloid 6 (hTRPV6) [10], a calcium-selective channel. Kovacs et al. found that a similar phenomenon also occurs in prostate cancer LnCaP cells. TRP ankyrin1 (TRPA1), another TRP family protein, is also a potential channel for cadmium [11].

**Cadmium Toxicity and Calcium Homeostasis**

*Mechanisms of Cadmium Uptake Disturb Calcium Homeostasis*

Cadmium has similar biochemical properties to calcium. Cadmium can cause disequilibrium of intracellular Ca²⁺ homeostasis, and disturb calcium-mediated signaling through several mechanisms.

Cadmium can inhibit the plasma membrane Ca²⁺-ATPase (PMCA), blocking Ca²⁺ efflux [12], and prevent calcium entering the endoplasmic reticulum and Golgi apparatus by inhibiting sarcoplasmic/endoplasmic reticulum Ca²⁺-ATPase (SERCA) or secretory pathway Ca²⁺-ATPase (SPCA) activities [13, 14]. Cadmium can increase the level of intracellular Ca²⁺; the increased Ca²⁺ induced by Cd²⁺ is released from the intracellular storage and/or from the extracellular space [15, 16].

Cadmium can also change the content of phosphatidylinositol (PI) in cell membrane lipids. Phospholipase C (PLC) can be activated through the interaction of G protein-coupled receptor with cadmium [17], and the activation of PLC can cause PI breakdown and the generation of inositol triphosphate (IP₃). Cadmium can thus induce an increase in IP₃, which then activates IP₃ receptors (IP3Rs) on the endoplasmic reticulum (ER) membrane, causing Ca²⁺ release from the ER into cytoplasm. Similarly, Ca²⁺ also may be released from ER through ryanodine receptors (RyRs), causing alterations in intracellular Ca²⁺ homeostasis [18], indicating that there is a close relationship between Ca²⁺ elevation and IP₃.

In cadmium-treated mouse, the lipid components can be altered markedly, with phosphatidylcholine (PC) being increased and phosphatidylethanolamine (PE) decreased [19]. The increasing ratio of PC and PE or increasing PC content in a membrane can inhibit the calcium transport activity of SERCA. With the inhibited protein expressions of SERCA2A and SERCA2B, the release process of Ca²⁺ stored in the ER is inhibited [20]. These results indicate that cadmium not only directly inhibits calcium channels or proteins, and thus influences calcium homeostasis, but also affects calcium homeostasis in an indirect way through cadmium-induced lipid metabolism disturbance.

*Mechanisms of Ca²⁺ Overload-Mediated Cell Apoptosis Induced by Cadmium*

Cadmium-induced Ca²⁺ overload induces cell apoptosis in various cells in several ways. In neuronal (PC12 and SH-SY5Y) cells, cadmium-induced intracellular Ca²⁺ elevation, leading to cell apoptosis, is due to activation of the MAPK and mTOR signaling pathways through stimulation of phosphorylation of JNK, Erk1/2, p38 MAPK, mTOR and calcium/calmodulin (CaM)-dependent protein kinase II (CaMKII) [16, 21–23]. The elevation of intracellular Ca²⁺ could activate CaM. CaM-mediated activation of MAPK and mTOR pathway plays an important role in Ca²⁺ homeostasis-mediated apoptosis induced by cadmium [16, 23].

By contrast, in mouse skin epidermal cells, JB6 Cl41 cells, cadmium disrupts intracellular Ca²⁺ homeostasis and leads to apoptosis through JNK- and p53-mediated signaling pathways. JNK-mediated pathways can activate the growth arrest and DNA damage inducible protein 45α (GADD45α) and eventually cause cell apoptosis [24].

Bcl-2 can prevent intracellular Ca²⁺ elevation but Bax promotes it, and both play an important role in apoptosis. Bcl-2 prevents Ca²⁺ release from the ER by binding to the BH4 domain in IP3R and RyR, and inhibiting their activities [25]. In cadmium-treated cerebral cortical neurons cells, cadmium decreases Bcl-2 and increases Bax, promoting Ca²⁺ overload and triggering cell apoptosis [26].

Increasing the intracellular Ca²⁺ level by cadmium could also activate calpain, an enzyme belonging to the family of Ca²⁺-dependent cysteine proteases, leading to apoptosis in HEK293 cells. This calpain-mediated apoptosis can be inhibited by PLC inhibitors [17].

The overload of intracellular calcium induced by cadmium can depolarize the mitochondrial membrane potential, and lead to apoptosis [27]. There are several different mechanisms in the various cell types. In hepatic cell Hep3B, cadmium-induced intracellular calcium elevation triggers nuclear translocation of endonuclease G and the apoptosis-inducing factor (AIF). This then leads to mitochondrial membrane potential depolarization and cell apoptosis [28]. Interestingly, AIF may be involved in anticancer drugs or environmental contaminants including cadmium in cells [29, 30].

Cadmium exposure can elevate intracellular calcium and activate CaM, leading to mitochondrial collapse and the release of cytochrome c into cytoplasm. The cytochrome binds to caspase-9 to form apoptosis bodies, which activate caspase-3 to cause cellular apoptosis in rat primary osteoblasts [31]. However, unlike the case in neuronal cells, in skin epidermal cells, dysfunction of mitochondrial cytochrome c and AIF release from mitochondria, but does not activate the caspases-9, -3, and -7 [24]. In rat hepatocytes, Drp1 has a pivotal role in cadmium-induced cell apoptosis [32]. The induction of Drp1 significantly enhances the release of cytochrome c, caspase-3 activation, and ROS generation, and decreases the mitochondrial transmembrane potential, eventually causing apoptosis in pancreatic β-cells [33] (fig. 1).

*Mechanisms of Ca²⁺ Homeostasis-Mediated Cell Autophagy Induced by Cadmium*

Many studies report that low-level cadmium exposure can cause cell autophagy in several types of cells such as PC12 cells, MES-13 mesangial cells, skin epidermal cells, and vascular endothelial cells [27, 34–36]. Low-level cadmium inhibits apoptosis by serum and basic fibroblast growth factor (bFGF) deprivation and promotes autophagy [35]. One mechanism of cadmium-induced autophagy is that of cadmium-induced autophagy mediated by Ca²⁺-extracellular signal-regulate kinase (ERK) signaling; elevation of intracellular Ca²⁺ could activate ERK, causing autophagy [27].
Calcium Homeostasis Disorder-Mediated Cancers Induced by Cadmium

Cadmium is reported as a risk factor for many types of cancers, and is classified as a carcinogen by the International Agency for Research on Cancer (IARC). According to some studies, cadmium exposure, either occupational or environmental, could elevate the risk for kidney [38], prostate [39, 40], breast [41–44], bladder [45], testis [39], endometrial [41], ovarian [41], gastrointestinal [46], and lung [47–49] cancers, or without a tropism for specific organs. However, the mechanism of how cadmium causes cancers is still unclear.

Calcium signaling is a key regulator of important pathways in tumor progression. Studies have been shown that 20 μM Cd2+ could stimulate cell proliferation by interference with intracellular calcium through enhancing the level of intracellular calcium. The elevated intracellular calcium induced the overexpression of oncogenes like c-fos, c-jun, and c-myc in BALB/c-3T3 cells. c-fos and c-jun constitute the AP-1 transcription factor, and activate several genes involved in cell growth and division, leading to cell transformation and tumorigenesis [50–54]. c-MYC contributes to the genesis of many human cancers, and there are several reviews on the pathways leading to cancers [55–57]. Elevated intracellular Ca2+ due to a low level cadmium can cause activation of the small guanine nucleotide-binding protein Ras [58], a protein which is related to some human cancers. Cadmium-elevated Ca2+ can stimulate phosphorylation of CaMKII [23]. CaMKII is a multi-functional serine/threonine protein kinase, and has been shown to be involved in processes that cause tumor progression, including cell cycle regulation, apoptosis, differentiation, and cancer cell metastasis [59]. These results suggest one mechanism through which cadmium may cause tumorigenesis is intracellular Ca2+ homeostasis deregulation mediated by activation of downstream oncogenes.

Many scientists believe that cadmium induces tumors mainly as a result of cadmium-induced ROS generation. Cadmium induces calcium overload, leading to ROS generation, and ROS can cause apoptosis and autophagy [33, 37]. Therefore, cadmium-induced calcium overload may play a role in apoptosis, autophagy and tumorigenesis crosstalk.

Summary

This review has demonstrated that cadmium is a potent toxicant, and the mechanisms underlying the way this heavy metal disturbs calcium homeostasis are multifactorial. Cadmium can permeate cells through several channels or with various proteins, and alter calcium homeostasis in a direct or indirect manner, inducing calcium homeostasis-mediated apoptosis. The major mechanisms by which Cd2+ alters Ca2+ homeostasis include inhibiting the plasma membrane Ca2+-ATPase (PMCA), SERCA or SPCA activities and altering the lipid components. The calcium channels or proteins related to calcium transportation could provide therapeutic targets for preventing cadmium-induced cancers.

Cadmium-induced calcium overload can activate the MAPK and mTOR signaling pathways, and promote apoptosis. Additionally, cadmium induces calcium-dependent apoptosis through GADD45-mediated JNK and P53 signaling pathways. However, GADD45 probably does not play a direct role in genotoxicity stress-induced apoptosis.

Cadmium-caused intracellular Ca2+ elevation represents one mechanism triggering cadmium-induced tumors. Cadmium can also change the concentration of phospholipids in the membrane systems, which then affects the cell biological function and cell signal transduction through altering calcium distribution. More in-depth studies are needed to establish the relationship between cadmium and calcium homeostasis, and the mechanism of calcium homeostasis disorder-induced apoptosis and tumorigenesis. Future studies will enrich the therapeutic strategies for combating cadmium-induced diseases.

Cadmium-caused intracellular Ca2+ homeostasis disruption can lead to apoptosis, autophagy and tumorigenesis. In some situations, calcium overload can induce both apoptosis and autophagy.
Cadmium-induced calcium overload stimulates phosphorylation of CaMKII, which is involved in tumor progression and cell apoptosis, and can also cause AIF release from mitochondria, which triggers cell apoptosis, representing one mechanism of action of anticancer drugs. The homeostatic relationship among apoptosis, autophagy and tumorigenesis during cadmium treatment presents a very interesting topic in the molecular relevance of the autophagy-apoptosis-tumorigenesis crosstalk.

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