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Review

Mechanisms of cadmium carcinogenesis ☆

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ABSTRACT

Cadmium (Cd), a heavy metal of considerable occupational and environmental concern, has been classified as a human carcinogen by the International Agency for Research on Cancer (IARC). The carcinogenic potential of Cd as well as the mechanisms underlying carcinogenesis following exposure to Cd has been studied using *in vitro* cell culture and *in vivo* animal models. Exposure of cells to Cd results in their transformation. Administration of Cd in animals results in tumors of multiple organs/tissues. Also, a causal relationship has been noticed between exposure to Cd and the incidence of lung cancer in human. It has been demonstrated that Cd induces cancer by multiple mechanisms and the most important among them are aberrant gene expression, inhibition of DNA damage repair, induction of oxidative stress, and inhibition of apoptosis. The available evidence indicates that, perhaps, oxidative stress plays a central role in Cd carcinogenesis because of its involvement in Cd-induced aberrant gene expression, inhibition of DNA damage repair, and apoptosis.

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Introduction

Cadmium (Cd), a toxic transition metal belonging to group IIB of the periodic table is found at concentrations ranging from 0.1 to 1 ppm in the earth's crust (IARC, 1993). Cadmium is ranked 67th in abundance among the 90 naturally occurring elements on earth. Significant quantities of Cd are introduced into the environment both by natural and anthropogenic activities, with anthropogenic activities contributing 3–10 times more Cd to the environment than natural activities. Volcanic activity, fossil fuel combustion, forest fires, and transportation of contaminated soil particles by wind constitute the major natural activities responsible for introducing Cd to the environment. Cadmium has several industrial applications, and the worldwide production of Cd in 2005 was estimated to be 20,000 metric tons. Cd is frequently used in electroplating, pigments, paints (as a stabilizer), welding and Ni-Cd batteries; and it is estimated that workers in certain occupations are exposed to Cd at significantly higher levels than the general public. Similarly, people living in areas contaminated with Cd are exposed to higher amounts of the metal. Food is often reported as a source for human exposure to Cd. Cd is selectively taken up by certain edible plants, and certain food items, such as crab, contains Cd as high as 30–50 ppm (Schwartz and Reis, 2000). In the United States, it is estimated that the average person consumes approximately 30 μg Cd per day through food materials (Schwartz and Reis, 2000). Another major source for human exposure to Cd is cigarette smoke. Tobacco plants selectively accumulate Cd from the soil, and it is fairly well established that the blood level of Cd in smokers is significantly higher than that of non-smokers (Mannino et al., 2004). In the last few decades, a significant decline in the production and use of Cd has been reported, especially in the United States and in countries of the European Union. However, Cd continues to be a major health concern primarily because of its long half-life and its persistence in the environment and in tissues.

The amount of Cd absorbed in the body following its exposure varies depending on the route of entry. Approximately 3–10% of ingested Cd is absorbed from the gastrointestinal system, whereas 50% of inhaled Cd is absorbed (Sahmoun et al., 2005). Upon absorption, Cd is rapidly transported by blood to different organs in the body where its estimated half-life in humans is 15–20 years (Jin et al., 1998). The amount of Cd stored in various organs varies considerably largely due to a small protein, metallothionein (MT), which exhibits high binding affinity to Cd. The organs that store Cd include the liver, kidney, testis, spleen, heart, lungs, thymus, salivary glands, epididymis, and prostate; however, approximately 50% of the Cd found in the body is stored in liver and kidney due to their high MT concentration (Waalkes and Klaassen, 1985).

The potential for Cd to cause toxicity has been demonstrated by the results of numerous experimental and epidemiological studies. In general, exposure of cells to low, micromolar concentrations of Cd results in significant toxicity (Othumpangat et al., 2005; Badisa et al., 2008). Similarly, the potential of Cd exposure to cause toxicity has been established in experimental animals. (Klaassen and Liu, 1998) and in humans (ATSDR, 1999). The target organs for Cd toxicity in

☆ The findings and conclusions in this report are those of the author(s) and do not necessarily represent the views of the National Institute for Occupational Safety and Health.

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animals include the liver (Koyuturk et al., 2007), kidney (Jamall et al., 1989), lungs (Boudreau et al., 1988), testes (Bonda et al., 2004), prostate (Alvarez et al., 2004), heart (Jamall et al., 1989), skeletal system (Blumenthal et al., 1995), nervous system (Minami et al., 2001) and immune system (Shippee et al., 1983). The toxicity of Cd in human subjects is not clearly understood primarily because of confounding factors such as co-exposure to other toxic chemicals and cigarette smoke. However, prolonged human exposure to Cd results in its accumulation in the body leading to diseases mainly affecting lungs and kidneys (IARC, 1993; ATSDR, 1999).

Strong evidence, based on experimental studies, exists to support the carcinogenic potential of Cd. Cell transformation, a routinely employed diagnostic *in vitro* test for the carcinogenic potential of chemicals, has been employed in several studies not only to demonstrate the carcinogenic potential of Cd but also to gain insight regarding the mechanisms potentially underlying Cd carcinogenesis (Abshire et al., 1996; Achanzar et al., 2001; Joseph et al., 2002a; Joseph et al., 2002b; Joseph et al., 2004a). Following various routes of exposure to Cd, experimental animals produce tumors of multiple organs (Waalkes et al., 1989a, 1989b, 1992b; Waalkes and Rehm, 1994; Waalkes et al., 1999a, 1999b, 2000). In addition, injection of Cd containing compounds in animals resulted in local tumors, typically sarcomas, at the site of injections (Waalkes et al., 1989b).

Epidemiological evidence is also available to document the carcinogenic potential of Cd. Although, the evidence is not very strong, cancers of the prostate (Waalkes and Rehm, 1994), kidney (Pesch et al., 2000), and pancreas (Schwartz and Reis, 2000) have been reported in populations who are exposed to Cd. It is, however, worth mentioning that, like any other epidemiological study, the results of studies involving Cd carcinogenesis should be interpreted with caution primarily because of confounding factors such as co-exposure to other toxic chemicals and life style factors, for example, cigarette smoking. Despite such limitations, a causal relationship has been observed between Cd exposure and the occurrence of lung cancer in human and the administration of compounds containing Cd have resulted in tumors of multiple organs/tissues in experimental animals prompting the International Agency for Research on Cancer (IARC) to classify Cd as a human carcinogen (IARC, 1993).

Several excellent reviews describing various aspects of Cd carcinogenesis have been published in the past (Waalkes et al., 1992a; Waalkes, 2000; Waalkes, 2003; Waisberg et al., 2003; Huff et al., 2007). The scope of this review article is limited to the possible mechanisms underlying Cd carcinogenesis. Most of our understanding with respect to the mechanisms of Cd carcinogenesis is derived from studies involving either *in vitro* cell culture or *in vivo* animal models. The major mechanisms involved in Cd carcinogenesis can be broadly categorized into four groups, aberrant gene expression, inhibition of DNA damage repair, inhibition of apoptosis, and induction of oxidative stress, with significant overlap among the groups. In addition, the ability of Cd to cause aberrant DNA methylation (Benbrahim-Tallaa et al., 2007a; Huang et al., 2008), endocrine disruption (Benbrahim-Tallaa et al., 2007b) and cell proliferation (Huang et al., 2008) may assume minor importance with respect to its carcinogenic potential.

Cadmium and aberrant gene expression

Recent developments in gene expression studies, especially those in toxicogenomics, have facilitated the identification of a large number of genes and, consequently, many novel mechanisms that are potentially involved in Cd carcinogenesis. The various genes whose expressions are influenced by Cd exposure and, therefore, may be involved in Cd toxicity and carcinogenesis are discussed under the following five categories: 1. immediate early response genes, 2. stress response genes, 3. transcription factors, 4. translation factors, and 5. miscellaneous genes. In addition, the potential mechanisms underlying Cd-induced differential gene expression are also discussed.

Immediate early response genes (IEGs)

Immediate early response genes (IEGs), a group of proto-oncogenes including, but not limited to, *c-fos*, *c-jun* and *c-myc* undergo early transcriptional activation in response to mitogenic stimuli and have been found overexpressed very often in cells in response to Cd exposure. This overexpression is believed to be responsible for Cd's carcinogenic potential mainly because IEGs are frequently found overexpressed in tumors and in cells undergoing proliferation and differentiation. The Cd-induced over-expression of one or more IEGs is found in a variety of cell lines, including rat L6 myoblasts (Abshire et al., 1996), rat kidney NRK-49 cells (Tang and Enger, 1993), rat and human mesangial cells (Wang and Templeton, 1998), human prostate epithelial cells (Achanzar et al., 2000), and in mouse embryonic fibroblasts (Joseph et al., 2001). Cd is a potent inducer of the IEGs — concentrations as low as 0.1 μM can cause significant induction in the expression of these genes (Ding and Templeton, 2000), and several-fold overexpressions of these genes are seen within a few hours post Cd exposure (Tang and Enger, 1993; Wang and Templeton, 1998; Achanzar et al., 2000; Ding and Templeton, 2000). The Cd-induced overexpression of the IEGs can be either transitional, lasting for a few hours (Wang and Templeton, 1998; Ding and Templeton, 2000), or sustained, such as in the case of cells that are transformed by exposure to Cd (Joseph et al., 2001; Qu et al., 2005).

Stress-response genes

Cells induce the expression of several genes collectively referred to as stress response genes in order to combat the stress induced by their exposure to Cd. The major classes of stress response genes which are induced in response to Cd exposure are those involved in the synthesis of MT, those encoding heat shock proteins, those responsible for glutathione (GSH) synthesis and homeostasis, and those involved in oxidative stress response.

MT, a low molecular weight protein containing approximately 30% cysteine, is encoded by more than one gene, and its role in Cd toxicity and carcinogenesis has been very well studied. Exposure of cells to very low concentrations of Cd results in significant induction of the MT genes in cell lines (Lee et al., 2002) and in animal tissues (Misra et al., 1997). Transcriptional activation of the genes involved in MT synthesis and the consequent increase in cellular level of MT protein resulting in enhanced sequestration and detoxification of Cd is considered an adaptive response of the cells against Cd toxicity. In general, an inverse relationship has been observed between the toxicity of Cd and the MT content in cells and in tissues. The best example to illustrate the protective role of MT in Cd carcinogenesis is the species-specific difference that has been observed in the incidence of lung cancer in mice and rats. Mouse lungs contain significantly higher amounts of MT compared to rat lungs, and the induction of lung cancer observed in mice exposed to Cd is significantly lower compared to that of rats (IARC, 1993). The general presumption regarding the protective role of MT in Cd toxicity and carcinogenesis is, however, contradicted by the potential of Cd to cause testicular toxicity in different strains of mice (Liu et al., 2001; Ren et al., 2003). Mice belonging to different strains vary with respect to their testicular metallothionein content (Liu et al., 2001). Strain-specific variations, rather than testicular MT content, were observed with respect to the testicular toxicity of Cd in mice (Liu et al., 2001). However, the lack of involvement of MT reported in the testicular toxicity induced by Cd in mice is contradicted by the results of studies conducted in rats (Ren et al., 2003). The enhanced testicular toxicity seen in rats in response to their exposure to Cd was found to be associated with a lack of induction of MT genes in the Sertoli and spermatogenic cells, further supporting a protective role for MT in Cd-induced testicular toxicity in rats.

GSH, the most important antioxidant molecule present in cells plays a major role in the detoxification of Cd and, therefore, in protecting cells against Cd toxicity and carcinogenicity. The protective role of GSH in the toxicity and carcinogenesis of Cd is mainly due to its role as an antioxidant molecule. Exposure of cells and animals to Cd is known to result in the induction of oxidative stress; and many of the reactive oxygen species (ROS) that are generated following exposure of cells to Cd are detoxified either by the action of GSH or the enzymes, such as GSH peroxidase and GSH reductase, that are involved in the GSH redox cycle. The role of ROS in chemical carcinogenesis is fairly well established, and, therefore, the GSH-mediated detoxification of the toxic molecules generated in response to cellular Cd exposure should be considered as the key mechanism underlying the GSH-mediated protection of Cd carcinogenesis. It has been demonstrated that exposure of cells to Cd results in the induction of γ-glutamyl cysteine synthetase facilitating enhanced GSH synthesis (Eneman et al., 2000). In addition, genes encoding GSH S-transferase are induced by Cd (Eneman et al., 2000). The enhanced cellular level of GSH as well as increased availability of enzymes involved in GSH-mediated detoxification processes facilitate the detoxification and elimination of Cd and its toxic by-products, potentially preventing their interaction with key cellular targets to result in toxicity and carcinogenesis. However, prolonged exposure to high concentrations of Cd may overwhelm the GSH-mediated detoxification processes; and this, in turn, may facilitate Cd-induced toxicity and carcinogenesis.

Heat shock proteins are another class of stress response proteins that are induced in cells in response to exposure to physical and chemical factors. In general, the induction of these proteins is considered as an adaptation of the cells to perform functions essential for survival under conditions of stress. Exposure of cells and animals to Cd is known to induce several genes encoding heat shock proteins (Lee et al., 2002). It has been hypothesized that protein denaturation or any other type of protein damage caused by Cd serves as the stimulus for induction of the genes encoding heat shock proteins (Morimoto et al., 1994).

Transcription factors

Some of the genes that are induced by the exposure of cells to Cd encode transcription factors which can result in transcriptional de-regulation of their target genes. For example, *c-fos* and *c-jun*, two of the Cd-responsive IEGs, constitute the AP-1 transcription element that is present in the promoter regions of several genes involved in cell growth and division (Angel and Karin, 1991). The Cd-induced overexpression of *c-fos* and *c-jun* has been observed in cells transformed upon exposure to Cd and has been suggested as a possible mechanism responsible for Cd carcinogenesis (Joseph et al., 2001). Other transcription factors that are activated by the exposure of cells to Cd are the metal regulatory transcription factor 1 (MTF1) (Smirnova et al., 2000), upstream stimulator factor (USF) (Li et al., 1998), nuclear factor κB (NFκB) (Misra et al., 2002), and NF-E2-related factor 2 (NRF2) (Alam et al., 2000). In contrast to these reports, a significant suppression of the DNA binding activities of the HIF1 and SP1 transcription factors has been observed in cells treated with Cd (Obara et al., 2003; Watkin et al., 2003).

Translation factors

Translation factors are a group of genes involved in the regulation of initiation, elongation and termination of peptide chain synthesis. Overexpression of several of the translation factors, especially those of eukaryotic translation initiation factor 4E (eIF4E) and eukaryotic translation elongation factor 1A2 (eEF1A2), has been identified in cancer cell lines and tumor samples suggesting their potential involvement in carcinogenesis (Joseph et al., 2004b). The involvement of specific translation factors in Cd-induced cell transformation and tumorigenesis was first reported in 2002 (Joseph et al., 2002a, 2002b). Differential display analysis of BALB/c-3T3 cells transformed by their exposure to $CdCl_2$ demonstrated the overexpression of translation initiation factor 3 (Joseph et al., 2002a) and translation elongation factor 1 delta (Joseph et al., 2002b) suggesting their possible involvement in Cd-induced cell transformation and tumorigenesis. Transfection of cells with cDNAs encoding these Cd-responsive translation factors resulted in cell transformation and tumorigenesis further supporting the oncogenic potential of the Cd-responsive translation factors. The antisense mRNA for both of these translation factors were capable of blocking the anchorage independent growth and tumorigenic potential of the Cd transformed cells confirming their role as novel Cd-responsive proto-oncogenes (Joseph et al., 2004a). At least one of the novel Cd-responsive translation factors has been found overexpressed in patients with esophageal carcinoma (Ogawa et al., 2004) even though the exposure history of the patients to Cd or to cigarette smoke, which is a major source for Cd, was not investigated in the study.

Miscellaneous genes

Recent developments in genomics, especially those in toxicogenomics, have facilitated the identification of a large number of genes which are differentially expressed in response to exposure of cells and tissues to Cd (Liao and Freedman, 1998; Momose and Iwahashi, 2001; Yamada and Koizumi, 2002; Li et al., 2008). In spite of the identification of a large number of genes that are differentially expressed in response to exposure of cells and animals to compounds containing Cd, the actual involvement of many of these genes, if any, in Cd carcinogenesis remains to be demonstrated.

Mechanisms of Cd-induced differential gene expression

There is ample evidence to suggest the involvement of multiple mechanisms for Cd-induced differential gene expressions. The induction of genes such as MT and heme-oxygenase by Cd involve the binding of the transcription factor MTF1 to the metal response element (MRE) sequence present in the promoter regions of these genes (Hamer, 1986; Muller et al., 1987). The involvement of indirect mechanisms, possibly involving secondary messengers such as ROS and Ca^{2+}, have also been suggested in the case of Cd-induced overexpression of the immediate early response genes (Joseph et al., 2001). Exposure of BALB/c-3T3 cells to $CdCl_2$ resulted in the overexpression of the IEGs, *c-fos*, *c-jun* and *c-myc*, and this was associated with elevated cellular level of Ca^{2+} (Joseph et al., 2001). Treating the cells with BAPTA/AM, a chelator of Ca^{2+}, blocked the Cd-induced overexpression of IEGs in the cells suggesting a definite role for Ca^{2+} in the Cd-induced overexpression of these genes. The Ca^{2+} can interact with specific response elements such as the serum response element (SRE) or cAMP-response element binding protein (CREB) that are present in the promoter/enhancer regions of the IEGs (Hardingham et al., 1997) to result in their overexpression in response to exposure of cells to Cd. Alternatively, elevated Ca^{2+} levels in the cells can act as the trigger to activate specific kinases which in turn can catalyze the phosphorylation of transcription factors resulting in the deregulation of expression of their target genes (Livneh and Fishman, 1997). Involvement of protein kinase C (PKC) in the overexpression of *c-fos* and *c-jun* in response to cellular Cd exposure has been demonstrated by the use of RO-31-8220, a PKC specific inhibitor (Joseph et al., 2001). Involvement of kinases other than PKC in the Cd-induced de-regulation of gene expression has also been demonstrated (Wang and Templeton, 1998; Joseph et al., 2001). It has been suggested that the Cd-induced mitogenic signals may converge at the kinases, which phosphorylate signaling proteins stimulating the activation of IEGs, to result in increased cell proliferation leading to cell transformation and tumorigenesis.

Reactive oxygen species (ROS), which are generated in response to exposure of cells to Cd, can also function as secondary messengers and, thus, may be involved in the Cd-induced de-regulation of gene expression (Kamata and Hirata, 1999). The potential of Cd exposure to result in the generation of ROS and oxidative stress in the cells is very well known (see the section "Cadmium and oxidative stress" in this review for more details). Similarly, the expressions of a large number of genes in the cells are known to be regulated by oxidative stress resulting from the generation of ROS. However, there are only a few studies that have investigated the potential involvement of ROS as a mechanism responsible for the Cd-induced de-regulation of gene expression, especially with respect to the carcinogenic potential of the metal. The transcription factor AP-1, consisting of *c-fos* and *c-jun*, is a redox-sensitive transcription factor that can be activated under both pro-oxidative and anti-oxidative conditions (Abate et al., 1990). A definite role for ROS in the Cd-induced overexpression of *c-fos*, *c-jun* and *c-myc*, genes involved in mitogenesis and cell proliferation, has been demonstrated in the case of rat PTE cells (Maki et al., 1992) and BALB/c-3T3 cells (Joseph et al., 2001).

Cadmium and the inhibition of DNA damage repair

In general, chemicals induce cancer either by genotoxic or non-genotoxic mechanisms. The potential of Cd to cause genotoxicity has been studied, mainly, by employing *in vitro* test systems. Exposure to Cd results in chromosomal aberrations, sister chromatid exchange, DNA strand breaks, and DNA–protein crosslinks in a variety of cell lines (Ochi and Ohsawa, 1985; Misra et al., 1998; Fatur et al., 2002). The potential for Cd to cause mutations, predominantly large chromosomal deletions, in the CD59 locus in a human-hamster hybrid (AL) cell model, which is highly proficient in detecting mutations involving large deletions, has been observed (Filipic and Hei, 2004). A good correlation has also been identified between Cd level and genotoxicity in peripheral blood mononuclear cells obtained from people occupationally exposed to Cd (Palus et al., 2003). In spite of these observations, it needs to be emphasized that Cd was not genotoxic in common bacterial mutation test systems and was only weakly mutagenic in mammalian cell systems (Rossman and Roy, 1992). Furthermore, very high concentrations of Cd ranging up to 1 mM were required to observe the genotoxicity of Cd in certain cases. The general consensus, therefore, is that Cd is, at best, a weak genotoxic agent.

Several reports support the view that genotoxicity induced by Cd is not a direct effect of the metal, but rather due to the generation of reactive oxygen free radicals and the resulting oxidative stress. The single strand DNA break caused by Cd in cultured V-79 cells was observed only under aerobic conditions (Ochi and Ohsawa, 1985). Likewise, the genotoxicity of Cd was significantly inhibited by scavengers/modulators of free radicals (Ochi and Ohsawa, 1985; Filipic and Hei, 2004). Exposure of cells to Cd resulted in the generation of 8-OHdG — a reliable marker for oxidative DNA damage (Mikhailova et al., 1997; Filipic and Hei, 2004). Pre-treating human-hamster hybrid A_L cells with buthionine sulfoximine resulted in the depletion of cellular GSH and a concomitant increase in the genotoxicity of Cd (Filipic and Hei, 2004) further supporting a role for ROS in Cd-induced genotoxicity.

In spite of being a weak genotoxic chemical, Cd exhibits remarkable potential to inhibit DNA damage repair, and this has been identified as a major mechanism underlying the carcinogenic potential of Cd (Giaginis et al., 2006). Both internal and external factors contribute to DNA damage in cells, which, if not repaired in a timely manner may result in genotoxic and carcinogenic consequences. The potential of Cd to inhibit DNA damage repair has been demonstrated by several investigators. Exposure of alveolar epithelial cells to Cd significantly reduced the activity of formamidopyrimidine DNA glycosylase, an enzyme involved in the recognition and removal of oxidative DNA damage such as 8-hydroxyguanine and 8-hydroxyadenine (Potts et al., 2001). Adaptation of alveolar epithelial cells to Cd was found associated with a significant loss in the ability to repair oxidative DNA damage (Potts et al., 2001). The repair of 8-oxoG in lymphocytes of Cd exposed workers was inversely correlated with the dose of exposure and level of DNA strand breaks (Lewinska et al., 2007). The cellular GSH level has been shown to be a determining factor influencing the effect of Cd on the DNA damage repair. Cd-induced GSH depletion in rat testis was associated with increased 8-oxoG formation as well as decreased rates of 8-oxoG repair suggesting that in the absence of efficient detoxification processes, ROS accumulate in the cells resulting in the inhibition of DNA damage repair (Hirano et al., 1997). Failure to repair DNA damage can result in the accumulation of damaged DNA contributing to mutation and carcinogenesis. Inhibition of DNA damage repair by Cd is also significant with respect to the incidences of spontaneous cancers and those induced by other genotoxic chemicals. Under conditions of Cd-induced inhibition of DNA damage repair, spontaneously occurring DNA damage in cells may remain unrepaired and may result, ultimately, in mutations and spontaneous cancers.

It is worth investigating the role of Cd as an inhibitor of DNA damage repair in cases of cancers induced by cigarette smoking. Since cigarette smoke contains a significant amount of Cd and a large number of potentially genotoxic chemicals, it is possible that the Cd-induced inhibition of DNA damage repair in people exposed to cigarette smoke may facilitate the accumulation of DNA damage brought about by the various genotoxic chemicals present in the cigarette smoke; resulting mutations may ultimately lead to cancer. By similar mechanisms, Cd co-exposure may enhance the carcinogenic potential of other genotoxic chemicals commonly found in the environment and in workplaces, or it may enable the genotoxic chemicals to cause cancer at concentrations less than those required to induce cancer following their individual exposures.

The Cd-induced inhibition of DNA damage repair is believed to be due to its effects on the enzymes that play key roles in the repair process. Several of these enzymes are members of the zinc finger family of proteins. Cd can be substituted for zinc in these enzymes, but the proteins with substituted Cd do not perform their functions as efficiently as DNA damage repair enzymes (O'Connor et al., 1993).

Cadmium and apoptosis

Apoptosis is an evolutionarily conserved and genetically regulated form of cell death which plays an important role in the development and maintenance of tissue homeostasis in multicellular organisms. Apoptosis plays an essential role in the elimination of mutated or transformed cells from the body. Thus, in order to survive, cancer cells and their precursors must develop highly efficient mechanisms to avoid apoptosis. In fact the avoidance of apoptosis is considered as a hallmark of cancer cells.

The potential involvement of apoptosis as a mechanism for Cd carcinogenesis has been demonstrated by a series of studies reported from the Waalkes Laboratory (Achanzar et al., 2000; Yuan et al., 2000; Achanzar et al., 2002). Exposure of normal human prostate epithelial cells to 10 μM $CdCl_2$ resulted in their malignant transformation (Achanzar et al., 2001). The Cd-transformed cells exhibited loss of contact inhibition and formed highly invasive and occasionally malignant adenocarcinomas when injected subcutaneously in immune-deficient mice. In another study reported from the same group of investigators, it was demonstrated that exposure of RWPE-1 cells to $CdCl_2$ resulted in the activation of genes, such as *c-jun* and *c-myc*, that are involved in cell proliferation (Achanzar et al., 2000). The expression of the *p53* tumor suppressor gene was significantly lower in the Cd-treated cells compared to the control at time intervals beyond 48-hour post-exposure to the chemical. A vast majority (approximately 65%) of the Cd exposed cells died following their exposure to Cd, and induction of apoptosis was one of the mechanisms

responsible for their death. Interestingly, the authors observed the survival of approximately 35% of the cells, and these cells appeared normal and were resistant to Cd-induced apoptosis. The Cd-resistant population of cells possessed 2.5-fold more MT content compared to the normal, untreated RWPE-1 cells. Therefore, it appears that in spite of its ability to induce apoptosis and cell death, Cd may facilitate the selective enrichment of a population of genetically damaged and apoptosis resistant cells with enhanced proliferative capacity so as to result in malignant transformation.

The proposed role of Cd as an inhibitor of apoptosis also deserves special mention with respect to its carcinogenic potential. Cd has been reported as an inhibitor of apoptosis induced by other metallic and non-metallic toxic agents (Shimada et al., 1998; Achanzar et al., 2002). The ability of Cd to block or inhibit apoptosis induced by hexavalent chromium, a metal known for its carcinogenic potential in experimental animals and human, has been studied (Yuan et al., 2000). Chinese hamster ovary (CHO K1-BH4) cells were treated with $CdCl_2$ (5–20 μM), hexavalent chromium (350 μM) or chromium (350 μM) plus $CdCl_2$ (5–20 μM) for three hours, and apoptosis was determined 48-hour post-exposure to the chemical(s). Exposure of the cells to chromium, but not to $CdCl_2$, resulted in the induction of apoptosis as evidenced from the appearance of DNA fragmentation and apoptotic nuclei in the cells. However, Cd was able to block or inhibit chromium-induced apoptosis when the cells were co-exposed to both metals. Similar inhibitory effects of $CdCl_2$ on apoptosis induced by cisplatin and etoposide have been reported in RWPE-1 cells following their transformation by $CdCl_2$ (Achanzar et al., 2002). The role of Cd in the apoptosis induced by benzo(a)pyrene-7,8-diol epoxide (BPDE), was investigated recently (Mukherjee et al., 2008). Exposure of the mouse epidermal JB6 Cl 41 cells to 10 μM $CdCl_2$ resulted in a significant inhibition of apoptosis induced by BPDE in the cells. The ability of Cd to inhibit the apoptosis induced by BPDE may represent a possible mechanism for its co-carcinogenic function. This view is further strengthened by the synergistic enhancement in the frequency of morphological transformation observed in mammalian cells treated with Cd and BPDE (Rivedal and Sanner, 1981). The inhibitory effect of Cd on apoptosis induced either by itself or by other carcinogenic chemicals may represent a major non-genotoxic mechanism for its role as a carcinogen and/or co-carcinogen. The inhibition of apoptosis by Cd may allow a greater portion of genetically damaged cells to survive or give selective growth advantages to pre-neoplastic cells to result in malignant transformation and carcinogenesis.

There is considerable evidence to demonstrate the involvement of multiple mechanisms underlying Cd-induced apoptosis. Both caspase-dependent (Kondoh et al., 2002) and independent (Shih et al., 2003) mechanisms involving mitochondria have been reported in Cd-induced apoptosis. In the case of murine macrophages, induction of oxidative stress has been demonstrated as the major mechanism responsible for Cd-induced apoptosis (Kim and Sharma, 2006). The Cd-induced oxidative stress in the macrophages influenced apoptosis indirectly by modulating the cellular level of Ca^{2+} and the activities of caspases and mitogen activated protein kinases (MAPKs) in the cells. Inhibition of Cd-induced apoptosis by the antioxidants, N-acetyl cysteine (NAC), glutathione, and catalase, further support the role of oxidative stress in Cd-induced apoptosis (Galan et al., 2001; Watjen and Beyersmann, 2004). In the human lymphoblastoid cell line Boleth, Cd induced apoptosis through caspase-dependent and -independent pathways, independent of activation of major MAPKs (Coutant et al., 2006). In mitochondrial DNA depleted cells, Cd-induced apoptosis was independent of caspases but dependent on mechanisms involving Ca^{2+} (Shih et al., 2005). Suppression of the activity of the transcription factor NFκB has been suggested as a mechanism responsible for the Cd-induced apoptosis in the rat kidney epithelial cells, NRK-52E (Xie and Shaikh, 2006). Involvement of the tumor suppressor gene, *p53*, in Cd-induced apoptosis has also been reported. A negative correlation has been observed between Cd-induced apoptosis and the induction of *p53* gene expression in rat testis (Xu et al., 1999). However, in contrast to testis, alveolar cells from rat lungs exhibited increased *p53* gene expression in response to Cd exposure (Lag et al., 2002). The inhibitory effect of Cd on chromium-induced apoptosis in CHO K1-BH4 cells is mediated through the inhibition of caspase 3 activity, a central mediator of apoptosis (Yuan et al., 2000). Studies conducted to determine the potential mechanisms involved in the Cd-mediated inhibition of BPDE-induced apoptosis demonstrated that the effect of Cd was not mediated through AP-1, but probably through a different mechanism involving the up-regulation of extracellular signal related kinase (ERK) (Mukherjee et al., 2008).

Gene expression profiling was employed to determine the molecular mechanisms responsible for resistance to apoptosis seen in RWPE-1 cells transformed by exposure to $CdCl_2$ (Achanzar et al., 2002). As mentioned above, transformation of RWPE-1 cells with $CdCl_2$ resulted in the selection of a population of apoptotic resistant cells with significantly higher MT content. Results of cDNA microarray analysis of the gene expression profile of the apoptosis resistant cells demonstrated the down-regulation of genes encoding several members of the caspase family of apoptotic proteases. Furthermore, the expression of *bax*, an important pro-apoptotic gene, was significantly reduced in the transformed cells compared with the control cells. In addition, the anti-apoptotic gene, *bcl2*, was significantly overexpressed in the transformed cells compared to the controls.

Cadmium and oxidative stress

Cadmium, in spite of not being a Fenton metal, is capable of inducing oxidative stress in cell culture models (Joseph et al., 2001; Shih et al., 2004) and in experimental animals (Nigam et al., 1999). Approximately 10,000 adults who participated in the third U.S. National Health and Nutrition Survey were examined for associations between urinary Cd levels and oxidative stress markers in serum such as γ-glutamyltransferase (GGT), vitamin C, carotenoids and vitamin E (Lee et al., 2006). Results of this study demonstrated graded associations of urinary Cd levels, positive with serum GGT and inverse to serum vitamin C, carotenoids and vitamin E, among the participants of the survey. The strong association observed between the urinary Cd levels and the markers of oxidative stress warrants that oxidative stress be considered in the pathogenesis of Cd-related diseases, including cancer. The ability of Cd to deplete GSH to inhibit the mitochondrial electron transport chain so as to cause the generation of superoxide anion radical, and to modulate the cellular level of proteins involved in the generation/detoxification of various ROS are thought to contribute to the oxidative stress observed in response to Cd exposure. Exposure of cultured cells and animals to compounds containing Cd has resulted in the generation of ROS (Bolduc et al., 2004; Shih et al., 2004). Similarly, the expression of various detoxification enzymes that are known to play a key role in the cellular response to the generation of ROS and the resulting toxicity, are affected by exposure to Cd (Casalino et al., 2006). Additional evidence to support the capability of Cd to induce oxidative stress comes from the observations of increased lipid peroxidation (Jurczuk et al., 2004), depletion of glutathione (Nigam et al., 1999) and induction of several stress response genes (Badisa et al., 2008) in response to Cd exposure.

Generation of ROS resulting in oxidative stress is believed to play a prominent central role in Cd carcinogenesis. Many of the cellular and molecular events taking place in the cells that have relevance to Cd-induced carcinogenesis are mediated, directly or indirectly, by oxidative stress. As mentioned above, Cd is very weak with respect to its ability to cause genotoxicity and most of the genotoxic events observed in cells in response to Cd exposure are mediated mainly by the generation of ROS. The genotoxicity induced by Cd exhibits hallmarks of oxidative stress and scavengers/modulators of ROS are capable of significantly reversing the Cd-induced genotoxicity (Bertin and Averbeck, 2006). Cd-induced oxidative stress causes the

production of typical oxidatively generated mutagenic lesions such as 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG) adducts in the DNA of human lymphoblastoid cells (Mikhailova et al., 1997). Cd-induced DNA strand breaks and the formation of 8-oxodG adducts were suppressed by DMSO — a scavenger of hydroxyl radicals (Filipic and Hei, 2004). Antioxidant enzymes such as superoxide dismutase and catalase were able to block the DNA strand breaks (Ochi and Ohsawa, 1985; Liu and Jan, 2000), chromosomal aberrations (Ochi and Ohsawa, 1985) and gene mutations (Yang et al., 1996) induced by Cd. Similarly, Cd-induced oxidative stress is known to play a major role in its potential to inhibit DNA damage repair and to induce apoptosis (Xie and Shaikh, 2006).

Cd-induced oxidative stress is also known to play an important role in aberrant gene expression — a key event involved in Cd toxicity and carcinogenesis. The expression of *c-fos* and *c-jun*, members of the redox-sensitive AP-1 transcription factor, is regulated by Cd-induced oxidative stress. Both *c-fos* and *c-jun* are found overexpressed in Cd transformed cells (Joseph et al., 2001; Qu et al., 2005). Furthermore, antioxidants are able to block the Cd-induced overexpression of *c-fos* and *c-jun* suggesting the involvement of ROS and the resulting oxidative stress in the Cd-induced overexpression of these genes (Joseph et al., 2001).

In spite of the demonstrated potential for Cd to induce oxidative stress through the generation of ROS, the actual involvement of oxidative stress in Cd-induced carcinogenesis remains debatable. Exposure of the mouse embryonic fibroblast cell line, BALB/c-3T3, to 9–12 μM $CdCl_2$ for 72 h resulted in cell transformation, and the transformed cells exhibited tumorigenicity when tested in immune-deficient nude mice (Keshava et al., 2000). The transformed cells exhibited significantly higher levels of hydrogen peroxide and superoxide anion, compared to their normal counterparts (Joseph et al., 2001), suggesting a potential role for the generation of ROS and the resulting oxidative stress in Cd carcinogenesis. However, cell transformation and tumorigenesis studies conducted in the rat liver epithelial cell line, TRL 1215, did not support any significant role for Cd-induced oxidative stress in the cell transformation and tumorigenesis induced by the metal (Qu et al., 2005). Accordingly, chronic exposure to low level Cd (1 μM $CdCl_2$ for up to 28 weeks) resulted in malignant cell transformation of the non-tumorigenic rat liver epithelial cell line, TRL 1215. Furthermore, inoculation of the Cd-transformed cells in mice resulted in tumorigenesis. Analysis of the cells for the presence of ROS demonstrated that the cellular level of hydrogen peroxide and superoxide anion were significantly lower in the transformed cells compared to the time-matched, non-transformed, control cells. Furthermore, the transformed cells, compared to the control cells, exhibited significant tolerance to the generation of both superoxide anion and hydrogen peroxide following their exposure to higher concentrations of $CdCl_2$ (10 and 50 μM concentrations). Based on their findings, these authors concluded that the generation of ROS resulting in oxidative stress did not play an important role in the low dose, long-term Cd-induced malignant transformation model used in their study (Qu et al., 2005).

Conclusions

It is unequivocally accepted that Cd is a carcinogen. Cd exposure results in cell transformation, induces cancers of various organs in experimental animals, and causes at least lung cancer in humans. The potential of Cd to cause cancer in target organs other than the lung, especially kidney and prostate, has been suggested in humans. However, further epidemiological evidence is required to support the reports that Cd causes cancer in target organs other than lungs in humans.

Most of our current understanding regarding the mechanisms of Cd carcinogenesis is derived from experiments conducted by employing *in vitro* cell culture and *in vivo* animal models. These studies have demonstrated that Cd is a complex carcinogen, and the mechanisms underlying Cd carcinogenesis are multifactorial. As summarized in Fig. 1, the major mechanisms of Cd carcinogenesis include aberrant gene expression, inhibition of DNA damage repair and apoptosis, and induction of oxidative stress. Application of new techniques such as differential gene and protein expression profiling in experimental animals exposed to Cd may facilitate a better understanding of the mechanisms involved in Cd toxicity and carcinogenesis. Such studies, in turn, have the potential to result in the development of molecular markers for Cd exposure, toxicity and carcinogenesis. Application of these molecular markers in epidemiological studies may facilitate establishing a better linkage between Cd exposure and the incidence of cancer in humans.

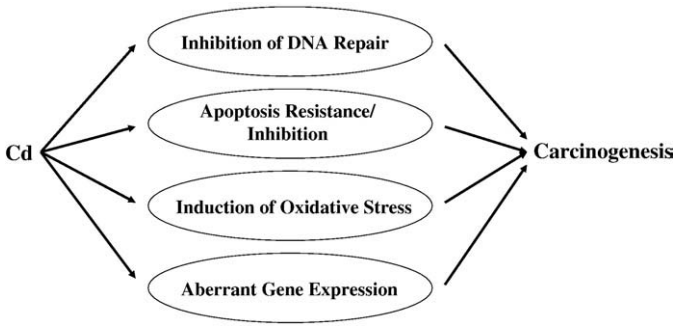

Fig. 1. A model summarizing the major mechanisms involved in cadmium carcinogenesis.

The persistent nature of Cd in the environment and in biological tissues may have serious consequences, especially, with respect to the role of Cd as a human carcinogen. Cd is almost ubiquitously present in the environment, in many food materials and in specific workplaces. Therefore, almost everyone is at potential risk of exposure to Cd and the resulting toxicity including cancer. As discussed above, Cd exposure can result in the inhibition of normal DNA damage repair processes in cells. Also, there is experimental evidence to suggest that Cd is inhibitory to apoptosis and may contribute to the development of apoptosis-resistant cells. These features of Cd (persistence in the environment and in biological tissues, inhibition of DNA damage repair processes and apoptosis) may have implications in carcinogenesis resulting from human exposure to other genotoxic chemicals. For example, Cd present in the cells may prevent the repair of DNA damage caused by other genotoxic chemicals to which humans are routinely exposed either from the environment, through food materials, or occupationally. Similarly, the Cd-induced inhibition of apoptosis may prevent the elimination of genetically damaged cells resulting in their accumulation in the body. In addition, Cd may stimulate the proliferation of such genetically damaged and apoptosis-resistant cells in the body. Through all of these processes, Cd may facilitate other carcinogenic compounds to cause cancer at significantly lower concentrations than what would be normally required. Therefore, in spite of the advances made in the past few decades regarding our understanding of the mechanisms of Cd carcinogenesis, further research is required to fully understand the mechanisms of Cd carcinogenesis, as well as, the involvement of Cd in the incidence of human carcinogenesis, particularly in populations where significant exposure to Cd has been observed.

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