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Review

Cadmium carcinogenesis

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Abstract

Cadmium is a heavy metal of considerable environmental and occupational concern. Cadmium compounds are classified as human carcinogens by several regulatory agencies. The most convincing data that cadmium is carcinogenic in humans comes from studies indicating occupational cadmium exposure is associated with lung cancer. Cadmium exposure has also been linked to human prostate and renal cancer, although this linkage is weaker than for lung cancer. Other target sites of cadmium carcinogenesis in humans, such as liver, pancreas and stomach, are considered equivocal. In animals, cadmium effectively induces cancers at multiple sites and by various routes. Cadmium inhalation in rats induces pulmonary adenocarcinomas, in accord with its role in human lung cancer. Cadmium can induce tumors and/or preneoplastic lesions within the rat prostate after ingestion or injection. At relatively high doses, cadmium induces benign testicular tumors in rats, but these appear to be due to early toxic lesions and loss of testicular function, rather than from a specific carcinogenic effect of cadmium. Like many other metals, cadmium salts will induce mesenchymal tumors at the site of subcutaneous (s.c.) or intramuscular (i.m.) injections, but the human relevance of these is dubious. Other targets of cadmium in rodents include the liver, adrenal, pancreas, pituitary, and hematopoietic system. With the exception of testicular tumors in rodents, the mechanisms of cadmium carcinogenesis are poorly defined. Cadmium can cause any number of molecular lesions that would be relevant to oncogenesis in various cellular model systems. Most studies indicate cadmium is poorly mutagenic and probably acts through indirect or epigenetic mechanisms, potentially including aberrant activation of oncogenes and suppression of apoptosis.

Keywords: Cadmium; Carcinogenesis; Humans; Rodents; Mechanisms

1. Introduction

Cadmium is a toxic transition metal of continuing occupational and environmental concern [1–4]. Cadmium exposure leads to a variety of adverse effects [1–4]. The extremely long biological half life of cadmium essentially makes it a cumulative toxin, so long past exposures could still result in direct toxic effects of the residual metal [1]. Unfortunately, there are no proven effective treatments for chronic cadmium intoxication [1]. The long residence time of cadmium is in part attributable to metallothionein (MT), a metal-binding protein that is induced at the transcriptional level by cadmium and tightly binds the metal [1,3,4]. Cadmium accumulates primarily in the liver and kidney where it is bound to MT, and it is felt that cadmium bound to MT is essentially detoxicated, at least temporarily, through this high affinity sequestration [1,3]. The body has limited capacity to respond to cadmium exposure, as the metal cannot undergo metabolic degradation to less toxic species and is only poorly excreted, making long-term storage a viable option for dealing with this toxic element.

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The toxic effects of cadmium often stem from interference with various zinc mediated metabolic processes, while zinc treatments frequently reduce or abolish the adverse effects of cadmium [1]. This might be viewed as molecular mimicry, as these two elements are closely located in the periodic table and favor similar bioligands. For instance, cadmium competes with zinc for binding to MT and blocks cellular zinc accumulation. Excess zinc can antagonize many of the adverse effects of cadmium, including tumor formation [4], indicating a mechanistic role for cadmium-zinc interaction in cadmium toxicity.

There are several sources of human exposure to cadmium, including employment in primary metal industries, production of certain batteries, some electroplating processes and consumption of tobacco products [5,6]. Smoking tobacco is thought to double the life time body burden of cadmium in non-occupationally exposed persons. Environmental exposure to cadmium is also not uncommon [2]. The most frequently observed chronic toxic effect of the metal in humans is chronic nephropathy characterized by proximal tubular necrosis and proteinurea [1–3]. A debilitating osteoporosis has been associated with high levels of environmental cadmium, possibly produced in concert with nutritional deficiencies [1].

Cadmium has been designated a human carcinogen by the World Health Organization's International Agency for Research on Cancer and the United States National Toxicology Program [5,6]. Multiple studies have linked occupational exposure to cadmium with pulmonary cancer in humans [4–6]. Several studies indicate a role for cadmium in human prostatic [4–6] and renal [4–9] cancers, while a few studies have associated cadmium exposure with human cancer of the liver, hematopoietic system, urinary bladder and stomach [4–6,9]. There is some indication that cadmium might be important in pancreatic cancer [10], but this is yet to be established. The role of cadmium as a pulmonary carcinogen in occupationally exposed populations is largely the basis for its declaration by regulatory agencies as a human carcinogen [5,6], while other target sites in humans, potentially including the prostate and kidney, are not definitively established [4–10].

On the other hand, cadmium is clearly an effective, multi-tissue animal carcinogen [4–6,9,11,12]. In clear support of human data, rodent studies show that chronic inhalation of cadmium causes pulmonary adenocarcinomas [4–6,9]. Cadmium can also cause prostatic proliferative lesions, including adenocarcinomas, after systemic or direct exposure in rats [4–6,9,11,12]. Systemic exposure to cadmium can also induce lung tumors [9]. Other target tissues of cadmium carcinogenesis in rodents include repository injection sites, testes, adrenals, liver, kidneys, pancreas and the hemopoietic system [4–6,9,11,12]. Treatments with zinc can modify cadmium carcinogenicity and prevents cadmium-induced injection site and testicular tumors while facilitating prostatic tumor formation [4,9]. Zinc deficient diets increase the progression of testicular tumors but reduce the progression of prostatic tumors [4,9]. There are definite species- and strain-related differences in sensitivity to cadmium carcinogenicity [4,9].

The potential mechanism or mechanisms of cadmium carcinogenesis are unknown. Various cellular models have been developed to help define potential mechanisms. Relatively speaking, cadmium binds DNA in a weak fashion, indicating this is not a primary mode of action. Cadmium is not a redox active metal, although it does produce oxidative stress [1], which could indirectly result in attack on DNA, but this has not been absolutely established as a mechanism. Cadmium may well act as a epigenetic or indirectly geneotoxic carcinogen since it is, in general, poorly mutagenic [4,9]. Potential contributing factors to cadmium oncogenicity include aberrant gene activation, suppressed apoptosis, and/or altered DNA repair. Additional work clearly is required to define the mode action of this important inorganic carcinogen. A more complete knowledge of mechanism would allow better assessment of the risk associated with this common environmental contaminant.

2. Cadmium metabolism

Metabolism of toxicant metals often is dictated by the essential elements they may mimic. Cadmium appears to mimic zinc and to a lesser extent calcium [1]. Cadmium absorption shows marked route dependency [1] as only ~5% of an oral dose is absorbed by the gastrointestinal tract. Cadmium absorption from the lung is very high, with upwards of 90% of a dose being absorbed. There is a common pathway for

absorption of cadmium and iron through the divalent metal transporter-1 (DMT-1) which accounts for high accumulation of cadmium during iron deficiency [13]. Regardless of the route, once absorbed, cadmium is rapidly cleared from the blood and concentrates in several tissues. Hepatic and renal cadmium usually make up the bulk of the total body burden [1,3]. Accumulation in these tissues may be due to their ability to produce large amounts of MT [3]. Typically, the presence of MT within cells will markedly decrease cadmium toxicity [3]. Zinc, which inhibits many of the adverse effects of cadmium including, in some instances, carcinogenesis, likely does so in part by stimulating the production of MT [9]. The long residence time of cadmium in the body is probably as a consequence of its binding to MT. It would seem reasonable to suspect that a long residence time could enhance the probability of neoplastic transformation by any agent, and this may be true of cadmium, although no direct evidence supports this contention. The fact that cadmium can be carcinogenic in animals after only a single dose [9], lends credence to the notion that a protracted residence time in target tissue may contribute to eventual tumor formation.

3. Cadmium carcinogenesis in humans

Various regulatory agencies have concluded that there is adequate evidence that cadmium is a human carcinogen [5,6]. This designation was largely prompted by repeated findings of a link between occupational cadmium exposure and lung cancer, as well as very strong data in rodents showing the pulmonary system as a target site after cadmium inhalation [1,4,9]. The lung is clearly the most definitive target site in humans. Multiple studies have also linked cadmium exposure to cancers of the prostate and kidney [1,4–9], and there is concordant rodent data showing prostatic or renal cancer development after cadmium treatment [4,9,11,12]. Some evidence exists that environmental cadmium exposure can be associated with prostate cancers [6,9]. Human prostate cancer is an often deadly disease with a complex etiology, and linking a relatively small portion of all cases to a single factor could prove very difficult. Recent evidence indicates that human prostate epithelial cells can be malignantly transformed by cadmium in vitro [15,16], providing strong evidence that the human prostatic epithelium can be a direct target of the oncogenic effects of the metal. Two recent case–control studies indicate renal cell carcinoma development is associated with occupational cadmium exposure [7,8] supporting earlier epidemiologic work [4,6,9]. However, although evidence is perhaps increasing, particularly for the kidney, the link between human cadmium exposure and non-pulmonary tumors is probably best considered less than definitive. Perhaps genomic finger printing, as has been used to develop signatures for classes of hepatotoxic agents [14], may help provide a more definitive linkage between cadmium and prostatic or renal cancers. In some studies, human cadmium exposure has also been linked to cancers of the liver, hematopoietic system, urinary bladder and stomach [4–6,9]. A recent proposal that cadmium may be associated with pancreatic tumors in humans [10] is intriguing and potentially very important, as this is a very deadly form of cancer. Clearly, further work, including molecular epidemiology, is necessary to determine the target sites and nature of the carcinogenic risk in humans posed by cadmium exposure.

4. Cadmium carcinogenesis in animals

Haddow et al. [17] provided the earliest suspicion that cadmium might be carcinogenic in rodents. They gave rats and mice either subcutaneous (s.c.) or intramuscular (i.m.) injections of rat liver ferritin which had been prepared by precipitation with cadmium [17], then a widely used method of protein precipitation. Subsequently, these animals developed malignant sarcomas at the site of injection [17]. At the time, although suspected, it was unclear if cadmium was the active agent in this preparation [17]. Further studies based on this initial work helped established cadmium as a effective rodent carcinogen. The carcinogenic potential of cadmium was subsequently shown at repository-type injection sites (i.m. or s.c.), where it forms sarcomas at high incidence [4–6,9]. Early studies also showed cadmium to be an effective testicular tumorigen, with a single high dose producing a remarkable elevation in the incidence of benign testicular interstitial (Leydig) cell tumors [4–6,9]. Many later studies have duplicated these data, showing cadmium to be quite effective in producing

injection site sarcomas or testicular tumors [4–6,9]. However, the relevance of either of these target sites to human exposure situations is questionable [4–6,9].

Numerous studies have establish inhaled cadmium as an efficacious pulmonary carcinogen in the rat, where it produces adenocarcinoma after inhalation [4–6,9]. These data clearly support the findings that cadmium can act as a human lung carcinogen [4–6,9]. For instance, in the initial study with cadmium in rats, chronic inhalation of cadmium chloride aerosols induced dose-related increases in pulmonary carcinoma incidence to a maximum of over 70% [18]. Other forms of cadmium, including the oxide which is more relevant in occupational exposure, are also carcinogenic to the rat lung after inhalation [4–6,9]. In contrast to the rat, inhaled cadmium has not proven to be an effective pulmonary carcinogen in mice or hamsters [4–6,9]. Mouse strains vary widely in cadmium sensitivity, at least with regard to its acute toxic effects, and this appears to be based in genetic background [19]. It is possible that sensitive strains have yet to be tested in chronic inhalation studies in mice. Pulmonary tumors can be induced systemic cadmium exposure mice [9].

The rat testes are extremely sensitive to cadmium-induced tumorigenesis. Cadmium, when given at a sufficiently high parenteral dose, rapidly induces an extensive hemorrhagic necrosis of the testes [1]. After this initial toxic lesion, chronic degeneration sets in and eventually a high incidence of testicular interstitial cell tumors occur [1,4–6,9]. Oral cadmium exposure can also cause interstitial cell tumors in the rat [9]. Mice and hamsters will typically show interstitial cell hyperplasia, although some studies have indicated interstitial cell tumors can form in the testes of mice exposed to cadmium [4–6,9]. In a fashion similar to many organic carcinogens that induce interstitial cell tumors of the testes [20], cadmium-induced testicular tumors in rats are likely related to the chronic degenerative effects of the metal on the testes, which results in loss of androgen production, and a subsequent overstimulation of remnant testicular cells by the pituitary [21]. Interestingly, the ovary in rodents will undergo an acute phase necrosis with a high dose of cadmium, the extent of which depends on the point in the estrus cycle [22]. However, tumors of the ovary are not subsequently formed, at least in female hamsters [23].

Cancer of the prostate gland is an important and deadly human malignancy of essentially undefined etiology. Several studies show cadmium can induce tumors and preneoplastic (hyperplastic) lesions of the prostate in rats [1,4–6,9,11,12]. The ability of cadmium to induce prostate cancer does not follow a typical dose–response pattern as the response is lost at higher doses. This is due to the effects of the metal on the testes at high doses. For instance, a single s.c. injection of cadmium in rats, using a wide range of doses, indicates prostatic tumor incidence is increased only at doses below the threshold for significant cadmium-induced testicular toxicity ($\sim$5.0 μmol Cd/kg, s.c.), while any prostatic proliferative response is lost at higher doses of the metal [4,9]. At these lower doses, cadmium induces dose-related increases in prostatic tumors [4,9]. Testicular androgen production is essential for the growth and maintenance of the prostate and prostate tumors are often testosterone dependent [24,25]. In rodents, testosterone alone will increase the incidence of prostatic carcinoma [25]. High dose cadmium ($\geq$5.0 μmol Cd/kg, s.c.) causes a permanent reduction in the levels circulating of testosterone in rats of up to 80%, and induces a high incidence of prostatic atrophy [21]. This prostatic atrophy would likely counter any cadmium-induced proliferative stimulus. Thus, the toxic effects in the testes is likely responsible for the loss of prostatic response at high, testopathic doses of cadmium. Oral exposure to cadmium can also induce proliferative lesions in the rat prostate and direct cadmium injection into the rat prostate produces adenocarcinomas [4,9]. Cadmium exposure enhances the appearance of chemically-induced prostatic tumors in rats [26]. Other work shows rat prostatic epithelial cells, when exposed to cadmium in vitro, can become malignantly transformed [27]. Development of prostatic tumors in rats after cadmium treatment supports, but does not establish, a possible causative role in human prostate cancer.

As is the case with many metallic carcinogens [28], malignant tumors are induced by cadmium in rats or mice at the site of s.c. or i.m. injection [1,4–6,9,29]. The tumors produced by cadmium injection at such sites are typically fibrosarcomas [1,4–6,9,29]. Cadmium-induced injection site sarcomas appear to be related to the locally accumulated dose [1,4–6,9,29]. The strain of rat or mouse has a pronounced effect on

the eventual incidence of injection site sarcomas induced by cadmium, indicating a genetic basis for sensitivity to these malignancies [4,9,29]. Repeated s.c. injections of cadmium at the same location, although not remarkably increasing incidence, cause injection site sarcomas to develop more rapidly and to become more aggressive, showing an increased rate of local invasion and distant metastasis [29]. This action as a tumor "progressor" could be important in cases where cadmium exposure occurs in conjunction with other carcinogens in humans, as is the case with tobacco smoking, since smoking is a major source of human cadmium exposure [6] and is rich in organic carcinogens. The ability to enhance progression of injection site sarcomas is also related to strain, with some strains clearly more sensitive to these effects of cadmium [29], indicating a genetic component in susceptibility.

Cadmium can induce a variety of other tumors. For instance, cadmium can induce tumors of the hematopoietic systems in rats and mice [4,9]. Oral cadmium exposure induces dose-related increases in the incidence of leukemia in male Wistar rats [4,9]. Increases in lymphoma are induced by s.c. injections of cadmium in several strains of mice, including BALB/c, NFS and DBA [4,9]. When cadmium is injected s.c. concurrently with salts of calcium, an elevated incidence of islet cell tumors of the rat pancreas has been shown [4,9]. Induction of tumors of the adrenals has been observed after cadmium exposure in hamsters, rats and mice [4,9,12]. A single study each has related cadmium exposures and liver tumors in mice [30], and renal [12] and pituitary [11] tumors in rats.

Overall, cadmium exposure has been linked with tumors of the lung, testes, injection site, prostate, hematopoietic system, pancreas, adrenals, liver, kidney and pituitary. Several of these sites are concordant with potential human target sites of cadmium carcinogenesis (lung, liver, prostate, kidney). All major routes of cadmium exposure have been associated with carcinogenic effects including inhalation, and ingestion. Cadmium is an effective carcinogen in three rodent species (mouse, rat, hamster). Thus, accumulated data indicate cadmium is a effective, multi-route, multi-site, multi-species carcinogen in rodents.

Cadmium can also enhance the carcinogenic effects of organic carcinogens. For instance, when cadmium is given soon after diethylnitrosamine (DEN), it markedly enhances DEN-induced hepatic and renal tumors in rats [31]. Some classical initiation/promotion studies for cadmium effects in rodents have been conducted. Many of these have found cadmium, when used as a promoter after treatment with strong organic carcinogens, can actually block tumor formation in rodents [32,33]. This is a complex response perhaps dictated by the ability of the particular tumors to produce MT [32,33]. With regard to positive studies, Kurokawa et al. [34] found that oral cadmium increased the number of renal dysplastic foci (a pre-tumorous lesion), but had no effect on renal carcinomas, after initiation in rats by the organic carcinogen *N*-ethyl-*N*-hydroxyethylnitrosamine (EHEN).

5. Modification of the carcinogenic response to cadmium in rodents; some mechanistic considerations

It is suspected that toxic metals, such as cadmium, often act by molecular/atomic mimicry of essential nutrient metals. In this fashion, cadmium may gain cellular access and disrupt normal cellular metabolism. An additional component of this mimicry is that excess essential element, when given with the toxic metal it mimics, can reduce or eliminate its toxicity. In this regard, the essential nutrient transition metal, zinc has a remarkable impact on cadmium carcinogenesis. In the lung, testes, and at the injection site, zinc reduces the carcinogenic effects of cadmium in rodents [4,9]. This effect of zinc in blocking injection site and testicular cancers induced by cadmium was appreciated very early on by Gunn et al. [35] who gave cadmium and zinc by s.c. injection. Additional work has shown chronic dietary zinc deficiency will enhance the carcinogenic response to cadmium at the injection site, and promotes the carcinogenic progression of cadmium-induced testicular lesions [4,9]. Other metals tested so far, specifically calcium and magnesium, are relatively ineffective in reducing the carcinogenic effects of cadmium compared to zinc [4,9]. This selective antagonism by zinc of the carcinogenic effects of cadmium in several different target sites may reveal a basic mechanism of cadmium carcinogenesis, at least in some tissues. In this regard, cadmium can compete with zinc for a multitude of important binding sites within biomolecules, including, potentially, sites important in gene regulation or

enzyme activity [1,4,9]. The fact that zinc effectively induces MT synthesis likely plays a major role in reduction of cadmium toxicity [1,3]. Cadmium can displace zinc from MT, and this in effect reduces the amount of free (and likely toxic) cadmium [1,3]. The induction of MT could be an important component in zinc-induced inhibition of cadmium carcinogenesis, although this is yet to be established. The MT system could also play a role in species- and/or strain-related differential sensitivity to cadmium carcinogenicity. In this regard, cadmium is an effective pulmonary carcinogen after inhalation only in the rat, while inhaled cadmium has not been shown to be a pulmonary carcinogen after inhalation in mice [1,4–6,9]. Acute cadmium toxicity in the lungs after inhalation displays a similar species differences, as cadmium causes more severe acute damage in the rat lung than in the mouse [36,37]. Mice produce much higher levels of MT in the lung and the variation in acute toxicity and chronic carcinogenicity may be due to a higher production capacity for pulmonary MT in mice than rats [37].

In contrast to inhibition of cadmium carcinogenesis in some tissues, treatment with zinc can actually facilitate cadmium-induced prostatic tumor production [4,9]. The basis of this response lies in the ability of zinc to block the effects of cadmium on the testes. At appropriate levels, zinc essentially abolishes the acute toxic effects of cadmium in the testes which then allows unaltered production of testicular androgens [4,9]. The maintenance of testicular androgen output, which is critical to support of accessory sex tissues, prevents indirect cadmium-induced prostatic atrophy. Atrophy of the prostate would counter any direct growth stimulation by cadmium. Prevention of the loss of testicular androgen production provides a hormonal environment facilitatory prostate cancer development, as most tumors of the prostate require androgen for growth, at least in their early stages [4,9]. Indeed, within weeks of a single, otherwise well tolerated, injected dose of cadmium in rats circulating testosterone is reduced to 20% of control levels and about half of the prostate mass is lost [21]. In comparison bilateral orchiectomy in rats reduces circulating testosterone by about 90% [21]. Similarly, chronic dietary zinc deficiency reduces the carcinogenic potential of cadmium within the prostate likely because zinc deficiency itself induces prostatic atrophy due to a loss of testicular androgen secretion [4,9]. However, a diet deficient in zinc enhances the progression of cadmium-induced testicular proliferative lesions and increases the incidence of cadmium injection site sarcomas in rats [4,9]. Thus, the effect of zinc on cadmium carcinogenesis is multifaceted as it can facilitatory or inhibitory depending on the tissue in question and circumstances of metal exposure.

6. Possible mechanisms in rodent cancers induced by cadmium

No clear in vivo mechanisms of action for cadmium carcinogenesis have emerged, with the possible exception of the rodent testes (Fig. 1). Unfortunately, because of their nature and the requirement of high parenteral dose of cadmium to induce testicular tumors, it is doubtful that these benign neoplasia have much relevance to human cadmium exposure. The mechanism here appears to lie in the remarkable testicular necrosis induced by high doses of cadmium in rodents [4,9,21], a lesion never recorded in humans. Within a day of a sufficiently high dose of cadmium, the rodent testes show a remarkable hemorrhagic necrosis, comparable to infarct, with a collapse of the testicular capillary system. The exact basis of this vascular collapse is undefined but may reside in local endothelial cells. It does not, however, have to do with the ability to produce MT in the testes, as genetic background dictates sensitivity to cadmium-induced testicular necrosis more than the absence of MT as, for example, in genetically engineered MT-null mice [19]. The testicular lesions induced by cadmium rapidly proceed to testicular degeneration, atrophy and loss of function, particularly with regard to testosterone production, and circulating testosterone is reduced almost to the level seen with castration [21]. A loss of androgen producing interstitial cells occurs with cadmium-induced necrosis. The loss of androgen production has several important consequences. First, the negative feed-back loop through hypothalamic/pituitary axis that modulates circulating androgen by altering testicular production, is essentially lost which, in turn, causes the over-production of luteinizing hormone (LH). The degenerate testicular remnant tissue apparently still contains some interstitial cells, although they appear largely dysfunctional. These remaining interstitial cells are then overstimulated

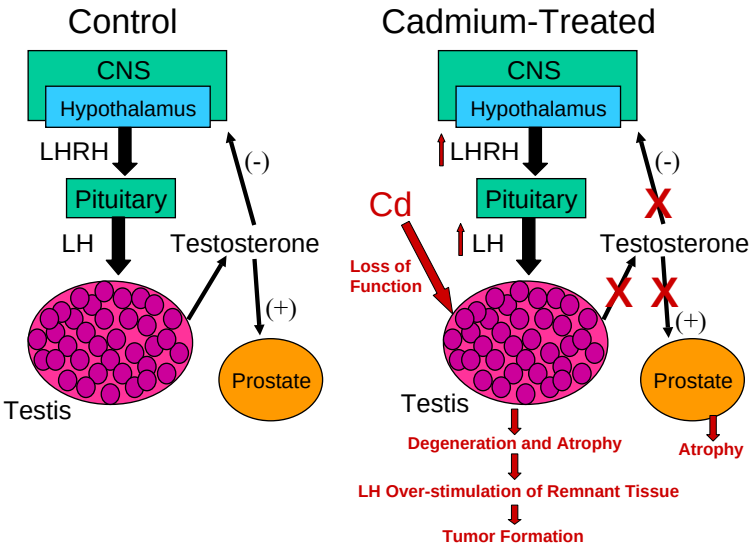

Fig. 1. Proposed mechanism for cadmium carcinogenesis in the rodent testes. See text for full description. Abbreviations: Cd, cadmium; CNS, central nervous system, LH, luteinizing hormone; LHRH, luteinizing hormone releasing hormone.

by LH, proliferate and finally form tumors. These tumors cells, although made up of cells that normally produce testosterone, are typically poor producers of androgens, so LH stimulation remains high despite proliferation of these remnant cells. A similar mechanism is thought to apply to spontaneously occurring testicular interstitial cell tumors in aging rats, where senescence reduces androgen producing capacity in interstitial cells [20,21]. In fact, supplementation with constant release testosterone preparations to produce circulating levels comparable to normal abolishes both cadmium-induced and spontaneously occurring testicular interstitial cell tumors in rats [21]. Testosterone supplementation does not alter the chronic degenerative effects of cadmium in the testes, which are secondary to the initial necrotic lesions [21]. So the underlying pathology is present but the tumors are not formed [21]. Similar mechanisms have been observed for a variety of non-genotoxic agents that induce these benign tumors [20]. As a secondary effect, the loss of testicular androgen production after cadmium exposure withdraws support for accessory sex tissues, including the prostate, which likely accounts for the loss of tumor response at high doses of cadmium in the prostate [21].

The interplay between the effects of cadmium on the testes and the metal's ability to produce other tumors (i.e. prostatic tumors) provides an interesting lesson in experimental design that deserves special mention. The testicular lesions and tumors are induced by parenteral cadmium at doses that could be considered the maximum tolerated dose (MTD) in rats, as based on survival and minimal weight loss [4,9,21]. In fact, these testicular lesions occur with cadmium even at a dose of one-half or one-quarter of the MTD [4,9,21]. Thus, a dose–response study designed that used the MTD, one-half of the MTD and one-quarter of the MTD, which is a common design, would produce testicular tumors, which likely have little to do with human exposure, and fail to produce androgen-dependent prostatic tumors, which may well be relevant to humans. So using high doses, although they may be tolerated, may not always fully reveal the carcinogenic potential of an agent. Wide range dose–response testing, that includes several lower doses, is always advisable.

7. Possible mechanisms defined in in vitro model systems of cadmium carcinogenesis

Many cellular model systems have been utilized to define the potential molecular events that are associated with the initiation phase of cadmium

carcinogenesis. It should be kept in mind that there is, in general, a significant bias towards publication of positive results with in vitro work. This is not to say that work in vitro is invalid, just that negative results, that may have important bearing on defining mechanisms of carcinogenesis, are rarely published. There is also the tendency to use higher concentrations of a toxicant if an effect is not observed at a lower level, particularly if cell death is not a considered as a qualifying factor in determining validity of the results. Thus, in analysis of in vitro data one must carefully look at the model system and concentrations used for an effect. For instance, cadmium-induced molecular events in tumor cell lines, which may be already malignantly transformed, may not reveal events important in tumor initiation. In addition, the results of in vitro studies that use concentrations of cadmium that would kill the majority of the cells present, yet produce a response purportedly linked to the carcinogenic process, need to be objectively analyzed. A dose that killed the majority of cells present in a human or animal would have no bearing on carcinogenesis, as survival is a critical aspect of cancer development. So experiments with in vitro model systems should be carefully designed in order to produce relevant data.

The molecular mechanisms of cadmium carcinogenesis are unknown. In this regard, there is no real reason to assume that cadmium acts the same way in all target tissues, and it is quite plausible that multiple, target tissue-specific mechanisms may apply. In vitro model systems should be designed with this in mind. On the molecular/cellular level, cadmium can cause a wide variety of geneotoxic and epigenetic effects in various model systems. However, the relevance of the models should always be a major concern in assigning plausibility to a given event as being etiologically important in vivo.

As a generalized basis of carcinogenesis, some metals can directly bind DNA or form crosslinks between DNA strands or between the surrounding protein and DNA [28]. These lesions could act as a prelude to mutation. However, cadmium binds only weakly to DNA in ex vivo systems [38], and there are many other cellular bioligands that cadmium will bind with very high affinity, including, for instance, MT [1,3]. In fact, cadmium exposure will activate the MT gene at the transcriptional level, resulting in a marked increase in the cellular content of this high affinity cadmium binding protein [1,3]. Indeed, it would seem quite difficult for an atom of cadmium to successfully negotiate the vast array of cellular binding sites, many with high affinity, presented to it prior to reaching DNA. This probably makes direct attack by cadmium on DNA leading to mutation a unlikely scenario.

Many metallic carcinogens can produce reactive oxygen species, such as nickel and chromium, which could become a indirect source of genotoxicity after attack on DNA [28,39]. Radical attack on DNA could produce altered bases which could lead to mutation and eventually tumor development [28,39]. However, cadmium, unlike many carcinogenic metals [28,39], does not participate in Fenton-type chemical reactions which produce reactive oxygen species capable of attack on DNA. Cadmium does induce oxidative stress [1], including lipid peroxidation. The exact basis of this production is unclear and may be indirectly due to the release of Fenton-type metals displaced by cadmium from their normal cellular binding sites. In the case of displacement, again getting the cadmium-displaced metal to where it could reasonable be expected produce a radical capable of attack on DNA could present a challenge. Furthermore, the production of oxidative stress by cadmium often can require high concentrations of the metal, although it can be exacerbated by manipulation of cellular redox status, such as by depletion of glutathione [40]. This might predispose a certain portion of a given cell population to cadmium-induced secondary reactive oxygen species production. Oxidative stress related genes are indeed activated by cadmium concurrently with apoptotic response in rat lung epithelial cells [41]. However, the concentrations of cadmium required to activate oxidative response genes eventually causes apoptosis in over 50% of the cells [41]. This does not mean a that there may be a small sub-population of cells that could be uniquely sensitive to cadmium-induced oxidative stress, and certainly inflammation would be an additional source of oxidative stress after cadmium inhalation [42]. This sub-population would need to have both a unique sensitivity and the ability to survive the cadmium insult. Therefore, although indirect mutational events from cadmium-induced reactive oxygen species are plausible, they have not been definitively established as the primary mode of action.

In fact, although cadmium can produce genotoxic and mutagenic events, these generally require high concentrations of the metal [4,9,43]. For instance, in a survey of four distinct cell lines, cadmium concentrations that were genotoxic, as assessed by DNA strand damage or DNA–protein crosslinks, in all cases also completely arrested cell growth [43]. This makes it unlikely that mutational events arising from these DNA lesions [43] would be sustained in the population and passed on by subsequent cell division. In a study using a shuttle-vector mutagenicity assay with host human Ad293 cells transfected with a pS189 vector that had been treated with 5-methylchrysene-1,2-dihydrodiol 3,4-epoxide (5-MCDE; an organic carcinogen producing bulky DNA adducts), cadmium treatment of host cells had little or no effect on mutations generated with the 5-MCDE-treated vector [44]. There was a significant increase in mutation frequency of cadmium exposed Ad293 cells transfected with untreated pS189 vector, including base substitutions and insertions/deletions, but there was no obvious effect on the spectrum or type of mutations that occurred [44]. This is quite different from the rather distinctive spectrum of mutations (large deletions, G:C to T:A transversions and G:C to A:T transitions) induced by reactive oxygen species in mammalian cells [45,46]. In a study investigating the role of reactive oxygen species in cadmium induction of mutations of the *hprt* gene in hamster cells, although the metal increased mutations and mutation frequency was reduced by catalase inhibition, analysis of the mutants generated revealed nearly 50% of cadmium-induced base substitutions occurred at T:A base pairs [47], which again indicates no preferential increase occurred in the mutations that are characteristic for reactive oxygen species [45,46]. Alternatively, cadmium can inhibit repair of DNA [48], which could be an indirect source of mutational events. Together with upregulation of mitogenic signaling, perturbed DNA repair and the resulting indirect genotoxicity could be key events in carcinogenesis [49]. Thus, although some mutations may be produced by cadmium, exactly how they may be produced is unknown and their impact on cadmium-induced carcinogenic initiation is unclear. In fact, a recent systematic review of the available studies concerning the cytogenetic effects of cadmium observed in exposed human populations found no clear association between cadmium exposure and any cytogenetic endpoint [50]. The absence of compelling data in humans [50] weakens the plausibility that cadmium genotoxicity is the primary mechanism by which the metal is carcinogenic.

Over the years, it has become quite clear that mutagenesis is not the only mechanism by which carcinogens can produce an inheritable alteration in cellular phenotype which in essence constitutes carcinogenic initiation [51,52]. Often termed epigenetic mechanisms of carcinogenesis [51], the fact that agents may act in this fashion makes them no less important as carcinogens. Epigenetic mechanisms may well apply with regard to the initiation phase of cadmium carcinogenesis. Cadmium exposures can effect cell proliferation, differentiation, apoptosis, cell signaling and a variety of other cellular activities that could have direct or indirect bearing on carcinogenesis. Such mechanisms could include aberrant gene expression, errors in DNA methylation, blockage of apoptosis, disruption of differentiation, etc. These events could result in carcinogenic transformation by cadmium in the absence of direct or indirect cadmium-induced genetic damage, and would be consistent with the generally poor ability of the metal to produce mutations at survivable dosages.

Cadmium can cause the aberrant activation of the expression of a wide variety of genes [49,53]. Although the mechanism of this aberrant expression is not always known, cadmium clearly activates transcription factors that normally require zinc, such as with the MT gene [1,3]. With regard to carcinogenesis, cadmium can activate oncogenes or genes associated with cell proliferation, such as c-*myc*, c-*jun* or c-*fos*, both in vivo or in vitro [54–59]. This activation may well enhance proliferation in a cell population and, assuming a basal level of cells with chemically or spontaneously damaged DNA, this could enhance the colonal expansion of such damaged cells. The ability of zinc to block cadmium-induced over-expression of c-*myc* or c-*jun* [54] could be seen as consistent with its ability to block cadmium-induced tumor formation in several tissues [4,9]. In addition, translation elongation factor-1 and 3, which are key components the cellular translation machinery, appear to act as cadmium-responsive oncogenes which are over-expressed in BALC/c-3T3 cells transformed by cadmium [60,61]. Cadmium can also induce up-regulation of signaling pathways resulting in increased mitogenesis, as, for instance, with AP-1 and MAP kinases [62]. The

suppression of DNA repair, as observed with cadmium [42,48], could potentially expand the population of cells with damaged DNA that is allowed to move forward through the next cycle of cell division. In fact, Hart et al. [42] have observed that lung alveolar cells adapted to cadmium show reduced DNA repair capacity. Thus, cadmium can enhance cellular proliferation, possibly through multiple mechanisms, which could help fix genetic errors that go unrepaired because of adaption to cadmium. Enhanced proliferation may also assist in the by-pass of apoptosis (see below) and thereby cause error accumulation.

As a model of its oncogenic properties in vivo, cadmium exposure can induce transformation of a variety of cells in vitro. In these sorts of studies, normally non-tumorigenic cells are exposed to cadmium, and their transformation is gauged by altered morphology, loss of contact inhibition, production of tumors upon inoculation into mice, etc. Recently, cadmium was shown to induce malignant transformation in human prostate epithelial cells [15,16], which is important because it shows cadmium can directly affect this cell population and fortifies the concept that it could be a target cell population in vivo in humans. In addition, genomic expression analysis of such transformants many allow development of a genetic signature of cadmium carcinogenesis, at least in the prostate, in a fashion similar to genomic analysis of hepatotoxicants [14]. This signature could then potentially be applied to human prostate tumors to define a possible etiological role for cadmium. Cadmium can also transform rodent prostate cells as well as other rodent cells [27,57,59,63]. In general, cellular transforms studies can assist in the definition of potential mechanisms through the determination alterations in genotype in cells displaying a carcinogen-induced altered phenotype. Cadmium-induced transformation has been shown to be associated with oncogene activation in several cases [57,59]. Recently, cadmium-induced transformation of rodent liver cells was found to be associated with errors in DNA methylation [64] that are often linked with aberrant gene expression during carcinogenesis [52]. This includes genomic DNA hypomethylation possibly due to cadmium inhibition of DNA methyltransferase in the early stages of exposure [64]. Other work indicates cadmium is an effective inhibitor of nuclear DNA methyltransferase isolated from rats [65].

Additional work indicates cadmium may reduce the number of gap junctions and inhibit intercellular communications concurrently with enhancing proliferation [66], characteristics that are consistent with a tumor promoting capability. It is thought that cadmium-induced disruption of E-cadherin dependent cell-to-cell junctions can trigger beta-catenin-mediated oncogene activation in epithelial cells [67]. The resulting enhancement of proliferation could contribute to tumor formation while the loss of cell-to-cell contacts could allow uninhibited growth that could contribute to both tumor promotion and progression [67].

Apoptotic cell death is perhaps best viewed as an ongoing, normal process in the control of cell populations and acts to eliminate cells with damaged genetic material [68]. In this regard, chemically-induced apoptosis can be very effectively blocked by cadmium [69,70], and this may involve inhibition of caspase-3 [70], a key enzyme in the dedication of a cell to apoptosis. The human prostate epithelial cell line RWPE-1, once transformed by cadmium, shows a marked resistance to apoptosis [71]. This acquired apoptotic resistance is likely through a global decrease in caspase expression, together with a decrease in the production of the pro-apoptotic regulatory protein, Bax and a corresponding over-expression of anti-apototic protein, Bcl-2 [71]. Early events after cadmium exposure in RWPE-1 cells indicate cadmium acts to select for apoptotic-defective cells [56], a factor which could clearly contribute to tumor formation. Interestingly, it has been shown that dsyplastic foci induced by cadmium exposure in the rat prostate show diminished apoptosis [72]. Others have found that cadmium-adapted alveolar cells show a significantly attenuated apoptotic response to oxidant-induced apoptosis [42]. So with cadmium adaption or transformation, the normal apoptotic response can be hindered. This suppression of apoptosis could presumably facilitate aberrant cell accumulation, allowing cells to survive that would otherwise not normally pass apoptotic checkpoints. In this fashion, the survival of pre-neoplastic or early neoplastic cells induced by cadmium could be favored and, with expansion of this cell population, this could ultimately result in tumor development [42,56]. Again, enhanced proliferation would only exacerbate the effects of perturbed apoptosis. Thus, for cadmium,

disorders of cell accumulation, potentially including enhanced proliferation and disrupted apoptosis, may be important events in carcinogenesis.

Functional inhibition of proteins critical to cell cycle control as a mode of carcinogenic initiation is an interesting possibility with cadmium [73]. In this regard, in MCF7 cells acute exposure to cadmium disrupts native (wild-type) p53 conformation, which in turn inhibits p53 binding to DNA and down regulates p53-mediated transcriptional activation [73]. Acute exposure of MCF7 cells to cadmium also impairs p53 induction in response to DNA damaging agents and suppresses p53-dependent cell cycle arrest induced by gamma-irradiation [73]. It is suspected that cadmium displaces a zinc within p53 that is critical to DNA binding and, thus, to its activity as a transcription factor [73]. It is not known if this dysfunction of p53 continues with protracted exposures to cadmium, where more p53 could be synthesized, or where proteins with very high affinity for cadmium, like MT, would be produced in response to the metal.

Although most mechanistic studies have been directed at the early, initiation phase of carcinogenesis, it is clear cadmium can potentially effect other stages of the carcinogenic process. This would include tumor promotion, the stage where transformed cells are stimulated to form early tumors, and tumor progression, where early tumors are stimulated to form more aggressive, advanced malignancies. Cadmium activation of oncogenes, such as c-*myc*, and inhibition of tumor suppressor genes, such as wild-type p53 and p27, can accelerate proliferation of cells previously initiated with organic carcinogens [74]. In this way cadmium can act in vitro in a fashion similar to an tumor promoter, which may be relevant to conditions of exposure to complex mixtures of cadmium and other carcinogens, as with inhalation of tobacco smoke. Myoblastic tumor cells exposed over a long period to cadmium in vitro, upon inoculation into nude mice form more rapidly larger, more aggressive tumors that more quickly kill the host animal and show a much more malignant morphology [75]. When human fibrosarcoma cells are chronically exposed to cadmium in vitro, the exposure promotes tumor cell invasion of reconstituted membranes, a characteristic taken to correspond to enhanced tumor invasiveness or metastatic capability [76]. There is some in vitro evidence that cadmium can modify normal host tissue in an unknown fashion such that tumors can more readily invade the tissue [77]. These in vitro results are consistent with the observation of enhanced progression of tumors observed in rats given repeated doses of cadmium [29]. Thus, cadmium could potentially effect all the various stages of the carcinogenic process, including initiation, promotion and progression.

8. Summary

A clear carcinogenic potential for cadmium exists in both humans and rodents. Further efforts are necessary to define more precisely the risks of cancer from cadmium exposure and its target sites in humans. Molecular profiling of the events associated with cadmium carcinogenesis in model systems may allow development expression signatures of cadmium-induced cancers. This in turn may assist in molecular epidemiological studies that could enable a much more definitive linkage to be made between cancer causation and cadmium exposure in humans, leading to a better definition of the risks involved. The mechanisms of cadmium carcinogenesis, with the possible exception of a target site that has limited relevance to humans (i.e. the testes in rats), remain largely unknown. Beyond this, there is no real rational to the belief that cadmium acts the same way in all target tissues, and it is quite plausible that multiple, target tissue specific mechanisms apply. For instance, cancer of the lung and of the prostate, although both the same general disease, are distinctive in a multitude of characteristics, not the least of which is response to circulating hormones. On the molecular level, cadmium can cause a wide variety of genotoxic and epigenetic effects in various model systems. Cadmium's mechanisms often appears to be related, in as yet some undefined way, to zinc metabolism because zinc can either perturb or enhance cadmium carcinogenesis in many rodent model systems. As yet there is no consensus on the molecular events associated with cadmium-induced malignant transformation but, since this metal is not strongly genotoxic, epigenetic and/or indirectly genotoxic mechanisms may apply. These mechanisms could include aberrant gene expression, enhanced cell proliferation, blocked apoptosis, and altered cell signaling, all of which could result in cell transformation in the absence of direct, cadmium-induced genetic

damage. Disruption of cell accumulation, perhaps by a combination of enhanced proliferation and blocked apoptosis, may be a crucial event in cadmium carcinogenesis. Cadmium-induced disruption of DNA repair, together with increased proliferation, could also result in tumor formation. It is quite possible that the mechanism of cadmium carcinogenesis could be multi-factorial, and therefore difficult to attribute to a single molecular event. Further research is required to define the mechanism of important human carcinogen.

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