Chapter 5
Cadmium and Its Impact on Genomic Stability

Andrea Hartwig

Abstract The carcinogenicity of cadmium for humans and experimental animals has been long established, most evident for tumors in the lung and kidney, but with increasing evidence also for other tumor locations. While cadmium does not interact directly with DNA, elevated levels of reactive oxygen species (ROS), the interference with the cellular response to DNA damage including all major DNA repair systems as well as the inactivation of tumor suppressor functions appear to be of major importance, thereby increasing the susceptibility towards exogenous and endogenous DNA damage. Furthermore, the deregulation of cell growth, the resistance to apoptosis, as well as epigenetic alterations have been demonstrated in diverse experimental systems. Particularly sensitive targets appear to be proteins with zinc-binding structures, present in many DNA repair proteins, transcription factors and in the tumor suppressor protein p53. The interaction with critical thiol groups and/or the enhanced generation of ROS may also provoke an interference with cellular redox regulation of critical signaling pathways. Especially the combination of these multiple mechanisms may give rise to a high degree of genomic instability in cadmium-adapted cells, relevant not only for tumor initiation but also for later steps in tumor development.

5.1 Introduction: Epidemiology and Animal Carcinogenicity

Cadmium is a natural element of the earth's crust, but its distribution in the environment and thus human exposure is greatly influenced by industrial use and agriculture. Significant exposure occurs via inhalation at the workplace. With respect to the general population, food and tobacco smoking are the main exposure sources. Mainly

This chapter has been partly adapted with permission from Ref. [1].

A. Hartwig (✉)
Food Chemistry and Toxicology, Karlsruhe Institute of Technology (KIT), 76131 Karlsruhe, Germany
e-mail: andrea.hartwig@kit.edu

© Springer Nature Switzerland AG 2018

F. Thévenod et al. (eds.), *Cadmium Interaction with Animal Cells*,
https://doi.org/10.1007/978-3-319-89623-6_5

based on sufficient evidence for an increased relative risk of lung cancer in workers occupationally exposed to cadmium, in 1993 and 2009, cadmium and its inorganic compounds were classified by the International Agency for Research on Cancer (IARC) as carcinogenic to humans (IARC Group 1) [2–4] and 2004 as carcinogens group 1 (carcinogenic to humans) by the German MAK Commission [5]. Cohorts in United Kingdom, Sweden and United States have been investigated, including extensive follow-up studies [6–9], revealing elevated risks of lung cancer in most cases. Some major constraints are, however, the small number of long-term, highly exposed workers and the lack of historical data on exposure to cadmium in some studies. Furthermore, confounding factors are cigarette smoke and simultaneous exposure to nickel and arsenic [4, 5]. Supportive evidence from environmental inhalative exposure provided a study in Belgium with subjects living near three smelters when compared to subjects not exposed to elevated levels of cadmium, investigated from 1985 until 2004. Based on urinary cadmium excretion and cadmium in garden soil as exposure indicators, elevated lung cancer risks were observed in the high-exposure group [10]. With respect to other cancer sites, especially the kidney may be of elevated risk due to high and persistent cadmium accumulation, going along with nephrotoxicity close to environmental exposure levels. No elevated risks for renal cancer due to cadmium exposure were observed in a Swedish or in a British cohort study [7, 9]. Nevertheless, case control studies elucidating the relative risk of kidney cancer due to occupational cadmium exposure, which have been conducted in the United States, in Finland, in Germany and in Canada and which estimated cadmium exposure via job-exposure-matrices (JEM), observed higher incidences of renal cancer upon cadmium exposure at the workplace [11–14]. Altogether, the German MAK Commission concluded that an increased relative risk of renal cancer has to be assumed [5] and also IARC stated a positive association with respect to renal and prostate cancer [4]. Finally, human cadmium exposure may also be associated with female breast and endometrial cancer, even though these tumor locations are not definitively established [15, 16]. Regarding data from experimental animals, cadmium did not induce lung tumors in the hamster [17] but at remarkably low concentrations in rats. In the latter species in long-term inhalation studies, several cadmium compounds ($CdCl_2$, $CdSO_4$, CdS, and CdO) caused lung cancer (mainly adenocarcinomas) [18, 19]. The lowest concentration inducing primary lung carcinoma in rats (15 versus 0% in controls) was 12.5 μg Cd/m^3, even though under an unusual exposure regimen (23 h/day, 7 days per week for 18 months exposure to $CdCl_2$ aerosols) [18]. In a later study, no lung tumors were induced when the rats were exposed continuously for 18 months to CdO fumes at a concentration of 10 μg Cd/m^3, whereas 21% of the animals developed tumors when exposed to 30 μg Cd/m^3 [19]. Considering oral exposure, adequately conducted studies revealed increased incidences of large granular lymphocytes, leukemia, prostate, and testis tumors in Wistar rats (summarized in [2, 5, 20]).

5.2 Direct and Indirect Genotoxicity

5.2.1 DNA Damage, Mutagenicity, and Clastogenicity

Cadmium does not cause DNA damage in cell extracts or in isolated DNA [21]. Furthermore, cadmium is not mutagenic in classical short-term test systems. Thus, in most bacterial assays, water-soluble cadmium compounds were not mutagenic, and in standard mammalian mutagenicity tests, the induction of point mutations was usually weak and/or restricted to comparatively high concentrations. In contrast, pronounced co-mutagenic effects in combination with DNA alkylating agents and with UVC radiation were observed both in bacteria and in mammalian cells, pointing towards an interaction with the cellular response to DNA damage (see below). In contrast to the missing mutagenicity, in mammalian cells cadmium compounds provoked clastogenic effects such as chromosomal aberrations and micronuclei [2, 5, 22–24]. This was also demonstrated by the pronounced positive effects of cadmium chloride in a modified mammalian test system capable of detecting large multi-locus deletions [25]. The clastogenicity is moreover evident in vivo in exposed rodents, while evidence for chromosomal damage in cadmium-exposed humans via environmental or workplace exposure is equivocal, partly due to simultaneous exposure to other metal compounds [2, 5, 26]. Recently, a reanalysis of the relationship between cadmium exposure and micronuclei in lymphocytes of exposed workers was performed, based on five studies published so far. While two studies with high exposure showed significant increases in micronuclei, three studies gave negative results, which may be due to lower exposure levels [27].

5.2.2 Oxidative Stress

Reactive oxygen species (ROS) such as superoxide anions (O_2^-) are continuously generated not only as by-products of mitochondrial respiration, due to incomplete reduction of oxygen to H_2O but also formed in large quantities during the immune defense against invading pathogens in phagocytes via NADPH oxidase. Also, hydrogen peroxide (H_2O_2) is produced in specific cellular compartments and acts as a “second messenger” to regulate many important biological processes (for review see [28]). To enable the use of oxygen for energy production and yet to minimize oxygen-derived toxicity, a complex antioxidant network has evolved, including the scavenging of reactive species by glutathione and vitamins, the enzymatic conversion of highly reactive oxygen species to less harmful ones by superoxide dismutase, catalase, and glutathione peroxidase, and finally the repair or elimination of damaged macromolecules. However, even under normal cellular conditions, protection is not complete and for example, a measurable amount of oxidatively induced DNA damage exists in mammalian cells. Oxidative stress occurs if the equilibrium between the generation of ROS and the efficiency of their detoxification is disrupted [29]. Elevated

levels of ROS have been implicated in the initiation as well as in later steps of carcinogenicity. Especially transition metal ions play an important role in the induction of oxidatively induced DNA damage. While neither superoxide anions nor hydrogen peroxide are able to react with DNA directly, in the presence of transition metals like iron, copper, cobalt, or nickel, they are converted into highly reactive hydroxyl radicals by Fenton-type reactions. DNA damage induced by ROS includes a range of lesions like DNA base modifications, sugar lesions, DNA single- and double-strand breaks, DNA–protein crosslinks, and abasic sites (for review see [30]). Among these, several oxidatively induced DNA base modifications such as 8-oxoG have miscoding and thus premutagenic properties and therefore may act as initiators in carcinogenesis [31]. In contrast, cadmium ions are not able to participate in redox reactions under physiological conditions, yet, oxidative stress and the interference with cellular redox regulation may be of high relevance in cadmium-induced carcinogenicity. Increased levels of ROS due to cadmium exposure have been observed both in vitro and in vivo [32]. Thus, different cadmium compounds have been shown to induce DNA strand breaks and oxidatively induced DNA base modifications in mammalian cells, but effects were usually small and/or restricted to comparatively high concentrations (e.g., [33, 34]). Similarly, the induction of DNA strand breaks and chromosomal aberrations by cadmium in mammalian cells was suppressed by antioxidants and antioxidant enzymes, indicating the involvement of ROS [35–37]. A pronounced oxidative stress response was also observed on the transcriptional level after exposure to BEAS-2B and A549 cells [38]. Even though cadmium ions themselves are not redox-active, several indirect effects may account for these observations, namely the release of Fenton-reactive metal ions from metallothioneins [39], the disturbance of the mitochondrial respiratory chain [40] and the inhibition of antioxidant enzymes, such as catalase, superoxide dismutase, glutathione reductase, and glutathione peroxidase [29]. Regarding oxidative DNA damage and resulting clastogenicity in cells and in vivo, an impaired DNA repair appears to be of major relevance (see below). Thus, ROS may be involved in cadmium-induced genotoxicity, but—perhaps more important—also in later steps of cadmium-induced carcinogenicity. With respect to the latter, moderately elevated levels of ROS have been implicated in later steps of tumor formation, such as cell proliferation due to mitotic stimuli and the activation of redox-sensitive transcription factors (see below). Furthermore, due to its reactivity towards thiol groups, cadmium may interfere directly with redox-controlled signaling pathways [37, 41, 42].

5.3 Interactions with DNA Repair and Tumor Suppressor Functions

Maintenance of genetic information is essential for basically all cellular processes and for the prevention of tumor development. However, many environmental agents as well as food mutagens have been identified which compromise genetic stability by

inducing different types of DNA lesions. They include ionizing radiation, UV radiation, alkylating agents, polycyclic aromatic hydrocarbons as well as heterocyclic aromatic amines. Furthermore, the DNA is also damaged by endogenous processes, such as ROS generation due to leakage of the electron transport chain in cellular respiration [37, 43]. DNA damage interferes with DNA transcription and replication; potential consequences are cell cycle arrest, programmed cell death, mutagenesis, genomic instability, and cancer. To maintain the integrity of the genome, a complex DNA damage response network has evolved, consisting of DNA repair systems, cell cycle control, and apoptosis in case of heavily damaged DNA [44–46]. Cadmium has been shown to impair almost all major DNA repair pathways. Convincing evidence is available for its interference with nucleotide excision repair (NER), base excision repair (BER), and mismatch repair (MMR), with some information also on its impact on DNA double-strand break repair; frequently, effects were observed at comparatively low, noncytotoxic concentrations (reviewed in [47–50]). Since DNA repair systems are not only required for the repair of DNA damage induced by environmental, workplace, and food mutagens but also for the elimination of DNA lesions due to endogenous processes and to keep replication errors low, the disturbance of DNA repair processes may explain co-mutagenic effects in combination with UVC radiation, benzo[*a*]pyrene and alkylating agents on one side but may also lead to genomic instability and thus contribute to cadmium-induced carcinogenicity on the other side (for reviews see [22, 23, 49]).

5.3.1 Nucleotide Excision Repair (NER)

NER is the most versatile repair system involved in the removal of structurally unrelated bulky base adducts which cause significant helical distortions. It can be subdivided into global genome repair (GG-NER) and, as a sub-pathway, transcription-coupled nucleotide excision repair (TC-NER), which removes preferentially transcription-blocking bulky DNA lesions. At least 30 different proteins and enzymes are required in mammalian cells, including those which are defective in patients suffering from the DNA repair disorder Xeroderma Pigmentosum (XP) complementation groups A through G. The most crucial step is the damage recognition, followed by the incision at both sides of the lesion and the repair polymerization leading to the displacement of the damaged oligonucleotide; repair is completed by the ligation of the repair patch (for reviews see [44–46, 51]). Cadmium has been shown to inhibit GG-NER in several studies and with respect to different DNA damaging agents. Thus, it interfered with the removal of benzo[*a*]pyrene- and UVC-induced DNA lesions in cultured mammalian cells [34, 52, 53]. As one underlying mechanism, an interaction with zinc-binding proteins has been identified. They comprise a family of proteins where zinc is complexed to four cysteine and/or histidine residues, folding a protein domain mostly involved in DNA-protein- or protein–protein interactions [54]. First discovered in transcription factors, similar structures have been identified in DNA repair proteins and tumor suppressor proteins like p53.

Examples for DNA repair proteins with zinc-binding structures include the bacterial formamidopyrimidine-DNA glycosylase (Fpg) involved in the removal of oxidative DNA base modifications and the mammalian Xeroderma Pigmentosum group A protein (XPA) essential for the formation of the DNA damage recognition complex during NER (reviewed in [55, 56]). In subcellular test systems, cadmium diminished DNA binding of XPA to an UVC-irradiated oligonucleotide [57, 58]. One molecular mechanism related to the inactivation of zinc-binding proteins appears to involve the displacement of zinc by cadmium, as evident from the reversal of cadmium-induced protein inactivation by excess of zinc as well as from structural investigations of XPA or a peptide resembling the zinc-binding domain of XPA [57–60]. Detailed studies in cadmium-treated A549 cells revealed an impaired assembly/disassembly of the DNA damage recognition proteins XPC and XPA at the repair complex after UVC irradiation [34].

5.3.2 *Base Excision Repair (BER)*

In contrast to the damage recognition complex in NER that detects a rather broad spectrum of DNA lesions, BER is initiated by glycosylases, which act specifically on one or few substrates. BER is mainly responsible for the removal of different types of endogenous DNA damage, including oxidative DNA base modifications like 8-oxoguanine (8-oxoG). This process generates abasic (AP) sites, which are further processed in a multistep process with slight differences depending on the type of damage [43–45, 51]. Regarding the impact of cadmium on this repair pathway, low concentrations of cadmium inhibited the activity of the bacterial Fpg [61] and disturbed the repair of oxidatively induced DNA base damage induced by visible light as well as DNA alkylation damage in mammalian cells [33, 62]. When compared with the induction of DNA base modifications such as 8-oxoG, inhibitory effects on the repair of this lesion were observed at much lower cadmium concentrations. This has been observed by direct comparison in HeLa cells: While the induction of DNA strand breaks by cadmium was restricted to 10 μM and higher, the removal of oxidatively induced DNA base modifications by visible light and recognized by the bacterial Fpg was inhibited starting at 0.5 μM cadmium, yielding complete inhibition at 5 μM, a noncytotoxic concentration in this test system [33]. With respect to isolated DNA repair enzymes, an inhibition of the murine 8-oxoguanine DNA glycosylase 1 (mOgg1), an enzyme responsible for recognition and excision of the premutagenic 8-oxoG during BER, as well as of 8-oxodG 5'triphosphate pyrophosphohydrolase (8-oxo-dGTPase), required for the removal of 8-oxo-dG from the deoxynucleotide pool, by cadmium have been described [63, 64]. Also, cadmium has been shown to inhibit the activity of the human 8-oxoguanine glycosylase (hOGG1) in mammalian cells [65–67]. Different mechanisms may be responsible, based on different experimental results, including the inactivation of the enzyme as such [66] or the diminished DNA binding of the zinc finger containing transcription factor SP1 to the OGG1 promotor [68], presumably due to the displacement of zinc by cadmium [69].

Inhibition of the repair of oxidatively induced DNA damage is also evident in vivo: When investigating, for example, the impact of cadmium on rat testis, a target organ for cadmium carcinogenesis, a gradual decrease in testicular 8-oxo-dGTPase activity was observed, accompanied with progressive increase of 8-oxo-dG levels in testicular DNA [70]. Therefore, increases in oxidatively induced DNA damage in vivo may at least in part be due to the repair inhibition and accumulation of endogenously induced DNA lesions. One other enzyme involved in DNA damage signaling, apoptosis and BER is poly(ADP-ribose) polymerase 1 (PARP-1). It contains three zinc fingers in its DNA binding domain involved in the recognition of DNA breaks and the subsequent synthesis of poly(ADP-ribose) [71, 72]. In HeLa cells, H_2O_2-induced PARP activity was decreased by cadmium chloride [73].

5.3.3 Mismatch Repair (MMR)

One other DNA repair system of particular relevance for maintaining genomic stability is MMR. This evolutionary conserved pathway is responsible for the repair of mismatched normal bases after DNA replication, contributing significantly to the extraordinary fidelity of DNA replication. Cells deficient in MMR exert a "mutator phenotype", in which the rate of spontaneous mutations is greatly elevated. Also, microsatellite instability (MSI) is a hallmark of MMR deficiency. Defects in MMR are associated with an increased risk of different types of cancer, including hereditary human colorectal cancer. The MMR system also plays a key role in cell killing in response to alkylating agents, and MMR deficient cells are about 100 times more resistant to the cytotoxicity of alkylating agents [74–77]. Finally, MMR also participates in the DNA damage response (DDR) system by activating ATM and ATR, which regulate cell cycle control and apoptosis upon elevated levels of DNA damage [78]. With respect to cadmium, exposure towards low concentrations resulted in pronounced hypermutability in yeast. Furthermore, in extracts of human cells, cadmium inhibited at least one step leading to mismatch repair [79]. Since then, different studies demonstrated the interference by cadmium with proteins involved in the initial step of MMR, i.e., damage recognition by MSH2-MSH6 and MSH2-MSH3. Also, the induction of MSI was demonstrated in mice testis [80]. As underlying mechanisms, cadmium affected ATP binding and hydrolysis of MMR enzymes, reducing their DNA binding activity and their ability to discriminate between mismatched and matched DNA base pairing in isolated systems and in mammalian cells in culture [48, 81, 82]. Furthermore, the induction of MSI concomitantly with elevated levels of oxidatively induced DNA damage has recently been shown in human colorectal cancer cells and in zebrafish at sublethal cadmium concentrations; interestingly, in both systems, N-acetyl-L-cysteine (NAC) suppressed cadmium-induced MSI, thus linking MMR inhibition to oxidative stress [83].

5.3.4 DNA Double-Strand Break Repair (DSB)

Only little is known about the impact of cadmium on DNA double-strand break repair. Current evidence suggests that cadmium leads to the formation of DSB and inhibits their repair via nonhomologous end-joining (NHEJ) via interaction with the DNA-PK protein, leading to an over-activation of the MRE11-dependent repair pathway which in turn may favor more misrepair [84]. The delay of NHEJ upon γ-irradiation was confirmed in another study; here, the authors demonstrated an altered phosphorylation of DNA-PKcs as well as reduced expression of XRCC4 and Ligase IV [85]. Also, a significant downregulation of genes coding for ATM and BRCA1 associated with DSB repair was observed via high-throughput RT-qPCR [38]. Finally, when assessing the impact of several metal compounds on DNA double-strand break repair outcomes, exposure towards cadmium was shown to increase mutagenic, nonallelic recombination [86].

5.3.5 P53 Tumor Suppressor Functions

Besides DNA repair systems, further DNA damage responses are activated upon genotoxic stress in mammalian cells. They include cell cycle control mechanisms, increasing the time for DNA repair, as well as apoptosis eliminating heavily damaged cells. The DNA damage response is strictly coordinated, for example by the tumor suppressor protein p53. P53 regulates cell cycle control and apoptosis by several coordinated pathways and thus exerts pronounced impact on the processing of DNA damage and on genomic stability [87]. Cadmium has been shown to interfere with structure and function of p53, but opposite effects have been reported. In some studies, a stabilization of p53 through phosphorylation followed by the induction of the p53-mediated stress response was observed [88–90]; others demonstrated an inactivation of p53 via structural changes [34, 91]. P53 contains a zinc-binding structure in its DNA binding domain, essential for its tumor suppressor functions and rendering the protein redox-sensitive. Exposure of either the isolated p53 protein or human breast cancer MCF7 cells to cadmium resulted in the disruption of the zinc-binding structure, yielding a so-called "mutant" conformation; consequences were the inhibition of DNA binding and the inhibition of the activation of p53 target genes including p21. Furthermore, suppression of the p53-mediated cell cycle arrest in response to DNA damage induced by γ-irradiation was observed [91]. Similar effects were demonstrated in A549 human lung tumor cells, where CdO and $CdCl_2$ induced structural alterations of the zinc-binding domain of p53, followed by diminished induction of the p53 regulated nucleotide excision repair gene XPC and diminished removal of UVC and benzo[*a*]pyrene induced DNA damage [34]. Thus, it appears that cadmium disrupts the zinc-binding structure of p53; whether or not this is due to the displacement of zinc is currently not clear.

5.4 Impact on Gene Expression Related to Genomic Instability and Deregulation of Cell Proliferation

Cadmium interacts with the expression of a large number of genes, including stress response genes and immediate early response genes. Major stress response genes induced by cadmium are those involved in the synthesis of metallothionein (MT), those encoding heat shock proteins, glutathione (GSH) synthesis and homeostasis and those mediating the oxidative stress response [23, 24, 42, 92–94]. Recently, the impact of cadmium was investigated via a high-throughput RT-qPCR test system specifically designed for the parallel and quantitative analysis of 95 selected genes crucial for genomic stability, including stress response as well as DNA repair, cell cycle control, apoptosis, and mitotic signaling. Gene expression analyses in cadmium-treated adenocarcinoma A549 and epithelial bronchial BEAS-2B cells revealed distinct dose- and time-dependent and also cell-type specific gene expression patterns, including the induction of genes coding for metallothioneins, the oxidative stress response, cell cycle control, mitotic signaling, and apoptosis. Interestingly, while genes coding for the DNA damage response were induced, distinct DNA repair genes were downregulated at the transcriptional level. Thus, this approach provided a comprehensive overview on the interaction by cadmium with distinct signaling pathways, also reflecting molecular modes of action in cadmium-induced carcinogenicity on the functional level, such as inhibition of DNA repair and tumor suppressor reactions [38]. On the molecular level, specific interactions with transcriptions factors, an interference with cellular redox regulation [42] as well as epigenetic alterations appear to be most relevant. With respect to transcription factors, cadmium exposure may lead to their activation or inactivation, depending on the actual transcription factor under investigation. Thus, c-fos and c-jun are overexpressed in cadmium-transformed cells; they constitute the AP-1 transcription factor, activating several genes involved in cell growth and division. Other transcription factors activated by cadmium are NF-κB and Nrf2. On the other hand, a suppression of the transcription factor SP1 has been observed in cadmium-treated cells (reviewed in [93, 94]). In support of a role for a redox-mediated mechanism in cadmium-induced activation of the MAPK-pathway, cadmium was shown to inhibit serine/threonine phosphatases 2A (PP2A) and 5 (PP5), leading to the activation of Erk1/2 and JNK. This effect was inhibited by N-acetyl-L-cysteine (NAC), and resembled the effects induced by hydrogen peroxide; therefore, the authors interpreted this effect being due to the enhanced generation of ROS [95, 96]. Nevertheless, since these phosphatases contain critical cysteine residues [97], the impact of cadmium may also be explained by a direct reaction with these cysteines. One other example is the cadmium-induced activation of Nrf2 [98, 99]. The Kelch-like ECH-associated protein (Keap1) serves as negative regulator of Nrf2. It contains critical cysteine residues as redox sensors; their oxidation results in the release of Nrf2 from the Keap1/cullin-3 E3-ubiquitin ligase (cul3) complex, preventing Nrf2 degradation and allowing for Nrf2 nuclear translocation. Additionally, Nrf2 contains a conserved cysteine located in the DNA binding domain (Cys-514) redox-regulated by Ref-1. After treatment of mouse embryo fibroblasts

(MEF) with cadmium, Nrf2-Keap1 were stabilized in the cytoplasm and translocated to the nucleus, where the components dissociated. This was followed by the induction of the ARE-dependent expression of HO-1 in MEF cells and in a respective reporter gene assay [98].

In addition to directly stimulating mitogenic signals, cadmium also inhibits negative controls of cell proliferation, for example, by inactivation of p53 (see above) [91]. Furthermore, long-term treatment of prostate epithelial cells resulted in cadmium-induced malignant transformation; transformed cells exerted an acquired resistance to apoptosis, which appeared to be linked to an increase in the antiapoptotic action of Bcl-2 that perturbs the JNK signal transduction pathway [100]. One other aspect related to cadmium-induced alterations in gene expression consists in epigenetic changes. Three levels of interaction appear to be relevant, namely interference with DNA methylation patterns, histone modifications and miRNAs, all of which may be affected by cadmium in a time- and cell-type specific manner (for review see [101]). The mechanism in cadmium-induced epigenetic alterations has been further investigated in a rat liver epithelial cell line as a model for cadmium-induced malignant transformation. Cadmium provoked the suppression of ApoE, a key factor of cell invasion during malignant transformation via 5-aza-2'-deoxycytidine-sensitive hypermethylation of the regulatory region of ApoE, together with the suppression of liver X receptor α (LXRα), a transcriptional regulator for ApoE [102].

5.5 Mechanistic Considerations

Cadmium appears to be involved in tumor initiation as well as in later steps of tumor development. Regarding genotoxicity, direct interactions of cadmium ions with DNA are of little importance, as evident also by the lack of relevant mutagenicity in classical bacterial and mammalian test systems. However, indirect genotoxicity leads to clastogenicity and elevated levels of oxidative DNA damage; in this context, interactions with proteins are of high significance. Especially the DNA repair inhibitions but also altered cell proliferation and/or diminished cell cycle control have frequently been observed at low, noncytotoxic concentrations of cadmium, pointing towards particular sensitive targets of cadmium ions. Relevant mechanisms include elevated levels of ROS, interactions with homeostasis and cellular functions of essential metal ions like zinc, calcium, and iron and the interference with cellular redox regulation.

Current evidence suggests that Cd^{2+} is the ultimate damaging species since water-soluble and particulate water-insoluble cadmium compounds exert similar effects in experimental cell culture systems and in experimental animals. Thus, both cadmium chloride and largely water-insoluble cadmium oxide induced oxidative DNA lesions and inhibited the removal of benzo[*a*]pyren-induced DNA lesions. Furthermore, cadmium-induced conformational changes of p53 were comparable when applying cadmium chloride or cadmium oxide. Repair inhibitory effects were strongly correlated with cadmium levels in the nuclei, indicating the bioavailability of both compounds [34]. While water-soluble cadmium compounds are taken up via ion

channels [103], particulate cadmium compounds may be taken up by phagocytosis and, due to the low pH, may dissolve gradually in lysosomes, yielding high concentrations of cadmium ions in the cytoplasm and in the nucleus, as described in detail for nickel compounds [104, 105]. This assumption is also supported by inhalation studies where water soluble cadmium sulfate, poorly water-soluble cadmium oxide and cadmium sulfide pigment with intermediate water solubility induced lung tumors in rats [106].

Since cadmium ions exert high affinity towards SH groups, potential targets are zinc-binding structures frequently found in transcription factors as well as in DNA repair and tumor suppressor proteins [55, 56]. As indicated above, one molecular mechanism related to the inactivation of zinc-binding proteins appears to involve the competition between zinc and cadmium. Compared to Zn^{2+}, the radius of Cd^{2+} ion is larger, but still, cadmium ions can substitute for zinc ions in many enzymes and transcription factors [24, 107]. Considering the example of the nucleotide excision repair protein XPA or a peptide resembling the zinc-binding domain of XPA, binding constants for cadmium were about 1000-fold higher as compared to zinc. Replacement of zinc by cadmium yielded only minor structural alterations [59, 60], but provoked a pronounced disturbance of XPA within the assembly and disassembly of the nucleotide excision repair complex [34]. In addition to direct interactions with DNA repair proteins, cadmium may disturb DNA repair processes via interaction with zinc-containing transcription factors. Thus, human OGG1 (hOGG1), a glycosylase responsible for recognition and excision of the premutagenic 8-oxoG during BER in mammalian cells, was inhibited by cadmium [108]. Even though hOGG1 contains no zinc-binding motif itself, its inhibition was shown to be due to diminished DNA binding of the zinc finger containing transcription factor SP1 to the *OGG1* promotor [68], presumably due to the displacement of zinc by cadmium [69]. Also, a downregulation of DNA repair genes like *XPC* has been observed recently in cultured cells [34] and in vivo in mouse testes [109], which may be due to a disturbed transcriptional activity of p53. However, whether or not the inactivation of the respective zinc-binding repair proteins are mediated via displacement of zinc by cadmium or whether interactions with other protein structures, such as critical thiols outside the zinc-binding structure, are relevant for the observed inhibitions has to be further elucidated. Furthermore, systematic investigations on the relevance of these mechanisms for in vivo situations are still missing. Multiple mechanisms appear to be involved in cadmium-induced alterations of gene expression. With respect to the induction of metallothionein, cadmium ions bind directly to the transcription factor MTF1 [110]. In some other cases, specific interactions have been identified. With respect to epigenetic effects, cadmium inhibited DNA-(cytosine-5) methyltransferase and lead to diminished DNA methylation during cadmium-induced cellular transformation, provoking augmented expression of cellular proto-oncogenes [111]. One example for a direct competition with calcium concerns the cadherin-mediated cell–cell adhesion system; here, cadmium specifically displaced calcium from the protein E-cadherin and impaired the cell–cell adhesion in kidney epithelial cells [112, 113]. A fast transient increase in levels of second messengers like Ca^{2+} and inositol-1,4,5-trisphosphate by low concentrations of cadmium may be due to its binding to G-protein coupled receptors in

the plasma membrane; however, cadmium affects also intracellular signaling mediated by mitogen-activated protein kinases (MAPK) as well as cAMP-dependent and calmodulin-dependent pathways (see Chap. 3). Even though zinc-binding structures are involved in many of these pathways, at present, it is unclear whether a direct replacement of zinc is the underlying mechanism [24, 94, 114]. One hypothesis integrating many mechanistic observations consists in the interference by cadmium with the cellular redox regulation [42] (see also Chap. 3). Thus, diverse signaling pathways have been identified to be redox-regulated via reversible oxidation and reduction of thiol groups [115–117]. Cadmium has been shown to induce several redox-regulated signal transduction pathways, such as NF-κB and Nrf2, but also mitotic signaling, which may be due to the increased formation of ROS or to direct interaction with redox-sensitive cysteines in signal transduction proteins. In most cases, the molecular interactions have not been fully explored experimentally, but are subject of current research activities.

5.6 Concluding Remarks and Perspectives

In summary, cadmium-induced carcinogenicity is likely based on multiple distinct mechanisms. As opposed to direct DNA damage, interactions with proteins appear to be more relevant for carcinogenicity, and several targets have been identified such as antioxidative defense systems, DNA repair processes as well as tumor suppressor and signal transduction proteins. All these features taken alone could contribute to carcinogenicity, but most likely their combination seems to be of particular importance. Thus, long-term exposure to low concentrations of cadmium leads to adapted cells exerting increased cadmium accumulation, increased proliferation, diminished DNA repair, and cell cycle control as well as resistance to apoptosis. The outcome is a severe decrease in genomic stability, which may play an important role in cadmium-induced tumor initiation and progression (summarized in Fig. 5.1).

One important question concerns specific mechanisms explaining the organ-specificity of cadmium-induced carcinogenicity. After inhalative exposure, the lung is a plausible target organ, but other organs like kidney, prostate, breast, and endometrium may be affected as well. Since tumors in prostate, breast, and endometrium are frequently hormone-dependent one aspect addressed by several groups concerns a potential impact of cadmium on steroid hormone-dependent signaling [118]. Nevertheless, respective experimental evidence is contradictory and needs to be further explored [49]. One other key issue in cadmium-induced carcinogenicity appears to be adaptation and the role of MT. Cadmium induces several genes for cadmium and ROS tolerance such as those coding for MT, GSH synthesis and function, catalase and superoxide dismutase. Hence, a condition for prolonged cell survival in the presence of cadmium is established, which may be beneficial in terms of protection from acute cadmium toxicity, also evident from comparative studies with MT-transgenic and MT-null mice [119]. However, adaptation may be a double-edged sword, since increased MT contents lead not only to cadmium accumulation

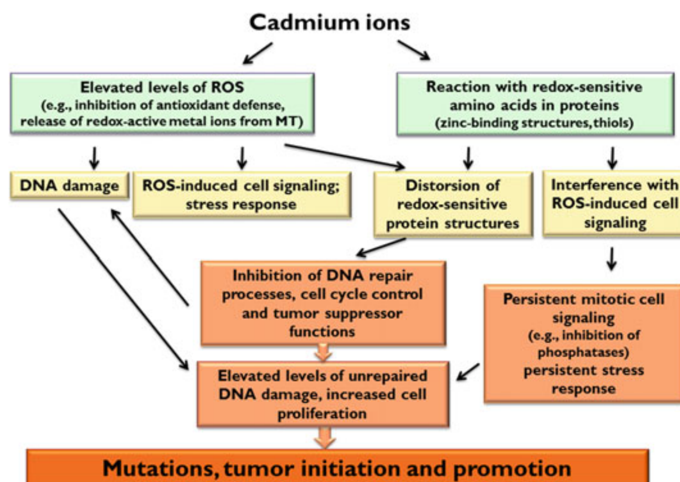

Fig. 5.1 Integrating concept of cadmium-induced carcinogenicity

and long half-lives but also to reduced DNA repair activities as well as suppressed apoptosis [92, 120].

Considering recent reports on cadmium-related carcinogenicity in different target organs under low exposure conditions, future research will have to focus on the relevance of the respective mechanisms in experimental animals and in exposed humans. Nevertheless, even though Cd blood levels are in the nanomolar concentration range and thus considerably lower as compared to low micromolar concentrations applied in most in vitro systems described above, it needs to be considered that far higher cadmium concentrations are observed for example in cadmium-exposed workers, reaching up to millimolar concentrations in the kidney cortex and high micromolar concentrations in the liver [121]. Also, indications for repair inhibition upon occupational cadmium exposure have been reported [122].

References

1. Hartwig A (2013) Cadmium and cancer. Met Ions Life Sci 11:491–507
2. IARC (1993) Beryllium, Cadmium, Mercury and exposures in the glass manufacturing industry, vol 58. Monographs for the evaluation of the carcinogenic risk to humans
3. IARC (1997) Supplement: Cadmium and Cadmium compounds. Monographs for the evaluation of the carcinogenic risk to humans
4. IARC (2012) Arsenic, metals fibres and dusts. IARC monographs 100C:121–145
5. Greim H (2006) Cadmium and its inorganic compounds. MAK Value Documentation 2006. The MAK Collection for Occupational Health and Safety 1–41
6. Stayner L, Smith R, Schnorr T, Lemen R, Thun M (1993) Lung cancer. Ann Epidemiol 3(1):114–116

7. Sorahan T, Esmen NA (2004) Lung cancer mortality in UK nickel-cadmium battery workers, 1947–2000. Occup Environ Med 61(2):108–116
8. Sorahan T (2009) Lung cancer mortality in arsenic-exposed workers from a cadmium recovery plant. Occup Med (Lond) 59(4):264–266
9. Jarup L, Bellander T, Hogstedt C, Spang G (1998) Mortality and cancer incidence in Swedish battery workers exposed to cadmium and nickel. Occup Environ Med 55(11):755–759
10. Nawrot T, Plusquin M, Hogervorst J, Roels HA, Celis H, Thijs L, Vangronsveld J, Van Hecke E, Staessen JA (2006) Environmental exposure to cadmium and risk of cancer: a prospective population-based study. Lancet Oncol 7(2):119–126
11. Pesch B, Haerting J, Ranft U, Klimpel A, Oelschlägel B, Schill W (2000) Occupational risk factors for renal cell carcinoma: agent-specific results from a case-control study in Germany. MURC Study Group. Multicenter urothelial and renal cancer study. Int J Epidemiol 29 (6):1014–1024
12. Hu J, Mao Y, White K (2002) Renal cell carcinoma and occupational exposure to chemicals in Canada. Occup Med (Lond) 52(3):157–164
13. Siemiatycki J (1991) Risk Factors for Cancer in the Workplace. CRC Press, Boca Raton, Florida
14. Kellen E, Zeegers MP, Hond ED, Buntinx F (2007) Blood cadmium may be associated with bladder carcinogenesis: the Belgian case-control study on bladder cancer. Cancer Detec Prev 31:77–82
15. McElroy JA, Shafer MM, Trentham-Dietz A, Hampton JM, Newcomb PA (2006) Cadmium exposure and breast cancer risk. J Natl Cancer Inst 98(12):869–873
16. Akesson A, Julin B, Wolk A (2008) Long-term dietary cadmium intake and postmenopausal endometrial cancer incidence: a population-based prospective cohort study. Cancer Res 68(15):6435–6441
17. Heinrich U, Peters L, Ernst H, Rittinghausen S, Dasenbrock C, König H (1989) Investigation on the carcinogenic effects of various cadmium compounds after inhalation exposure in hamsters and mice. Exp Pathol 37(1–4):253–258
18. Takenaka S, Oldiges H, Konig H, Hochrainer D, Oberdörster G (1983) Carcinogenicity of cadmium chloride aerosols in Wistar rats. J Natl Cancer Inst 70(2):367–373
19. Glaser U, Hochrainer D, Otto FJ, Oldiges H (1990) Carcinogenicity and toxicity of four cadmium compounds inhaled by rats. Toxicol Environ Chem 27:153–162
20. Huff J, Lunn RM, Waalkes MP, Tomatis L, Infante PF (2007) Cadmium-induced cancers in animals and in humans. Int J Occup Environ Health 13(2):202–212
21. Valverde M, Trejo C, Rojas E (2001) Is the capacity of lead acetate and cadmium chloride to induce genotoxic damage due to direct DNA-metal interaction? Mutagenesis 16(3):265–270
22. Beyersmann D, Hartwig A (2008) Carcinogenic metal compounds: recent insight into molecular and cellular mechanisms. Arch Toxicol 82(8):493–512
23. Filipic M (2012) Mechanisms of cadmium induced genomic instability. Mutat Res 733(1–2):69–77
24. Waisberg M, Joseph P, Hale B, Beyersmann D (2003) Molecular and cellular mechanisms of cadmium carcinogenesis. Toxicology 192(2–3):95–117
25. Filipic M, Hei TK (2004) Mutagenicity of cadmium in mammalian cells: implication of oxidative DNA damage. Mutat Res 546(1–2):81–91
26. Tapisso JT, Marques CC, Mathias Mda L, Ramalhinho Mda G (2009) Induction of micronuclei and sister chromatid exchange in bone-marrow cells and abnormalities in sperm of Algerian mice (Mus spretus) exposed to cadmium, lead and zinc. Mutat Res 678(1):59–64
27. Nersesyan A, Kundi M, Waldherr M, Setayesh T, Misik M, Wultsch G, Filipic M, Mazzaron Barcelos GR, Knasmueller S (2016) Results of micronucleus assays with individuals who are occupationally and environmentally exposed to mercury, lead and cadmium. Mutat Res 770(Pt A):119–139
28. Valko M, Jomova K, Rhodes CJ, Kuca K, Musilek K (2016) Redox- and non-redox-metal-induced formation of free radicals and their role in human disease. Arch Toxicol 90(1):1–37

29. Valko M, Rhodes CJ, Moncol J, Izakovic M, Mazur M (2006) Free radicals, metals and antioxidants in oxidative stress-induced cancer. Chem Biol Interact 160(1):1–40
30. Cadet J, Douki T, Ravanat JL (2010) Oxidatively generated base damage to cellular DNA. Free Radic Biol Med 49(1):9–21
31. Kryston TB, Georgiev AB, Pissis P, Georgakilas AG (2011) Role of oxidative stress and DNA damage in human carcinogenesis. Mutat Res 711(1–2):193–201
32. Liu J, Qu W, Kadiiska MB (2009) Role of oxidative stress in cadmium toxicity and carcinogenesis. Toxicol Appl Pharmacol 238(3):209–214
33. Dally H, Hartwig A (1997) Induction and repair inhibition of oxidative DNA damage by nickel(II) and cadmium(II) in mammalian cells. Carcinogenesis 18(5):1021–1026
34. Schwerdtle T, Ebert F, Thuy C, Richter C, Mullenders LH, Hartwig A (2010) Genotoxicity of soluble and particulate cadmium compounds: impact on oxidative DNA damage and nucleotide excision repair. Chem Res Toxicol 23(2):432–442
35. Ochi T, Ohsawa M (1985) Participation of active oxygen species in the induction of chromosomal aberrations by cadmium chloride in cultured Chinese hamster cells. Mutat Res 143(3):137–142
36. Stohs SJ, Bagchi D, Hassoun E, Bagchi M (2001) Oxidative mechanisms in the toxicity of chromium and cadmium ions. J Environ Pathol Toxicol Oncol 20(2):77–88
37. Valko M, Rhodes CJ, Moncol J, Izakovic M, Mazur M (2006) Free radicals, metals and antioxidants in oxidative stress-induced cancer. Chem Biol Interact 160(1):1–40
38. Fischer BM, Neumann D, Piberger AL, Risnes SF, Koberle B, Hartwig A (2016) Use of high-throughput RT-qPCR to assess modulations of gene expression profiles related to genomic stability and interactions by cadmium. Arch Toxicol 90(11):2745–2761
39. O'Brien P, Salacinski HJ (1998) Evidence that the reactions of cadmium in the presence of metallothionein can produce hydroxyl radicals. Arch Toxicol 72:690–700
40. Wang Y, Fang J, Leonard SS, Krishna Rao KM (2004) Cadmium inhibits the electron transfer chain and induces Reactive Oxygen Species. Free Radical Biol Med 36:1434–1443
41. Genestra M (2007) Oxyl radicals, redox-sensitive signalling cascades and antioxidants. Cell Signal 19(9):1807–1819
42. Hartwig A (2013) Metal interaction with redox regulation: an integrating concept in metal carcinogenesis? Free Radic Biol Med 55:63–72
43. Hakem R (2008) DNA-damage repair; the good, the bad, and the ugly. EMBO J 27(4):589–605
44. Camenisch U, Naegeli H (2009) Role of DNA repair in the protection against genotoxic stress. EXS 99:111–150
45. Christmann M, Tomicic MT, Roos WP, Kaina B (2003) Mechanisms of human DNA repair: an update. Toxicology 193(1–2):3–34
46. Fousteri M, Mullenders LH (2008) Transcription-coupled nucleotide excision repair in mammalian cells: molecular mechanisms and biological effects. Cell Res 18(1):73–84
47. Hartwig A (1994) Role of DNA repair inhibition in lead- and cadmium-induced genotoxicity: a review. Environ Health Perspect 102(Suppl 3):45–50
48. Giaginis C, Gatzidou E, Theocharis S (2006) DNA repair systems as targets of cadmium toxicity. Toxicol Appl Pharmacol 213(3):282–290
49. Hartwig A (2010) Mechanisms in cadmium-induced carcinogenicity: recent insights. Biometals 23(5):951–960
50. Koedrith P, Seo YR (2011) Advances in carcinogenic metal toxicity and potential molecular markers. Int J Mol Sci 12(12):9576–9595
51. de Boer J, Hoeijmakers JH (2000) Nucleotide excision repair and human syndromes. Carcinogenesis 21(3):453–460
52. Hartmann A, Speit G (1996) Effect of arsenic and cadmium on the persistence of mutagen-induced DNA lesions in human cells. Environ Mol Mutagen 27(2):98–104
53. Snyder RD, Davis GF, Lachmann PJ (1989) Inhibition by metals of X-ray and ultraviolet-induced DNA repair in human cells. Biol Trace Elem Res 21:389–398
54. Mackay JP, Crossley M (1998) Zinc fingers are sticking together. Trends Biochem Sci 23(1):1–4

55. Hartwig A (2001) Zinc finger proteins as potential targets for toxic metal ions: differential effects on structure and function. Antioxid Redox Signal 3(4):625–634
56. Witkiewicz-Kucharczyk A, Bal W (2006) Damage of zinc fingers in DNA repair proteins, a novel molecular mechanism in carcinogenesis. Toxicol Lett 162(1):29–42
57. Asmuss M, Mullenders LH, Hartwig A (2000) Interference by toxic metal compounds with isolated zinc finger DNA repair proteins. Toxicol Lett 112–113:227–231
58. Hartmann M, Hartwig A (1998) Disturbance of DNA damage recognition after UV-irradiation by nickel(II) and cadmium(II) in mammalian cells. Carcinogenesis 19(4):617–621
59. Buchko GW, Hess NJ, Kennedy MA (2000) Cadmium mutagenicity and human nucleotide excision repair protein XPA: CD, EXAFS and (1)H/(15)N-NMR spectroscopic studies on the zinc(II)- and cadmium(II)-associated minimal DNA-binding domain (M98-F219). Carcinogenesis 21(5):1051–1057
60. Kopera E, Schwerdtle T, Hartwig A, Bal W (2004) Co(II) and Cd(II) substitute for Zn(II) in the zinc finger derived from the DNA repair protein XPA, demonstrating a variety of potential mechanisms of toxicity. Chem Res Toxicol 17(11):1452–1458
61. Asmuss M, Mullenders LH, Eker A, Hartwig A (2000) Differential effects of toxic metal compounds on the activities of Fpg and XPA, two zinc finger proteins involved in DNA repair. Carcinogenesis 21(11):2097–2104
62. Fatur T, Lah TT, Filipic M (2003) Cadmium inhibits repair of UV-, methyl methanesulfonate- and N-methyl-N-nitrosourea-induced DNA damage in Chinese hamster ovary cells. Mutat Res 529(1–2):109–116
63. Bialkowski K, Kasprzak KS (1998) A novel assay of 8-oxo-2'-deoxyguanosine 5'-triphosphate pyrophosphohydrolase (8-oxo-dGTPase) activity in cultured cells and its use for evaluation of cadmium(II) inhibition of this activity. Nucleic Acids Res 26(13):3194–3201
64. Zharkov DO, Rosenquist TA (2002) Inactivation of mammalian 8-oxoguanine-DNA glycosylase by cadmium(II): implications for cadmium genotoxicity. DNA Repair (Amst) 1(8):661–670
65. Potts RJ, Watkin RD, Hart BA (2003) Cadmium exposure down-regulates 8-oxoguanine DNA glycosylase expression in rat lung and alveolar epithelial cells. Toxicology 184(2–3):189–202
66. Hamann I, König C, Richter C, Jahnke G, Hartwig A (2011) Impact of cadmium on hOGG1 and APE1 as a function of the cellular p 53 status. Mutat Res May 13 Epub ahead of print
67. Bravard A, Campalans A, Vacher M, Gouget B, Levalois C, Chevillard S, Radicella JP (2010) Inactivation by oxidation and recruitment into stress granules of hOGG1 but not APE1 in human cells exposed to sub-lethal concentrations of cadmium. Mutat Res 685(1–2):61–69
68. Youn CK, Kim SH, Lee DY, Song SH, Chang IY, Hyun JW, Chung MH, You HJ (2005) Cadmium down-regulates human OGG1 through suppression of Sp1 activity. J Biol Chem 280(26):25185–25195
69. Kothinti RK, Blodgett AB, Petering DH, Tabatabai NM (2010) Cadmium down-regulation of kidney Sp1 binding to mouse SGLT1 and SGLT2 gene promoters: possible reaction of cadmium with the zinc finger domain of Sp1. Toxicol Appl Pharmacol 244(3):254–262
70. Bialkowski K, Bialkowska A, Kasprzak KS (1999) Cadmium(II), unlike nickel(II), inhibits 8-oxo-dGTPase activity and increases 8-oxo-dG level in DNA of the rat testis, a target organ for cadmium(II) carcinogenesis. Carcinogenesis 20(8):1621–1624
71. Beneke S, Bürkle A (2007) Poly(ADP-ribosyl)ation in mammalian ageing. Nucleic Acids Res 35(22):7456–7465
72. Petrucco S (2003) Sensing DNA damage by PARP-like fingers. Nucleic Acids Res 31(23):6689–6699
73. Hartwig A, Asmuss M, Ehleben I, Herzer U, Kostelac D, Pelzer A, Schwerdtle T, Bürkle A (2002) Interference by toxic metal ions with DNA repair processes and cell cycle control: molecular mechanisms. Environ Health Perspect 110(Suppl 5):797–799
74. Hsieh P, Yamane K (2008) DNA mismatch repair: molecular mechanism, cancer, and ageing. Mech Ageing Dev 129(7–8):391–407
75. Kunkel TA, Erie DA (2015) Eukaryotic Mismatch Repair in Relation to DNA Replication. Annu Rev Genet 49:291–313

76. Modrich P (1994) Mismatch repair, genetic stability, and cancer. Science 266(5193):1959–1960
77. O'Brien V, Brown R (2006) Signalling cell cycle arrest and cell death through the MMR System. Carcinogenesis 27(4):682–692
78. Jiricny J (2006) The multifaceted mismatch-repair system. Nat Rev Mol Cell Biol 7(5):335–346
79. Jin YH, Clark AB, Slebos RJ, Al-Refai H, Taylor JA, Kunkel TA, Resnick MA, Gordenin DA (2003) Cadmium is a mutagen that acts by inhibiting mismatch repair. Nat Genet 34(3):326–329
80. Oliveira H, Lopes T, Almeida T, Pereira Mde L, Santos C (2012) Cadmium-induced genetic instability in mice testis. Hum Exp Toxicol 31(12):1228–1236
81. Lutzen A, Liberti SE, Rasmussen LJ (2004) Cadmium inhibits human DNA mismatch repair in vivo. Biochem Biophys Res Commun 321(1):21–25
82. Wieland M, Levin MK, Hingorani KS, Biro FN, Hingorani MM (2009) Mechanism of cadmium-mediated inhibition of Msh2-Msh6 function in DNA mismatch repair. Biochemistry 48(40):9492–9502
83. Wu CL, Huang LY, Chang CL (2017) Linking arsenite- and cadmium-generated oxidative stress to microsatellite instability in vitro and in vivo. Free Radic Biol Med 112:12–23
84. Viau M, Gastaldo J, Bencokova Z, Joubert A, Foray N (2008) Cadmium inhibits nonhomologous end-joining and over-activates the MRE11-dependent repair pathway. Mutat Res 654(1):13–21
85. Li W, Gu X, Zhang X, Kong J, Ding N, Qi Y, Zhang Y, Wang J, Huang D (2015) Cadmium delays non-homologous end joining (NHEJ) repair via inhibition of DNA-PKcs phosphorylation and downregulation of XRCC4 and Ligase IV. Mutat Res 779:112–123
86. Morales ME, Derbes RS, Ade CM, Ortego JC, Stark J, Deininger PL, Roy-Engel AM (2016) Heavy Metal Exposure Influences Double Strand Break DNA Repair Outcomes. PLoS ONE 11(3):e0151367
87. Hainaut P, Hollstein M (2000) p53 and human cancer: the first ten thousand mutations. Adv Cancer Res 77:81–137
88. Cao F, Zhou T, Simpson D, Zhou Y, Boyer J, Chen B, Jin T, Cordeiro-Stone M, Kaufmann W (2007) p53-Dependent but ATM-independent inhibition of DNA synthesis and G2 arrest in cadmium-treated human fibroblasts. Toxicol Appl Pharmacol 218(2):174–185
89. Chatterjee S, Kundu S, Sengupta S, Bhattacharyya A (2009) Divergence to apoptosis from ROS induced cell cycle arrest: effect of cadmium. Mutat Res 663(1–2):22–31
90. Yu X, Sidhu JS, Hong S, Robinson JF, Ponce RA, Faustman EM (2011) Cadmium induced p53-dependent activation of stress signaling, accumulation of ubiquitinated proteins, and apoptosis in mouse embryonic fibroblast cells. Toxicol Sci 120(2):403–412
91. Meplan C, Mann K, Hainaut P (1999) Cadmium induces conformational modifications of wild-type p53 and suppresses p53 response to DNA damage in cultured cells. J Biol Chem 274(44):31663–31670
92. Hart BA, Potts RJ, Watkin RD (2001) Cadmium adaptation in the lung—a double-edged sword? Toxicology 160(1–3):65–70
93. Joseph P (2009) Mechanisms of cadmium carcinogenesis. Toxicol Appl Pharmacol 238(3):272–279
94. Thevenod F (2009) Cadmium and cellular signaling cascades: to be or not to be? Toxicol Appl Pharmacol 238(3):221–239
95. Chen L, Liu L, Huang S (2008) Cadmium activates the mitogen-activated protein kinase (MAPK) pathway via induction of reactive oxygen species and inhibition of protein phosphatases 2A and 5. Free Radic Biol Med 45(7):1035–1044
96. Chen L, Liu L, Yin J, Luo Y, Huang S (2009) Hydrogen peroxide-induced neuronal apoptosis is associated with inhibition of protein phosphatase 2A and 5, leading to activation of MAPK pathway. Int J Biochem Cell Biol 41(6):1284–1295
97. Kim HS, Song MC, Kwak IH, Park TJ, Lim IK (2003) Constitutive induction of p-Erk1/2 accompanied by reduced activities of protein phosphatases 1 and 2A and MKP3 due to reactive oxygen species during cellular senescence. J Biol Chem 278(39):37497–37510

98. He X, Chen MG, Ma Q (2008) Activation of Nrf2 in defense against cadmium-induced oxidative stress. Chem Res Toxicol 21(7):1375–1383
99. Chen J, Shaikh ZA (2009) Activation of Nrf2 by cadmium and its role in protection against cadmium-induced apoptosis in rat kidney cells. Toxicol Appl Pharmacol 241(1):81–89
100. Qu W, Ke H, Pi J, Broderick D, French JE, Webber MM, Waalkes MP (2007) Acquisition of apoptotic resistance in cadmium-transformed human prostate epithelial cells: Bcl-2 overexpression blocks the activation of JNK signal transduction pathway. Environ Health Perspect 115(7):1094–1100
101. Wang B, Li Y, Shao C, Tan Y, Cai L (2012) Cadmium and its epigenetic effects. Curr Med Chem 19(16):2611–2620
102. Suzuki M, Takeda S, Teraoka-Nishitani N, Yamagata A, Tanaka T, Sasaki M, Yasuda N, Oda M, Okano T, Yamahira K, Nakamura Y, Kobayashi T, Kino K, Miyazawa H, Waalkes MP, Takiguchi M (2017) Cadmium-induced malignant transformation of rat liver cells: Potential key role and regulatory mechanism of altered apolipoprotein E expression in enhanced invasiveness. Toxicology 382:16–23
103. Thevenod F (2010) Catch me if you can! Novel aspects of cadmium transport in mammalian cells. Biometals 23(5):857–875
104. Costa M, Heck JD, Robison SH (1982) Selective phagocytosis of crystalline metal sulfide particles and DNA strand breaks as a mechanism for the induction of cellular transformation. Cancer Res 42(7):2757–2763
105. Evans RM, Davies PJ, Costa M (1982) Video time-lapse microscopy of phagocytosis and intracellular fate of crystalline nickel sulfide particles in cultured mammalian cells. Cancer Res 42(7):2729–2735
106. Heinrich U (1992) Pulmonary carcinogenicity of cadmium by inhalation in animals. IARC Sci Publ 118:405–413
107. Martelli A, Rousselet E, Dycke C, Bouron A, Moulis JM (2006) Cadmium toxicity in animal cells by interference with essential metals. Biochimie 88(11):1807–1814
108. Potts RJ, Bespalov IA, Wallace SS, Melamede RJ, Hart BA (2001) Inhibition of oxidative DNA repair in cadmium-adapted alveolar epithelial cells and the potential involvement of metallothionein. Toxicology 161(1–2):25–38
109. Zhou T, Jia X, Chapin RE, Maronpot RR, Harris MW, Liu J, Waalkes MP, Eddy EM (2004) Cadmium at a non-toxic dose alters gene expression in mouse testes. Toxicol Lett 154(3):191–200
110. Lichtlen P, Schaffner W (2001) Putting its fingers on stressful situations: the heavy metal-regulatory transcription factor MTF-1. BioEssays 23(11):1010–1017
111. Takiguchi M, Achanzar WE, Qu W, Li G, Waalkes MP (2003) Effects of cadmium on DNA-(Cytosine-5) methyltransferase activity and DNA methylation status during cadmium-induced cellular transformation. Exp Cell Res 286(2):355–365
112. Prozialeck WC, Lamar PC (1999) Interaction of cadmium (Cd(2+)) with a 13-residue polypeptide analog of a putative calcium-binding motif of E-cadherin. Biochim Biophys Acta 1451(1):93–100
113. Prozialeck WC, Lamar PC, Lynch SM (2003) Cadmium alters the localization of N-cadherin, E-cadherin, and beta-catenin in the proximal tubule epithelium. Toxicol Appl Pharmacol 189(3):180–195
114. Moulis JM (2010) Cellular mechanisms of cadmium toxicity related to the homeostasis of essential metals. Biometals 23(5):877–896
115. Brigelius-Flohe R, Flohe L (2011) Basic principles and emerging concepts in the redox control of transcription factors. Antioxid Redox Signal 15(8):2335–2381
116. Giles GI (2006) The redox regulation of thiol dependent signaling pathways in cancer. Curr Pharm Des 12(34):4427–4443
117. Ray PD, Huang BW, Tsuji Y (2012) Reactive oxygen species (ROS) homeostasis and redox regulation in cellular signaling. Cell Signal 24(5):981–990
118. Byrne C, Divekar SD, Storchan GB, Parodi DA, Martin MB (2009) Cadmium—a metallohormone? Toxicol Appl Pharmacol 238(3):266–271

119. Klaassen CD, Liu J, Diwan BA (2009) Metallothionein protection of cadmium toxicity. Toxicol Appl Pharmacol 238(3):215–220
120. Singh KP, Kumari R, Pevey C, Jackson D, DuMond JW (2009) Long duration exposure to cadmium leads to increased cell survival, decreased DNA repair capacity, and genomic instability in mouse testicular Leydig cells. Cancer Lett 279(1):84–92
121. Börjesson J, Bellander T, Jarup L, Elinder CG, Mattsson S (1997) In vivo analysis of cadmium in battery workers versus measurements of blood, urine, and workplace air. Occup Environ Med 54(6):424–431
122. Hengstler JG, Bolm-Audorff U, Faldum A, Janssen K, Reifenrath M, Gotte W, Jung D, Mayer-Popken O, Fuchs J, Gebhard S, Bienfait HG, Schlink K, Dietrich C, Faust D, Epe B, Oesch F (2003) Occupational exposure to heavy metals: DNA damage induction and DNA repair inhibition prove co-exposures to cadmium, cobalt and lead as more dangerous than hitherto expected. Carcinogenesis 24(1):63–73

Andrea Hartwig received her Diploma in Chemistry, and her Ph.D. as well as her Habilitation in Biochemistry at the University of Bremen. In 1998, she became Professor for Food Chemistry in Karlsruhe, Germany, followed by a professorship for Food Chemistry at the Technical University of Berlin, Germany. Since 2010, she is Full Professor and Chair of the Department of Food Chemistry and Toxicology at the Karlsruhe Institute of Technology (KIT). The main research area focuses on the impact of carcinogenic metal compounds including metal-based nanoparticles as well as essential trace elements on genomic stability, with special emphasis on DNA damage induction and DNA damage response systems.