Biometals (2010) 23:951–960
DOI 10.1007/s10534-010-9330-4

Mechanisms in cadmium-induced carcinogenicity: recent insights

Andrea Hartwig

Received: 20 January 2010 / Accepted: 23 March 2010 / Published online: 15 April 2010

Abstract Cadmium is an environmental pollutant, with relevant exposures at workplaces and in the general population. The carcinogenicity has been long established, most evident for tumors in the lung and kidney, but with increasing evidence also for other tumor locations. While direct interactions with DNA appear to be of minor importance, the interference with the cellular response to DNA damage, the deregulation of cell growth as well as resistance to apoptosis have been demonstrated in diverse experimental systems. With respect to DNA repair processes, cadmium has been shown to disturb nucleotide excision repair, base excision repair and mismatch repair; consequences are increased susceptibility towards other DNA damaging agents and endogenous mutagens. Furthermore, cadmium induces cell proliferation, inactivates negative growth stimuli, such as the tumor suppressor protein p53, and provokes resistance towards apoptosis. Particularly 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. Future research needs to clarify the relevance of these interactions for low exposure conditions in humans.

A. Hartwig (✉)
Institut für Lebensmitteltechnologie und Lebensmittelchemie, Fachgebiet Lebensmittelchemie und Toxikologie, Technische Universität Berlin, 13355 Berlin, Germany
e-mail: andrea.hartwig@tu-berlin.de

Keywords Cadmium · DNA repair · Gene expression · Cell cycle control · Apoptosis · Genomic instability

Introduction

Cadmium is ubiquitously distributed throughout the environment, attributable to natural sources, agriculture and manifold industrial uses. Relevant exposures occur at workplaces mainly via inhalation, but also in the general population predominantly via food, tobacco smoke and ambient air. Even though the toxicity of cadmium has been known for decades, during recent years there has been an ongoing discussion on cadmium-induced toxicity, even at comparatively low exposure conditions. For example, recently the European Food Safety Authority (EFSA) has lowered the Provisional Tolerable Weekly Intake (PTWI) of 7 μg/kg bw established previously by the Joint FAO/WHO Expert Committee on Food Additives to a TWI of 2.5 μg/kg bw based on cadmium-induced nephrotoxicity (EFSA 2009). Other endpoints of toxicological concern are bone demineralization and cardiovascular diseases (see contributions of Tim Nawrot and David Bernhard,

this volume). In addition, human cadmium exposure has been associated with increased incidences of lung tumors by the International Agency for Research on Cancer (IARC 1993, 1997) and also kidney tumors by the German MAK Commission (DFG 2006). A recent review of substances classified as human carcinogens by the IARC confirmed sufficient evidence for cadmium-induced lung tumors and limited evidence for kidney and prostate tumors in humans (Straif et al. 2009). Furthermore, new data indicate that human cadmium exposure may also be associated with female breast and endometrial cancer, even though the causalities are not definitively established (McElroy et al. 2006; Akesson et al. 2008). In animals, cadmium induces carcinomas of the lung after inhalation and cancers of the prostate and testis after ingestion or injection (Waalkes 2003; Goyer et al. 2004).

This raises the question about the underlying mechanisms for tumor induction, especially those relevant at comparatively low exposure conditions. Current evidence suggests that no direct genotoxicity but rather multiple indirect mechanisms are operative, including increased oxidative stress, interactions with the cellular DNA damage response systems, including DNA repair processes, cell cycle control and apoptosis as well as alterations in gene expression patterns (Beyersmann and Hartwig 2008; Joseph 2009). Within this review, the different mechanisms will be discussed, also in light of recent epidemiological findings and with respect to physico-chemical properties of cadmium ions.

DNA damage, mutagenicity and clastogenicity

In most bacterial assays soluble cadmium compounds were not mutagenic, and in standard mammalian mutagenicity tests effects of cadmium salts are usually weak and/or restricted to comparatively high concentrations. In contrast, pronounced comutagenic effects in combination with DNA alkylating agents and with UVC radiation were observed both in bacteria and in mammalian cells. In addition, in mammalian cells cadmium compounds provoke clastogenic effects such as chromosomal aberrations and micronuclei (IARC 1993; Waisberg et al. 2003; DFG 2006; Filipic et al. 2006). This was also demonstrated by the pronounced mutagenicity of cadmium chloride in a modified mammalian test system capable of detecting large multilocus deletions (Filipic and Hei 2004). The clastogenic activity of cadmium compounds 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 (IARC 1993; DFG 2006; Tapisso et al. 2009). Furthermore, cadmium compounds increase the extent of oxidative DNA damage in cultured cells and in vivo (see below). Since cadmium salts do not cause DNA damage in cell extracts or with isolated DNA (Valverde et al. 2001) but rather interact with proteins, the genotoxicity of cadmium is likely due to indirect mechanisms. Most promising hypothesis are (1) the increased formation of reactive oxygen species, (2) interactions with the cellular DNA damage response system, such as DNA repair processes, cell cycle control and apoptosis as well as (3) epigenetic changes in DNA methylation patterns, leading to a high degree of genomic instability.

Oxidative stress

Cadmium(II) is not able to participate in redox reactions under physiological conditions, but oxidative stress-related reactions appear to be relevant in cadmium-induced carcinogenicity. Increased levels of reactive oxygen species (ROS) have been observed both in vitro and in vivo (Liu et al. 2009), and their appearance is interpreted by the inhibitory effect of cadmium on antioxidant enzymes such as catalase, superoxide dismutase, glutathione reductase, and glutathione peroxidase (Stohs et al. 2001; Valko et al. 2006). ROS may be involved in cadmium-induced genotoxicity, but—perhaps more importantly—also in later steps of cadmium-induced carcinogenicity. Different cadmium compounds have been shown to induce DNA strand breaks and oxidative DNA base modifications in mammalian cells, but effects were usually small and/or restricted to comparatively high concentrations (e.g., Dally and Hartwig 1997; Schwerdtle et al. 2010). Concerning the involvement of ROS, the induction of DNA strand breaks and chromosomal aberrations by cadmium in mammalian cells were suppressed by antioxidants and antioxidative

enzymes (Ochi and Ohsawa 1985; Stohs et al. 2001; Valko et al. 2006). Nevertheless, oxidative DNA damage does not appear to be sufficient to explain the carcinogenicity of cadmium, and enhanced frequencies of oxidative DNA lesions in cells and in vivo may also be due to impaired removal of the respective lesions (see below). Additionally, especially moderately elevated levels of ROS have been implicated in further steps related to tumor formation, such as cell proliferation due to mitotic stimuli and the activation of redox-sensitive transcription factors (Valko et al. 2006; Genestra 2007). Therefore, enhanced oxidative stress may not only initiate tumor development by mutagenesis but also deregulate cell growth and promote tumor growth depending on dose and time of interference.

Interactions with the DNA damage response system

Maintenance of genetic information and thus the correct sequence of nucleotides in DNA is essential for replication, gene expression and protein synthesis. However, many environmental agents as well as food mutagens have been identified which compromise genetic stability by inducing different types of DNA damage. They include ionizing radiation, UV-light, alkylating agents, polycyclic aromatic hydrocarbons as well as heterocyclic aromatic amines. Furthermore, the DNA is also damaged by endogenous processes, such as reactive oxygen species arising for example due to leakage of ROS from the electron transport chain, but also due to other sources such as xanthine oxidase and activated macrophages (Valko et al. 2006; Hakem 2008). DNA lesions interfere 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 network of different repair systems has evolved. The major pathway eliminating DNA base damage is the excision repair pathway, subdivided into nucleotide excision repair (NER) and base excision repair (BER). NER is the most versatile repair system involved in the removal of structurally unrelated bulky base adducts which cause significant helical distortions. 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. Most of them are involved in the damage recognition and the incision at both sides of the lesion, followed by the repair polymerisation leading to the displacement of the damaged oligonucleotide and finally the ligation of the repair patch. Some forms of DNA base damage are recognized by a specific class of DNA repair enzymes called glycosylases, initiating BER. DNA glycosylases act specifically on one or few substrates, and BER is mainly responsible for the removal of different types of endogenous DNA damage, including oxidative DNA base modifications. This process generates abasic (AP) sites, which are further processed in a multistep process with slight differences depending on the type of damage (de Boer and Hoeijmakers 2000; Christmann et al. 2003; Hakem 2008; Camenisch and Naegeli 2009). One other DNA repair system essential for maintaining genomic stability is the DNA mismatch repair system (MMR). This evolutionary conserved system is responsible for the repair of normal bases mismatches after DNA replication, contributing significantly to the extraordinary fidelity of DNA replication. Defects in MMR are associated with an increased risk of different types of cancer as cells deficient in MMR exert a "mutator phenotype", in which the rate of spontaneous mutations is greatly elevated. 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 (O'Brien and Brown 2006; Hsieh and Yamane 2008). Finally, DNA double strand breaks (DSB) are repaired by either homologous recombination (HR) or by nonhomologous end-joining (NHEJ) (Shrivastav et al. 2008). Besides DNA repair systems, further DNA damage responses are operative 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 (Hainaut and Hollstein 2000). While DNA excision repair systems are predominantly error-free, DNA damage persisting

at the time of DNA replication may also be converted into mutations due to error-prone bypass mechanisms. Also, DNA double strand break repair may be highly error prone, especially NHEJ (de Boer and Hoeijmakers 2000; Christmann et al. 2003; Camenisch and Naegeli 2009; Kerzendorfer and O'Driscoll 2009).

Cadmium has been shown to impair almost all major DNA repair pathways. Multiple evidence is available for its interference with nucleotide excision repair, base excision repair and mismatch repair, providing a plausible explanation for its comutagenicity in combination with different DNA damaging agents, including UVC radiation and DNA alkylation agents (reviewed in Hartwig 1994; Giaginis et al. 2006). With respect to NER, impaired removal of UVC- and benzo[*a*]pyrene-induced DNA damage has been demonstrated in intact cells and by applying cadmium-treated cell extracts (Snyder et al. 1989; Fatur et al. 2003; Mukherjee et al. 2004; Schwerdtle et al. 2010). More detailed studies revealed a disturbance of the DNA damage recognition/incision step of the repair process (Hartmann and Hartwig 1998; Fatur et al. 2003) and an impaired assembly/disassembly of the DNA damage recognition proteins XPC and XPA at the repair complex after UVC irradiation in cells (Schwerdtle et al. 2010).

Regarding BER, low concentrations of cadmium inhibited the repair of oxidative DNA base damage as well as DNA alkylation damage in mammalian cells (Dally and Hartwig 1997, Fatur et al. 2003). When compared with the induction of oxidative DNA base modifications such as 8-oxoguanine (8-oxoG) as a relevant and frequently determined indicator of oxidative DNA damage, 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 oxidative DNA base modifications induced by visible light and recognized by the bacterial formamidopyrimidine DNA glycosylase (FPG) was inhibited starting at 0.5 μM cadmium, yielding complete inhibition at 5 μM, a completely non-cytotoxic concentration in this test system (Dally and Hartwig 1997). With respect to isolated DNA repair enzymes, an inhibition of the murine 8-oxoguanine DNA glycosylase 1 (mOgg1), an enzyme that removes 8-oxoG from DNA, 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 (Bialkowski and Kasprzak 1998; Zharkov and Rosenquist 2002). Inhibitory effects of enzymes involved in the defence against oxidative DNA damage are 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 (Bialkowski et al. 1999). Therefore, increases in oxidative DNA damage in vivo may at least in part be due to the repair inhibition of endogenously induced oxidative DNA lesions.

In addition to excision repair, cadmium has been shown to impair DNA mismatch repair in different systems. It was first reported by Jin and co-workers that exposure towards low concentrations of cadmium resulted in pronounced hypermutability in yeast, and the mutation specificity along with responses in proofreading-deficient and MMR-deficient mutants indicated a reduced capacity for MMR of small misalignments and base–base mismatches upon cadmium exposure. Furthermore, in extracts of human cells, cadmium inhibited at least one step leading to mismatch repair (Jin et al. 2003). 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. Even though the exact mechanism is still not known at present, 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 (Lutzen et al. 2004; Giaginis et al. 2006; Wieland et al. 2009). In addition to manifold interference with DNA repair systems, cadmium has also been shown to disrupt the DNA damage response system in further aspects. Thus, cadmium disturbs the function of the tumor suppressor protein p53 and thus interferes with cell cycle control in response to DNA damage (Meplan et al. 1999). Furthermore, cadmium-induced malignant transformation of human prostate epithelial cells acquired resistance to apoptosis (Qu et al. 2007). As a consequence, damaged cells could escape from

elimination by apoptosis, allowing them to replicate damaged DNA with high frequency of mutations, which may play an important role in cadmium-induced carcinogenicity (see below).

Impact on gene expression and deregulation of cell proliferation

Cadmium interacts with the expression of a large number of genes, including stress response genes, immediate early response genes, transcription factors and translation factors. Major stress response genes induced by cadmium are those involved in the synthesis of metallothionein (MT), as well as those encoding heat shock proteins, glutathione (GSH) synthesis and homeostasis and oxidative stress response (Hart et al. 2001; Waisberg et al. 2003; Joseph 2009). Immediate early response genes induced by cadmium include protooncogenes like *c-fos*, *c-jun* and *c-myc* activated in response to mitotic stimuli and frequently found overexpressed in tumors. With respect to transcription factors, cadmium exposure may lead to activation or inactivation, depending on the actual transcription factor under investigation. Thus, c-fos and c-jun constitute the AP-1 transcription factor, activating several genes involved in cell growth and division. Other transcription factors activated by cadmium are nuclear factor κB (NFκB) and NF-E2-related factor (NRF2). On the other hand, a suppression of the transcription factor SP1 has been observed in cadmium-treated cells (Joseph 2009). In addition to directly stimulating mitogenic signals, cadmium also inhibits negative controls of cell proliferation, for example by inactivation of p53 (Meplan et al. 1999).

Molecular mechanisms

Independent of the actual cadmium compound applied, Cd^{2+} appears to be the ultimate damaging species. Even though most mechanistic studies have been conducted with water soluble cadmium compounds, some investigations are available comparing water soluble and largely water insoluble, particulate cadmium compounds. In a recent study, 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 (Schwerdtle et al. 2010). While water soluble cadmium compounds are taken up via ion transporters and channels (Thevenod 2010), particulate cadmium compounds may be taken up by phagocytosis and, due to the low pH, may be gradually dissolved in lysosomes, yielding high concentrations of cadmium ions in the cytoplasm and in the nucleus, as described in detail for nickel compounds (Costa et al. 1982; Evans et al. 1982). 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 (Heinrich 1992).

Since especially the DNA repair inhibitions but also altered cell proliferation and/or diminished cell cycle control have frequently been observed at low, non-cytotoxic concentrations of cadmium, this raises the question why for example DNA damage response pathways are particularly sensitive towards cadmium ions. As one plausible explanation, many biological effects may be due to the interaction between cadmium and the essential elements calcium and zinc. The Cd^{2+} ion easily substitutes for Ca^{2+} in biological systems, since it carries the same charge and has a similar radius. 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 (Waisberg et al. 2003; Martelli et al. 2006).

Since cadmium ions exert high affinity towards SH groups, potential targets are so-called zinc finger proteins (Hartwig 2001; Witkiewicz-Kucharczyk and Bal 2006). They comprise a family of proteins where zinc is complexed through four invariant cysteine and/or histidine residues forming a zinc finger domain, which is mostly involved in DNA binding, but also in protein-protein-interactions (Mackay and Crossley 1998). Even though most zinc finger structures have been described as DNA binding motifs in transcription factors, they have also been identified in several DNA repair and cell cycle control proteins. They include the bacterial Fpg

involved in the removal of oxidative DNA base modifications and the mammalian xeroderma pigmentosum group A protein (XPA) essential for DNA damage recognition during NER. One other example is poly(ADP-ribose) polymerase 1 (PARP-1), which plays a complex role in DNA damage signalling, BER and in drug-induced and spontaneous apoptosis. This enzyme contains three zinc fingers involved in the recognition of DNA breaks and the subsequent synthesis of poly(ADP-ribose) (Petrucco 2003; Beneke and Bürkle 2007). Also, p53 contains a zinc binding structure in its DNA binding domain, essential for its tumor suppressor functions (Hainaut and Hollstein 2000). All of these proteins have been shown to be inhibited by cadmium in different experimental systems. Thus, cadmium disturbed the activity of the isolated Fpg and diminished DNA binding of XPA to an UVC-irradiated oligonucleotide (Hartmann and Hartwig 1998; Asmuss et al. 2000). Furthermore, in HeLa cells, H_2O_2-induced PARP activity was decreased by cadmium(II) (Hartwig et al. 2002). Finally, cadmium induced a conformational shift in the zinc binding domain of the tumor suppressor protein p53. Here, DNA binding is mediated by a rather complex structure brought together by tetrahedral coordination of zinc to three cysteines and one histidine, and exposure of either the isolated p53 protein or human breast cancer MCF7 cells to cadmium resulted in disruption of native p53 conformation and inhibition of DNA binding; furthermore, suppression of the p53-mediated cell cycle arrest in response to DNA damage by cadmium was observed in the cellular system (Meplan et al. 1999).

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 (Buchko et al. 2000; Kopera et al. 2004). Whether or not the other protein inactivations described above are due to interactions with the respective zinc finger structures as well and/or whether these effects occur in vivo has to be further investigated.

In addition to direct interactions with DNA 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-oxodG during BER in mammalian cells, was inhibited by cadmium (Potts et al. 2003). 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 (Youn et al. 2005), presumably due to displacement of zinc by cadmium (Kothinti et al. 2010). Also, a downregulation of DNA repair genes like XPC has been observed recently in cultured cells (Schwerdtle et al. 2010) and in vivo (Zhou et al. 2004), which may be due to a disturbed transcriptional activity of p53.

With respect to interactions with gene expression, multiple mechanisms appear to be operative as well, including direct binding to transcription factors, as evident for MTF1 responsible for MT induction, as well as several indirect mechanisms, such as cadmium-mediated elevated levels of second messengers like ROS, Ca^{2+} and inositol-1,4,5-trisphosphate. For example, elevated levels of Ca^{2+} can activate mitotic protein kinases such as protein kinase C, which in turn can phosphorylate transcription factors resulting in the expression of cellular proto-oncogenes (reviewed by Waisberg et al. 2003). Cadmium also exerts epigenetic effects which may contribute to tumor development. It inhibited DNA-(cytosine-5) methyltransferase and diminished DNA methylation during cadmium-induced cellular transformation (Takiguchi et al. 2003). Decreased DNA methylation is thought to have a tumor promoting effect, since it was associated with augmented expression of cellular proto-oncogenes. A unique mechanism by which cadmium deregulates cell proliferation is the disruption of the cadherin-mediated cell–cell adhesion system and of cell–cell communication. Cadmium specifically displaced calcium from the protein E-cadherin (Prozialeck et al. 1999) and impaired the cell-cell adhesion in kidney epithelial cells (Prozialeck et al. 2003). Also, elevated levels of ROS can affect redox-sensitive transcription factors and thus stimulate proliferation and enhanced survival as shown in a wide variety of cell types (Valko et al. 2006). Finally, the acquired resistance to apoptosis in cadmium-transformed human prostate cells appeared to be linked to an increase in the anti-apoptotic action of Bcl-2 that perturbs the JNK signal transduction pathway (Qu et al. 2007).

In conclusion it is evident that cadmium interferes with cellular controls of proliferation in several ways, which all can contribute to the observed deregulation of cell growth by this metal.

Mechanistic considerations with respect to organ-specific carcinogenicity of cadmium and the role of adaptation

The mechanisms described above could well explain the carcinogenicity of cadmium, but an open question remains concerning the organ-specificity of cadmium-induced carcinogenicity. After inhalative exposure, the lung is a plausible target organ. Nevertheless, as described above, other organs are also potentially affected, including kidney, prostate, breast and endometrium. In principle, target organ specificity could be explained either by mechanisms leading to cadmium accumulation, by selective sensitivity or by interactions occurring specifically in target organs. 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 signalling (Byrne et al. 2009). Nevertheless, respective experimental evidence is contradictory. Thus, for example, cadmium has been shown to activate the estrogen receptor α (ERα) in a reporter gene assay (Stoica et al. 2000), which was not observed in another study (Silva et al. 2006). Alternatively, cadmium has been shown to transform normal human breast epithelial cells through a mechanism not requiring ERα. The latter study supports a mechanistic model for hormone-independent cadmium-related breast cancer, showing DNA hypomethylation and oncogene overexpression in a pattern typical in aggressive breast cancers (Benbrahim-Tallaa et al. 2009). One 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 (Stohs et al. 2001). 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 (Klaassen et al. 2009). However, accumulating evidence suggests that adaptation may be a double-edged sword, since hallmarks of cadmium adapted cells are increased MT contents with a consequence of cadmium accumulation, reduced DNA repair activities as well as suppressed apoptosis, evident for example in an in vivo pulmonary rat model (Hart et al. 2001). Similarly, in humans, breast tumor MT overexpression is associated with a poorer prognosis, accompanied by exaggerated cadmium accumulation and retention (summarized in Benbrahim-Tallaa et al. 2009).

Conclusions 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. 1). DNA repair and other DNA damage response systems do not only provide pronounced protection against environmental mutagens, but also against endogenous DNA damage which occurs continuously due for example to leakage of ROS from the electron transport chain. Therefore, their disturbance leads to an increase in mutations and carcinogenesis. This is evident for example in the high tumor frequency in patients with the DNA repair disorder Xeroderma pigmentosum and in the hypermutability of mismatch repair deficient cells (de Boer and Hoeijmakers 2000). Furthermore, mismatch-deficient cells escape from cell cycle arrest and apoptosis (O'Brien and Brown 2006). The impairment of DNA repair by cadmium may be particularly deleterious in cadmium-adapted

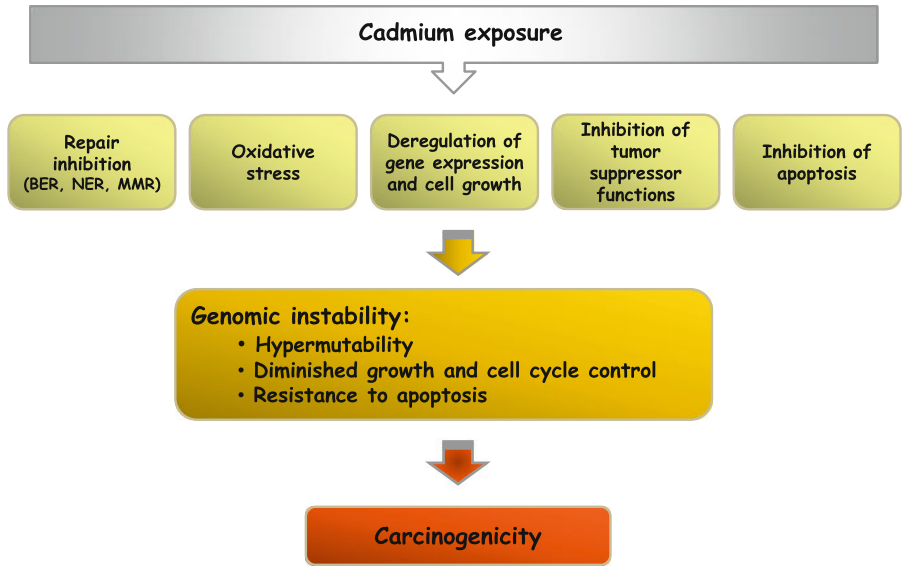

Fig. 1 Proposed mechanisms involved in cadmium-induced carcinogenicity

cells; concurrently, tolerance to cadmium toxicity may constitute an extended chance for the induction of further critical mutations (Achanzar et al. 2002).

Altogether, it appears that cadmium interferes with multiple targets in the DNA damage response network; together with growth deregulation and apoptotic resistance, highly instable phenotypes may arise. Considering recent indications of 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, especially with respect to effective concentrations. Furthermore, the role of MT in cadmium-induced carcinogenicity needs to be further explored.

Acknowledgements The author gratefully acknowledges Nancy Sobier-Maier for critically proof-reading the manuscript. Research conducted in the author's laboratory was supported by the Deutsche Forschungsgemeinschaft and by BWPLUS.

References

Achanzar WE, Webber MM, Waalkes MP (2002) Altered apoptotic gene expression and acquired apoptotic resistance in cadmium-transformed human prostate epithelial cells. Prostate 52:236–244

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 68:6435–6441

Asmuss M, Mullenders LH, Eker A et al (2000) Differential effects of toxic metal compounds on the activities of Fpg and XPA, two zinc finger proteins involved in DNA repair. Carcinogenesis 21:2097–2104

Benbrahim-Tallaa L, Tokar EJ, Diwan BA et al (2009) Cadmium malignancy transforms normal human breast epithelial cells into a basal-like phenotype. Environ Health Perspect 117:1847–1852

Beneke S, Bürkle A (2007) Poly(ADP-ribosyl)ation in mammalian ageing. Nucleic Acids Res 35:7456–7465

Beyersmann D, Hartwig A (2008) Carcinogenic metal compounds: recent insight into molecular and cellular mechanisms. Arch Toxicol 82:493–512

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 26:3194–3201

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 20:1621–1624

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 21:1051–1057

Byrne C, Divekar SD, Storchan GB et al (2009) Cadmium—a metallohormone? Toxicol Appl Pharmacol 238:266–271

Camenisch U, Naegeli H (2009) Role of DNA repair in the protection against genotoxic stress. EXS 99:111–150

Christmann M, Tomicic MT, Roos WP et al (2003) Mechanisms of human DNA repair: an update. Toxicology 193:3–34

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 42:2757–2763

Dally H, Hartwig A (1997) Induction and repair inhibition of oxidative DNA damage by nickel(II) and cadmium(II) in mammalian cells. Carcinogenesis 18:1021–1026

de Boer J, Hoeijmakers JH (2000) Nucleotide excision repair and human syndromes. Carcinogenesis 21:453–460

DFG (2006) Cadmium and its compounds (in the form of inhable dusts/aerosols). The MAK collection for occupational health and safety, vol 22. D. Forschungsgemeinschaft, Wiley-VCH, Weinheim

EFSA (2009) Scientific opinion of the panel on contaminants in the food chain on a request from the European Commission on cadmium in food. EFSA J 980:1–139

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 42:2729–2735

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 529:109–116

Filipic M, Hei TK (2004) Mutagenicity of cadmium in mammalian cells: implication of oxidative DNA damage. Mutat Res 546:81–91

Filipic M, Fatur T, Vudrag M (2006) Molecular mechanisms of cadmium induced mutagenicity. Hum Exp Toxicol 25:67–77

Genestra M (2007) Oxyl radicals, redox-sensitive signalling cascades and antioxidants. Cell Signal 19:1807–1819

Giaginis C, Gatzidou E, Theocharis S (2006) DNA repair systems as targets of cadmium toxicity. Toxicol Appl Pharmacol 213:282–290

Goyer RA, Liu J, Waalkes MP (2004) Cadmium and cancer of prostate and testis. Biometals 17:555–558

Hainaut P, Hollstein M (2000) p53 and human cancer: the first ten thousand mutations. Adv Cancer Res 77:81–137

Hakem R (2008) DNA-damage repair; the good, the bad, and the ugly. EMBO J 27:589–605

Hart BA, Potts RJ, Watkin RD (2001) Cadmium adaptation in the lung—a double-edged sword? Toxicology 160:65–70

Hartmann M, Hartwig A (1998) Disturbance of DNA damage recognition after UV-irradiation by nickel(II) and cadmium(II) in mammalian cells. Carcinogenesis 19:617–621

Hartwig A (1994) Role of DNA repair inhibition in lead- and cadmium-induced genotoxicity: a review. Environ Health Perspect 102 Suppl 3:45–50

Hartwig A (2001) Zinc finger proteins as potential targets for toxic metal ions: differential effects on structure and function. Antioxid Redox Signal 3:625–634

Hartwig A, Asmuss M, Blessing H et al (2002) Interference by toxic metal ions with zinc-dependent proteins involved in maintaining genomic stability. Food Chem Toxicol 40:1179–1184

Heinrich U (1992) Pulmonary carcinogenicity of cadmium by inhalation in animals. IARC Scientific Publications, Lyon, pp 405–413

Hsieh P, Yamane K (2008) DNA mismatch repair: molecular mechanism, cancer, and ageing. Mech Ageing Dev 129:391–407

IARC (1993) Beryllium, cadmium, mercury, and exposures in the glass manufacturing industry. IARC monographs on the evaluation of carcinogenic risks to humans. IARC, Lyon

IARC (1997) Supplement: cadmium and cadmium compounds. IARC monographs on the evaluation of carcinogenic risks to humans. IARC, Lyon

Jin YH, Clark AB, Slebos RJ et al (2003) Cadmium is a mutagen that acts by inhibiting mismatch repair. Nat Genet 34:326–329

Joseph P (2009) Mechanisms of cadmium carcinogenesis. Toxicol Appl Pharmacol 238:272–279

Kerzendorfer C, O'Driscoll M (2009) Human DNA damage response and repair deficiency syndromes: linking genomic instability and cell cycle checkpoint proficiency. DNA Repair (Amst) 8:1139–1152

Klaassen CD, Liu J, Diwan BA (2009) Metallothionein protection of cadmium toxicity. Toxicol Appl Pharmacol 238:215–220

Kopera E, Schwerdtle T, Hartwig A et al (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 17:1452–1458

Kothinti RK, Blodgett AB, Petering DH et al (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 244:254–262

Liu J, Qu W, Kadiiska MB (2009) Role of oxidative stress in cadmium toxicity and carcinogenesis. Toxicol Appl Pharmacol 238:209–214

Lutzen A, Liberti SE, Rasmussen LJ (2004) Cadmium inhibits human DNA mismatch repair in vivo. Biochem Biophys Res Commun 321:21–25

Mackay JP, Crossley M (1998) Zinc fingers are sticking together. Trends Biochem Sci 23:1–4

Martelli A, Rousselet E, Dycke C et al (2006) Cadmium toxicity in animal cells by interference with essential metals. Biochimie 88:1807–1814

McElroy JA, Shafer MM, Trentham-Dietz A et al (2006) Cadmium exposure and breast cancer risk. J Natl Cancer Inst 98:869–873

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 274:31663–31670

Mukherjee JJ, Gupta SK, Kumar S et al (2004) Effects of cadmium(II) on (+/−)-anti-benzo[a]pyrene-7,8-diol-9,10-epoxide-induced DNA damage response in human fibroblasts and DNA repair: a possible mechanism of cadmium's cogenotoxicity. Chem Res Toxicol 17:287–293

O'Brien V, Brown R (2006) Signalling cell cycle arrest and cell death through the MMR System. Carcinogenesis 27:682–692

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 143:137–142

Petrucco S (2003) Sensing DNA damage by PARP-like fingers. Nucleic Acids Res 31:6689–6699

Potts RJ, Watkin RD, Hart BA (2003) Cadmium exposure down-regulates 8-oxoguanine DNA glycosylase expression in rat lung and alveolar epithelial cells. Toxicology 184:189–202

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 1451:93–100

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 189:180–195

Qu W, Ke H, Pi J et al (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 115:1094–1100

Schwerdtle T, Ebert F, Thuy C et al (2010) Genotoxicity of soluble and particulate cadmium compounds: impact on oxidative DNA damage and nucleotide excision repair. Chem Res Toxicol 23:432–442

Shrivastav M, De Haro LP, Nickoloff JA (2008) Regulation of DNA double-strand break repair pathway choice. Cell Res 18:134–147

Silva E, Lopez-Espinosa MJ, Molina-Molina JM et al (2006) Lack of activity of cadmium in in vitro estrogenicity assays. Toxicol Appl Pharmacol 216:20–28

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

Stohs SJ, Bagchi D, Hassoun E et al (2001) Oxidative mechanisms in the toxicity of chromium and cadmium ions. J Environ Pathol Toxicol Oncol 20:77–88

Stoica A, Katzenellenbogen BS, Martin MB (2000) Activation of estrogen receptor-alpha by the heavy metal cadmium. Mol Endocrinol 14:545–553

Straif K, Benbrahim-Tallaa L, Baan R et al (2009) A review of human carcinogens—part C: metals, arsenic, dusts, and fibres. Lancet Oncol 10:453–454

Takiguchi M, Achanzar WE, Qu W et al (2003) Effects of cadmium on DNA-(cytosine-5) methyltransferase activity and DNA methylation status during cadmium-induced cellular transformation. Exp Cell Res 286:355–365

Tapisso JT, Marques CC, Mathias Mda L et al (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 678:59–64

Thevenod F (2010) Catch me if you can! Novel aspects of cadmium transport in mammalian cells. Biometals. doi: 10.1007/s10534-010-9309-1

Valko M, Rhodes CJ, Moncol J et al (2006) Free radicals, metals and antioxidants in oxidative stress-induced cancer. Chem Biol Interact 160:1–40

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 16:265–270

Waalkes MP (2003) Cadmium carcinogenesis. Mutat Res 533:107–120

Waisberg M, Joseph P, Hale B et al (2003) Molecular and cellular mechanisms of cadmium carcinogenesis. Toxicology 192:95–117

Wieland M, Levin MK, Hingorani KS et al (2009) Mechanism of cadmium-mediated inhibition of Msh2-Msh6 function in DNA mismatch repair. Biochemistry 48:9492–9502

Witkiewicz-Kucharczyk A, Bal W (2006) Damage of zinc fingers in DNA repair proteins, a novel molecular mechanism in carcinogenesis. Toxicol Lett 162:29–42

Youn CK, Kim SH, Lee DY et al (2005) Cadmium down-regulates human OGG1 through suppression of Sp1 activity. J Biol Chem 280:25185–25195

Zharkov DO, Rosenquist TA (2002) Inactivation of mammalian 8-oxoguanine-DNA glycosylase by cadmium(II): implications for cadmium genotoxicity. DNA Repair (Amst) 1:661–670

Zhou T, Jia X, Chapin RE et al (2004) Cadmium at a non-toxic dose alters gene expression in mouse testes. Toxicol Lett 154:191–200