

## Epigenetic Effects of Cadmium in Cancer: Focus on Melanoma

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**Abstract:** Cadmium is a highly toxic heavy metal, which has a destroying impact on organs. Exposure to cadmium causes severe health problems to human beings due to its ubiquitous environmental presence and features of the pathologies associated with prolonged exposure. Cadmium is a well-established carcinogen, although the underlying mechanisms have not been fully understood yet. Recently, there has been considerable interest in the impact of this environmental pollutant on the epigenome. Because of the role of epigenetic alterations in regulating gene expression, there is a potential for the integration of cadmium-induced epigenetic alterations as critical elements in the cancer risk assessment process. Here, after a brief review of the major diseases related to cadmium exposure, we focus our interest on the carcinogenic potential of this heavy metal. Among the several proposed pathogenetic mechanisms, particular attention is given to epigenetic alterations, including changes in DNA methylation, histone modifications and non-coding RNA expression. We review evidence for a link between cadmium-induced epigenetic changes and cell transformation, with special emphasis on melanoma. DNA methylation, with reduced expression of key genes that regulate cell proliferation and apoptosis, has emerged as a possible cadmium-induced epigenetic mechanism in melanoma. A wider comprehension of mechanisms related to this common environmental contaminant would allow a better cancer risk evaluation.

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### INTRODUCTION

Heavy metals are the most common environmental pollutants all over the world. Heavy metal contamination provoked by natural and anthropogenic sources has become a global issue. The ubiquitous and indestructible nature of heavy metals turns them into one of the main causes of environmental pollution.

Environmental pollution has become a matter of grave importance in relation with industrial development, provoking pedosphere, hydrosphere and atmosphere deterioration. Unsuitable disposal practices related to industrial production are considered to be one of the main reasons for environmental pollution [1]. It has been demonstrated that industrial effluents contain high concentrations of metals, such as zinc, copper, iron, manganese, lead, nickel and cadmium [2]. Such effluents deposit heavy metals in soil and change their intake in the food chain, causing bioaccumulation. Although it is

well known that heavy metals can disturb fundamental cellular processes, e.g. by interfering with intracellular redox state regulation, their potential to provoke disease is still not fully understood [3, 4]. The same goes for their role in the onset and progression of tumors, despite the fact that environmental metal carcinogenesis is considered a major public health problem. In particular, while the association of heavy metal pollution with cancer has been known for some time, the mechanisms underlying malignant transformation remain to be clarified.

Cadmium is considered to be the most toxic metal present in industrial effluents. The pollutant is led into human beings by water, metal plating, batteries, fertilizers, mining, pigments, stabilizers, alloy material and sewage sludge [5]. Non-occupational exposure is mainly due to diet and smoking [6]. In fact, tobacco plants can absorb high quantities of cadmium from the environment and smoking contributes considerably to cadmium exposure. A cigarette contains about 2.0 µg of cadmium [7]. Cadmium levels in blood and body fluids are double in people who smoke relative to non-smokers [8]. More recently, cadmium exposure has been also associated with airborne particulate CdO and with cadmium-containing quantum dots in medical applications [8, 9]. Cadmium, as it has been said, is extremely toxic and tends to lay in tissues [10]. The average cadmium blood level is 0.315 µg/L, whereas the mean urine level of is 0.193 µg/g

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creatinine (0.185 µg/L) [11]. As soon as blood absorbs it, most of cadmium binds with proteins, such as albumin and metallothionein (MT). Liver is the first organ to be reached and there cadmium activates MT production. After having triggered hepatocyte necrosis and apoptosis, cadmium-metallothionein groups move into sinusoidal blood and enters the enterohepatic cycle through secretion into the biliary tract in the form of cadmium-glutathione conjugates. Once it has been enzymatically degraded to cadmium-cysteine complexes in the biliary tree, cadmium reaches the small intestine, from which it can be reabsorbed [12]. Kidney is the main organ for long-term cadmium accumulation [13]. Here the half-life for cadmium is approximately 10 years. A life-long intake can therefore lead to a cadmium accumulation in the kidney, thus provoking tubulus cell necrosis. The presence of high cadmium levels in the blood and urine indicates a recent and past exposure, respectively [14]. Cadmium is excreted via faeces and urine. (Fig. 1) shows a scheme on the handling of cadmium in the human body. Several studies took into exam the role of cadmium in smokers' chronic obstructive pulmonary diseases (COPD) [15]. Smokers, or former ones, show higher levels of cadmium relative to non smokers and the presence of cadmium is linked to lung diseases provoked by smoking [7], suggesting that cadmium plays a pivotal role in lung disease and in lung cancer [16, 17]. A massive exposure to cadmium, for example in the workplace, provokes respiratory diseases, such as rhinitis, olfactory epithelium destruction, anosmia and bronchitis [15, 18].

Foods which have been grown in areas contaminated by cadmium are generally the main source of cadmium exposure. People take up about 30-50 micrograms per day [19, 20], but they usually absorb orally only small quantities (1-10%) [21].

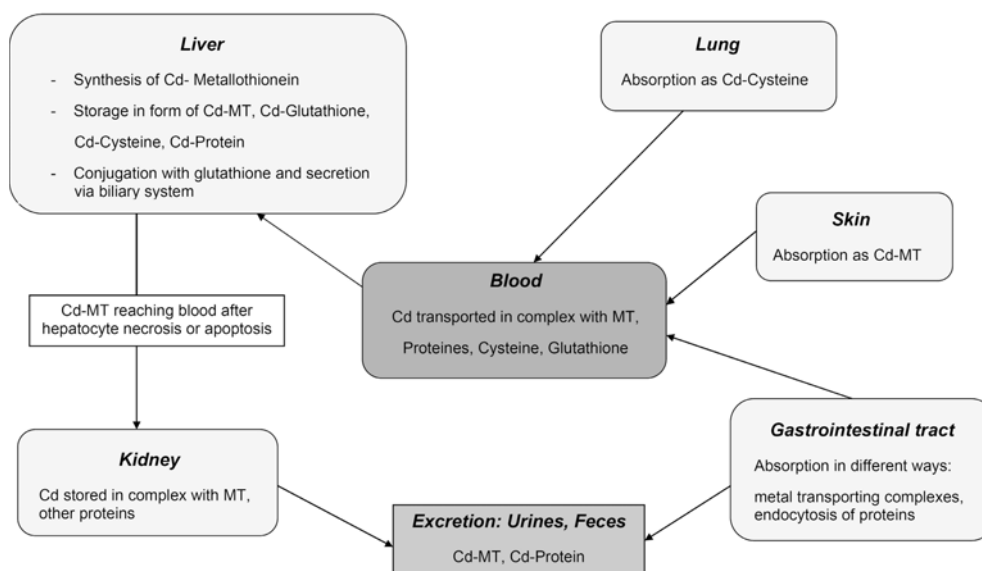
A low intake of vitamin D, calcium and the presence of metals, such as zinc and copper, increase cadmium absorption. In the case of zinc and calcium, their molecular homol-

ogy could be one of the mechanisms underlying higher cadmium absorption [22]. Foulkes showed that, in rat jejunum, cadmium uptake was depressed by high concentrations of polyvalent cations, including Pb, Ni, Cr3+, Sr, and Mg [23].

Cadmium intake is increased by diets rich in fibers and poor in iron [24]. People with low iron intakes have a higher cadmium absorption relative to people with well-balanced iron supplies [25]. For example, patients suffering from anaemia and iron lack, such as children and menstruating women, absorb higher levels of cadmium. Interestingly, low iron levels in blood stimulate a divalent cation transporter, which is employed for cadmium absorption in the gastrointestinal tract [26].

### CADMIUM AND DISEASES

In the world, there are areas showing high levels of cadmium in their soils and people who live there are exposed to cadmium absorption. For example, in the Jinzu and Kakehashi river basins in Japan, soils are contaminated and rice absorbs cadmium. Eating it for prolonged periods of time leads to serious kidney illnesses and provokes a bone disorder called "Itai-Itai", which mainly affect women [27, 28]. Patients show symptoms resulting from low levels of bone mineralization, such as fractures, osteoporosis and pains in their bones. In a study on this topic, patients showed the latter symptoms after having eaten rice grown on fields irrigated with highly cadmium-polluted water. Fractures provoked by osteomalacia and skeleton decalcification were also noticed. These studies were criticised as mostly post-menopausal women were involved [29] who had a high percentage of osteoporosis [30]. In animals, continuous cadmium absorption causes high systolic blood pressure even in absence of renal disease. These effects have been linked to depressed levels of atrial natriuretic peptide, increased blood amounts of aldosterone, and retention of sodium and water [15]. Cadmium exposure in human beings might be also related to hypertension. There are several stud-



**Fig. (1). Handling of cadmium in human body** (from Johannes Godt, Franziska Scheidig, Christian Grosse-Siestrup, Vera Esche, Paul Brandenburg, Andrea Reich, David A Groneberg. The toxicity of cadmium and resulting hazards for human health. *J Occup Med Toxicol.*, 2006, 1:22.)

ies on this topic. The Cadmibel study found no relationship between cadmium and blood pressure [31]. This study was carried out on people that underwent low-level exposure to cadmium. The follow-up of the original Cadmibel cohort, the PheeCad study, confirmed this result [32]. However, other studies on the same topic [33, 34] have shown a role for cadmium in peripheral artery disease. In particular, a correlation was observed between the levels of cadmium exposure and the pathological effects exerted by smoke on peripheral arteries.

People who work with cadmium can have problems if proper industrial hygiene measures are not applied. Occupational exposure occurs predominantly via inhalation of dusts in metallurgic industries, where batteries are produced and electroplating processes [5, 35]. Cadmium has a very long biological life, which makes it a cumulative toxin, so even a past exposure to such a metal can provoke damages after a long time [36]. Cadmium toxic properties have been known for over 150 years [37], but the issue regarding low-level exposure to it is still on.

Cadmium severe intoxication provokes injuries to the testes, liver and lungs [37, 38]. Chronic exposure provokes respiratory diseases, emphysema, end-stage renal failure, diabetic and renal complications, deregulated blood pressure, bone problems and immune suppression [35, 24, 40]. The main organ affected by cadmium exposure is the kidney. Cadmium inhalation and ingestion may lead to nephrotoxicity. Data from human studies suggest renal damage due to cadmium exposure can be seen after 10 years. In animals, massive cadmium exposure provokes renal toxicity, but few data are available in people.

Increasing cadmium exposure can be related to growing renal tubular dysfunction. Renal malfunction can be evidenced at levels of 2  $\mu\text{g/g}$  creatinine by microscopic tubular proteinuria or using biomarkers such as  $\beta_2$ -microglobulin and  $\alpha_1$ -microglobulin. At urinary cadmium levels of 4  $\mu\text{g/g}$  creatinine, N-acetyl-B-glucosaminidase (NAG) is elevated in urine and signs of glomerular damage are observed. In the final stages of cadmium intoxication it is possible to evidence nephropathy, glycosuria, calcium and phosphate loss, and altered calcium metabolism with osteoporosis and osteomalacia [41, 42]. Some studies argue that microproteinuria due to cadmium exposure is not progressive and that cadmium nephropathy becomes progressive and irreversible when it reaches cadmium levels higher than 4  $\mu\text{g/g}$  creatinine or a  $\beta_2$ -microglobulinuria higher than 1,000  $\mu\text{g/g}$  creatinine [43, 27]. Toxic effects on kidneys depend on cadmium doses [44]. Some researches state that renal tubular dysfunction related to cadmium is irreversible [45]. Cadmium nephropathy is one of the main causes of mortality in people who work in contaminated environments. The risk of nephropathy in people who work in cadmium-contaminated places was high when total airborne exposure was higher than 300  $\text{mg/m}^3$ , urine cadmium levels were higher than 10  $\mu\text{g/g}$  creatinine, and renal cortex levels were higher than 200 ppm [41]. People show early signs of kidney damage with urine levels between 2-4 nmol/mmol creatinine, as shown by a considerable literature on this topic [27, 42, 43, 46-49]. These studies show that even small amounts of cadmium can provoke kidney diseases. The World Health Organiza-

tion (WHO) has stated that 200  $\mu\text{g/g}$  levels wet weight in kidney cause detrimental changes in 10% of the population [50]. As mentioned above, literature on the topic showed that the threshold for renal damage was urinary cadmium levels of 2-4 nmol/mmol creatinine [46]; however, the OSCAR study showed that people who exhibited a urine cadmium level of 1 nmol/mmol creatinine had a threefold risk of increased  $\alpha_1$ -microglobulin [42]. However, it has not been determined yet if changes in kidney biomarkers, as seen in conditions of low-level cadmium exposure, have any correlation with the impairment of renal function [49]. At the moment, researchers are at work to define the amount of cadmium which provokes kidney diseases. Recent studies in polluted areas suggest that a dose of cadmium is 2.0 grams can be tolerated in men and women [51, 52]. In smokers there is a slight margin between critical renal concentration and body burdens [6]. Some researchers also suggests that children might be especially susceptible to cadmium [51]. Early signs of glomerular damage provoked by cadmium are increased excretion of albumin and transferrin. Glomerular damage is dose-dependent and can be irreversible [53]. Glomerular filtration rate (GFR) decreases slowly but progressively and it's possible that cadmium accelerates the normal age-related decline in kidney function. Uremia is not common, but people with normal baseline GFR and serum creatinine, who work in environments contaminated by cadmium, show a decreased filtration reserve capacity [46]. Medium-level exposure to cadmium can cause decreased GFR and chronic renal failure, which manifest itself with aminoaciduria, glycosuria, hypercalciuria, hyperphosphaturia, polyuria, and reduced buffering capacity for acids [53]. Kidney stones are common in people exposed to cadmium, above all in those with occupational exposure. Stone formation, that is secondary to kidney damage, can be related to hypercalciuria and hyperphosphaturia. Other factors which contribute to stone formation are uric aciduria, reduced urinary citrate, and renal tubular acidosis. Cadmium also accumulates in bones where it might promote pathological changes. However, bone disease resulting from excessive cadmium exposure is believed to be mainly secondary to changes in calcium metabolism due to cadmium-induced renal damage [19]. Bone lesions occur in cases of severe cadmium poisoning and include pseudo-fractures and other effects of osteomalacia and osteoporosis. Spontaneous fractures can occur anywhere in the skeleton or in places where the main arteries cross the bone. In Sweden the OSCAR study, which we have mentioned before, has examined the relationship between cadmium exposure and risk for reduced density in bone [42, 54, 55]. Researchers found out a negative correlation between cadmium in urines and bone density. Skeletal effects appeared to be secondary to increased urinary calcium and phosphorus losses due to cadmium-induced effects on kidney [42]. These included decreased renal hydroxylation of vitamin D which provokes deficiency of its active form. Moreover, an inhibitory effect on calcium absorption from the gastrointestinal tract can be exerted by cadmium [56]. When animals are pregnant, cadmium crosses the placenta inducing malformations in the newborns. Amounts of cadmium exceeding 2.5 mg/kg cause severe placental damage and lead to fetal death. In the case of pregnant women, it has been reported that cadmium exposure in the working place does not induce malformations in

newborns. However, in Japan, women with higher urinary cadmium levels have increased rates of preterm delivery than mothers with lower levels. Infant weight was also lower in the former group [57]. However, other studies did not show that cadmium causes pre-term labour [58]. At this time, the evidence of cadmium's effects on pregnancy is inconsistent and requires further investigation. Cadmium can seriously damage the reproductive system [59] as it can mimic estrogen function by virtue of its binding with high affinity to estrogen receptors (ER) [60], leading to gene activation [61]. Alternatively, estrogen receptors can exert their effects through other mechanisms, such as the activation of secondary messengers and enzymatic pathways [62]. Likely, the high incidence of tumors of the genital sphere in industrialized countries can be related to pollutants, such as cadmium, interfering with the hormonal network [63, 64]. In some, but not all, cell culture systems cadmium exerts either genomic or non-genomic effects [65-67]. The different hormonal effects exerted by cadmium have been attributed to the presence of different pools of ER-alpha with different reactivity to cadmium, as shown in (Fig. 2) [68, 69]. A recent review evaluated the effects on neurodevelopmental disorders of pre- or post-natal exposure to arsenic, cadmium, and manganese in children up to 16 years of age. Very little evidence was found about effects of cadmium that seemed consistent with neurodevelopmental and/or behavioral disorders. [70]. No significant differences have been observed between children and adults and developmental or behavioral changes have not been generally reported in populations exposed to cadmium. Therefore further studies are needed to carefully examine these potential effects of cadmium exposure. A limited number of animal studies suggest that young animals may absorb more cadmium than adults and may therefore display increased bone loss and fragility [11]. Recent studies have indicated that cadmium, even at non-toxic concentrations, can induce inflammatory changes in the airways [71] and in the vascular bed [72], and that these effects might be mediated by its ability to induce CXCL1 and CXCL2 chemokines [73]. These chemokines have central role in promoting neutrophil infiltration, which, in turn, can further amplify the production of not only CXCL1 and CXCL2 but also and of the potent pro-inflammatory cytokine IL-1 $\beta$  [74, 75]. Further studies will be needed to ascertain whether cadmium can directly potentiate the activation of receptors of the innate immune system, including the Toll-like receptors [76-78] and Nod-like receptors [79], leading to the induction of proinflammatory cytokines. All adverse effects caused by cadmium are due to its low excretion rate (half-life as long as 15–20 years) [80], and its consequent accumulation in the organism. In (Fig. 3) are reported the effects of cadmium on several organ systems.

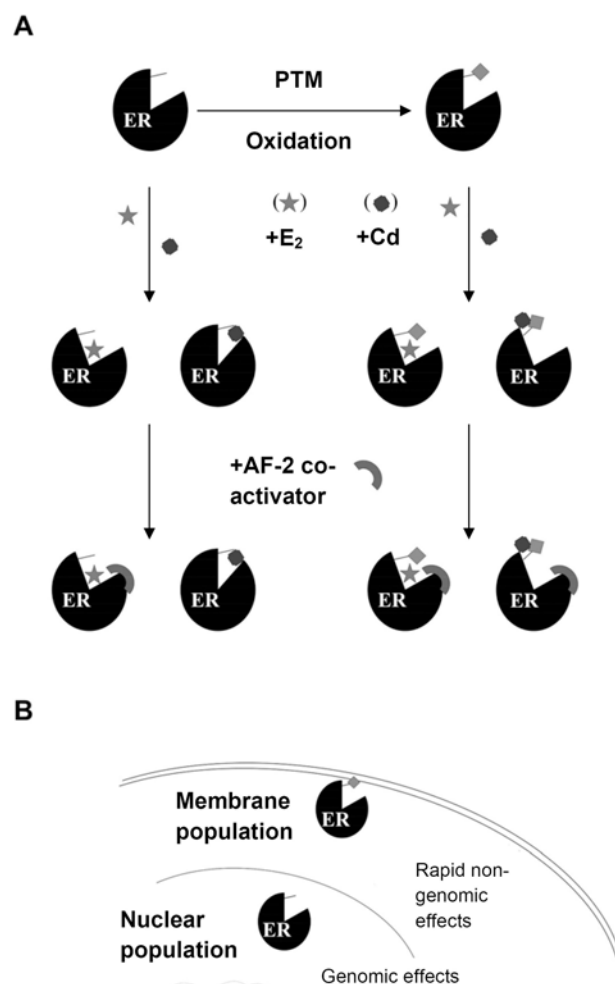
## CADMIUM AND CANCER

Studies have shown an increasing incidence of cancer in association with increasing contamination of the environment by cadmium. The effects of cadmium at the cellular level can result in a variety of metabolic derangements, alterations in the proliferative activity with reduced or increased cell growth, perturbations of apoptosis programs, modifications in the homeostasis of redox systems, cytotoxic as well as genotoxic responses. Along with all these

activities, cadmium can also act as a carcinogen, making it extremely dangerous to human health. The mechanisms involved could include aberrant gene expressions, errors in DNA methylation, apoptosis blockage and differentiation disruption, to cite a few. These phenomena can result in cadmium-induced carcinogenic transformation in the absence of direct or indirect genetic damage, which is consistent with the metal's poor ability to produce mutations at sub-lethal dosages. Cadmium can activate oncogenes or genes associated with cell proliferation, such as *c-myc*, *c-jun* or *c-fos*, both *in vivo* and *in vitro* [81] and it can also induce up-regulation of signal pathways resulting in increased mitogenesis, such as, for example, the AP-1 and MAP kinase pathways [82]. A direct action of cadmium on the expression of E-cadherin, a transmembrane Ca(II)-binding glycoprotein, has not been demonstrated yet, but the few available data indicate that cadmium may disrupt E-cadherin-dependent cell communication and promote cell division [83]. Such event may be one of the different mechanisms by which cadmium promotes tumour formation. Another mechanism may involve suppression of DNA repair that can allow damaged cells to enter the S phase of the cell cycle. Enhanced proliferation may by-pass apoptosis with a consequent accumulation of cells harbouring serious DNA impairment and development of pre-neoplastic or early neoplastic cells [84, 85]. Thus, cadmium-induced cell growth enhancement and reduced apoptosis may together cooperate in increasing cell population and promoting cancerogenesis.

Moreover, cadmium effects may synergize with those of the other pro-neoplastic agents, such as carcinogens present in cigarette smoke, with whom it is often associated. The understanding the mechanisms of cadmium resistance involved in the acute and chronic responses to low, non cytotoxic concentrations of this pollutant may be particularly useful, since they may provide a linkage between human health and environmental, occupational, and every-day life conditions. Although cadmium was associated with the development of prostate and testicular tumours in rats [86], no studies have definitively proved that cadmium can cause prostate cancer in humans [87]. But, nevertheless, the IARC (International Agency for Research on Cancer) classified cadmium as a group I carcinogen by virtue of its effect in inducing renal cancer [88, 89]. Some evidences indicate that cadmium may be tumorigenic only when it is absorbed through the respiratory tract [14]. The molecular mechanisms of cadmium transformation have not yet been well defined, although several signal transduction pathways have been implicated. Either increased proliferation rate or decreased apoptosis have been linked to cadmium-induced neoplasia. Alterations in DNA mismatch repair genes have also been described. Moreover, cadmium was shown to interfere with the transcriptional machinery by substituting for zinc in some transcriptional factors [90]. Epidemiological studies have provided further evidence that in addition to lung cancer, other cancers can be induced by cadmium [35, 91, 92]. Altogether, the German MAK Commission concluded that an increased relative risk of renal cancer has to be assumed [93] and also International Agency for Research on Cancer IARC stated a positive association with respect to renal and prostate cancer [94]. Finally, an association was also found between cadmium toxicity and breast and endometrial cancer [95, 63]. As mentioned above, cadmium ap-

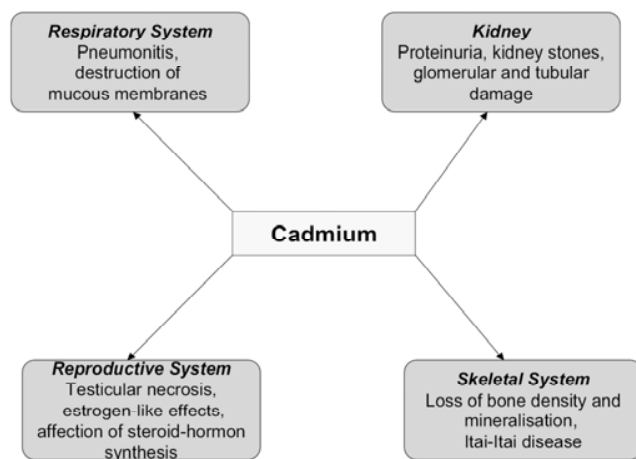
pears not to be a direct carcinogen, as it weakly binds to DNA. However cadmium can induce perturbations of genome stability through several indirect mechanisms, such as the production of reactive oxygen species (ROS), the disturbance of cellular signalling processes, DNA repair, mitotic cycle regulation, induction of apoptosis as well as epigenetic alterations, which may act together and lead to carcinogenesis [96]. The biological effects of cadmium are strictly dose-dependent. Cadmium exposure induces mitogenic signaling at concentrations above 1 $\mu$ M by increasing the levels of kinases of the RAS pathway: RAF-1, MAPK, MEK1/2, ERK1/2, p38 MAPK and JNK, beyond its effects on the protooncogenes *c-fos*, *c-myc*, and *c-jun* [97]. At lower concentrations, it enhances cell proliferation through distinct mechanisms [98]. The effects of cadmium on apoptosis are complex and both pro-apoptotic and anti-apoptotic consequences can be observed under different conditions of exposure to the metal. Several groups of researchers showed that cadmium-induced apoptosis is mediated by up-regulation and activation of p53 [99, 100, 85]. However, the implication of p53 is not always found in all biological systems [101, 102], being oxidative stress often involved in the activation of apoptosis by cadmium. Moreover, interactions with the DNA damage response systems [36] and the induction of oxidative stress appear to be relevant in cadmium-induced genotoxicity. It is worth to note that the increase in ROS levels is not direct, as cadmium lacks redox activity and it is not able to direct Fenton type-reaction. Furthermore, several studies show that cadmium associates with proteins, such as metallothionein or albumin, rather than with DNA [103, 104] and this is probably why cadmium-induced genotoxicity is mostly due to indirect processes [35, 105]. It is well known that cellular redox homeostasis, which is maintained by an elaborate endogenous system of enzymatic and non-enzymatic antioxidant biological molecules [96], is heavily involved in the regulation of apoptosis. Decreased antioxidant activities induce apoptosis and overproduction of ROS leads to inhibition of apoptosis [106-112]. In any case, it has not been well established whether suppression of apoptosis contributes to carcinogenesis induced by cadmium, particularly in the lung [113, 114]. Another issue is the increased resistance to high doses of cadmium and the cross-tolerance to an otherwise lethal dose of oxygen observed in animals subjected to cadmium inhalation [115, 116]. Moreover, lung cells adapted to cadmium by repeated exposure, *in vitro* or *in vivo*, showed greater resistance to oxidation, mainly through modifications in cellular thiols, such as MT and GSH [117]. However, although exerting a protective effect, this response may give to the cells the opportunity to evade from apoptosis and to clonally expand. This would be particularly damaging in a stem cell like the type II pneumocyte that is the putative target of carcinogenesis caused by cadmium [118]. Recently, it has been shown that cadmium is able to induce apoptosis in naive alveolar epithelial cells [119], likely by a mechanism of oxidative stress [120]. Indeed, cadmium may also affect antioxidative proteins, such as catalase and glutathione reductase, which have a crucial role in ROS elimination and induce, thereby, a reduction in cellular antioxidants and release of ROS by mitochondria [121]. Cadmium is believed to impair not only the repair of DNA damage caused by oxidative stress [92, 122, 123] but also the repair of DNA double-strand breaks and DNA interstrand crosslinks.



**Fig. (2).** A working hypothesis. (A) Different cellular pools of Era might have different reactivity towards cadmium depending on the cysteine residues (marked with a stick). The cysteine residues may become unavailable for interaction with cadmium for instance by posttranslational modifications (PTM) or oxidation. When a total population of ERA is exposed to cadmium, the metal binds close the ligand binding pocket of unmodified ERA and prevents the formation of active conformation recognized by AF-2 interacting co-activators, but in the case of modified ERA (the diamond indicates modification of the cysteine residues) cadmium binds outside the ligand binding pocket and pulls the receptor towards the active conformation that allows interaction with co-activators. (B) In living cells, membrane-associated ERA and nuclear unmodified ERA could be examples of receptor pools with different reactivity towards cadmium. (From Peter Fechner, Pauliina Damdimopoulou, Günter Gauglitz, *Biosensors Paving the Way to Understanding the Interaction between Cadmium and the Estrogen Receptor Alpha*, *Plos One*, 2011, Open Access).

It is noteworthy that cadmium has been shown to act as a tumour “progressor” in mouse models of sarcomas [124]. Therefore, when cadmium exposure occurs in the presence of other human carcinogens, such as those contained in tobacco smoking, or with UV, synergistic effects may be observed. For example, cadmium interfered with the removal of thymine dimers following UV irradiation [125]. Moreover smoking delays epithelial wound healing [126] and disrupts

the antioxidant/oxidant balance [127, 128]. Several findings indicate that cadmium is a strong multi-route, multi-site, multi-species carcinogen in rodents. Cadmium exposure has been associated with tumors of the lung, testes, injection site, prostate, hematopoietic system, pancreas, adrenals, liver, kidney and pituitary and several of these sites concur with potential target sites of cadmium carcinogenesis in humans [8]. The *in vivo* mechanisms of action for cadmium carcinogenesis is still not clear, with the exception of rodent testes. Given that high doses of cadmium are required to induce testicular tumors in rodent, it is uncertain whether these malignancies are relevant to human cadmium exposure. On the other hand, there is evidence that multiple, target tissue-specific mechanisms of cadmium carcinogenesis may apply and no correlation was demonstrated between cadmium exposure and cytogenetic effects in humans [129]. Therefore, the lack of convincing evidence in humans reduces the likelihood that genotoxicity is the main mechanism of cadmium carcinogenesis.



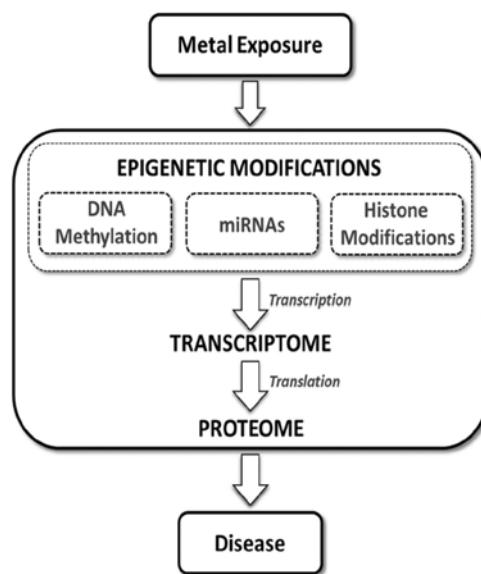
**Fig. (3).** Effects of cadmium on several organ systems (from Johannes Godt, Franziska Scheidig, Christian Grosse-Siestrup, Vera Esche, Paul Brandenburg, Andrea Reich, David A Groneberg. The toxicity of cadmium and resulting hazards for human health. *J Occup Med Toxicol.*, 2006, 1:22.)

### EPIGENETIC EFFECTS OF CADMIUM

Over the years it has become increasingly evident that mutagenesis is not the only mechanism underlying the activities of carcinogens [130, 131]. Instead, epigenetic events appear to be involved in the starting phase of heavy metal carcinogenesis by “writing” and “erasing” epigenetic signals at the promoter regions of several cancerous genes.

Epigenetic alterations regulate key events in cellular homeostasis, including transcriptional and translational regulation of gene expression. The most well studied epigenetic alterations are DNA methylation, the covalent, post-translational modification of histones, and non-coding small RNAs (miRNAs) [132-135]. Epigenetic alterations can be induced by environmental stimuli, and much attention has been given to the role of the epigenome in human disease [136]: a role that arises primarily from the control that the epigenome exerts over the transcriptome and the proteome. The transcriptome, which is the total transcribed RNA of a cell at a given point in time, is regulated primarily through

transcription and mRNA stability and degradation. DNA methylation, histone modifications, and miRNA all regulate the transcriptome through transcriptional processes. The proteome, the total proteins of the cell, is the functional mediator between the genome and the cell and is regulated primarily through post-transcriptional regulation of mRNA transcripts. Epigenetic regulation of the proteome occurs primarily through the action of miRNAs on mRNA transcript stability and translation. In (Fig. 4), the main epigenetic effects induced by heavy metals are reported. The recent scientific interest aroused by cadmium for its effects on human carcinogenesis has been enhanced by its ability to interfere with gene expression despite its inability to alter gene structure. At the moment, cadmium is predominantly considered as an epigenetic carcinogen. Many studies converge on its ability to induce epi-mutations, mostly by modifying the DNA methylation pattern of target genes, although the definition of ways and mechanisms involved is still far from being achieved. The carcinogenic potential of cadmium through epigenetic changes includes silencing of DNA repair and tumor-suppressor genes.



**Fig. (4).** The role of the epigenome in toxic metal-induced disease pathways. Epigenetic alterations classified broadly as effects to the “epigenome” have the potential to regulate mRNAs/transcripts (i.e., the transcriptome) and ultimately impact protein expression (i.e., the proteome) within cells. Exposure to toxic metals can impact various components of the epigenetic machinery. These toxic metal-mediated epigenetic alterations may directly impact gene transcription and subsequently regulate protein translation, leading to aberrant expression of key mediators of disease processes (from Ray PD, Yosim A, Fry RC. *Incorporating epigenetic data into the risk assessment process for the toxic metals arsenic, cadmium, chromium, lead, and mercury: strategies and challenges.* *Front Genet.*, 2014, 5:201.)

### DNA Methylation

Both DNA hypomethylation and hypermethylation were found according to the time of exposure to the metal, but while some researchers have shown that hypermethylation appears to be an early signature for cadmium-induced cancer [137], other studies have highlighted that the acute exposure

to cadmium prevents DNA methyltransferase activity leading to global DNA hypomethylation, while extended exposure results in increased DNA methyltransferase activity and DNA hypermethylation. Likely, the discrepancies may be due to the different species examined, humans or animals, and to the different cell types used.

Recently, it was found that cadmium-induced transformation of rodent liver cells is associated with DNA hypomethylation, likely due to inhibition of DNA methyltransferases in the early stages of cadmium exposure [138]. Another work indicates that cadmium is an effective inhibitor of nuclear DNA methyltransferases in rats [139].

There is evidence also in humans that cadmium exposure can, under certain circumstances, alter DNA methylation, which may represent an additional mechanism for cadmium-induced carcinogenesis [140]. For example, life-long cadmium exposure in women, as evidenced by low-level urinary cadmium concentrations, was inversely associated with a marker of global DNA methylation, such as *long interspersed nuclear element-1 (LINE-1)* methylation, and with the expression of *DNMT3B*, which is involved in *de novo* CpG methylation. These data are of concern, since genome-wide methylation loss from *LINE-1* promoter sites, which are normally heavily methylated at CpG sites, is considered as a frequent epigenetic change in malignancies and may play a role in carcinogenesis [141-143]. However, urinary cadmium was not associated with alterations in the expression of *DNMT1*, a factor that plays a role in maintaining CpG methylation patterns in the genome. The association between urinary cadmium and decreased *LINE-1* methylation was stronger among carriers of the rare alleles of *DNMT1* rs10854076 and rs2228611, indicating that cadmium effects can vary according to *DNMT1* polymorphisms. A recent work links arsenite and cadmium exposure with agglomerative DNA methylation, confirming that this may be one avenue by which these chemicals drive carcinogenesis [144]. Agglomerative DNA methylation, in which many differentially methylated regions (DMRs) are concentrated within a certain region of a chromosome, can be part of long-range epigenetic silencing (LRES), which is implicated in cancer progression and associated with changes in histone modifications [145]. While the role of LRES in cancers continues to gain supporting evidence, which environmental factors trigger this kind of epigenetic change, if any, has been unclear. Agglomerative DNA hypermethylation has been observed in different kinds of human tumors in association with a reduction in plasticity-associated gene transcription of consecutive genes [145]. Moreover, agglomerative DNA hypermethylation was found in toxicant-transformed cell lines RWPE-1 and UROtsa (derived, respectively, from the prostate and ureter and used as models to study epigenetic dysfunction related to malignant transformation induced by arsenic or cadmium), thus providing a link between *in vitro* chronic exposure to both arsenic and cadmium and hypermethylation of large chromosomal regions [144]. Over the course of cadmium-induced transformation, global DNA methylation and activity of DNMTs increased, along with *DNMT1* and *DNMT3a* expression. Moreover, four key genes related to DNA repair, *hMSH2*, *ERCC1*, *XRCC1*, and *hOGG1*, were hypermethylated at the

promoter and suppressed at the mRNA and protein levels in cadmium-transformed cells compared to controls. Tying these findings together, DNA demethylation treatment partially restored mRNA and protein expression of the DNA repair genes, brought down elevated DNMTs levels and suppressed the growth of tumor-derived cells and of cells passaged 35 times in the presence of cadmium chloride. Hypermethylation and repression of DNA repair genes, therefore, appear to be an early signature for cadmium-induced cancer and may also constitute part of the mechanism by which the toxicant induces tumorigenesis [137]. Another study has shown that following acute exposure (1 week) to cadmium, TRL 1215 rat liver cells exhibited decreased DNA methyltransferase activity and global DNA hypomethylation, whereas extended exposure (10 week) resulted in increased DNA methyltransferase activity and DNA hypermethylation [138]. A study using human prostate cells was in line with these results by showing that a 10-week exposure to cadmium led to malignant transformation in association with DNA hypermethylation at the global level, overexpression of *DNMT3b* and increased DNMT activity, promoter hypermethylation and decreased expression of the tumor suppressor genes *RASSF1A* and *p16<sup>INK4A</sup>* [146]. Recently, it has been shown that the level of genomic DNA methylation markedly increased in long-term cadmium-treated human B lymphoblasts [147]. The methylation status of *p16<sup>INK4A</sup>* in the cadmium-exposed cells was significantly increased compared with control cells. Moreover the expression of *p16<sup>INK4A</sup>* protein in the cadmium-treated cells was consistent with *p16<sup>INK4A</sup>* mRNA expression both in the presence and in the absence of 5-aza-dC treatment. These data provided evidence that DNA hypermethylation of *p16<sup>INK4A</sup>* gene contributes to cadmium-enhanced cell proliferation of human lymphocytes and spurred further investigations about the epigenetic mechanism for cadmium-induced carcinogenesis. However, another research group reported that cadmium-induced global DNA hypomethylation led to increased cell proliferation in the chronic myelogenous leukemia K562 cell line [148]. Recently, a causal association was demonstrated between caspase 8 promoter hypermethylation, pathological changes in the liver of rats and mice exposed to cadmium, and loss of the apoptotic pathway [149]. The functional loss of caspase 8, an apoptosis-related cysteine protease [150], was previously attributed to DNA hypermethylation in approximately 61% of human neuroblastoma specimens [151]. In addition, caspase 8 promoter was aberrantly methylated in all liver tissues from patients with hepatic cell carcinoma (HCC) while it was unmethylated in all the normal liver tissues. These data suggest that the hypermethylation-mediated inactivation of caspase 8 contributes to HCC by regulation of apoptotic cell death [152]. Moreover, it has been shown that the hypermethylation of caspase 8 promoter is a common feature of relapsed glioblastoma multiforme [153]. Although some studies have indicated that acute exposure to cadmium alone induces apoptotic cell death through different cell death pathways [154, 101], other *in vitro* studies showed that cadmium's inhibition of apoptotic cell death induced by other stressors, such as chromium, occurs through caspase-3 repression [155]. Therefore, it has been considered that the inhibition of apoptosis may be the main feature of cadmium carcinogenic

mechanism. The relationships between altered activity of DNA methyltransferases and cadmium-induced cell proliferation needs to be better investigated.

### Histone Tail Modification

As far as we know, very few studies have shown post-translational modifications of N-terminal tails of histones as a result of cadmium poisoning. Acute exposure of non-transformed human urothelial cells (UROtsa) to both arsenicum and cadmium markedly increased the expression of enolase (ENO) 2, a useful biomarker for identifying tumoral neuroendocrine differentiation. It has been demonstrated that the expression of ENO2 mRNA was markedly elevated following treatment with the histone deacetylase inhibitor MS-275 or in cadmium-and arsenicum-transformed UROtsa cells and tumour transplants [156]. A similar mechanism has been proposed for MT-3 gene expression, but in reverse. MT-3 gene is silent either in cadmium or arsenicum-transformed cell lines derived from UROtsa cells [157] through a mechanism that implies histone changes [158]. After treatment with MS-275, higher expression of MT-3 mRNA was found in the cadmium and arsenicum-transformed cell lines as compared with the parental UROtsa cells. Further studies are recommended to explore whether cadmium can alter gene expression by inducing histone changes at a global or gene-specific level.

### Modulation of miRNA Expression

miRNAs are small, non-coding RNAs transcripts of ~21–23 nucleotides in length that regulate gene expression at the post-transcriptional level through complementary binding of 3' or 5' untranslated regions (3' or 5' UTR) of mRNA. miRNAs target the transcripts for degradation or to prevent translation, resulting in decreased target protein expression, though it has been reported that miRNAs may activate translation [159, 160]. miRNAs may also regulate transcription by directly binding to gene promoter sequences and inducing chromatin remodeling [161]. miRNA sequences are located in specifically associated promoters, or even embedded in host genes [160]. After transcription miRNAs are processed in the nucleus to yield hairpin structures known as pre-miRNA. Pre-miRNAs are then exported into the cytoplasm where they undergo further processing into mature miRNAs. Mature miRNAs involved in gene silencing are incorporated into RNA-induced silencing complexes (RISC) [162]. At present, over 17,000 different miRNAs sequences have been identified in approximately 140 species [163]. Aberrant expression of miRNAs has been associated with several human disease states, including cancer, cardiovascular disease, and genetic disorders [164]. A number of studies provide hints about the involvement of miRNAs in the toxic mechanism induced by cadmium. Cadmium has been shown to alter miRNA levels *in vitro*. In the human hepatocellular carcinoma cell line HepG2, a useful *in vitro* model for metal toxicity studies, miRNA expression was analyzed after cadmium exposure [165]. A large percentage of modified microRNAs belonged to the let-7 family, which is reported to have oncosuppressor functions, as evidenced by its ability to regulate DNA repair and cell division [166]. Another potential tumor suppressor that might be down-modulated by cadmium is mir-15b, an element that can

target Bcl-2, thereby regulating apoptosis [167]. Several pathways connected to cancer were regulated at the transcriptional level, and miRNAs had a potential impact on the modulation of this regulation. At the low cadmium concentration (2  $\mu\text{M}$ ) only eleven genes belonging to the metallothionein family were regulated. At the higher concentration (10  $\mu\text{M}$ ) the pathway enrichment analysis for the 536 up-regulated genes showed a large number of pathways related to cancer, whereas the 424 down-regulated genes were enriched on pathways correlated to liver function. Focal adhesion (hsa04510) and the MAPK signaling pathway (hsa04010) were the two top pathways in the analysis of enrichment in KEGG pathways of the downregulated miRNAs targets. Focal adhesion is a fundamental mechanism regulating a number of crucial cell functions, such as gene expression, proliferation, differentiation, survival and motility. Metastasis and local invasion in cancer cells are facilitated by motility events requiring the regulation of focal adhesion and the deregulation of the actin cytoskeleton [168, 169]. Cell proliferation and migration are associated with increased signaling through the MAPK cascade, a highly conserved activation pathway [170]. In the same hepatoma cell line, very recently, Urani and his colleagues [171] showed that mir-372 and mir-138, both connected to carcinogenesis [172, 173], were upregulated after exposure to 10  $\mu\text{M}$  cadmium. Cell cycle regulation can be affected by mir-372 that binds to the 3'UTR of p21<sup>Cip1/WAF-1a</sup>, a downstream protein of the p53 network [174]. Accordingly, mir-372 can promote cell-cycle progression and cell proliferation [173]. Oncosuppression was recently linked with increased mir-138 expression and, conversely, its downregulation was associated with the development of head and neck squamous cell carcinoma (HNSCC). However, although miR-138 downregulation is associated with HNSCC and other tumors, the exact contribution of miR-138 in oncogenesis and the mechanisms underlying this effect remain unclear [175].

In studying the role of airway pollutants in cystic fibrosis, Hassan *et al.* showed that cadmium reduced the expression of the cystic fibrosis transmembrane conductance regulator (CFTR) in human bronchial epithelial cells through upregulation of miR-101 and miR-144 [176]. Notably, miR-101 might promote inflammation by downregulating the expression of MAPK phosphatase-1 (MKP-1). Indeed, this dual specific phosphatase inactivates MAPKs, and thereby acts as an important negative regulator of innate immunity responses [177, 178]. Furthermore, miR-144 targets the hedgehog (Hh) signaling pathway that plays an important role in glioblastoma development [179], as well as the isocitrate dehydrogenase 2 (IDH2) pathway, that has been shown to contribute to atherosclerosis progression. Indeed, the identification of the latter pathway, which involves 7-ketocholesterol (7-KC) in addition to miR-144, has provided new insight into 7-KC function and microRNA biology in cardiovascular disease [180]. Moreover, miR-144 expression levels are inversely related with cell proliferation. For example, bladder cancer cell lines or cancer tissue samples display decreased miR-144 levels. In addition, blocking the expression of endogenous miR-144, promotes cell proliferation. Conversely, miR-144 overexpression results in blockade of cell proliferation, whereas miR-144 downregulation mitigates miR-144-mediated repression of zeste homolog 2 (EZH2) and subsequent cell proliferation [181].



Therefore, it can be speculated that cadmium is implicated in a lot of disparate disease states through alterations of miRNA levels and that such alterations may be useful in the risk characterization steps.

### CADMIUM AND MELANOMA

Melanoma incidence has been on the rise over the last years. Among the risk factors identified so far for the development and the progression of the disease are some trace elements of nutritional and toxicological interest. Data obtained from trace element determination in toenails of 58 cases and 58 controls from the Modena province of Italy showed higher levels of copper and lower concentrations of iron in patients with melanoma of the skin, whereas no differences were observed for cadmium. Therefore, the authors excluded an involvement of cadmium exposure in this disease [182]. However, an involvement of cadmium in the pathogenesis of skin melanoma cannot be excluded, as little is known on cadmium absorption through the skin. In 1991, Wester *et al.* showed a higher adsorption of cadmium from contaminated soil and water into the skin rather than in plasma [183]. Importantly, rats (shaved skin) subjected to percutaneous administration of cadmium chloride solutions showed dermal hyperkeratosis and acanthosis with occasional ulceration, increased mitotic index of the skin cells and elevated cadmium levels in blood, liver and kidney [184]. Cadmium may be absorbed by the skin through its binding to sulfhydryl radicals of cysteine in epidermal keratins, or its complexing with metallothionein [185]. The analysis of mouse B16 melanoma cell lines with high and low constitutive MT expression revealed that cadmium resistance was associated with constitutive MT accumulation in the absence of heavy-metal induction [186]. Resistance to anticancer drugs and radiotherapy, and thereby a poor prognosis, have been associated with MT overexpression in a variety of cancers, according to a number of recent studies. For example, a tendency towards disease progression is associated with MT overexpression in primary melanoma. This feature is useful to identify thin melanomas (< 1.5 mm) carrying a high risk of progression, since MT expression is independent from Breslow thickness [187]. It has been known for many years that MT expression in malignant melanoma is significantly associated with progressive disease and might therefore be a useful prognostic indicator, as shown in paraffin-embedded tissues from 63 cases of malignant melanoma and 13 secondary deposits [188]. An association has been found between exposure to cadmium and the development of uveal melanoma [189]. Moreover, in uveal melanoma cultures, an increased resistance to cadmium-induced cytotoxicity characterizes the vasculogenic mimicry (VM)-forming tumor cells. VM patterns are present in a wide variety of malignant tumours and are associated with a poor prognosis, since they reflect a marked ability of tumour cells to form perfusion pathways [190-194]. C918 uveal melanoma cells have been studied in both traditional two-dimensional (2D) and extracellular matrix (ECM, Matrigel)-containing 3D cultures. While growing as a monolayer in 2D cultures, C918 cells formed complex patterns in 3D cultures, involving morphologically distinct cell subpopulations. For example, monolayers were formed both on Matrigel surfaces and on the bottom of the culture dish, whereas VM patterns were pro-

duced in other areas. At 4 to 5 days after the beginning of culture, 2D and 3D cultures were exposed to various concentrations of cadmium chloride, ranging from 20 to 1000  $\mu\text{M}$  [195] and observed daily for toxicity. Under these conditions, cadmium-exposed cells showed dose-dependent toxicity, while control cultures remained viable. Interestingly, however, VM-forming tumour cells showed increased survival following cadmium exposure relative to other subpopulations in 3D cultures or cells cultured in 2D. Moreover, when exposure to cadmium was stopped after initiation of cisplatin treatment, residual viable cells in VM patterns could function as foci for new growth in 3D cultures [196]. These data suggest that VM-forming cells in uveal melanoma cultures are less susceptible to cisplatin or cadmium toxicity. Moreover, such increased resistance may represent a mechanism whereby VM-forming melanoma cells might contribute to a poor prognosis [196]. In addition, several years ago it was well documented that the mouse melanoma cell invasion of organ samples obtained from syngeneic mice who had been administered heavy metals, including cadmium, was enhanced through the induction of MTs. Therefore, it has been suggested that heavy metal-induced MTs serve as host-derived factors in malignant disease and are strictly associated with metastasis [197]. Thus from a review of the literature, a picture emerges of conflicting results on the involvement of cadmium in melanoma. Some studies could not find any role of the metal in the pathogenesis of this tumor, while increased resistance to cadmium was associated with some features of malignant melanomas. Indeed, more attention should perhaps be paid to the effects of non-toxic doses of cadmium on normal and transformed melanocytes. Indeed, this issue has never been studied in depth, despite cadmium was shown to be comutagenic with UV, the leading cause of the onset of melanoma. For example, as previously indicated, cadmium interferes with nucleotide excision repair by interfering with the removal of thymine dimers after UV irradiation [125]. However, to the best of our knowledge, the possible role of cadmium in inducing epigenetic modifications of key genes in the regulation of proliferation and apoptosis in transformed melanocytes has not been studied. As mentioned, investigations of this type could be of importance, in view of the recognized role of cadmium as a cancer promoter in combination with ultraviolet radiations. Moreover, patterns of epigenetic modifications could serve as markers of gene activity and expression [198-200]. To get initial insights into this issue, we evaluated epigenetic effects on genes involved in apoptosis and proliferation after acute cadmium exposure. In other transformed cell systems, inhibition of the expression of tumor suppressor genes responsible for the regulation of the cell cycle, such as p16<sup>INK4A</sup>, and of apoptosis, such as caspase 8, was demonstrated following exposure to cadmium. Similarly, we could document that short-term cadmium exposure had a critical impact on the behavior of melanoma cells through epigenetic modifications of p16<sup>INK4A</sup> and caspase 8, resulting in their altered expression and in aberrant cell proliferation and apoptosis. Indeed, after a short cadmium exposure we observed an increase in cell population in either cutaneous or uveal melanoma. However, the mechanisms appeared to be quite different. In skin melanoma the extrinsic pathway of apoptosis was affected and caspase 8 hypermethylation and

inactivation was indicated as the main pathogenic mechanism. In uveal melanoma, instead, p16<sup>INK4A</sup> aberrant methylation and silencing was called as the determinant cause [201]. It is worth of notice that in some cell lines the effects were more pronounced after 48 hours of exposure to cadmium than those observed after 72 hours of treatment, even when using low concentrations of the metal. The other genes investigated did not undergo any modification of their expression following exposure to cadmium regardless of whether they were expressed or not. Our results show that cadmium-treated uveal melanoma cell lines have >80% methylated clones (i.e., severe hypermethylation and complete methylation) in the p16<sup>INK4A</sup> promoter versus >60% unmethylated clones (i.e., severe hypomethylation and complete unmethylation) in the same gene region of the corresponding untreated cells. Clones of cutaneous melanoma cell lines with <20% methylation of the caspase 8 promoter (i.e., completely unmethylated and mildly hypermethylated) were found in control untreated cells, whereas clones with >70% methylation (i.e., moderately-to-severely hypermethylated) occurred in the same cell lines exposed to cadmium. Integrative analysis of DNA methylation and RNA expression data revealed a positive association between DNA methylation and transcript expression of p16<sup>INK4A</sup> and caspase 8 either before or after treatment with cadmium. Inquiring about the role of DNMTs evidenced a prevalent role for DNMT3a and DNMT1 in the methylation of, respectively, caspase 8 and p16<sup>INK4A</sup> while DNMT3b seemed to have much less impact. These results are in agreement with those of others, highlighting that during malignant transformation cadmium is able to alter total DNMTs activities and to progressively increase global DNA methylation [137, 138, 142]. The methylation profile of the other genes that we have studied revealed generally hypomethylated or mildly hypomethylated clones with no significant difference between the untreated and cadmium-treated cells. The silencing of p16<sup>INK4A</sup> and caspase 8 by cadmium in melanoma cells is of particular importance in light of the negligible role played by constitutional epimutations of CDKN2A (p16<sup>INK4A</sup> and p14<sup>ARF</sup>) genes in melanoma families, whereby it was concluded that it is unlikely that they play a role in susceptibility to the disease in families without CDKN2A mutations [202]. These findings are coherent with those of others which aimed at identifying germline epimutations of genes that were found to be mutated in familial cutaneous malignant melanoma. No association was found between the risk of cutaneous malignant melanoma and peripheral blood mononuclear cell methylation levels of p16<sup>INK4A</sup> [203]. These data confirm the ones presented in this study, and highlight a crucial role of the environment in inducing epigenetic changes in a key gene in melanoma development, such as p16<sup>INK4A</sup>, in absence of inherited mutations. The relevance of the occurrence of p16<sup>INK4A</sup> promoter methylation in melanoma was well established by a very recent work which has shown that hypermethylation of the p16<sup>INK4A</sup> promoter was greater in the vertical growth-phase (60%) melanomas than in the radial (40%, P = 0.063) melanomas and in those displaying epidermal involvement (P = 0.046). Notably, p16<sup>INK4A</sup> methylation well correlated with increased melanoma thickness and marginally with increased Clark level. Low (1-30% p16<sup>INK4A</sup> expression was detected in the majority (19 of 54 or 35%)) of melanoma cases [204]. And more, loss of

CDKN2A expression was shown to frequently occur in primary invasive melanoma [205] and absent p16<sup>INK4A</sup> expression was associated with adverse prognostic markers of the cancer [206]. For these reasons, p16<sup>INK4A</sup> immunohistochemical expression is believed to be an independent prognostic biomarker of potential value in routine melanoma diagnostic practice and was found to be useful in confirming the benign nature of mildly and moderately atypical cutaneous blue nevi (CBN) compared to severely atypical CBN and melanomas [207]. Absence or downregulation of caspase 8 is a frequent event in cancer and is correlated with an unfavourable outcome [208, 209], since it may cause resistance to apoptosis [146, 210]. Several studies have demonstrated that expression of caspase 8 may be controlled by epigenetic modifications, which differ according to the type of cancer cells [211-213]. Hypermethylation of the promoter region has been recognized as the most recurrent mechanism causing functional loss of caspase 8 gene. However, although an association between aberrant DNA methylation of caspase 8 and carcinogenesis has been demonstrated in several studies [214, 215], the functional relevance of this epigenetic trait in melanomagenesis has not yet been fully understood [216]. Our findings, showing for the first time that caspase 8 expression is impaired by DNA hypermethylation following cadmium treatment in cutaneous melanoma cell lines, highlight the potential relevance of such a mechanism in the context of this tumor and extend earlier *in vitro* and *in vivo* observations linking exposure to cadmium with decreased expression of caspase 8 in tumours different from melanoma [217, 218]. The pivotal role of caspase 8 overexpression in reactivating the apoptosis program in cutaneous melanoma cells exposed to cadmium with hypermethylated caspase 8 promoter suggests that cadmium might act as a tumor promoter for melanoma development. Based on evidence that both the programmed re-expression of caspase 8 by demethylation and caspase 8 gene transfer can sensitize tumour cell lines for drug-induced apoptosis [216, 219, 220], it is possible to hypothesize that this pollutant may prevent drug-induced apoptosis and interfere with therapeutic strategies targeting cutaneous melanoma. Therefore, these findings may be of interest because they: i) highlight the crucial role of acute cadmium exposure in silencing key genes involved in the control of cell cycle and apoptosis in melanoma; ii) discriminate between cutaneous and uveal melanoma on the basis of the genes epigenetically modified by cadmium; iii) indicate cadmium pollution as one of the potential causes of inactivation of p16<sup>INK4A</sup>, a previously recognized biomarker of malignant melanoma; iv) show for the first time the significance of caspase 8 methylation and silencing in cutaneous melanoma and the role of cadmium in this effect. In conclusion, our data suggest that aberrant DNA methylation of p16<sup>INK4A</sup> and caspase 8 gene promoters is associated with single-phenotype defects in melanoma cells after acute exposure to low non cytotoxic doses of cadmium. Further studies are warranted to address the role of epigenetic mechanisms induced by environmental pollutants in worsening the melanoma progression.

## CONCLUSION

Cadmium is a heavy metal, widely distributed in the environment, that affects human health in a multiple ways. In

mammals it exerts toxic effects on several organs, including kidney, liver, stomach, respiratory tract, brain, and bones, although the underlying mechanisms are far from being elucidated.

Cadmium was classified as a human carcinogen by the International Agency for Research on Cancer, since many studies have linked exposure to cadmium with pulmonary, prostatic, and renal cancer in humans and some studies have associated cadmium exposure with human cancer of the liver, hematopoietic system, urinary bladder and stomach. This review emphasizes the importance for applying the study of cadmium carcinogenesis to melanomagenesis, as there is some indication that cadmium might be important in melanoma despite the limited number of studies on the subject.

Relying on available evidence, cadmium apparently has little direct genotoxic activity and acts apparently through various indirect mechanisms, including ROS generation, DNA repair inhibition, impairment of the cellular antioxidant defence system, interference with signal transduction, and possibly the disruption of cell–cell adhesion with the consequent initiation of cancer.

The evidence described in this review highlights the importance of considering epigenetics as a possible mechanism underlying the toxicity and cell-transforming ability of this metal by demonstrating that exposure to cadmium perturbs DNA methylation levels, global and gene-specific histone posttranslational modification markers, and miRNA expression levels. As most of the epigenetic changes are reversible, insights into this topic might provide novel therapeutic strategies based on molecules that modify the activities of epigenetic enzymes, such as DNMTs and HDACs, and/or affect miRNA expression. Thus, epigenome modification might be used to restore normal gene transcription levels. Results presented here indicate that the effects of cadmium on melanomagenesis may be more important than previously thought. Moreover, they highlight the role of epigenetics in melanoma progression following exposure to cadmium. It is speculated that in cutaneous melanoma cadmium affects the extrinsic apoptotic pathway, that is prevalent in this kind of tumor, through methylation and inactivation of caspase 8, while in uveal melanoma the metal alters the cell cycle through methylation and silencing of p16<sup>INK4A</sup>. It's possible that in a near future well characterized epigenetic modifications will aid in the molecular diagnosis and treatment of a variety of cancers and other disorders induced by cadmium.

## CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflict of interest.

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## REFERENCES

- [1] Salnikow, K.; Zhitkovich, A. Genetic and epigenetic mechanisms in metal carcinogenesis and cocarcinogenesis: nickel, arsenic and chromium. *Chem. Res. Toxicol.*, **2008**, *21*, 28-44.
- [2] Singh, V.; Singh Chandel, C.P. Analytical study of heavy metals of industrial effluents at Jaipur, Raasthan (India). *Ind. J. Environ. Sci. Eng.*, **1993**, *4*, 374-379.
- [3] Andrew, A.S.; Klei, L.R.; Barchowsky, A. Nickel requires hypoxia-inducible factor-1 alpha, not redox signaling, to induce plasminogen activator inhibitor-1. *Am. J. Physiol. Lung. Cell Mol. Physiol.*, **2001**, *281*, 607-15.
- [4] Thomson, S.; Hollis, A.; Hazzalin, C.A.; Mahadevan, L.C. Distinct stimulus-specific histone modifications at hsp70 chromatin targeted by the transcription factor heat shock factor-1. *Mol. Cell.*, **2004**, *15*, 585-94.
- [5] National Toxicology Program, Tenth Report on carcinogenesis. Department of Health and Human Health Services. *Research Triangle Park NC*, **2000**, III-42-III-44.
- [6] Satarug, S.; Moore, M.R. Adverse health effects of chronic exposure to low-level cadmium in foodstuffs and cigarette smoke. *Environ. Health Perspec.*, **2004**, *112*(10), 1099-1103.
- [7] Mannino, D.M.; Holguin, F.; Greves, H.M.; Savage-Brown, A.; Stock, A.L.; Jones, R.L. Urinary cadmium levels predict lower lung function in current and former smokers: data from the Third National Health and Nutritional Examination Survey. *Thorax*, **2004**, *59*(3), 194-8.
- [8] Waalkes, M.P. Cadmium carcinogenesis. *Mut. Res.*, **2003**, *533*, 107-120.
- [9] Schwerdtle, T.; Ebert, F.; Thuy, C.; Richter, C.L.; Mullenders, H.F.; Hartwig, A. Genotoxicity of soluble and particulate cadmium compounds: impact on oxidative DNA damage and nucleotide excision repair. *Chem. Res. Toxicol.*, **2010**, *23*(2), 432-442.
- [10] Othman Z.A.; Hashem A.; Habila M.A. Kinetic, equilibrium and thermodynamic studies of cadmium (II) adsorption by modified agricultural wastes. *Molecules*, **2011**, *16*(12), 10443-56.
- [11] Agency for Toxic Substances and Disease Registry Toxic guide for cadmium. Department of Human and Health services. *ATSDR*, **2012**. <http://www.atsdr.cdc.gov/toxprofiles/index.asp>.
- [12] Zalups, R.K.; Ahmad, S. Molecular handling of cadmium in transporting epithelia. *Toxicol. Appl. Pharmacol.*, **2003**, *186*, 163-188.
- [13] Orłowski, C.; Piotrowski, J.K. Biological levels of cadmium and zinc in the small intestine of non-occupationally exposed human subjects. *Hum. Exp. Toxicol.*, **2003**, *22*, 57-63.
- [14] Jin, T.; Nordberg, M.; Frech, W.; Dumont, X.; Bernard, A.; Ye, T.T.; Kong, Q.; Wang, Z.; Li, P.; Lundstrom, N.G.; Li, Y.; Nordberg, G.F. Cadmium biomonitoring and renal dysfunction among a population environmentally exposed to cadmium from smelting in China (ChinaCad). *Biomaterials*, **2002**, *15*, 397-410.
- [15] Agency for Toxic Substances and Disease Registry. Toxicological Profile for Cadmium. US Department of Human and Health Services. *ATSDR*, **1999**.
- [16] Sorahan, T.; Esmen, N.A. Lung cancer mortality in UK nickel-cadmium battery workers, 1947-2000. *Occup. Environ. Med.*, **2004**, *61*(2), 108-16.
- [17] Verougstraete, V.; Lison, D.; Hotz, P. Cadmium, lung and prostate cancer: a systematic review of recent epidemiological data. *J. Toxicol. Environ. Health B. Crit. Rev.*, **2003**, *6*(3), 227-55.
- [18] Dressler, J.; Schulz, K.; Klemm, M.; Schütting, R.; Beuthin, A.; Felscher, D. Lethal manganese-cadmium intoxication. A case report. *Arch. Toxicol.*, **2002**, *76*, 449-451.
- [19] Satarug, S.; Baker, J.R.; Urbenjapol, S.; Haswell-Elkins, M.; Reilly, P.E.; Williams, D.J.; Moore, M.R. A global perspective on cadmium pollution and toxicity in non-occupationally exposed population. *Toxicol. Lett.*, **2003**, *137*(1-2), 65-83.
- [20] National Toxicology Program. Report on Carcinogenesis, Eleventh edition, Carcinogen profiles. Research Triangle Park, North Carolina; US Department of Health and Human Services, Public Health Services. *Nat. Toxicol. Program, NTP*, **2004**.
- [21] Horiguchi, H.; Oguma, E.; Sasaki, S.; Miyamoto, K.; Ikeda, Y.; Machida, M.; Kayama, F. Dietary exposure to cadmium at close to the current provisional tolerable weekly intake does not affect renal function among female Japanese farmers. *Environ. Res.*, **2004**, *95*(1), 20-31.
- [22] Taylor, W.R. Permeation of barium and cadmium through slowly inactivating calcium channels in cat sensory neurones. *J. Physiol.*, **1988**, *407*, 433-452.
- [23] Foulkes, E.C. Interactions between metals in rat jejunum: implications on the nature of cadmium uptake. *Toxicology*, **1985**, *37*, 117-125.
- [24] Jarup, L.; Berglund, M.; Elinder, C.G.; Nordberg, G.; Vahter, M.

- Health effects of cadmium exposure--a review of the literature and a risk estimate. *Scand. J. Work Environ. Health*, **1998**, 24 Suppl 1, 1-51.
- [25] Flanagan, P.R.; McLellan, J.S.; Haist, J.; Cherian, G.; Chamberlain, M.J.; Valberg, L.S. Increased dietary cadmium absorption in mice and human subjects with iron deficiency. *Gastroenterology*, **1978**, 74, 841-846.
- [26] Gunshin, H.; Mackenzie, B.; Berger, U.V.; Gunshin, Y.; Romero, M.F.; Boron, W.F.; Nussberger, S.; Gollan, J.L.; Hediger, M.A. Cloning and characterization of a mammalian proton-coupled metal-ion transporter. *Nature*, **1997**, 388, 482-488.
- [27] Kobayashi, E.; Suwazono, Y.; Uetani, M.; Kito, T.; Nishijo, M.; Nakagawa, H.; Nogawa, H.; Nogawa, K. Tolerable level of lifetime cadmium intake estimated as a benchmark dose low, based on excretion of  $\beta_2$ -Microglobulin in the cadmium-polluted regions of the Kakehashi River Basin, Japan. *Bull Environ. Contam. Toxicol.*, **2006**, 76, 8-15.
- [28] Ezaki, T.; Tsukahara, T.; Moriguchi, J.; Furuki, K.; Fukui, Y.; Ukai, H.; Okamoto, S.; Sakurai, H.; Honda, S.; Ikeda, M. No clear-cut evidence for cadmium-induced renal tubular dysfunction among over 10,000 women in the Japanese general population: a nationwide large-scale survey. *Int. Arch. Occup. Environ. Health*, **2003**, 76(3), 186-196.
- [29] Nogawa, K.; Kobayashi, E.; Okubo, Y.; Suwazono, Y. Environmental cadmium exposure, adverse effects and preventive measures in Japan. *Biomaterials*, **2004**, 17, 581-587.
- [30] Kazantzis, G. Cadmium, osteoporosis and calcium metabolism. *Biomaterials*, **2004**, 17, 493-498.
- [31] Staessen, J.A.; Kuznetsova, T.; Roels, H.A.; Emelianov, D.; Fagard, R. Exposure to cadmium and conventional and ambulatory blood pressures in a prospective population study. Public Health and Environmental Exposure to Cadmium Study Group. *Am. J. Hypertens*, **2000**, 13(2), 146-56.
- [32] Staessen, J.A.; Roels, H.A.; Emelianov, D.; Kuznetsova, T.; Thijs, L.; Vangronsveld, J.; Fagard, R. Environmental exposure to cadmium, forearm bone density, and risk of fractures: prospective population study. Public Health and Environmental Exposure to Cadmium (PhecCad) Study Group. *Lancet*, **1999**, 353(9159) 1140-1144.
- [33] Navas-Acien, A.; Selvin, E.; Sharrett, A.R.; Calderon-Aranda, E.; Silbergeld, E.; Guallar, E. Lead, cadmium, smoking, and increased risk of peripheral arterial disease. *Circulation*, **2004**, 109, 3196-3201.
- [34] Navas-Acien, A.; Silbergeld, E.K.; Sharrett, R.; Calderon-Aranda, E.; Selvin, E.; Guallar, E. Metals in Urine and Peripheral Artery Disease. *Environ. Health Perspec.*, **2005**, 113(2), 164-169.
- [35] International Agency for Research on Cancer (IARC). Beryllium, Cadmium, Mercury and Exposures in the Glass Industry. *IARC monographs*, **1993**, 58, 119-238.
- [36] Goering, P.L.; Waalkes, M.P.; Klaassen, C.D. *Toxicology of Cadmium*. In: *Handbook of Experimental Pharmacology: Toxicology of Metals, Biochemical Effects*, Goyer, R.A.; Cherian M.G. Eds.; Springer-Verlag: New York, **1994**, pp. 189-214.
- [37] Nordberg, G.F. Historical perspectives on cadmium toxicology. *Toxicol. Appl. Pharmacol.*, **2009**, 238, 192-200.
- [38] Friberg, L.; Kjellstrom, T.; Nordberg, G.F.; Piscator, M. *Cadmium*. In: *"Handbook on the Toxicology of Metals"*, 2nd ed.; Friberg, L.; Nordberg, G.F.; Voul, V.B. Eds.; Elsevier: Oxford, **1986**, 130-184.
- [39] Kasuya, M.; Teranishi, H.; Aoshima, K.; Katoh, T.; Horiguchi, H.; Morikawa, Y.; Nishijo, M.; Iwata, K. Water pollution by cadmium and the onset of Itai-itai disease. *Water Sci. Technol.*, **2000**, 25, 149-156.
- [40] Jin, T.; Kong, Q.; Ye, T.; Wu, X.; Nordberg, G.F. Renal dysfunction of cadmium-exposed workers residing in a cadmium-polluted environment. *Biomaterials*, **2004**, 17, 513-518.
- [41] Roels, H.A.; Hoet, P.; Lison, D. Usefulness of biomarkers of exposure to inorganic mercury, lead, or cadmium in controlling occupation and environmental risks of nephrotoxicity. *Renal. Failure*, **1999**, 21(3-4), 251-62.
- [42] Järup, L.; Hellström, L.; Alfvén, T.; Carlsson, M.D.; Grubb, A.; Persson, B.; Pettersson, C.; Spång, G.; Schütz, A.; Elinder, C.G. Low level exposure to cadmium and early kidney damage: the OSCAR study. *Occup. Environ. Med.*, **2000**, 57(10), 668-672.
- [43] Ikeda, M.; Ezaki, T.; Moriguchi, J.; Fukui, Y.; Ukai, H.; Okamoto, S.; Sakurai, H. The threshold cadmium level that causes a substantial increase in urine of general populations. *Tohoku. J. Exp. Med.*, **2005**, 205(3), 247-261.
- [44] Mueller, P.W.; Paschal, D.C.; Hammel, R.R.; Klinczewicz, S.L.; MacNeil, M.L.; Spierto, B.; Steinberg, K.K. Chronic renal effects in three studies of men and women occupationally exposed to cadmium. *Arch. Environ. Contaminat. Toxicol.*, **1992**, 23(1), 125-136.
- [45] Iwata, K.; Saito, H.; Moriyama, M.; Nakano, A. Renal tubular function after reduction of environmental cadmium exposure: a ten year follow up. *Arch. Environ. Health.*, **1993**, 48(3), 157-63.
- [46] Buchet, J.P.; Lauwerys, R.; Roels, H.; Bernard, A.; Bruaux, P.; Claeys, F.; Ducoffre, G.; de Plaen, P.; Stessen, J.; Amery, A.; Lijnen, p.; Thijs, L.; Rondia, D.; Sartor, F.; Saint Remi, A.; Nick, L. Renal effects of cadmium body burden of the general population. *Lancet*, **1990**, 336, 699-702.
- [47] Ikeda, M.; Ezaki, T.; Tsukahara, T.; Moriguchi, J.; Furui, K.; Fukui, Y.; Ukai, H.; Okamoto, S.; Sakurai, H. Threshold levels of urinary cadmium in relation to increases in urinary  $\beta_2$ -microglobulin among general Japanese populations. *Toxicol. Lett.*, **2003**, 137(3), 135-141.
- [48] Ikeda, M.; Ezaki, T.; Moriguchi, J.; Fukui, Y.; Okamoto, S.; Ukai, H.; Sakurai, H. No meaningful increase in urinary tubular dysfunction markers in a population with 3 microg cadmium/g creatinine in urine. *Biol. Trace. Elem. Res.*, **2006**, 113(1), 35-44.
- [49] Noonan, C.W.; Sarasua, S.M.; Campagna, D.; Kathman, S.J.; Lybarger, J.A.; Mueller, P.W. Effects of exposure to low levels of environmental cadmium on renal biomarkers. *Environ. Health Perspect.*, **2002**, 110(2), 151-155.
- [50] Satoh, M.; Koyama, H.; Kaji, T.; Kito, H.; Tohyama, C. Perspectives on cadmium toxicity research. *Tohoku. J. Exp. Med.*, **2002**, 196(1), 23-32.
- [51] Trzcinka-Ochcicka, M.; Jakubowski, M.; Razniewska, G.; Halatek, T.; Gazewski, A. The effects of environmental cadmium exposure on kidney function: the possible influence of age. *Environ. Res.*, **2004**, 95(2), 143-150.
- [52] Watanabe, k.; Kobayashi, E.; Suwazono, Y.; Okubo, Y.; Kito, T.; Nogawa, K. Tolerable lifetime cadmium intake calculated from the inhabitants living in the Jinzu River basin, Japan. *Bull. Environ. Contam. Toxicol.*, **2004**, 72(6), 1091-1097.
- [53] Jarup, L. Cadmium overload and toxicity. *Nephrol. Dialysis Transplanta.*, **2002**, 17(Suppl 2), 35-39.
- [54] Alfvén, T.; Järup, L.; Elinder, C.G. Cadmium and lead in blood in relation to low bone mineral density and tubular proteinuria. *Environ. Health Perspec.*, **2002**, 110(7), 699-702.
- [55] Alfvén, T.; Elinder, C.G.; Hellström, L.; Lagarde, F.; Järup, L. Cadmium exposure and distal forearm fractures. *J. Bone Mine. Res.*, **2004**, 19(6), 900-908.
- [56] Nogawa, K.; Tsuritani, I.; Kido, T.; Honda, R.; Yamada, Y.; Ishizaki, M. Mechanism for bone disease found in inhabitants environmentally exposed to cadmium: decreased serum 1 $\alpha$ , 25-dihydroxyvitamin D level. *Int. Arch. Occup. Environ. Health*, **1987**, 59, 21-30.
- [57] Nishijo, M.; Natagawa, H.; Honda, R.; Tanebe, K.; Saito, S.; Teranishi, H.; Tawara, K. Effects of maternal exposure to cadmium on pregnancy outcome and breast milk. *Occup. Environ. Med.*, **2002**, 59(6), 394-6.
- [58] Zhang, Y.L.; Zhao, Y.C.; Wang, J.X.; Zhu, H.D.; Liu, Q.F.; Fan, Y.G.; Wang, N.F.; Zhao, J.H.; Liu, H.S.; Ou-Yang, L.; Liu, A.P.; Fan, T.Q. Effect of environmental exposure to cadmium on pregnancy outcome and fetal growth: a study on healthy pregnant women in China. *J. Environ. Sci. Health A Tox. Hazard. Ubst. Environ. Eng.*, **2004**, 39(9), 2507-15.
- [59] Safe, S. Cadmium's disguise dupes the estrogen receptor. *Nat. Med.*, **2003**, 9, 1000-1
- [60] Johnson, M.D.; Kenney, N.; Stoica, A.; Hilakivi-Clarke, L.; Singh, B.; Chepko, G.; Clarke, R.; Sholler, P.F.; Lirio, A.A.; Foss, C.; Reiter, R.; Trock, B.; Paik, S.; Martin, M.B. cadmium mimics the *in vivo* effects of estrogen in the uterus and mammary gland. *Nature Med.*, **2003**, 9(8), 1081-1084.
- [61] Brzozowski, AM.; Pike, A.C.; Dauter, Z.; Hubbart, R.E.; Bonn, T.; Engström, O.; Ohman, L.; Greene, G.L.; Gustafsson, J.A.; Carlquist, M. Molecular basis of agonism and antagonism in the oestrogen receptor. *Nature*, **1997**, 389, 753-758.
- [62] Norman, A.W.; Mizwicki, M.T.; Norman, D.P. Steroid-hormone rapid actions, membrane receptors and a conformational ensemble model. *Nat. Rev. Drug Discov.*, **2004**, 3(1), 27-41.
- [63] Akesson, A.; Julin, B.; Work, A. Long-term dietary cadmium intake and postmenopausal endometrial cancer incidence: a popula-

- tion-based prospective cohort study. *Cancer Res.*, **2008**, *68*(15), 6435-6441.
- [64] Strumylaite, L.; Bogusevicius, A.; Abdrachmanovas, O.; Baranauskienė, D.; Kregzdyte, R.; Pranys, D.; Poskiene, L. Cadmium concentration in biological media of breast cancer patients. *Breast Cancer Res. Treat.*, **2011**, *125*(2), 511-517.
- [65] Siewit, C.L.; Gengler, B.; Vegas, E.; Puckett, R.; Louie, M. C. cadmium Promotes Breast Cancer Cell Proliferation by Potentiating the Interaction between ER{alpha} and c-Jun. *Mol. Endocrinol.*, **2010**, *24*(5), 981-992.
- [66] Zang, Y.; Odwin-Dacosta, S.; Yager, J.D. Effects of cadmium on estrogen receptor mediated signaling and estrogen induced DNA synthesis in T47D human breast cancer cells. *Toxicol. Lett.*, **2009**, *184*(2), 134-138.
- [67] Denier, X.; Hill, E.M.; Rotchell, J.; Minier, C. Estrogenic activity of cadmium, copper and zinc in the yeast estrogen screen. *Toxicol. In Vitro.*, **2009**, *23*(4), 569-573.
- [68] Ishitobi, H.; Mori, K.; Yoshida, K.; Watanabe, C. Effects of perinatal exposure to low-dose cadmium on thyroid hormone-related and sex hormone receptor gene expressions in brain of offspring. *Neurotoxicology*, **2007**, *28*(4), 790-7.
- [69] Fechener, P.; Damdimopoulou, P.; Gauglitz, G. Biosensors Paving the Way to Understanding the Interaction between Cadmium and Estrogen Receptor Alpha. *PLoS One*, **2011**, *6*(8), e23048.
- [70] Rodríguez-Barranco, M.; Lacasaña, M.; Aguilar-Garduño, C.; Alguacil, J.; Gil, F.; González-Alzaga, B.; Rojas-García, A. Association of arsenic, cadmium and manganese exposure with neurodevelopment and behavioural disorders in children: a systematic review and meta-analysis. *Sci. Total Environ.*, **2013**, *454-455*, 562-77.
- [71] Kundu, S.; Sengupta, S.; Chatterjee, S.; Mitra, S. Bhattacharyya A. Cadmium induces lung inflammation independent of lung cell proliferation: a molecular approach. *J. Inflamm. (Lond)*, **2009**, *12*, 6-19.
- [72] Knoflach, M.; Messner, B.; Shen, Y.H.; Frotschnig, S.; Liu, G.; Pfaller, K.; Wang, X.; Matosevic, B.; Willeit, J.; Kiechl, S.; Lauffer, G.; Bernhard, D. Non-toxic cadmium concentrations induce vascular inflammation and promote atherosclerosis. *Circ. J.*, **2011**, *75*(10), 2491-5.
- [73] Lee, V.; McMahan, R.S.; Hu, X.; Gao, X.; Faustman, E.M.; Griffith, W.C.; Kavanagh, T.J.; Eaton, D.L.; McGuire, J.K.; Parks, W.C. Amphiphilic polymer-coated CdSe/ZnS quantum dots induce pro-inflammatory cytokine expression in mouse lung epithelial cells and macrophages. *Nanotoxicology*, **2014**, *1*, 1-8.
- [74] Biondo, C.; Mancuso, G.; Midiri, A.; Signorino, G.; Domina, M.; Lanza Cariccio, V.; Mohammadi, N.; Venza, M.; Venza, I.; Teti, G.; Bennati, C. The Interleukin-1β/CXCL1/2/Neutrophil Axis Mediates Host Protection against Group B Streptococcal Infection. *Infect. Immun.*, **2014**, *82*(11), 4508-17.
- [75] Biondo, C.; Mancuso, G.; Midiri, A.; Signorino, G.; Domina, M.; Lanza Cariccio, V.; Venza, M.; Venza, I.; Teti, G.; Beninati, C. Essential role of interleukin-1 signaling in host defenses against group B streptococcus. *MBIO.*, **2014**, *5*(5), e01428-14.
- [76] Biondo, C.; Mancuso, G.; Beninati, C.; Iaria, C.; Romeo, O.; Cascio, A.; Teti, G. The role of endosomal toll-like receptors in bacterial recognition. *Eur. Rev. Med. Pharmacol. Sci.*, **2012**, *16*(11), 1506-12.
- [77] Signorino, G.; Mohammadi, N.; Patanè, F.; Buscetta, M.; Venza, M.; Venza, I.; Mancuso, G.; Midiri, A.; Alexopoulou, L.; Teti, G.; Biondo, C.; Beninati, C. Role of TLR13 in innate immune recognition of group B streptococci. *Infect. Immun.*, **2014**, pii: IAI.02282-14.
- [78] Biondo, C.; Malara, A.; Costa, A.; Signorino, G.; Cardile, F.; Midiri, A.; Galbo, R.; Papasergi, S.; Domina, M.; Pugliese, M.; Teti, G.; Mancuso, G.; Beninati, C. Recognition of fungal RNA by TLR7 has a nonredundant role in host defense against experimental candidiasis. *Eur. J. Immunol.*, **2012**, *42*(10), 2632-43.
- [79] Costa, A.; Gupta, R.; Signorino, G.; Malara, A.; Cardile, F.; Biondo, C.; Midiri, A.; Galbo, R.; Trieu-Cuot, P.; Papasergi, S.; Teti, G.; Henneke, P.; Mancuso, G.; Golenbock, D.T.; Beninati, C. Activation of the NLRP3 inflammasome by group B streptococci. *J. Immunol.*, **2012**, *188*(4), 1953-60.
- [80] Jin, T.; Lu, J.; Nordberg, M. Toxicokinetics and biochemistry of cadmium with special emphasis on the role of metallothioneins. *Neurotoxicology*, **1998**, *19*, 529-535.
- [81] Spruill, M.D.; Song, B.; Whong, W.Z.; Ong, T. Proto-oncogene amplification and overexpression in cadmium-induced cell transformation. *J. Toxicol. Environ. Health*, **2002**, *65*, 2131-2144.
- [82] Huang, C.; Zhang, Q.; Li, J.; Shi, X.; Castrinova, V.; Ju, G.; Coata, M.; Dong, Z. Involvement of Rrks activation in cadmium-induced AP-1 transactivation *in vitro* and *in vivo*. *Mol. Cell. Biochem.*, **2001**, *222*, 141-147.
- [83] Jeon, S.H.; Cho, M.H.; Cho, J.H. Effects of cadmium on gap junctional intercellular communications in WB-F344 rat liver epithelial cells. *Hum. Exp. Toxicol.*, **2001**, *20*, 577-583.
- [84] Hart, B.A.; Potts, R.J.; Watkin, R.D. Cadmium adaptation in the lung—a double-edged sword? *Toxicology*, **2001**, *160*, 65-70.
- [85] Achanzar, W.E.; Achanzar, K.B.; Lewis, J.G.; Webber, M.M.; Waalkes, M.P. Cadmium induces *c-myc*, *p53*, and *c-jun* expression in normal human prostate epithelial cells as a prelude to apoptosis. *Toxicol. Appl. Pharmacol.*, **2000**, *164*, 291-300.
- [86] Waalkes, M.P.; Rehm, S.; Riggs, C.W.; Bare, R.M.; Devor, D.E.; Poirier, L.A.; Wenk, M.L.; Henneman, J.R.; Balaschak, M.S. Cadmium carcinogenesis in male Wistar [CrI:(WJ)BR] rats: dose-response analysis of tumor induction in the prostate and testes and at the injection site. *Cancer Res.*, **1988**, *48*, 4656-4663.
- [87] Sahmoun, A.E.; Case, L.D.; Jackson, S.A.; Schwartz, G.G. Cadmium and prostate cancer: a critical epidemiologic analysis. *Cancer Invest.*, **2005**, *23*, 256-263.
- [88] Kolonel, L.N. Association of cadmium with renal cancer. *Cancer*, **1976**, *37*, 1782-1787.
- [89] Il'yasova, D.; Schwartz, G.G. Cadmium and renal cancer. *Toxicol. Appl. Pharmacol.*, **2005**, *207*, 179-186.
- [90] Goyer, R.A.; Liu, J.; Waalkes, M.P. Cadmium and cancer of prostate and testis. *Biometals*, **2004**, *17*, 555-558.
- [91] Waalkes, M.P. Metal carcinogenesis. In: *Handbook of Heavy Metals in the Environment*, Sarkar, B.; ed.; Marcel Dekker: New York **2002**; pp.121-146.
- [92] Waalkes, M.P. Cadmium carcinogenesis in review. *J. Inorg. Biochem.*, **2000**, *79*, 241-244.
- [93] *Cadmium and Its Compounds (in the form of inhalable dusts/aerosols)*, The MAK Collection for Occupational Health and Safety, Greim, H.; Ed.; Wiley-VCH: Weinheim, Germany, **2006**; 22, pp. 119-146.
- [94] International Agency for Research on Cancer (IARC). Arsenic, Metals, Fibres, and Dusts. A Review of Human Carcinogens. **2012**, *100C*, 121-145.
- [95] McElroy, J.A.; Shafer, M.M.; Trentham-Dietz, A.; Hampton, J.M.; Newcomb, P.A. Cadmium exposure and breast cancer risk. *J. Natl. Cancer Inst.*, **2006**, *98*, 869-873.
- [96] Hartwig, A. Cadmium and cancer. *Met. Ions Life Sci.*, **2013**, *11*, 491-507.
- [97] Misra, U.K.; Gawdi, G.; Pizzo, S.V. Induction of mitogenic signaling in the 1LN prostate cell line on exposure to submicromolar concentrations of cadmium+. *Cell. Signal*, **2003**, *15*, 1059-1070.
- [98] Von Zglinicki, T.; Edwall, C.; Ostlund, E.; Lind, B.; Nordberg, M.; Ringertz, N.R.; Wroblewski, J. Very low cadmium concentrations stimulate DNA synthesis and cell growth. *J. Cell Sci.*, **1992**, *103*, 1073-1081.
- [99] Hainaut, P.M. Hollstein, M. p53 and human cancer: the first ten thousand mutations. *Adv. Cancer Res.*, **2000**, *77*, 81-137.
- [100] Lag, M.; Westly, S.; Lerstad, T.; Bjornrud, C.; Refsnes, M.; Schwarze, P.E. Cadmium-induced apoptosis of primary epithelial lung cells: involvement of Bax and p53, but not of oxidative stress. *Cell Biol. Toxicol.*, **2002**, *18*, 29-42.
- [101] Oh, S.H.; Choi, J.E.; Lim, S.C. Protection of betulin against cadmium-induced apoptosis in hepatoma cells. *Toxicology*, **2006**, *220*, 1-12.
- [102] Pathak, N.; S. Khandelwal, S. Oxidative stress and apoptotic changes in murine splenocytes exposed to cadmium. *Toxicology*, **2006**, *220*, 26-36.
- [103] Waalkes, M.P.; Poirier, L.A. *In vitro* cadmium-DNA interactions: cooperativity of cadmium binding and competitive antagonism by calcium, magnesium, and zinc. *Toxicol. Appl. Pharmacol.*, **1984**, *75*, 539-546.
- [104] Hu, J.; Mao, Y.; White, K. Renal cell carcinoma and occupational exposure to chemicals in Canada. *Occupat. Med.*, **2002**, *52*, 157-164.
- [105] Dally, H.; Hartwig, A. Induction and repair inhibition of oxidative DNA damage by nickel(II) and cadmium(II) in mammalian cells. *Carcinogenesis*, **1997**, *18*, 1021-1026.
- [106] Briehl, M.M.; Baker, A.F. Modulation of the antioxidant defence as

- a factor in apoptosis. *Cell. Death Differ.*, **1996**, 3(1), 63-70.
- [107] Kayanoki, Y.; Fujii, J.; Islam, K.N.; Suzuki, K.; Kawata, S.; Matsuzawa, Y.; Taniguchi, N. The protective role of glutathione peroxidase in apoptosis induced by reactive oxygen species. *J. Biochem.*, **1996**, 119(4), 817-22.
- [108] Slater, A.F.; Stefan, C.; Nobel, I.; van den Dobbelsteen, D.J.; Orrenius, S. Intracellular redox changes during apoptosis. *Cell. Death Differ.*, **1996**, 3(1), 57-62.
- [109] Kondo, T.; Reaume, A.G.; Huang, T.T.; Carlson, E.; Murakami, K.; Chen, S.F.; Hoffman, E.K.; Scott, R.W.; Epstein, C.J.; Chan, P.H. Reduction of CuZn-superoxide dismutase activity exacerbates neuronal cell injury and edema formation after transient focal cerebral ischemia. *J. Neurosci.*, **1997**, 17(11), 4180-9.
- [110] Zucker, B.; Bauer, G. Intercellular induction of apoptosis of transformed cells is modulated by their intracellular glutathione concentration. *Int. J. Oncol.*, **1997**, 10(1), 141-6.
- [111] Deng, D.X.; Chakrabarti, S.; Waalkes, M.P.; Cherian, M.G. Metallothionein and apoptosis in primary human hepatocellular carcinoma and metastatic adenocarcinoma. *Histopathology*, **1998**, 32(4), 340-7.
- [112] Manna, S.K.; Zhang, H.J.; Yan, T.; Oberley, L.W.; Aggarwal, B.B. Overexpression of manganese superoxide dismutase suppresses tumor necrosis factor-induced apoptosis and activation of nuclear transcription factor-kappaB and activated protein-1. *J. Biol. Chem.*, **1998**, 273(21), 13245-54.
- [113] Manning, F.C.; Patierno, S.R. Apoptosis: inhibitor or instigator of carcinogenesis? *Cancer Invest.*, **1996**, 14(5), 455-65.
- [114] Shimada, H.; Shiao, Y.H.; Shibata, M.; Waalkes, M.P. Cadmium suppresses apoptosis induced by chromium. *J. Toxicol. Environ. Health A.*, **1998**, 54(2), 159-68.
- [115] Hart, B.A.; Voss, G.W.; Willean, C.L. Pulmonary tolerance to cadmium following cadmium aerosol pretreatment. *Toxicol. Appl. Pharmacol.*, **1989**, 101(3), 447-60.
- [116] Hart, B.A.; Voss, G.W.; Shatos, M.A.; Doherty, J. Cross-tolerance to hyperoxia following cadmium aerosol pretreatment. *Toxicol. Appl. Pharmacol.*, **1990**, 103(2), 255-70.
- [117] Hart, B.A.; Prabhu, R.M.; Eneman, J.D.; Durieux-Lu, C.C.; Jansen, A.M.; Borm, P.J. Oxidant resistance of cadmium-adapted human lung fibroblasts. *Toxicology*, **1995**, 98(1-3), 1-13.
- [118] Takenaka, S.; Oldiges, H.; König, H.; Hochrainer, D.; Oberdörster, G. Carcinogenicity of cadmium chloride aerosols in W rats. *J. Natl. Cancer Inst.*, **1983**, 70(2), 367-73.
- [119] Hart, B.A.; Lee, C.H.; Shukla, G.S.; Shukla, A.; Osier, M.; Eneman, J.D.; Chiu, J.F. Characterization of cadmium-induced apoptosis in rat lung epithelial cells: evidence for the participation of oxidant stress. *Toxicology*, **1999**, 133(1), 43-58.
- [120] Yang, C.F.; Shen, H.M.; Shen, Y.; Zhuang, Z.X.; Ong, C.N. Cadmium induced oxidative cellular damage in human fetal lung fibroblasts (MRC-5 cells). *Environ. Health Perspect.*, **1997**, 105(7), 712-6.
- [121] Waisberg, M.; Joseph, P.; Hale, B.; Beyersmann, D. Molecular and cellular mechanisms of cadmium carcinogenesis. *Toxicology*, **2003**, 192, 95-117.
- [122] Hartwig, A.; Asmuss, M.; Blessing, H.; Hoffmann, S.; Jahnke, G.; Khandelwal, S.; Pelzer, A.; Burkle, A. Interference by toxic metal ions with zinc-dependent proteins involved in maintaining genomic stability. *Food Chem. Toxicol.*, **2002**, 40, 1179-1184.
- [123] Waalkes, M.P.; Misra, R.R. *Cadmium carcinogenicity and genotoxicity. In: Toxicology of Metals*, Chang, L.; Ed.; CRC Press: Boca Raton, FL, **1996**, 231-244.
- [124] Waalkes, M.P.; Rehm, S.; Cherian, M.G. Repeated cadmium exposures enhance the malignant progression of ensuing tumors in rats. *Toxicol. Sci.*, **2000**, 54, 110-120.
- [125] Hartwig, A.; Schwerdtle, T. Interactions by carcinogenic metal compounds with DNA repair processes: toxicological implications. *Toxicol. Lett.*, **2002**, 127(1-3), 47-54.
- [126] Roszkowska, A.M.; De Grazia, L.; Visalli, M.; Mondello, M.; Teti, D.; Venza, M.; Venza, I. Contact lens wearing and chronic cigarette smoking positively correlate with TGF- $\beta$ 1 and VEGF tear levels and impaired corneal wound healing after photorefractive keratectomy. *Curr. Eye Res.*, **2013**, 38(3), 335-41.
- [127] Venza, I.; Visalli, M.; Oteri, R.; Teti, D.; Venza, M. Combined effect of cigarette smoking and alcohol consumption on antioxidant/oxidant balance in age-related macular degeneration. *Ageing. Clin. Exp. Res.*, **2012**, 24(5), 530-6.
- [128] Venza, I.; Visalli, M.; Cucinotta, M.; Teti, D.; Venza, M. Association between oxidative stress and macromolecular damage in elderly patients with age-related macular degeneration. *Ageing. Clin. Exp. Res.*, **2012**, 24(1), 21-7.
- [129] Verougstraete, V.; Lison, D.; Holtz, P. A systematic review of cytogenetic studies conducted in human populations exposed to cadmium compounds. *Mutat. Res.*, **2002**, 511, 15-43.
- [130] Watson, R.E.; Goodman, J.I. Epigenetics and DNA methylation come of age in toxicology. *Toxicol. Sci.*, **2002**, 67, 11-16.
- [131] Venza, I.; Visalli, M.; Tripodo, B.; De Grazia, G.; Loddo, S.; Teti, D.; Venza, M. FOXE1 is a target for aberrant methylation in cutaneous squamous cell carcinoma. *Br. J. Dermatol.*, **2010**, 162(5), 1093-7.
- [132] Venza, M.; Visalli, M.; Catalano, T.; Fortunato, C.; Oteri, R.; Teti, D.; Venza, I. Impact of DNA methyltransferases on the epigenetic regulation of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) receptor expression in malignant melanoma. *Biochem. Biophys. Res. Commun.*, **2013**, 441(4), 743-50.
- [133] Venza, I.; Visalli, M.; Oteri, R.; Cucinotta, M.; Teti, D.; Venza, M. Class II-specific histone deacetylase inhibitors MC1568 and MC1575 suppress IL-8 expression in human melanoma cells. *Pigment. Cell Melano. Res.*, **2013**, 26(2), 193-204.
- [134] Venza, I.; Visalli, M.; Fortunato, C.; Ruggeri, M.; Ratone, S.; Caffo, M.; Caruso, G.; Alataci, C.; Tomasello, F.; Teti, D.; Venza, M. PGE2 induces interleukin-8 derepression in human astrocytoma through coordinated DNA demethylation and histone hyperacetylation. *Epigenetics*, **2012**, 7(11), 1315-30.
- [135] Venza, M.; Dell'Aversana, C.; Visalli, M.; Altucci, L.; Teti, D.; Venza, I. Identification of microRNA expression patterns in cutaneous and uveal melanoma cell lines. *Tumori*, **2014**, 100(1), e4-7.
- [136] Skinner, M.K. Environmental epigenomics and disease susceptibility. *EMBO Rep.*, **2011**, 12, 620-622.
- [137] Zhou, Z.H.; Lei, Y.X.; Wang, C.X. Analysis of aberrant methylation in DNA repair genes during malignant transformation of human bronchial epithelial cells induced by cadmium. *Toxicol. Sci.*, **2012**, 125(2), 412-7.
- [138] Takiguchi, M.; Achanzar, W.E.; Qu, W.; Li, G.; Waalkes, M.P. Effects of cadmium on DNA-(cytosine-5) methyltransferase activity and DNA methylation status during cadmium-induced cellular transformation. *Exp. Cell Res.*, **2003**, 286(2), 355-65.
- [139] Poirier, L.A.; Vlasova, T.I. The prospective role of abnormal methyl metabolism in cadmium toxicity. *Environ. Health Perspect.*, **2002**, 110 (Suppl. 5), 793-795.
- [140] Wright, R.O.; Schwartz, J.; Wright, R.J.; Bollati, V.; Tarantini, L.; Park, S.K.; Hu, H.; Sparrow, D.; Vokonas, P.; Baccarelli, A. Biomarkers of lead exposure and DNA methylation within retrotransposons. *Environ. Health Perspect.*, **2010**, 118(6), 790-95.
- [141] Cho, Y.H.; Yazici, H.; Wu, H.C.; Terry, M.B.; Gonzalez, K.; Qu, M.; Dalay, N.; Santella, R.M. Aberrant promoter hypermethylation and genomic hypomethylation in tumor, adjacent normal tissues and blood from breast cancer patients. *Anticancer Res.*, **2010**, 30(7), 2489-2496.
- [142] Choi, S.H.; Worswick, S.; Byun, H.M.; Shear, T.; Soussa, J.C.; Wolff, E.M.; Douer, D.; Garcia-Manero, G.; Liang, G.; Yang, A.S. Changes in DNA methylation of tandem DNA repeats are different from interspersed repeats in cancer. *Int. J. Cancer*, **2009**, 125(3), 723-729.
- [143] Wilhelm, C.S.; Kelsey, K.T.; Butler, R.; Plaza, S.; Gagne, L.; Zens, M.S.; Andrew, A.S.; Morris, S.; Nelson, H.H.; Schned, A.R.; Karagas, M.R.; Marsit, C.J. Implications of LINE 1 methylation for bladder cancer risk in women. *Clin. Cancer Res.*, **2010**, 16(5), 1682-1689.
- [144] Severson, P.L.; Tokar, E.J.; Vrba, L.; Waalkes, M.P.; Futscher, B.W. Agglomerates of aberrant DNA methylation are associated with toxicant-induced malignant transformation. *Epigenetics*, **2012**, 7(11), 1238-48.
- [145] Coolen, M.W.; Stirzaker, C.; Song, J.Z.; Statham, A.L.; Kassir, Z.; Moreno, C.S.; Young, A.N.; Varma, V.; Speed, T.P.; Cowley, M.; Lacaze, P.; Kaplan, W.; Robinson, M.D.; Clark, S.J. Consolidation of the cancer genome into domains of repressive chromatin by long-range epigenetic silencing (LRES) reduces transcriptional plasticity. *Nat. Cell Biol.*, **2010**, 12(3), 235-46.
- [146] Benbrahim-Tallaa, L.; Waterland, R.; Dill, A.; Webber, M.; Waalkes, M. Tumor suppressor gene inactivation during cadmium-induced malignant transformation of human prostate cells correlates with overexpression of *de novo* DNA methyltransferase. *Environ. Health Perspect.*, **2007**, 115, 1454-1459.

- [147] Yuan, D.; Ye, S.; Pan, Y.; Bao, Y.; Chen, H.; Shao, C. Long-term cadmium exposure leads to the enhancement of lymphocyte proliferation via down-regulating p16 by DNA hypermethylation. *Mutation Res.*, **2013**, *757*, 125-131.
- [148] Huang, D.; Zhang, Y.; Qi, Y.; Chen, C.; Weihong, J. Global DNA hypomethylation, rather than reactive oxygen species (ROS), a potential facilitator of cadmium-stimulated K562 cell proliferation. *Toxicol. Lett.*, **2008**, *179*, 43-47.
- [149] Wang, B.; Li, Y.; Tan, Y.; Miao, X.; Xu-Dong Liu, Chen Shao, Xiao-Hui Yang, Subat Turdi, Li-Jie Ma mail, Jun Ren, Lu Cai. Low-Dose Cd Induces Hepatic Gene Hypermethylation, along with the Persistent Reduction of Cell Death and Increase of Cell Proliferation in Rats and Mice. *PLoS One*, **2012**, *7*(3), e33853.
- [150] Venza, I.; Visalli, M.; Oteri, R.; Teti, D.; Venza, M. Class I-specific histone deacetylase inhibitor MS-275 overrides TRAIL-resistance in melanoma cells by downregulating c-Flip. *Int. Immunopharmacol.*, **2014**, *21*(2), 439-446.
- [151] Teitz, T.; Wei, T.; Valentine, M.B.; Vanin, E.F.; Grenet, J.; Valentine, V.A.; Behm, F.G.; Look, A.T.; Lahti, J.M.; Kidd, V.J. Caspase 8 is deleted or silenced preferentially in childhood neuroblastomas with amplification of MYCN. *Nat. Med.*, **2000**, *6*(5), 529-535.
- [152] Yu, J.; Ni, M.; Xu, J.; Zhang, H.; Gao, B.; Gu, J.; Chen, J.; Zhang, L.; Wu, M.; Zhen, S.; Zhu, J. Methylation profiling of twenty promoter-CpG islands of genes which may contribute to hepatocellular carcinogenesis. *BMC Cancer*, **2002**, *2*, 29.
- [153] Martinez, R.; Setien, F.; Voelter, C.; Casado, S.; Quesada, M.P.; Schackert, G.; Esteller, M. CpG island promoter hypermethylation of the pro-apoptotic gene caspase-8 is a common hallmark of relapsed glioblastoma multiforme. *Carcinogenesis*, **2007**, *28*(6), 1264-1268.
- [154] Kondoh, M.; Araragi, S.; Sato, K.; Higashimoto, M.; Takiguchi, M.; sato, M. Cadmium induces apoptosis partly via caspase-9 activation in HL-60 cells. *Toxicology*, **2002**, *170*(1-2), 111-7.
- [155] Yuan, C.; Kadiiska, M.; Achanzar, W.E.; Mason, R.P.; Waalkes, M.P. Possible role of caspase-3 inhibition in cadmium-induced blockage of apoptosis. *Toxicol. Appl. Pharmacol.*, **2000**, *164*, 321-329.
- [156] Soh, M.; Dunlevy, J.R.; Garretta, S.H.; Allen, C.; Donald A. Sens, D.A.; Zhou, X.D.; Sens, M.A.; Somji, S. Increased Neuron Specific Enolase Expression by Urothelial Cells Exposed to or Malignantly Transformed by Exposure to Cd<sup>2+</sup> or As<sup>3+</sup>. *Toxicol. Lett.*, **2012**, *212*(1), 66-74.
- [157] Zhou, X.D.; Sens, M.A.; Garrett, S.H.; Somji, S.; Park, S.; Gurel, V.; Sens, D.A. Enhanced expression of metallothionein isoform 3 (MT-3) protein in tumor heterotransplants derived from As<sup>3+</sup> and Cd<sup>2+</sup> transformed human urothelial cells. *Toxicol. Sci.*, **2006**, *93*, 322-330.
- [158] Somji, S.; Garrett, S.H.; Toni, C.; Zhou, X.D.; Zheng, Y.; Ajji-maporn, A.; Sens, M.A.; Sens, D.A. Differences in the epigenetic regulation of MT-3 gene expression between parental and Cd<sup>2+</sup> or As<sup>3+</sup> transformed human urothelial cells. *Cancer Cell Int.*, **2011**, *11*(1), 2.
- [159] Vasudevan, S.; Tong, Y.; Steitz, J.A. Switching from repression to activation: microRNAs can up-regulate translation. *Science*, **2007**, *318*(5858), 1931-4.
- [160] Breving, K.; Esquela-Kerscher, A. The complexities of microRNA regulation: mirandering around the rules. *Int. J. Biochem. Cell Biol.*, **2010**, *42*(8), 1316-29.
- [161] Zardo, G.; Ciolfi, A.; Vian, L.; Billi, M.; Racanicchi, S.; Grignani, F.; Nervi, C. Transcriptional targeting by microRNA-polycomb complexes: a novel route in cell fate determination. *Cell Cycle*, **2012**, *11*(19), 3543-9.
- [162] Palanichamy, J.K.; Rao, D.S. miRNA dysregulation in cancer: towards a mechanistic understanding. *Front. Genet.*, **2014**, *18*, 5-54.
- [163] Kozomara, A.; Griffiths-Jones, S. miRBase: integrating microRNA annotation and deep-sequencing data. *Nucleic. Acids Res.*, **2011**, *39*(Database issue), D152-7.
- [164] Mendell, J.T.; Olson, E.N. MicroRNAs in stress signaling and human disease. *Cell*, **2012**, *148*(6), 1172-87.
- [165] Fabbri, M.; Urani, C.; Sacco, M.G.; Procaccianti, C.; Grimaldo, L. Whole genome analysis and microRNAs regulation in HepG2 cells exposed to cadmium. *ALTEX*, **2012**, *29*(2), 173-82.
- [166] Boyerinas, B.; Park, S.M.; Hau, A.; Murmann, A.E.; Peter, M.E. The role of let-7 in cell differentiation and cancer. *Endocr. Relat. Cancer*, **2010**, *17*, F19-36.
- [167] Guo, C.J.; Pan, Q.; Li, D.G.; Sun, H.; Liu, B.W. miR-15b and miR-16 are implicated in activation of the rat hepatic stellate cell: An essential role for apoptosis. *J. Hepatol.*, **2009**, *50*, 766-778.
- [168] Jiang, P.; Enomoto, A.; Takahashi, M. Cell biology of the movement of breast cancer cells: intracellular signalling and the actin cytoskeleton. *Cancer Lett.*, **2009**, *284*, 122-130.
- [169] Lambrechts, A.; Van Troys, M.; Ampe, C. The actin cytoskeleton in normal and pathological cell motility. *Int. J. Biochem. Cell Biol.*, **2004**, *36*, 1890-1909.
- [170] Imajo, M.; Tsuchiya, Y.; Nishida, E. Regulatory mechanisms and functions of MAP kinase signaling pathways. *IUBMB Life*, **2006**, *58*(5-6), 312-7.
- [171] Urani, C.; Melchiorretto, P.; Fabbri, M.; Bowe, G.; Macerati, E.; Grigaldo, L. Cadmium Impairs p53 Activity in HepG2 Cells. *ISRN Toxicol.*, **2014**, *2014*, 976428.
- [172] Jin, Y.; Chen, D.; Cabay, R.J.; Wang, A.; Crowe, D.L.; Zhou, X. Role of microRNA-138 as a potential tumor suppressor in head and neck squamous cell carcinoma. *Inter. Rev. Cell Mole. Biol.*, **2013**, *303*, 357-385.
- [173] Gu, H.; Guo, X.; Zou, L.; Zhu, H.; Zhang, J. Upregulation of microRNA-372 associates with tumor progression and prognosis in hepatocellular carcinoma. *Mole. Cell Biochem.*, **2013**, *375*, 23-30.
- [174] Wu, S.; Huang, S.; Ding, J.; Zhao, Y.; Liang, L.; Liu, T.; Zhang, R.; He, X. Multiple microRNAs modulate p21Cip1/WAF1 expression by directly targeting its 3' untranslated region. *Oncogene*, **2010**, *29*, 2302-2308.
- [175] Jin, Y.; Chen, D.; Cabay, R.J.; Wang, A.; Crowe, D.L.; Zhou, X. Role of microRNA-138 as a potential tumor suppressor in head and neck squamous cell carcinoma. *Inter. Rev. Cell Mole. Biol.*, **2013**, *303*, 357-385.
- [176] Hassan, F.; Nuovo, G.J.; Crawford, M.; Boyaka, P.N.; Kirkby, S.; Nana-Sinkam, S.P.; Cormet-Boyaka, E. MiR-101 and miR-144 regulate the expression of the CFTR chloride channel in the lung. *PLoS One*, **2012**, *7*(11), e50837.
- [177] Zhu, Q.Y.; Liu, Q.; Chen, J.X.; Lan, K.; Ge, B.X. MicroRNA-101 targets MAPK phosphatase-1 to regulate the activation of MAPKs in macrophages. *J. Immunol.*, **2010**, *185*, 7435-7442.
- [178] Valente, A.J.; Yoshida, T.; Gardner, J.D.; Somanna, N.; Delafontaine, P.; Chandrasekar, B. Interleukin-17A stimulates cardiac fibroblast proliferation and migration via negative regulation of the dual-specificity phosphatase MKP-1/DUSP-1. *Cell Signal.*, **2012**, *24*: 560-568.
- [179] Gu, W.; Shou, J.; Gu, S.; Sun, B.; Che, X. Identifying hedgehog signaling specific microRNAs in glioblastomas. *Int. J. Med. Sci.*, **2014**, *11*(5): 488-93.
- [180] Fu, X.; Huang, X.; Li, P.; Chen, W.; Xia, M. 7-Ketocholesterol inhibits isocitrate dehydrogenase 2 expression and impairs endothelial function via microRNA-144. *Free Radic. Biol. Med.*, **2014**, *71*, 1-15.
- [181] Guo, Y.; Ying, L.; Tian, Y.; Yang, P.; Zhu, Y.; Wang, Z.; Qiu, F.; Lin, J. miR-144 downregulation increases bladder cancer cell proliferation by targeting EZH2 and regulating Wnt signaling. *FEBS J.*, **2013**, *280*(18), 4531-8.
- [182] Bergomi, M.; Pellacani, G.; Vinceti, M.; Bassissi, S.; Malagoli, C.; Alber, D.; Sieri, S.; Vescovi, L.; Seidenari, S.; Vivoli, R. Trace elements and melanoma. *J. Trace Elements Med. Biol.*, **2005**, *19*(1), 69-73.
- [183] Wester, R.C.; Maibach, H.I.; Sedik, L.; Melendres, J.; DiZio, S.; Wade, M. *In vitro* percutaneous absorption of cadmium from water and soil into human skin. *Fundam Appl. Toxicol.*, **1992**, *19*, 1-5.
- [184] Lansdown, A.B.; Sampson, B. Dermal toxicity and percutaneous absorption of cadmium in rats and mice. *Lab. Anim. Sci.*, **1996**, *46*, 549-554.
- [185] Fasanya-Odewumi, C.; Latinwo, L.M.; Ikediobi, C.O.; Gilliard, L.; Sponholtz, G.; Nwoga, J.; Stino, F.; Hamilton, N.; Erdos, G.W. The genotoxicity and cytotoxicity of dermally-administered cadmium: effects of dermal cadmium administration. *Int. J. Mol. Med.*, **1998**, *1*, 1001-1006.
- [186] Koropatnick, J.; Pearson, J. Zinc treatment, metallothionein expression, and resistance to cisplatin in mouse melanoma cells. *Somat. Cell Mol. Genet.*, **1990**, *16*(6), 529-37.
- [187] Weinlich, G.; Bitterlich, W.; Mayr, V.; Fritsch, P.O.; Zelger, B. Metallothionein overexpression as a prognostic factor for progression and survival in melanoma. A prospective study on 520 patients. *Br. J. Dermatol.*, **2003**, *149*(3), 535-41.
- [188] Zelger, B.; Hittmair, A.; Schir, M.; Ofner, C.; Ofner, D.; Fritsch, P.O.; Böcker, W.; Jasani, B.; Schmid, K.W. Immunohistochemi-



- cally demonstrated metallothionein expression in malignant melanoma. *Histopathology*, **1993**, *23*(3), 257-63.
- [189] Behrens, T.; Lyngge, E.; Cree, I.; Lutz, J.M.; Eriksson, M.; Guénel, P.; Merletti, F.; Morales-Suarez-Varela, M.; Afonso, N.; Stengrevics, A.; Stang, A.; Févotte, J.; Sabroe, S.; Llopis-González, A.; Gorini, G.; Hardell, L.; Ahrens, W. Occupational exposure to endocrine-disrupting chemicals and the risk of uveal melanoma. *Scand. J. Work Environ. Health*, **2012**, *38*(5), 476-483.
- [190] Maniotis, A.J.; Folberg, R.; Hess, A.; Seftor, E.A.; Gardner, L.M.; Pe'er, J.; Trent, J.M.; Meltzer, P.S.; Hendrix, M.J. Vascular channel formation by human melanoma cells *in vivo* and *in vitro*: vasculogenic mimicry. *Am. J. Pathol.*, **1999**, *155*(3), 739-52.
- [191] Folberg, R.; Hendrix, M.J.; Maniotis, A.J. Vasculogenic mimicry and tumor angiogenesis. *Am. J. Pathol.*, **2000**, *156*(2), 361-81.
- [192] Hendrix, M.J.; Seftor, E.A.; Hess, A.R.; Seftor, R.E. Vasculogenic mimicry and tumour-cell plasticity: lessons from melanoma. *Nat. Rev. Cancer*, **2003**, *3*(6), 411-21.
- [193] Folberg, R.; Maniotis, A.J. Vasculogenic mimicry. *APMIS*, **2004**, *112*(7-8), 508-25.
- [194] Döme, B.; Hendrix, M.J.; Paku, S.; Tóvári, J.; Timár, J. Alternative vascularization mechanisms in cancer: Pathology and therapeutic implications. *Am. J. Pathol.*, **2007**, *170*(1), 1-15.
- [195] Yokouchi, M.; Hiramatsu, N.; Hayakawa, K.; Kasai, A.; Takano, Y.; Yao, J.; Kitamura, M. Atypical, bidirectional regulation of cadmium-induced apoptosis via distinct signaling of unfolded protein response. *Cell Death Differentiat.*, **2007**, *14*, 1467-1474.
- [196] Valyi-Nagy, K.; Voros, A.; Gagyi E.; Valyi-Nagy, T. Increased Resistance of Vasculogenic Mimicry-Forming Uveal Melanoma Cells Against Cytotoxic Agents in Three-Dimensional Cultures. Research on Melanoma- A Glimpse into Current Directions and Future Trends, **2011**, *18*, 377-392.
- [197] Haga, A.; Nagase, H.; Kito, H.; Sato, T. Enhanced invasiveness of tumour cells after host exposure to heavy metals. *Eur. J. Cancer*, **1996**, *32A*(13), 2342-7.
- [198] Bernstein, B.E.; Meissner, A.; Lander, E.S. The mammalian epigenome. *Cell*, **2007**, *128*, 669-81.
- [199] Jenuwein, T.; Allis, C.D. Translating the histone code. *Science*, **2001**, *293*, 1074-80.
- [200] Berger, S.L. The complex language of chromatin regulation during transcription. *Nature*, **2007**, *447*, 407-12.
- [201] Venza, M.; Visalli, M.; Biondo, C.; Oteri, R.; Agliano, F.; Morabito, S.; Teti, D.; Venza I. Epigenetic marks responsible for cadmium-induced melanoma cell overgrowth. *Toxicol In Vitro*, **2014**, *29*(1), 242-250.
- [202] Hyland, P.L.; Burke, L.S.; Pfeiffer, R.M.; Rotunno, M.; Sun, D.; Patil, P.; Wu, X.; Tucker M.A.; Goldstein A.M.; Yang, X.R. Constitutional promoter methylation and risk of familial melanoma. *Epigenetics*, **2014**, *9*(5), 685-92.
- [203] Pergoli, L.; Favero, C.; Pfeiffer, R.M.; Tarantini, L.; Calista, D.; Cavalieri, T.; Angelici, L.; Consonni, D.; Bertazzi, P.A.; Pesatori, A.C.; Landi, M.T.; Bollati, V. Blood DNA methylation, nevi number, and the risk of melanoma. *Melanoma Res.*, **2014**, *24*(5), 480-7.
- [204] Kostaki, M.; Manona, A.D.; Stavraka, I.; Korkolopoulou, P.; Levidou, G.; Trigka, E.A.; Christofidou, E.; Champsas, G.; Stratigos, A.J.; Katsambas, A.; Papadopoulos O.; Piperi, C.; Papavassiliou, A.G. High-frequency p16(INK) (4A) promoter methylation is associated with histone methyltransferase SETDB1 expression in sporadic cutaneous melanoma. *Exp. Dermatol.*, **2014**, *23*(5), 332-8.
- [205] Young, R.J.; Waldeck, K.; Martin, C.; Foo, J.H.; Cameron, D.P.; Kirby, L.; Do, H.; Mitchell, C.; Cullinane, C.; Liu, W.; Fox, S.B.; Dutton-Regester, K.; Hayward, N.K.; Jene, N.; Dobrovic, A.; Pearson, R.B.; Christensen, J.G.; Randolph, S.; McArthur, G.A.; Sheppard, K.E. Loss of CDKN2A expression is a frequent event in primary invasive melanoma and correlates with sensitivity to the CDK4/6 inhibitor PD0332991 in melanoma cell lines. *Pigment Cell Melanoma Res.*, **2014**, *27*(4), 590-600.
- [206] Lade-Keller, J.; Riber-Hansen, R.; Guldborg, P.; Schmidt, H.; Hamilton-Dutoit, S.J.; Steiniche, T. Immunohistochemical analysis of molecular drivers in melanoma identifies p16 as an independent prognostic biomarker. *J. Clin. Pathol.*, **2014**, *67*(6), 520-8.
- [207] Chang, L.M.; Cassarino, D.S. p16 Expression Is Lost in Severely Atypical Cellular Blue Nevi and Melanoma Compared to Conventional, Mildly, and Moderately Atypical Cellular Blue Nevi. *ISRN Dermatol.*, **2014**, *2014*, 348417.
- [208] Pingoud-Meier, C.; Lang, D.; Janss, A.J.; Rorke, L.B.; Phillips, P.C.; Shalaby, T.; Grotzer, M.A. 2003. Loss of caspase-8 protein expression correlates with unfavorable survival outcome in childhood medulloblastoma. *Clin. Cancer Res.*, **2003**, *9*, 6401-6409.
- [209] Yang, Q.; Kiernan, C.M.; Tian, Y.; Salwen, H.R.; Chlenski, A.; Brumback, B.A.; London, W.B.; Cohn, S.L.; 2007. Methylation of CASP8, DCR2, and HIN-1 in neuroblastoma is associated with poor outcome. *Clin Cancer Res.*, **2007**, *13*, 3191-3197.
- [210] Van Geelen, C.M.; Pennarun, B.; Ek, W.B.; Le, P.T.; Spierings, D.C.; De Vries, E.G.; De Jong, S. 2010. Downregulation of active caspase 8 as a mechanism of acquired TRAIL resistance in mismatch repair-proficient colon carcinoma cell lines. *Int. J. Oncol.*, **2010**, *37*(4), 1031-41.
- [211] Chen, S.; Zhao, Y.; Gou, W.F.; Zhao, S.; Takano, Y.; Zheng, H.C. The anti-tumor effects and molecular mechanisms of suberoylanilide hydroxamic acid (SAHA) on the aggressive phenotypes of ovarian carcinoma cells. *PLoS One*, **2013**, *8*(11), e79781.
- [212] Kikuchi, H.; Yuan, B.; Yuhara, E.; Takagi, N.; Toyoda, H. Involvement of histone H3 phosphorylation through p38 MAPK pathway activation in casticin-induced cytotoxic effects against the human promyelocytic cell line HL-60. *Int. J. Oncol.*, **2013**, *43*(6), 2046-56.
- [213] Lin, Z.; Guo, Z.; Xu, Y.; Zhao, X. Identification of a secondary promoter of CASP8 and its related transcription factor PURa. *Int. J. Oncol.*, **2014**, *45*(1), 57-66.
- [214] Sánchez-Vega, F.; Gotea, V.; Petrykowska, H.M.; Margolin, G.; Krivak, T.C.; DeLoia, J.A.; Bell, D.W.; Elnitski, L. Recurrent patterns of DNA methylation in the ZNF154, CASP8, and VHL promoters across a wide spectrum of human solid epithelial tumors and cancer cell lines. *Epigenetics*, **2013**, *8*(12), 1355-72.
- [215] Liedtke, C.; Zschemisch, N.H.; Cohrs, A.; Roskams, T.; Borlak, J.; Manns, M.P.; Trautwein, C. Silencing of caspase-8 in murine hepatocellular carcinomas is mediated via methylation of an essential promoter element. *Gastroenterology*, **2005**, *129*(5), 1602-15.
- [216] Fulda, S.; Küfer, M.U.; Meyer, E.; van Valen, F.; Dockhorn-Dworniczak, B.; Debatin, K.M. Sensitization for death receptor- or drug-induced apoptosis by re-expression of caspase-8 through demethylation or gene transfer. *Oncogene*, **2001**, *20*(41), 5865-77.
- [217] Asara, Y.; Marchal, J.A.; Carrasco, E.; Boulaiz, H.; Solinas, G.; Bandiera, P.; Garcia, M.A.; Farace, C.; Montella, A.; Madeddu, R. Cadmium modifies the cell cycle and apoptotic profiles of human breast cancer cells treated with 5-fluorouracil. *Int. J. Mol. Sci.*, **2013**, *14*(8), 16600-16.
- [218] Gunawardana, C.G.; Martinez, R.E.; Xiao, W.; Templeton, D.M. Cadmium inhibits both intrinsic and extrinsic apoptotic pathways in renal mesangial cells. *Am. J. Physiol. Renal. Physiol.*, **2006**, *290*(5), F1074-82.
- [219] Teitz, T.; Lahti, J.M.; Kidd, V.J. Aggressive childhood neuroblastomas do not express caspase-8: an important component of programmed cell death. *J. Mol. Med. (Berl.)*, **2001**, *79*(8), 428-36.
- [220] Kaminsky, V.O.; Surova, O.V.; Vaculova, A.; Zhivotovsky, B. Combined inhibition of DNA methyltransferase and histone deacetylase restores caspase-8 expression and sensitizes SCLC cells to TRAIL. *Carcinogenesis*, **2011**, *32*(10), 1450-8.