

Heavy metals in the cell nucleus — role in pathogenesis

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People are exposed to heavy metals both in an occupational and natural environment. The most pronounced effects of heavy metals result from their interaction with cellular genetic material packed in form of chromatin. Heavy metals influence chromatin, mimicking and substituting natural microelements in various processes taking place in the cell, or interacting chemically with nuclear components: nucleic acids, proteins and lipids. This paper is a review of current knowledge on the effects of heavy metals on chromatin, exerted at the level of various nuclear components.

Key words: heavy metal, nucleus, chromatin, DNA, RNA, telomere

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INTRODUCTION

The progress in industrialization taking place in the last century was reflected by increased environmental release of xenobiotics, and resultant increase in the risk of human exposure, especially in the industrialized areas. Several groups of chemicals associated with occupational exposure were included in the Fourth National Report on Human Exposure to Environmental Chemicals (CDC, 2009, updated February 2013) and the number of xenobiotics encompassed increased significantly since then. Only between 2003 and 2004, 75 new chemicals were added (CDC, 2013), including a number of pesticides (fungicides, herbicides, insecticides), environmental phenols, perfluorochemicals, phthalates, polybrominated and polychlorinated diphenyls, dioxins, polycyclic aromatic hydrocarbons, volatile organic compounds, metals and metalloids. The latter two pose a particular threat for human population as they act as micronutrients or mimic thereof, and thus often utilize specialized pathways to enter human body. According to the European Environmental Agency (EEA, No 5/2013), heavy metals severely impair human health, may contribute to increased incidence of cancer, cardiovascular and respiratory diseases, exert toxic effect on reproduction and display endocrine disrupting properties. Also the nephrotoxic, neurotoxic and immunotoxic effects of heavy metals are well established (Nordberg, *et al.* 2007). Due to development of novel diagnostic techniques, metals, such as lead (Pb), mercury (Hg), cadmium (Cd) and arsenic (As), as well as metalloids (Sb, antimony; Ba, barium; Be, beryllium; Cs, caesium; Co, cobalt; Tl, thallium; Pt, platinum; W, tungsten and U, uranium) can be accurately determined in various biological materials. Moreover, the progress in life sciences allows us to examine the effect of exposure

to heavy metals with an aid of modern techniques from the field of molecular biology and epigenetics. This allows to recognize the symptoms of heavy metal intoxication even in the case of long-term, low-level exposure which usually remains undetected due to the lack prominent somatic symptoms typical for high-dose poisoning (e.g. plumbism, Minamata disease, Itai-Itai disease, organophosphate poisoning).

Heavy metals may enter cells via many different pathways, typically utilizing constitutive components of the cell membrane, such as protein transporters and ion channels. Some metals, being important micronutrients at physiological concentrations, e.g. Zn²⁺ or Fe²⁺, cross the membrane *via* specialized ion-specific channels. In contrast, most of known toxic metals enter cells mimicking natural ions and utilizing their transporters. After crossing the barrier of plasma membrane, the metals can reach cell nucleus and thus interfere with nucleic acids and other nuclear compounds. In this paper, we review various mechanisms due to which heavy metals can interact with nucleic acids and impair chromatin functioning.

IMPACT OF HEAVY METALS ON CHROMATIN PHYSIOLOGY

Heavy metals and DNA

The individual DNA strands of eukaryotic cell nucleus, i.e. chromosomes, are complexed with proteins. Due to its negative charge, DNA interacts with metal ions which in turn modulate its conformational state (Eichhorn *et al.*, 1985). An *in vitro* analysis of the effect of ion partner on DNA conformation showed that heavy metals may induce severe conformational changes of the double helix (Duguid *et al.*, 1993). There is also an evidence that interaction of heavy metals with the double helix may impair relationships between DNA and nuclear proteins; for example, Cr³⁺ complexes are able to bind to large groove in DNA, blocking its availability for transcription factors and thus inhibiting gene expression (Raja & Nair, 2008).

Interactions with heavy metals may also have more severe consequences. A study of Chinese battery manufacturing plant workers showed that exposure to Pb²⁺ results in telomere shortening (Wu *et al.*, 2012). Telomeres are terminal parts of chromosomes, composed of a double strand array of tandem repetitive sequences (TTAGGG) reach in guanidine residues, followed by ter-

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Abbreviations: DNA, deoxyribonucleic acid; NB, nuclear bodies; RNA, ribonucleic acid; mRNA, messenger ribonucleic acid; tRNA, transfer ribonucleic acid; rRNA, ribosomal ribonucleic acid; PML, promyelocytic leukaemia bodies

minimal 3' G-rich single-stranded overhangs. Due to replication problems, the telomeres are shortened with every cell division. Plausibly, the telomere DNA adopts a T-loop structure, and thus the telomere end folds back on itself. Basically, 3' G-rich single strand overhang invades the upstream double-stranded DNA, probably forming G-quadruplexes, in which each G base serves as both the donor and the acceptor for hydrogen bond formation. Available data suggest that the telomeric G-quadruplex structures of humans may be involved in telomere protection, suppression of recombination, and inhibition of telomerase-dependent telomere extension (for review see: Lu *et al.*, 2013). Pottier and coworkers (2013) tried to explain the mechanism by which lead impacts the telomere length. They showed that Pb^{2+} may displace physiological ions, e.g. K^+ or Na^+ , in previously formed G-quadruplexes and induce formation of additional G-quadruplex structures. The authors suggested that such G-quadruplexes may persist during telomere replication which results in telomere instability and perturbations in their maintenance (Pottier *et al.*, 2013). Similar results were reported by Liu and coworkers (2004), who studied the effects of heavy metals on hepatocytes. However, the treatment of hepatoma cells was reflected by an increase in telomere length, suggesting that Pb^{2+} and Cr^{3+} may play a role in "immortalization" of cancer cells. Nonetheless, these findings should not be generalized on all heavy metals; for example, Zn^{2+} -treated normal cells maintain their telomere length, while the exposure of cancer cells to Zn^{2+} results in telomere shortening. This may be caused by the direct inhibition of telomerase activity and/or result from the inhibition of Zn^{2+} -binding transcription factors (Liu *et al.*, 2004).

Heavy metals are also able to chemically react with DNA. Pb^{2+} was shown to bind DNA; this is reflected by the two contradictory effects: while binding of Pb^{2+} to phosphate groups stabilizes the structure of DNA, the interaction between this metal and bases leads to denaturation of the double helix (Rabbani-Chadegani *et al.*, 2009). While binding of Al^{3+} to phosphate groups was shown to change the topology of DNA from B to Z in $(CCG)_{12}$ repeat regions, the Mg^{2+} to Ni^{2+} substitution within the DNA backbone resulted in condensation of chromatin and thus, in silencing of the active genes (Mishra *et al.*, 2010). Also Cr^{6+} was demonstrated to bind to both DNA backbone and purine (adenine, guanidine) residues, which was reflected by generation of Cr^{3+} adducts and resultant DNA–DNA or DNA–protein-crosslinks (Blankert *et al.*, 2003; Arakawa *et al.*, 2006; Arakawa *et al.*, 2012). Noticeably, these adducts may become mutagenic, acting as substrates for nucleotide excision repair (Salnikow & Zhitkovich, 2008). Furthermore, the Cr^{3+} -DNA adducts may undergo replication, albeit with decreased fidelity, leading to increase in the telomere length and/or promoting carcinogenesis (Liu *et al.*, 2004). Moreover, Cr^{6+} is able to induce local formation of reactive oxygen species (ROS), when reduced to Cr^{3+} . Due to their high reactivity, ROS induce DNA–DNA and DNA–protein cross-links, single strand DNA breaks and alkaline labile sites (Nickens *et al.*, 2010; Fowler *et al.*, 2011), which may significantly impair the ability of nuclear proteins to execute their action over DNA (Bridgewater *et al.*, 1994). Similar influence on the DNA utilizing potential to generate ROS was also postulated in the case of other heavy metals, such as Cd^{2+} , Fe^{2+} , Ni^{2+} and Cu^{2+} , that are known to induce ROS via Fenton or Fenton-type reactions (Nackerdien *et al.*, 1991; Altman *et al.*, 1995; Lloyd & Phillips, 1999; Fowler *et al.*, 2011). The metal ions involved in the Fenton reaction

may be bound to macromolecules, such as DNA, or to the surface of the proteins. Such metals, also referred to as "loosely bound metals" due to their properties and easy removal, may undergo the reduction-oxidation processes and generate ROS. This exerts detrimental effect in the close proximity to DNA, e.g. leading to formation of single and double strand breaks, apurinic sites, DNA bases and deoxyribose modifications (Kohen & Nyska, 2002; Olewińska *et al.*, 2010). The impact of ROS on DNA should not be underestimated as the former may impair each aspect of DNA physiology, not only causing its damage but also affecting the proteins involved in such important processes of DNA maintenance as double strand repair (Hartwig, 2013).

Virtually all previously examined heavy metals were shown to interfere with DNA on the epigenetic level (Fragou *et al.*, 2011; Cheng *et al.*, 2012). Although the interaction is likely a consequence of an indirect influence of heavy metals on various nuclear proteins, also their direct effect on DNA cannot be excluded. Takiguchi and coworkers (2003) showed that Cd^{2+} acts as an inhibitor of mammalian methyltransferases, and a short-term exposure to Cd^{2+} results in DNA hypomethylation. Interestingly, an opposite effect, namely DNA hypermethylation and increased activity of methyltransferases, was observed in the case of prolonged exposure to Cd^{2+} (Takiguchi *et al.*, 2003). As^{3+} and As^{5+} act *via* a different mechanism. As the processes of As^{3+} and As^{5+} detoxification are based on methylation, these ions were postulated to destabilize methyl group balance, thus leading to global hypomethylation of DNA (Zhao *et al.*, 1997; Chen *et al.*, 2004). However, it should be noted that some authors claimed on the phenomenon of As-induced gene hypermethylation (Reichard & Puga, 2010).

Heavy metals and nucleosome structure

Nucleosome constitutes the main building block of chromatin. It is formed of DNA helix wrapped around the histone octamer (referred to as nucleosome core). Nucleosomes are linked by linker DNA into so called beads on a string, making the first step of packing DNA into chromatin fibres. While an association with proteinaceous histone octamer is thought to protect DNA, the liners are generally considered to be more susceptible to the influence of exogenous factors (Hayes & Hansen, 2001; Liu *et al.*, 2011). DNA and histones interact with each other within the nucleosome, which is crucial for positioning DNA on the nucleosome core, and thus for its availability for nuclear proteins. Nucleosomes are extremely dynamic structures, undergoing reorganization in response to different stimuli, including DNA–histone and nucleosome–nucleosome interactions (Luger, 2006; Liu *et al.*, 2011). Epigenetic modifications are one of the main factors influencing organization of nucleosomes. Depending on a functional group linked to histones and/or DNA, the structure of nucleosomes may become looser or tighter, which directly affects the availability of genetic information for nuclear proteins (Rohlf *et al.*, 2012).

Various metals may affect nucleosome structure either directly or indirectly, modulating the activity of proteins involved in chromatin remodelling. Mohideen *et al.* (2010) showed that interaction between Co^{2+} or Ni^{2+} and nucleosome is reflected by changes in the histone–DNA register and resultant distortions of the double helix; this may affect compaction and dynamics of chromatin, as well as its interactions with various nuclear proteins. Moreover, on the basis of heavy metal coordination

observed within the nucleosome, the abovementioned authors suggested that multiple histone sites may serve as an active source of reactive species generated proximal to the DNA (Mohideen *et al.*, 2010). Also Pb^{2+} was shown to interact with histone proteins and impair their structure and function. Histones are thiol ($-SH$) rich proteins able to bind Zn^{2+} . Pb^{2+} is known to mimic Zn^{2+} and was postulated to substitute Zn^{2+} in its interaction with histones (Rabbani-Chadegani *et al.*, 2009). Some metals, e.g. Cr^{6+} , may induce cross-links between DNA and DNA or DNA and proteins, potentially leading to joining neighbouring nucleosomes and thus, fixing their mutual conformation (Macfie *et al.*, 2009).

An interaction between heavy metals and nucleosomal core proteins may also lead to truncation of the latter. Bal and coworkers (2000) and Karaczyn and coworkers (2005; 2009) showed that Ni^{2+} can truncate C- and N- termini of H2B histone and cleaves octapeptide from the C-terminus of the H2A histone. This is reflected by changes in the expression of wide variety of genes (Karaczyn *et al.*, 2009). This effect of Ni^{2+} may be associated with changes in nucleosome structure and the ability of this metal to interact with various nuclear proteins involved in control of chromatin dynamics and condensation (Vogler *et al.*, 2010). Moreover, due to their ability to generate reactive oxygen species, heavy metals may also cause damage to histone proteins, participating in their oxidation (Hartwig, 2013).

Moreover, heavy metals are known to affect the organization of nucleosomes indirectly, changing their histone code (Pawlak & Deckert, 2007; Davidson *et al.*, 2011; Fragou *et al.*, 2011; Arita *et al.*, 2012; Cheng *et al.*, 2012). This mainly results from functional modification of various enzymes involved in chromatin remodelling. Many of these enzymes have $-SH$ groups in their structure or utilize metal cofactors, which makes them susceptible to heavy metal-induced damage. Ni^{2+} was shown to displace Fe^{2+} in the active centre of H3K9 demethylase (Chen *et al.*, 2006). Moreover, it can inhibit histone acetylases, leading to hypo-acetylation and resultant silencing of genes (Broday *et al.*, 2000; Yan *et al.*, 2003). Furthermore, Ni was shown to impact epigenetic marks, such as histone methylation, ubiquitylation and phosphorylation; this effect is at least partially exerted indirectly, via interaction with certain signal transduction pathways (Karaczyn *et al.*, 2006; Zhou *et al.*, 2009). Ke *et al.* (2008) showed that Ni^{2+} induces phosphorylation of H3Ser10 in human cell lines due to interaction with upstream components of JNK/SAPK pathway.

Cr^{6+} acts *via* a different mechanism; this metal was shown to modulate histone methylation and acetylation (Schnekenburger *et al.*, 2007; Sun *et al.*, 2009). Due to its cross-linking activity, chromium may attach chromatin remodelling enzymes to DNA, thus enforcing their action over local chromatin. This phenomenon was documented by Schnekenburger *et al.* (2007), who studied the influence of Cr^{6+} on deacetylase-1-DNA methyltransferase 1 (HDAC1-DNMT1) enzyme complex and histone modifications in 5'-flanking region of inducible member of cytochrome P450 family, *Cyp1a1* gene. They showed that Cr^{6+} is able to induce cross-links within the HDAC1-DNMT1, thus enforcing the complex to occupy the proximal promoter region of the analysed gene. The presence of HDAC1 resulted in the loss of histone marks associated with the gene. Moreover, the enzymatic complex physically blocked underlying chromatin, thus preventing acetylation of critical histone residues associated with activation of the gene, and impaired recruitment of the basal transcriptional complex. The authors

suggested that long-term exposure to Cr^{6+} may lead to chronic cross-linking of the inhibitory complexes and resultant increase in histone de-acetylation. This may be reflected by methylation of histones in specific positions involved in silencing of the gene, as well as by hypermethylation of DNA, changing the organization of chromatin to compact and silent state, affecting gene expression and possibly leading to carcinogenesis (Schnekenburger *et al.*, 2007). Similar mechanisms as those described in the case of Ni^{2+} and Cr^{6+} may be also utilized by other heavy metals that influence epigenetic modifications, e.g. As^{3+} (Zhou *et al.*, 2008; Ren *et al.*, 2011), Pb^{2+} (Bihagi *et al.*, 2011) and Co^{2+} (Li *et al.*, 2009; Verma *et al.*, 2011).

Heavy metals and RNA

RNA is involved in most of the processes associated with the genome function, i.e. transcription (mRNA) and protein synthesis (rRNA, tRNA) (Krebs *et al.*, 2013), gene silencing (Djupedal & Ekwall, 2009; Ghildiyal & Zamore, 2009; Siomi & Siomi, 2009), chromatin organisation (van Wolfswinkel & Ketting, 2010; Engreitz *et al.*, 2013), functioning of nuclear bodies (Dundr, 2013) and telomere maintenance (Podlevsky & Chen, 2012). It is noteworthy that the action of RNA depends not only on its sequence but also on its conformation (Hendrix *et al.*, 2005). Proper folding is vital for interaction between RNA and its various target molecules. RNA is a polyanionic molecule and its appropriate folding requires the presence of metal cations, neutralizing the repulsion effects in RNA chain (Feig & Uhlenbeck, 1999; Woodson, 2005; Tan & Chen, 2012). The two types of metal-RNA interactions were described: (1) diffusive ions, that maintain their hydration sphere, accumulate near RNA due to presence of electrostatic field, and (2) chelated ions directly interact with RNA chain at specific location, and at least some of their hydration water is displaced due to coordination with polar RNA atoms (Hendrix *et al.*, 2005). The two types of metal-RNA interactions are crucial for function of this nucleic acid. Diffuse ions were postulated to play a crucial role in stabilising tertiary structures of RNA and linking (in an energetic sense) all the ions associated with the RNA molecule (Draper, 2004). As for chelated ions, eight metal binding motifs were recognised in the RNA structure. A metal may be bound directly to preformed sites and motifs, or stimulate large structural rearrangements leading to formation of a specific binding pocket (Wu & Tinoco, 1998; Penedo *et al.*, 2004). Therefore, metal ions may stabilize a specific 3D structure of the RNA molecule and/or play a catalytic role (Hendrix *et al.*, 2005).

These are monovalent Na^{+} and K^{+} ions or divalent Mg^{2+} ions that play main role during physiological ion-RNA interactions. However, these ions may be displaced by heavy metals (Feig & Uhlenbeck, 1999). Due to their chemical properties, some metals, such as Cd^{2+} , Pb^{2+} and Mn^{2+} , may bind nucleic acids with greater affinity than Mg^{2+} and K^{+} , and show distinct coordination preferences, thus affecting RNA folding or even disrupting its structure. After binding to RNA, some metals, e.g. Eu^{3+} and Pb^{2+} , may induce cleavage of the nucleic acid, causing deprotonation of a nearby 2'-OH group which subsequently attracts the adjacent phosphodiester bond (Feig & Uhlenbeck, 1999).

Apart from the direct interactions, heavy metals influence RNA indirectly, interfering with the proteins involved in metabolism of nucleic acids and generating reactive oxygen species. The scale of these interactions

is hard to predict as they are unspecific and affect various RNAs involved in different processes. Available data point to the pluripotency of possible effects of these interactions. For example, different As^{3+} compounds may influence gene expression and resultant synthesis of mRNA (Chen *et al.*, 2001; Zheng *et al.*, 2003) or expression of microRNA (miRNA) (Cui *et al.*, 2012) in various human cell lines. Similar effects were also observed for Cd^{2+} (Badisa *et al.*, 2008; Fabbri *et al.*, 2012) and other metals (Hou *et al.*, 2011).

HEAVY METAL-MEDIATED CHANGES IN CHROMATIN ORGANISATION

It is generally accepted that, influencing epigenetic modifications, heavy metals may change the degree of chromatin compaction and induce abnormalities in chromatin condensation. However, the data directly documenting the impact of metals on local organisation of chromatin are sparse (Cheng *et al.*, 2012). Lee and coworkers (1995) showed that treatment with Ni^{2+} results in condensation of gtp transgene *locus*, which is associated with DNA hypermethylation. Moreover, binding preferentially to heterochromatin, Ni^{2+} is able to induce condensation of chromatin, spreading from euchromatin–heterochromatin junctions onto genes present in adjacent euchromatin regions (Sen *et al.*, 1987; Ellen *et al.*, 2009).

When present at an appropriate concentration, heavy metals may induce severe changes in overall chromatin organisation. Cd^{2+} may induce overall condensation of chromatin as shown in cheomocytes, *Sinopotamon benanense* (Qin *et al.*, 2012). This effect is particularly evident in the case of active cells in the case of which changes in chromatin condensation are directly coupled with cell cycle phases. Treatment of several cell lines with Cd^{2+} and Pb^{2+} was reflected by increased chromatin stickiness, its ribboned arrangement, formation of perichromatin bodies, and obstructed condensation (Banfalvi *et al.*, 2012). Moreover, the treatment with these metals induced apoptotic-like changes in Chinese hamster cells, namely premature chromatin condensation, presence of early chromosomal forms and sticky, incompletely folded chromosomes, and nuclear fragmentation during the early S-phase (Banfalvi *et al.*, 2005). Apoptotic-like and necrotic-like changes in chromatin organisation were also observed in human cells exposed to $[CH_3Hg]^+$ (Farkas *et al.*, 2010).

All the aforementioned effects of heavy metals on nucleic acids and chromatin fibres lead to reorganisation of chromatin inside the nucleus. This effect should not be underestimated as most of the recently published data suggest that the activity of chromatin is determined by the way in which it is distributed inside the nucleus (Hou & Corces, 2012). Chromatin is a dynamic structure, and some of its domains change their localization in response to both endogenous and exogenous stimuli. Genes were shown to loop out of their chromosome territory upon activation (Hou & Corces, 2012). Also movement of either chromosome domains or whole chromosomes was observed (Marshall *et al.*, 1997; Edelman *et al.*, 2001; Chuang *et al.*, 2006). The movements of chromatin are postulated to be underlied by myosin and/or actin-dependent mechanisms. Available data suggest that heavy metals may affect functioning and organisation of those proteins (Peysner *et al.*, 1996; Wang *et al.*, 1996; Wang & Templeton, 1996; DalleDonne *et al.*, 1997). Thus, it is tempting to speculate that heavy metals

may also disturb organisation of chromatin, interfering with the mechanisms responsible for its movement.

Many authors showed that treatment with heavy metals may lead to disruption of nuclear membrane (Banfalvi *et al.*, 2005; Farkas *et al.*, 2010; Banfalvi *et al.*, 2012). Available data suggest that various trace elements may interact with lipid and protein components of the membrane, thus influencing its properties and fluidity. This may result from a direct interaction between metals and membrane components, or from the destructive effect of ROS (Garcia *et al.*, 2005; Patra *et al.*, 2011). Nuclear envelope is reach in various proteins, among them nuclear pore complex — a key gate allowing entry of various molecules into the nucleus (Holaska *et al.*, 2002; Starr, 2009). Consequently, disrupting the membrane, metals may also deregulate nuclear – cytosol transport. This effect may be even more detrimental as the interactions between nuclear envelope and chromatin are known to influence the expression of genes. A number of previous studies showed that the interaction between heavy metals and membrane proteins or nuclear lamina, a proteinaceous layer found at the interface between chromatin and the inner nuclear membrane, may result in activation or silencing of genes (Ahmed *et al.*, 2010; Dieppois & Stutz, 2010; Mewborn *et al.*, 2010). Moreover, nuclear envelope seems to be also involved in the maintenance of telomeres; the telomeres of many species are often located peripherally, next to the nuclear envelope (Parada & Misteli, 2002; Meldi & Brickner, 2011). Therefore, interacting with the nuclear envelope, heavy metals may also affect the telomeres which are known to play an important role in aging (Martinez & Blasco, 2011; Lu *et al.*, 2013).

Heavy metals and nuclear bodies

Analysing the effect of heavy metals on chromatin organisation, also the influence on nuclear bodies (NB) should be considered. Nuclear bodies are inner nuclear structures that remain in a dynamic steady state, constantly exchanging their components with nuclear matrix. Most of known nuclear bodies, e.g. nucleolus, Cajal bodies, speckles and promyelocytic leukaemia (PML) bodies, contain specific proteins and RNAs. The contents of nuclear bodies reflect their function; depending on their type, NBs may be involved in modification of snRNAs and snoRNA, synthesis and maturation of rRNA, and stress response (Zimber *et al.*, 2004; Mao *et al.*, 2011). PML bodies seem to be specifically involved in stress response; they were shown to undergo severe reorganisation under stress, including the heavy metal-induced stress (Eskiw *et al.*, 2003; Eskiw *et al.*, 2004). Due to their high concentration of protein and RNA, NBs seem to be particularly prone to heavy metal attack; however, we lack the data on the influence of heavy metals on these structures.

Micronuclei

One should also mention micronuclei as an outcome of clastogenic and/or aneugenic action of heavy metals. Micronuclei are small, extra-nuclear structures, formed as a result of chromosome fragmentation and/or dysfunction of mitotic spindle during the cell cycle. They may consist of acentric chromosome fragments or whole chromosomes that lagged during mitosis and became separately enwrapped by the nuclear membrane during telophase, thus forming a nuclear-like structure (Kashyap & Reddy, 2012). The exposure to lead, even at low en-

vironmental levels, was shown to result in an enhanced micronuclei formation (Kapka *et al.*, 2007).

The micronuclei that contain chromosome fragments were suggested to be a product of ROS-induced double strand breaks formed in chromatin fibres during the interphase. In turn, the micronuclei with whole chromosomes are supposed to be formed due to mitotic spindle failure, kinetochore damage, hypomethylation of centromeric DNA or impaired cell cycle control (Luzhna *et al.*, 2013). Thus, the epigenetic mechanisms engaged in DNA methylation may be also involved in micronuclei formation. Several heavy metals, including arsenic, zinc and cadmium, were shown to induce formation of the whole-chromosome micronuclei (Dulout *et al.*, 1996; Tapisso *et al.*, 2009).

INDIRECT MECHANISMS OF HEAVY METAL ACTION

Heavy metals may also affect chromatin by interfering with various nuclear proteins. Some metals were shown to activate or inactivate various proteins, changing their activity profiles. This may *inter alia* lead to carcinogenesis. One example comes from a study of *bTERT* gene expression; this gene encodes telomerase, an enzyme involved in telomere replication. Arsenic was shown to induce the expression of two genes: *c-myc* and *egf*, both known to positively regulate transcription of *bTERT* (Ferrario *et al.*, 2009). Moreover, exposure to As may result in an increase in telomere length, an established symptom of carcinogenesis. (Li *et al.*, 2012).

Some heavy metals were shown to interact with various representatives of zinc finger protein family. These proteins play vital role in crucial nuclear processes, such as transcription and DNA repair (e.g. XPA enzyme). Some metals, e.g. As³⁺, Cd²⁺, Ni²⁺ and Co²⁺, were shown to actively interact with the zinc finger proteins, especially with zinc finger motif, impairing their functioning (Hartwig *et al.*, 1999; Kopera *et al.*, 2004; Witkiewicz-Kucharczyk & Bal, 2006; Tabrez *et al.*, 2014).

Interestingly, heavy metals were also shown to mimic natural signalling molecules, e.g. Ca²⁺. Moreover, Al³⁺ and Cd²⁺, the so-called metallo-oestrogens, may substitute natural oestrogens and interact with nuclear oestrogen receptors, changing the transcription process (Darbre, 2006; Silva *et al.*, 2012).

CONCLUSIONS

Heavy metals may play important roles in the nuclear environment, especially at lower levels. Their action should be always analysed in a holistic manner, with attention paid to all their potential effects. Mimicking microelements, heavy metals access the cell and enter physiological pathways. Moreover, they exert their toxic effects due to chemical interactions with nucleic acids and proteins, generation of ROS and changes in physical conformation of nuclear macromolecules. This results in deregulation of vital nuclear processes, such as transcription, replication and DNA repair, often introducing unexpected and irreversible changes in the cell program and leading to carcinogenesis.

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