Remodeling of heterochromatin induced by heavy metals in extreme old age

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Abstract The levels of chromosome instability and heat absorption of chromatin have been studied in cultured lymphocytes derived from blood of 80–93- and 18–30-year-old individuals, under the effect of heavy metal Cu(II) and Cd(II) salts. The analysis of the results obtained indicates that 50 μ M Cu(II) induced a significantly higher level of cells with chromosome aberrations in old donors ($13.8 \pm 1.5\%$ vs control, $3.8 \pm 1.7\%$), whereas treatment with 100 μ M Cd(II) did not induce any changes in the background index. Analysis of the lymphocyte melting curves showed that Cu(II) ions caused more effective condensation of heterochromatin in old healthy individuals compared with young donors, which was expressed by the increase of the T_m of elderly chromatin by $\sim 3^\circ\text{C}$ compared with the norm. Treatment of lymphocyte chromatin of old individuals with 100 μ M Cd(II) caused decondensation (deheterochromatinization) of both the facultative and constitutive domains of heterochromatin. The deheterochromati-

nization T_m was decreased by $\sim 3\text{--}3.5^\circ\text{C}$ compared with the T_m observed for young individuals. Thus, the chromatin of cultured lymphocytes from the old-aged individuals underwent modification under the influence of copper and cadmium salts. Cu(II) caused additional heterochromatinization of heterochromatin, and Cd(II) caused deheterochromatinization of facultative and constitutive heterochromatin. Our data may be important as new information on the remodeling of constitutive and facultative heterochromatin induced by heavy metals in aging, aging pathology, and pathology linked with metal ions.

Keywords Aberration · Aging · Cadmium · Chromosome · Copper · Heterochromatin · Heterochromatinization · Microcalorimetry

Introduction

In recent years, the leading role of the 3d transition metal ions (Mn, Fe, Ni, Cu, Cd, Co, Cr, and Mg) has become increasingly evident in the conformational rearrangement of genetic material (chromatin) at the subcellular level, which plays a principal role in the regulation of genome activity. Metal ions penetrate cells in the form of cations, and their absorption and concentration in cells are controlled by specific protein systems that mediate their transport to a specific location to participate in the appropriate enzymatic system (Valko et al. 2005,

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2006). In addition to their participation in normal biological processes, transition metals belong to a group of biologically active substances. They not only affect the conformational state of the double helix of DNA or chromatin but also determine significantly the key processes of the gene expression and induce mutagenesis and carcinogenesis (Favier et al. 2008; Lezhava and Jokhadze 2007; Lezhava et al. 2008). In turn, mutagenesis mediated by heavy metals depends largely on several factors. In particular, it is dramatically increased in model systems of aging (Jokhadze and Lezhava 1994; Lezhava 2006). Heavy metals affect the human genome and provoke the development of different senile pathological processes (Durham and Snow 2006). It was shown that the interactions of Cu(II) and Cd(II) ions with DNA and chromatin are distinct (Jokhadze and Lezhava 1994; Valko et al. 2005). This difference is associated with the stabilization of DNA at an extremely low concentration of these ions, strong destabilization of DNA at middle and high concentrations of these ions, exclusive interaction with the GC base pair of the DNA duplex (Berger and Eichhorn 1971; Henson and Chedrese 2004), and their connection with G-quadruplexes.

Practically all cytostatic drugs are characterized by reversible binding, e.g., transition metals and non-histone proteins causing local unfolding of the DNA helix (Nemeth and Langst 2004).

According to this view, we considered it expedient to determine whether the system of chromatin domains (eu- and heterochromatin regions) in lymphocyte cultures from old individuals undergoes changes when exposed to Cu(II) and Cd(II) ions. In particular, our aim was to study the variability of the levels of chromosome and eu- and heterochromatin instability under the effect of low concentrations of CuCl₂ and CdCl₂ salts during the process of aging.

Materials and methods

Materials and chemicals

We studied chromosomes 30 lymphocyte cultures obtained from 10 healthy individuals aged 80–93 years and 30 cultures from 10 young individuals (age, 18–30 years). Peripheral blood was collected between 10:00 and 11:00 hours from residents of the

Tbilisi Boarding House for Aged People. Three cultures (intact, treated with CuCl₂, and treated with CdCl₂) that were prepared from each individual allowed us to compare the indices of the treated cultures with their own control values. Metal salts at the concentrations 50 μM Cu(II) and 100 μM Cd(II) were added to the cultures at the onset of culture and were left for the entire period of incubation (48 h). The parameters of the chromatin heat absorption defined using the differential scanning heat absorption defined by the differential scanning microcalorimetry (DSC) method were studied lymphocytes obtained from seven young and six old donors.

Mutation

Studies were carried out on the PHA-stimulated cultures of peripheral blood lymphocytes (without serum and antibiotics) obtained from 20 healthy individuals aged from 80 to 93 years and from 18 to 30 years. Inorganic salts of copper (CuCl₂) and cadmium (CdCl₂) were added to the cultures, so that their concentrations were 50 and 100 μM per 1 ml of cell cultures, respectively. The substances were injected into the 48 h lymphocyte cultures (obtained from middle- and old-aged individuals). The material was fixed in the usual manner. Chromosomal aberrations (single and paired fragments) we analyzed 30–30 metaphases for both age groups, on samples of intact cultures and cultures treated with metal. The experimental data were evaluated using the nonparametric method according to the X^2 criterion, which does not require knowledge of species distribution.

Differential scanning microcalorimetry

The sensitivity of DSC was 0.1 μW. The volume the measuring vessel was 0.30 cm³, the heating rate was 0.55°C/min, and the temperature range of measurements was from 2°C to 140°C. The precision of temperature measurements was not less than 0.1°C. The error in determination of chromatin melting enthalpy (Hm) was not more than 15%. Chromatin concentration in calorimetric measurements was from 0.05 to 0.08 mg/ml. The DNA quantity in the measuring vessels was from 80 to 120 μg. By DSC was studied on lymphocyte cultures from 13 individuals aged of 20–30 (seven individuals-control) and

80–86 years (six individuals). Each 48-h cell culture of young and old persons, and the relevant suspension of cells incubated with CuCl_2 and CdCl_2 were measured two or three times. The maxima (T_m) of heat absorption peaks for one and the same individuals differed from each other not more by $\pm 0.2^\circ\text{C}$ in the same condition, but the heat absorption peaks (T_m) obtained for different individuals differed by not more than 1.0°C . It was determined that the clearly expressed shoulder of the heat absorption curve in the temperature interval from 40°C to 50°C with $T_m(\text{I}) = 45 \pm 1^\circ\text{C}$ corresponds to melting of membranes and some cytoplasm proteins, maximum at $T_m(\text{II}) = 55 \pm 1^\circ\text{C}$ correspond to melting (denaturation) of non-histone nuclei proteins, maximum at $T_m(\text{IV}) = 70 \pm 1^\circ\text{C}$ corresponds to the ribonucleoprotein complex, and maximums at $T_m(\text{III}) = 63 \pm 1^\circ\text{C}$ and $T_m(\text{V}) = 83 \pm 1^\circ\text{C}$ correspond to cytoplasm proteins. Other clearly expressed peaks at $T_m(\text{VI}) = 96 \pm 1^\circ\text{C}$ and $T_m(\text{VII}) = 104 \pm 1^\circ\text{C}$ correspond to the chromatin denaturation (Cardellini et al. 2000; Monaselidze et al. 2006, 2008).

Results

Chromosome instability

Analysis of the results (Fig. 1) showed that $\text{Cu}(\text{II})$ and $\text{Cd}(\text{II})$ treatment of the short-term 48-h lymphocyte cultures from young donors (aged 18–30 years) caused a certain increase in the frequency of cells with chromosome aberrations. The frequency of processing of $\text{Cu}(\text{II})$ ions by the aberrant cells was $5.9 \pm 0.9\%$, whereas it was $5.2 \pm 0.6\%$ under the influence of $\text{Cd}(\text{II})$ (control, $1.7 \pm 0.7\%$). The tested metal ions showed a significant ability to induce chromosomal aberrations. In general, the spectrum of chromosome disturbances was from single and paired fragments to terminal chromatin deletions. In short-term lymphocyte cultures of individuals aged 80–93 years, the presence of $\text{Cu}(\text{II})$ caused a significant increase in the number of cells with chromosome aberrations ($13.8 \pm 1.5\%$ vs control, $3.94 \pm 1.06\%$). However, no significant effect of $\text{Cd}(\text{II})$ on the level of aberrant cells was detected in lymphocytes from old individuals; the frequency of cells with chromosome aberrations was $4.0 \pm 0.98\%$, which remained at the control level (Fig. 1).

Fig. 1 Percent of metaphase with chromosome aberration induced by heavy metals $\text{Cu}(\text{II})$ and $\text{Cd}(\text{II})$ in human 48 h lymphocytes cultures from 18–30- and 80–93-year-old individuals

In summary, we can conclude that the effect of the two metals ($\text{Cu}(\text{II})$ and $\text{Cd}(\text{II})$) tested in lymphocyte cultures of old people was variable. The different effects of these metals can be explained by the different modifications of chromatin structure. $\text{Cu}(\text{II})$ ions caused a considerable increase in the number of cells with aberrant chromosomes in aged individuals, whereas $\text{Cd}(\text{II})$ did not raise the level of cells with chromosome alterations.

Thermal denaturation of chromatin

Figure 2 shows the heat absorption microcalorimetric curve of lymphocytes prepared from young donors (48 h cell culture). The calculation was made per gram of biomass. The curve shows that the cell denaturation process started at 40°C and ended at 115°C . The curve was complex and consisted of seven transitions at $T_m(\text{I}) = 45 \pm 1^\circ\text{C}$, $T_m(\text{II}) = 55 \pm 1^\circ\text{C}$, $T_m(\text{III}) = 63 \pm 1^\circ\text{C}$, $T_m(\text{IV}) = 70 \pm 1^\circ\text{C}$, $T_m(\text{V}) = 83 \pm 1^\circ\text{C}$, $T_m(\text{VI}) = 96 \pm 1^\circ\text{C}$, and $T_m(\text{VII}) = 104 \pm 1^\circ\text{C}$. The integral heat was $26.5 \pm 3 \text{ J/g}$ dry biomass, which was calculated from the area between the heat absorption curve and its baseline.

The two main structural domains of the chromatin of human lymphocytes (eu- and heterochromatin), which are influenced by heat, melted cooperatively and independently of each other at $T_m = 96 \pm 1^\circ\text{C}$ and $104 \pm 1^\circ\text{C}$, respectively.

According to Fig. 2, $50 \mu\text{M}$ $\text{Cu}(\text{II})$ did not cause any significant change in the thermo stability of membranes, nuclei, cytoplasmic proteins, and RNA complexes compared with untreated normal cells.

Fig. 2 The excess of heat capacity ($\Delta C_p = dQ/dT$) as function of temperature for lymphocytes cultures from young donors (*straight line*) intact; (*dotted line*) treated with 50 μM CuCl_2 , 87 μg DNA, 8.5 mg dry biomass; (*dashed line*) treated with 100 μM CdCl_2 ; 82 μg DNA, 8.8 mg dry biomass

Namely, the maxima of the endotherms (T_m) I to V and their corresponding heats (Q_m) coincided with the same parameters detected in untreated lymphocytes, within the experimental error. As for the chromatin heat absorption peaks VI and VII, they changed significantly.

As seen in Figs. 2 and 3, 50 μM Cu(II) caused alterations in the profiles of the heat absorption curves. In particular, Cu(II) shifted endotherm VII to higher temperatures, by 2°C in the case of young donors and by 3°C in the case of old individuals,

Fig. 3 The excess of heat capacity ($\Delta C_p = dQ/dT$) as function of temperature for lymphocytes cultures from old donors (*straight line*) intact; (*dotted line*) treated with 50 μM CuCl_2 , 87 μg DNA, 8.5 mg dry biomass; (*dashed line*) treated with 100 μM CdCl_2 ; 82 μg DNA, 8.8 mg dry biomass

compared with untreated lymphocytes. At this, the thermostability of the euchromatin did not change.

Treatment with 100 μM CdCl_2 caused significant changes in the stability of euchromatin and heterochromatin (Fig. 3), as their melting temperatures decreased by 3°C and 3.5°C, respectively. At this, the integral heat did not change and was equal to 91.0 ± 11 J/g DNA. This value was in good agreement with data obtained for chromatin extracted from humans and animals, which exhibits a denaturation heat (Q_d) of 75.6 ± 10 J/g.

Discussion

Cations are important for the functionality of chromatin in old people. Our results indicate that heavy metals cause remodeling of heterochromatin in cultured lymphocytes from aged individuals. In particular, 50 μM Cu(II) caused an increase in chromosome aberrations and led to an additional level of heterochromatin heterochromatinization, whereas 100 μM Cd(II) caused deheterochromatinization of facultative and constitutive heterochromatin.

Changes in chromosome structure are key aspects in the epigenetic regulation of gene expression. Hypermethylation may cause heterochromatinization and, thus, result in gene silencing (Mazin 1994, 2009). It was demonstrated that progressive heterochromatinization of chromosomes (condensation of eu- and heterochromatic regions) occurs during aging, followed by inactivation of the genes that functioned actively at younger ages (Lezhava 2001, 2006). Using the DSC method, our data support the statement that lymphocytes from old individuals exhibit increased chromosome heterochromatinization compared with the level of heterochromatin in young individuals.

There is abundant experimental evidence indicating that structural lesions caused by mutagens develop more frequently in heterochromatic than in the euchromatic regions. To explain the prevalence of accumulation of damage in heterochromatin and in regions of heterochromatinization, it has been assumed that the repair of lesions that are capable of causing aberration is possible only in areas of DNA that are actively involved in transcription and are physically accessible to reparative enzymes, i.e., euchromatin areas. It is known that the condensed (heterochromatic) regions of chromosomes are phys-

ically inaccessible to repair enzymes (Prokofieva-Belgovskaya 1986).

Heterochromatinization of chromatin by 3d transition metals is a potential mechanism of metal-mediated gene regulation (Ellen et al. 2009). Aluminum and copper accumulate in the tissues of old humans and animals and presumably act as binding agents that mediate eu- and heterochromatin condensation. Thus, they may explain rising mutation rates and structural changes of chromosomes (Jokhadze and Lezhava 1994; Kawanishi et al. 2002; Theophanides and Anastassopoulou 2002). Cu(II) induces conformational changes (condensation) in the chromosomes exposed on the cells of Chinese hamster ovaries. This decreases the availability of antibodies to the chromosomal histone H2b (Turner and Koehane 1986). Chromosome condensation requires posttranslational modification and the action of an ATP-dependent complex termed “condensing,” which introduces positive DNA supercoils into DNA substrates, in the presence of topoisomerases (Uhlmann 2001).

Our data pertaining to the increase of chromosome aberrations and the presence of additional heterochromatinization of heterochromatin induced by Cu(II) in old individuals are in good agreement with these findings. We explain the additional heterochromatinization of heterochromatin by the Cu(II)-induced additional crosslinking of the chromatin 30-nm fiber with the nuclear matrix.

Regarding the mutagenic action of Cd(II) ions, chromosomal changes under the influence of this element have not been observed (Jokhadze and Lezhava 1994). However, it was shown that exposure of cells to CdCl₂ for 6 h causes decondensation of chromosomes in bone-marrow cells of rodents (Muramatsu et al. 1980). Moreover, Cd(II) has been shown to cause a significant effect, as extremely low doses reportedly stimulate progesterone biosynthesis, whereas high doses inhibit this process (Henson and Chedrese 2004). Our studies demonstrated that low doses of Cd(II) induced the deheterochromatinization of eu- and heterochromatin regions in individuals aged 80–93 years.

Thus, our results indicate that the transition metals Cu(II) and Cd(II) induce remodeling of heterochromatin in cultured lymphocytes of aged individuals. Cu(II) causes additional heterochromatinization of heterochromatin, and Cd(II) causes deheterochromatinization of the facultative and constitutive heterochromatin.

The proposed remodeling of the heterochromatin induced by the heavy metals Cu(II) and Cd(II) in aging, aging pathology, and pathology linked with metal ions may help evaluate the importance of external and internal factors in the development of diseases and enable the development of proper therapeutic tools.

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