Evaluation of the Dependence of Mutagenesis Intensity on Activity of Nucleolus Organizer Regions of Chromosomes in Aboriginal Population of Kursk Region

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Visual semiquantitative method of silver staining of nucleolus organizer regions in peripheral blood leukocytes was applied for evaluation of the correlation between activity of nucleolus organizer regions and the level of chromosome aberrations in 241 residents of Kursk region. Significant differences in the percent of chromosome aberrations were revealed between three groups differing by the number of active nucleolus organizer regions, which can be explained by different proliferative activity in these groups. The lowest level of chromosome aberrations was found in individuals with high transcription activity of the nucleolus organizer regions (due to high proliferative activity in this group and due to more intensive protein biosynthesis, *e.g.* synthesis of reparation enzymes). The maximum level of chromosome aberrations was observed in the group with medium number of active nucleolus organizer regions (adaptive norm).

Key Words: *nucleolus organizer regions; silver staining; Ag-polymorphism; chromosome aberrations*

Methods of peripheral blood leukocyte culture recommended by World Health Organizations [11] are widely used for evaluation of the effects of adverse environmental factors on humans. The use of this test provides valuable information on the frequency of chromosome aberrations (CA) in populations exposed (or presumably exposed) to adverse environmental factors [1,2].

The involvement of NOR into processes of spontaneous mutagenesis affecting the level of CA [2,7] cannot be evaluated on the basis of published data on phenotypic manifestations of transcription activity of nucleolus organizer regions (NOR) in chromosome abnormalities [7] and data on the size of NOR in individual chromosomes under the action of mutagenic factors [1].

NOR in human chromosomes are located in short arms (secondary constrictions) of 5 pairs of acrocentric chromosomes (13-15 and 21-22). The method of selective silver staining of chromosomes makes it possible to evaluate transcription activity of NOR at the cytogenetic level [13]. However, in humans, phenotypic manifestations of NOR at the subcellular level in parameters of spontaneous mutagenesis are little studied.

Here we studied phenotypic manifestation of transcription activity of NOR at the subcellular level by the level of CA.

MATERIALS AND METHODS

A random sampling including 241 residents of Ponyrovskii, Oktyabr'skii, and Kurskii districts of the

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Kursk region was examined. Peripheral blood for cytogenetic studies was obtained from the cubital vein.

Blood cells were cultured and metaphase plates were prepared [2,4,11]. The cells were fixed in Carnoy fluid (3:1 methanol-acetic acid mixture) for 3 h or longer. Seeding and culturing of blood lymphocytes and preparation of metaphase plates were performed strictly standardly in all cases. The preparations were stained with $AgNO_3$ at room temperature for 7-14 days.

The number of active NOR was determined using a Biolam microscope (×90). The number of stained NOR was determined in each analyzed metaphase plate.

Activity of NOR was determined by the amount of Ag precipitate in individual acrocentric chromosomes [14]. Visual estimation of this parameter was performed according to a 5-point scale: 0 — absence of staining (NOR is inactive); 1 — weak staining (Ag grains on satellite stands narrower than chromatid width); 2 — medium intensity of staining (Ag grains correspond to chromatid width); 3 — intensive staining (Ag grains wider than chromatid); 4 — high-intensity staining (Ag grains on each chromatid are considerably wider than the chromatid and form agglomerates).

For comparison of Ag-stained chromosomes we used an integral parameter (total Ag-staining intensity for all NOR in chromosomes of metaphase plate). To this end, the total staining score for all (usually 20) metaphase plates was divided by the number of analyzed metaphase plates. The summary size of 10AgNOR characterizes the number of active NOR in cell and serves as the basis for comparison of individual genomes by this parameter (Ag-polymorphism). In normal, 10AgNOR varied from 15 to 23 arb. units [9,10].

The chromosome preparations were stained with Romanowsky—Giemsa dye in water (1:50) for 10 min without pretreatment. Properly stained metaphase plates without overlaid chromosomes were analyzed [2,4,12].

The preparations were examined under a microscope, 100 metaphase plates were analyzed for each individual, the type of aberration, group of chromosome, and coordinates of metaphase plate were specified in the protocol. The level of CA was expressed as the percent of damaged cells from the total number of analyzed metaphases [2,4,12]. Cells with CA were counted, number of CA per 100 cells, number of fragments, single and paired fragments, and chromosome and chromatid exchanges were determined (Table 1).

The data were processed using GEN 1 software [12]. The distribution was verified using Statgraphics 3.0, Systat 4.0, and Statistica 6.0 software.

TABLE 1. Total Level of CA in Residents of Kursk Region (n=234; $M\pm m$)

Parameter	Value
Number of	
cells with CA	1.07±0.08
CA per 100 cells	1.11±0.09
fragments	1.22±0.08
exchanges	0.12±0.03
single fragments	0.56±0.06
paired fragments	0.43±0.05
chromosome exchanges	0.07±0.02
chromatid exchanges	0.05±0.03

Statistical hypothese were verified using parametric Student and Fisher tests at $p<0.05$. Discriminant and regression analyses of multivariate statistics were used [9].

RESULTS

The total level of CA in residents of Kursk region was 1.11±0.09. According to previous studies performed in Kursk, control level of CA was 0.97± 0.15 [7]. Predominant CA in our study were single fragments (0.56±0.06) and paired fragments (0.43± 0.05), while in the previous work the levels of single and paired fragments were 0.75±0.13 and 0.18±0.06, respectively [7]. The number of chromosome and chromatid exchanges in our study was insignificant (Table 1). The sex- and age-related differences in CA level were absent except chromatid exchange, which were more often seen in women and young individuals, but the data on these sampling were pooled because of low total number of these abnormalities (Table 1).

The maximum level of CA was observed in the group with medium number of 10AgNOR, and the minimum level in the group with the highest number of 10AgNOR (Table 2). In all variants of the number of 10AgNOR we observed different levels of CA reflecting the effects of environmental factors manifesting by different ways in the phenotype of individuals of different groups. Thus, individuals with low and medium numbers of 10AgNOR had different number of cells with CA (t=2.18), number of CA (t=2.01), fragments (t=2.09), and single fragments (t=2.22).

The differences between individuals with low and medium numbers of 10AgNOR were significant by the number of cells with CA (t=3.25), number of CA (t=3.88), fragments (t=3.50), single fragments (t=5.02) and paired fragments (t=3.50).

The differences between individuals with medium and high numbers of 10AgNOR were significant by the number of cells with CA (t=6.01), number of CA (t=6.0), fragments (t=5.60), single fragments (t=5.04) and paired fragments (t=2.91). We revealed no differences by the number of chromosome and chromatid exchanges because of their low incidence.

Comparison of dispersions in the groups of individuals with low and medium number of 10AgNOR revealed high heterogeneity of the group with medium number of 10AgNOR by the number of cells with CA (F=2.04), number of CA (F=1.58), fragments (F=1.61), exchanges (F=1.42), single fragments (F=1.54); heterogeneity of the group with low number of 10AgNOR by the number of chromosome exchanges (F=1.75).

Comparison of dispersions in the groups of individuals with low and high number of 10AgNOR revealed high heterogeneity of the group with low number of 10AgNOR by the number of cells with CA (F=1.25), paired fragments (F=2,18), and chromatid exchanges (F=2.0), and heterogeneity of the group with high number of 10agNOR by the number of CA (F=1.41), fragments (F=1.25), exchanges (F=1.57), and chromosome exchanges (F=2.25). Comparison of dispersions in the groups of individuals with medium and high number of 10AgNOR revealed high heterogeneity of the group with medium number of 10AgNOR by the number of CA (F=2.25), fragments (F=1.27), single (F=1.79) and paired fragments (F=1.81), and heterogeneity of the group with high number of 10AgNOR by the number of chromosome exchanges (F=1.28; Table 2).

Regression equations showed that negative correlations exist between AgNOR and almost all parameters of CA except for chromosome and chromatid exchanges, but they did not attain the level of significance by all parameters of CA except the total number of fragments and paired fragments.

Reliable differences between the groups with different number of 10AgNOR can be explained by different proliferative activity in these groups. In the group with high number of 10AgNOR we observed the lowest level of CA, which can be determined by higher proliferative activity leading to accelerated elimination of CA and more intensive protein biosynthesis, which leads to acceleration of repair processes (due to intensive synthesis of repair enzymes). Transitions of inactive NOR to acti-

TABLE 2. Comparison of CA Levels in Groups with Different Transcription Activity of NOR (*n*=213; *M*±*m*)

Parameter	I (*n*=55)	II (*n*=82)	III (*n*=56)
Number of cells with CA	0.96±0.12	1.2±0.14	0.70±0.11
Number of CA	1.01±0.12	1.25±0.12	0.70±0.13
Fragments	1.11±0.12	1.35±0.12	0.84±0.13
Exchanges	0.09±0.03	0.10±0.03	0.08±0.06
Single fragments	0.49±0.09	0.69±0.09	0.24±0.08
Paired fragments	0.43±0.09	0.51±0.07	0.22±0.07
Chromosome exchanges	0.06±0.02	0.07±0.03	0.06±0.04
Chromatid exchanges	0.03±0.02	0.04±0.02	0.02±0.01

Parameter	Significant values					
	t>1.98			F>1.25		
	I-II	I-III	II-III	I-II	I-III	II-III
Number of cells with CA	2.18	3.25	6.01	2.04	1.25	2.55
Number of CA	2.01	3.88	6.00	1.58	1.41	—
Fragments	2.09	3.50	5.60	1.61	1.26	1.27
Exchanges	—	—	—	1.42	3.43	2.40
Single fragments	2.22	5.02	5.03	1.54	—	1.79
Paired fragments	—	3.5	2.91	—	2.18	1.81
Chromosome exchanges	—	—	—	1.75	2.25	1.28
Chromatid exchanges	—	—	—	—	2.00	2.50

Note. I-III: groups with different transcription activity of NOR.

ve state under the action of some adverse environmental factors cannot also be excluded, which can lead to an increase in the number of 10AgNOR.

It is currently accepted that low doses of radiation induce an adaptive response in most cells and autoinduction of chromosome abnormalities; this phenomenon represents a kind of genetic adaptation aimed at the formation of cells of evolution reserve ensuring (after selection) survival of genetically changed cell population adapted to new conditions [3, 5,6]. These adaptation mechanisms can include CA. There are published data that CA are a manifestation of sensitization, *i.e.* an adaptive response [5]. It was also noted that the level of chromatid exchanges increases during cross-adaptation [6]. In our case, the numbers of chromosome and chromatid exchanges were similar.

The group with medium number of 10AgNOR can be considered as the adaptive norm and hence, these individuals are characterized by the maximum number of CA, *i.e*. the best adaptive response. According to some reports, it consists in amplification of some genes which can activate transcription of genes encoding inducible enzymes [3,5,6]. The group with low number of 10AgNOR is intermediate by the level of CA, which can reflect less intensive proliferation and lower adaptation capacities. We revealed no linear dependence between AgNOR and CA and only a tendency to a decrease in the level of CA with increasing the number of 10AgNOR was noted.

Thus, our experiments showed that activity of NOR affects the formation of CA in humans and confirmed adaptation nature of chromatid abnormalities in chromosomes, which predominate in the group with medium activity of NOR (adaptive norm) and are less pronounced in other groups. We demonstrated the relationship between functional Ag-polymorphism and CA level in humans in the absence of linear dependence between these parameters.

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