GENETICS

Evaluation of the Relationship between Chromosome Aberrations and Transcription Activity of Nucleolus Organizer Regions in Indigenous Population of the Kursk Region

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The relationship between activity of chromosomal nucleolus-organizer regions and levels of chromosome aberrations was studied in 215 residents of the Kursk region by visual semi-quantitative method (silver staining of the nucleolus-organizer regions, NOR) in chromosomes of peripheral blood lymphocytes [13]. The levels of chromosome aberrations differed significantly in three groups differing by the levels of 10AgNOR, which can be explained by different proliferative activity in these groups. The lowest level of chromosome aberrations was found in subjects with high transcription activity of NOR, presumably due to high proliferative activity in this group and more intensive synthesis of proteins, including the reparation enzymes. The highest level of chromosome aberrations was detected in the group with the medium level of 10AgNOR.

Key Words: *chromosomal nucleolus-organizer regions; Ag-method; Ag-polymorphism; chromosome aberrations*

Peripheral blood lymphocyte culture was recommended by the WHO [11] for evaluation of aftereffects of unfavorable environmental factors on humans and now is widely used for this purpose. This method is used for evaluation of the incidence of chromosome aberrations (CA) in populations [1,2] and activity of the nucleolus organizer regions (NOR) located in the short arms (secondary constrictions) of five pairs of acrocentric chromosomes (13-15 and 21-22); transcription activity of NOR is studied by selective silver staining [13].

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Studies of phenotypical manifestation of NOR transcription activity in chromosome aberrations [7] under the effect of mutagenic factors [1] were described. The data on the impact of NOR activity for spontaneous mutagenesis processes are extremely scanty [2].

We studied the regularities of the effects of NOR transcription activity on the level of CA in indigenous residents of the Kursk region.

MATERIALS AND METHODS

We examined 215 indigenous residents (86 men and 129 women) of Ponyrovskii, Oktyabrskii, and Kursk districts of the Kursk region. The peripheral blood for cytogenetic studies was collected from the ulnar vein.

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Blood culturing was carried out and metaphase chromosome preparation were made by the standard methods [2,4,11]. The cells were fixed with Carnoy's fixative (methanol+acetic acid) in 3:1 ratio for 3 h and longer. Inoculation and culturing of blood lymphocytes and preparation making were carried out by the standard methods in all cases. The preparations were exposed at ambient temperature for 7-14 days for staining with silver nitrate.

Transcription-active NOR were detected as described previously [13]. Active NOR were counted under the Biolam light microscope (10×90 magnification) for each analyzed metaphase plate. NOR activity was evaluated by the content of silver precipitate in 10 individual acrocentric chromosomes (10AgNOR). This parameter was visually scored by a 5-point (0-4) scale: 0 points: no staining (inert NOR); 1: slight staining (precipitated silver grains on satellite strands are narrower than the chromatid width); 2: medium staining (silver grains correspond to the chromatid width); 3: intense staining (silver grain precipitate wider than the chromatid width); and 4: highly intense staining (silver grains which precipitated on each chromatid are much wider and stick one to the other, forming a common conglomeration). Silver-stained chromosomes were compared using a comprehensive indicator of summary staining intensity for all NOR in the metaphase plate. The sum of staining intensity scores for all metaphase plates (20 in our study) was divided by the number of the analyzed metaphase plates. The summary size of 10 AgNOR was characterized by the quantity of active NOR in the cell and served as the basis for evaluation of individual genome activities by Ag-polymorphism. Normally the size of 10AgNOR varies from 15 to 23 arb. units [9,10].

For studies of mutagenesis, the chromosome preparations were stained with Romanowsky–Giemsa dye (1:50, aqueous solution) for 10 min without pretreatment. Only well-stained metaphase plates without chromosome superimposition onto each other were evaluated [2,4,12]. At least 100 metaphase plates were examined per subject and the results were recorded in the protocol; the type of chromosome aberrations (chromosome or chromatid), chromosome group (A, B, C, D, E, F, G), and metaphase plate coordinates were recorded. The CA level was expressed in percent of damaged cells to the total number of examined metaphases [2,4,12].

The results were statistically processed on IBM PC/AT (486) using GEN 1 software [12] and applied software. The normality of distribution was verified using Statgraphics 3.0 and Systat 4.0 (Statistica 6.0) software. Statistical hypotheses were verified using Student's and Fisher's parametric tests [8].

RESULTS

The total level of CA in indigenous population of the Kursk region was 1.11±0.09; single (0.56±0.06) and paired (0.43±0.05) fragments predominated (Table 1). Chromosome and chromatid exchanges were represented negligibly in our sampling (0.12±0.03). No gender- and age-associated differences in the groups with CA were detected, except the chromatid exchanges, which were somewhat more incident in women and young men, but the sample was united because of low (0.05±0.03) incidence of these exchanges.

Comparative analysis of groups with different content of 10AgNOR among the residents of the Kursk region was carried out (Table 2). The highest level of CA was detected in the group with the medium content of 10AgNOR, the least in the group with its high level.

Groups of subjects with low and medium levels of 10AgNOR differed by the numbers of cells with CA (t=2.18), numbers of CA (t=2.01), total numbers of fragments (t=2.09) and single fragments (t=2.22).

The differences between the groups of subjects with low and high levels of 10AgNOR were most pronounced for the counts of cells with CA (t=3.25), numbers of CA (t=3.88), total numbers of fragments (t=3.50), single (t=5.02) and paired (t=3.50) fragments.

The groups with medium and high content of 10AgNOR differed significantly by the number of cells with CA (t=6.01), number of CA (t=6.0), total number of fragments (t=5.60), single (t=5.04) and paired (t=2.91) fragments.

Comparative analysis of dispersions in the studied groups with low and medium levels of 10AgNOR showed the highest heterogeneity of the latter group by the number of cells with CA (F=2.04), number of CA (F=1.58), total number of fragments (F=1.61), exchanges (F=1.42), and single fragments (F=1.54),

TABLE 1. Levels of CA in Residents of the Kursk Region (*n*=215)

Parameter	The mean and its error (*X*±*SE*)
Numbers of	
cells with CA	1.07±0.08
CA (per 100 cells)	1.11±0.09
fragments (total)	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

while the group with a low number of 10AgNOR was highly heterogeneous by chromosome exchanges (F=1.75) (Table 2).

Comparative analysis of dispersions between the groups with low and high levels of 10AgNOR showed the highest heterogeneity of the former group by the number of cells with CA (F=1.25), paired fragments (F=2.18), and chromatid exchanges (F=2.0), while the latter group was characterized by the highest heterogeneity by the numbers 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 with the medium and high content of 10AgNOR showed the highest heterogeneity of the former group by the numbers of CA (F=2.25), total number of fragments (F=1.27), single (F=1.79) and paired (F=1.81) fragments, while the group with high content of 10AgNOR was most heterogeneous by the number of chromosome exchanges (F=1.28) (Table 2).

Analysis of the multiple correlation matrix showed statistically significant correlations between the NOR activity and CA numbers in the total sampling. The 10AgNOR in all the examined subjects formed significant correlations with several parameters of CA: cell counts (r=0.20), number of exchanges (r=0.42), total number of fragments (r= 0.16), single fragments (r=0.16), chromosome exchanges (r=0.19), and chromatid exchanges (r=0.14). The relationship between 10AgNOR and number of exchanges was most pronounced (r=0.42; Table 3). NOR activity in the groups with low level of 10AgNOR formed significant correlations with the counts of cells with CA (r=0.25) and number of CA (r=0.26) (Table 4).

The impact of NOR activity was most pronounced in the group of subjects with medium content of 10AgNOR. Activity of NOR in this group formed significant correlations with all CA parameters except chromosome exchanges, the correlation coefficients varying from 0.27 to 0.99. The 10AgNOR formed the highest correlations with number of cells with CA (r=0.94), CA (r=0.92), and number of fragments (r=0.91). In the group with a high level of 10AgNOR the AgNOR formed significant correlations with the number of cells with CA (r=0.25) and number of CA (r=0.26).

Hence, the relationship between CA parameters and NOR activity in indigenous population of the Kursk region was demonstrated at a statistically significant level.

The results of the study indicating that CA characteristics are not gender-associated are confirmed by the results of studies carried out on a greater sample (n=1172), which proved that all cytogenetic parameters were similar for males and females [1].

In order to detect the relationship between NOR activity and CA formation, comparative analysis of groups of residents of the Kursk region with different content of 10AgNOR was carried out. It was expected that individuals with different content of 10AgNOR could have different levels of CA formation as a result of different capacity to resist the common environmental factors.

Several generations of ancestors of the subjects participating in the study lived in the same region and were regularly exposed to certain environmental factors, including chemical, biological, and radiation components. These factors caused a certain (eventu-

TABLE 2. Comparative Analysis of CA Levels in Groups with Different Transcription Activity of Chromosomal NOR (n=215)

NOR/CA	Mean and its mean error (X±SE)			t			F		
	I (n=64)	II (n=90)	III (n=61)	I-II	I-III	II-III	I-II	I-III	II-III
Numbers of									
cells with CA	0.96±0.12	1.2±0.14	0.70±0.11	2.18	3.25	6.01	2.04	1.25	2.55
CA	1.01±0.12	1.25±0.12	0.70±0.13	2.01	3.88	6.00	1.58	1.41	*
fragments	1.11±0.12	1.35±0.12	0.84±0.13	2.09	3.50	5.60	1.61	1.26	1.27
exchanges	0.09±0.03	0.10±0.03	0.08±0.06	*	*	*	1.42	3.43	2.40
single fragments	0.49±0.09	0.69±0.09	0.24±0.08	2.22	5.02	5.03	1.54	*	1.79
paired fragments	0.43±0.09	0.51±0.07	0.22±0.07	*	3.5	2.91	*	2.18	1.81
chromosome exchanges	0.06±0.02	0.07±0.03	0.06±0.04	*	*	*	1.75	2.25	1.28
chromatid exchanges	0.03±0.02	0.04±0.02	0.02±0.01	*	*	*	*	2.00	2.50

Note. Significant differences at t>1.98; F>1.25. *I*) group with low content of 10AgNOR (17.23±0.21); D-NOR: 10.53±0.12; G-NOR: 6.69±0.18; *II*) group with medium content of 10AgNOR (19.22±0.12); D-NOR: 11.60±0.13; G-NOR: 7.61±0.12; *III*) group with high level of 10AgNOR (21.77±0.19); D-NOR: 13.12±0.19; G-NOR: 8.55±0.16. Here and in Tables 3, 4: *the differences were statistically insignificant.

TABLE 3. Correlation Matrix between NOR Transcription Activity and CA Parameters in the Total Sampling of Examined Subjects (n=215; R5%=0.13)

10AgNOR		1.00				10AgNOR — 19.54±0.23 arb. units				
Numbers of	cells with CA	0.20	1.00			D-NOR — 12.28±0.23 arb. units				
	CA	*	0.77	1.00		G-NOR — 7.26±0.18 arb. units				
	fragments	*	0.73	0.96	1.00					
	exchanges	0.42	0.20	0.44	0.36	1.00				
	single fragments	0.16	0.58	0.68	0.67	*	1.00			
	paired fragments	*	0.48	0.68	0.69	0.30	*	1.00		
	chromosome exchanges	0.19	0.20	0.22	0.27	0.73	*	*	1.00	
	chromatid exchanges	0.14	*	*	*	0.28	*	*	*	1.00

TABLE 4. Correlation Matrix between NOR Transcription Activity and CA Parameters in the Group of Subjects with Low Level of 10AgNOR (n=64; R5%=0.24)

10AgNOR			1.00			10AgNOR — 16.93±0.11 arb. units				
Numbers of	cells with CA	0.22	1.00			D-NOR — 10.28±0.13 arb. units				
	CA	0.22	0.77	1.00		G-NOR — 6.65±0.07 arb. units				
	fragments	0.24	0.95	0.95	1.00					
	exchanges	*	*	*	*	1.00				
	single fragments	*	0.64	0.64	0.63	*	1.00			
	paired fragments	*	0.65	0.65	0.68	*	*	1.00		
	chromosome exchanges	*	*	*	*	0.80	*	*	1.00	
	chromatid exchanges	*	0.27	0.27	*	0.56	*	*	*	1.00

ally adaptive) response in the major part of body cells, associated with autoinduction of chromosome aberrations, a sort of genetic adaptation to these environmental factors. Due to this adaptation, the cells and the population in general survived (after selection). Chromosome aberrations can be considered as a manifestations of this adaptation [1].

The study has revealed significant differences between three groups differing by the content of 10AgNOR, which can be attributed to the peculiarities of proliferative activity in these groups. The highest level of CA was observed in the group with medium content of 10AgNOR and the lowest level of CA was found in the group with their highest level. The lowest level of CA in the group with high content of 10AgNOR can be due to several facts: higher proliferative activity leading to rapid elimination of CA, more intense protein synthesis stimulating reparative processes because of intense synthesis of reparation enzymes, and possible mechanisms of stimulation of inert NOR under the effect of unfavorable environmental factors, which leads to an increase in 10AgNOR level in the subjects.

It was reported that medium content of 10AgNOR in humans is associated with amplification of some genes paralleled by the increase in CA level and promoting adaptation of the individual to the environment [3,5,6]. The intermediate level of CA in the group with low content of 10AgNOR can be due to less intense protein synthesis and proliferation [1].

Hence, the study proved the relationship between chromosomal NOR activity and level of CA formation in humans. High transcription activity of chromosomal NOR causes the lowest level of CA, predominating in the group with the medium activity of NOR.

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