GENETICS

Transcriptional Activity of Chromosome Nucleolar Organizing Regions in Population of Kursk Region

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We studied population polymorphism of active nucleolar organizing regions in residents of the Kursk region and the association of activity of chromosomal nucleolar organizing regions with intensity of the synthesis of erythrocyte membrane protein. We revealed a tendency towards increasing of the amount of the major erythrocyte membrane proteins (primarily, spectrins and band 5 protein) with increasing total transcriptional activity of nucleolar organizing regions of chromosomes. The intensity of protein synthesis affects cell proliferation, determines the rate of tissue growth and its function, which determines its participation in the development of various diseases.

Key Words: *nucleolar organizing regions; Ag-method; Ag-polymorphism; erythrocyte membrane proteins*

Nucleolar organizing regions (NORs) of human choromosomes are localized in short arms (secondary constrictions) of 5 pairs of acrocentric chromosomes (13, 14, 15, 21, and 22). In humans, NORs contain tandemly arranged ribosomal genes [4]. Investigation of transcriptional activity of these genes led to discovery of a new type of human chromosome polymorphism, Ag-polymorphism. The total size of AgNOR in 10 acrocentric chromosomes (10AgNOR) expressed in arbitrary units (arb. units) is a measure of NOR activity.

The aim of the study was to investigate the effect of transcriptional activity of NOR on the amount of structural proteins, which do not depend on the body status, *e.g.* erythrocyte membrane proteins (EMP), and on Ag-polymorphism in an isolated population (marriages between residents within the same isolated region, radius of 30 km, for at least 3 generations).

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MATERIALS AND METHODS

Peripheral blood of volunteers was studies. The sample was random and consisted of 215 residents of Kurskii, Oktyabrskii, and Ponyrovskii districts of the Kursk region.

Peripheral blood lymphocytes were cultured and metaphase chromosome were prepared routinely [2]. The following reagents were used in the study: heparin (Richter), phytohemagglutinin (Difko-P), medium 199, cattle serum, colchicine, potassium chloride, methanol, glacial acetic acid, strong sulfuric acid, potassium dichromate, silver nitrate, gelatin, sodium citrate, potassium dihydroorthophosphate, and Giemsa dye (Merck). Reagents of Russian origin were chemically pure or of high purity.

Cells were fixed with Carnoy fixative (methanol+ acetic acid, 3:1) for 3 h or longer. Seeding and culturing of blood lymphocytes and preparation of metaphase chromosomes were strictly standardized in all cases. The preparations were stained with silver nitrate for 7-14 days at room temperature.

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The number of active NORs was estimated under a Biolam microscope (×900). The number of stained NORs was counted in each metaphase plate.

NOR activity was determined by the size of silver precipitate of individual acrocentric chromosomes. This parameter was visually estimated and scored using a 5-point scale: from 0 (no stain, NOR is inactive) to 4.

Integral intensity of silver staining of all NORs of metaphase plate chromosomes was used for comparison of silver-stained chromosomes [3]. The total size of 10AgNOR characterizes the number of active NORs in the cells and is used for comparison of individual genomes by this feature (Ag-polymorphism). Normally, 10AgNOR varies from 15 to 23 arb. units

Erythrocytes were obtained from heparinized blood [2]. Then membrane proteins from purified erythrocyte mass were fractionated using one-dimension electrophoresis in PAAG after Laemmli. Electrophoregrams were stained with Coomassie R-250 in the following solution: 10% acetic acid, 24% isopropanol, and 0.05% Coomassie R-250. Sixteen protein fractions were identified on electrophogergrams. Electrophoregram densitometry was conducted using laser densitometer Ultrascan XL. The following reagents were used: dextran T-500 (Sigma), HBS-cellulose (Sigma), sodium hydrophosphate, sodium chloride, urea (Bio-Rad), tris, 2-mercaptoethanol, ammonium persulphate (Reanal), sodium dodecylsulphate (Dia-Pharm), acrylamide (Sigma), N,N'-methylenebisacrylamide (Fluka), Coomassie G-200 (Serva), bromphenol blue, TEMED, glycine, and MS-2 set (molecular weight marker proteins).

Statistical analysis was conducted using parametric Student and Fisher tests and significance level of 0.05.

RESULTS

Functional state of NOR in choromosomes of indigenous residents of the region was 19.46±0.13 arb. units. D-NOR was 11.68±0.09 arb. units and G-NOR was 7.78±0.07 arb. units. On the basis of total NOR activity individual values were divided into groups with low (15-17.99 arb. units), mean (18-20.49 arb. units) and high amount of 10AgNOR (>20.5 arb. units) [4]. In group 1 (29.3% of the studied sample), 10AgNOR was 17.26±0.11 arb. units and D-NOR and G-NOR were 10.47±0.09 and 6.81±0.08 arb. units, respectively; in group 2 (41.4%), 10AgNOR was 19.36±0.07 arb. units and D-NOR and G-NOR were 11.59±0.08 and 7.74±0.07 arb. units, respectively; in group 3 (29.3%), the corresponding values were 21.70±0.12, 13.09±0.11, and 8.61±0.11 arb. units. The distribution of NOR activity in the analyzed sample is presented in Table 1.

Regions with a size of 1 and 3 arb. units prevailed among D-NOR and G-NOR, respectively. Analysis of 10AgNOR revealed the prevalence of NORs with a size of 3 arb. units, whereas NORs of 0 and 4 arb. units were practically absent.

All data, obtained on Ag-polymorphism, fit normal distribution and were in line with published reports [1], but had a specific feature of a low presence of NORs of 0 and 4 arb. units.

The most abundant protein fractions in cell membranes were α- and β-spectrins, band 3 protein (BP3) and BP4.5 (glucose transporter); these fractions were also most heterogeneous (Table 2).

The quantity of all EMP increased with increasing transcriptional activity of NOR. In most cases the difference was statistically significant. Differences in the content of spectrin and BP6 proteins (Table 2) were most pronounced, which is probably due to influence of active NOR on the quantitative representation of these proteins.

Comparative analysis of the groups of individuals with different 10AgNOR amount revealed significant difference in some parameters (Table 2). The most marked differences were established when groups with low and high amount of 10AgNORs were compared.

AgNOR variants of each acrocentric chromosome are inherited in generations as independent Mendelian

TABLE 1. NOR Activity in Residents of Kursk Region (*n*=215)

NOR, arb. units	*n*	D-NOR, arb. units	*n*	G-NOR, arb. units	*n*
15.60-15.99	2	9.3-9.9	8	5.41-5.99	2
16.00-16.99	14	10.0-10.9	44	6.00-6.99	39
17.00-17.99	19	11.0-11.9	68	7.00-7.99	72
18.00-18.99	46	12.0-12.9	48	8.00-8.99	62
19.00-19.99	44	13.0-13.9	41	9.00-9.99	37
20.00-20.99	42	14.0-14.7	6	10.00-10.62	3
21.00-21.99	27				
22.00-22.99	12				
23.00-23.52	9				

TABLE 2. Comparative Analysis of EMP Content (mg/10^{12} erythrocytes) in Groups of Individuals with Different Transcriptrional Activity of Chromosome NORs ($X \pm SE$; n=193)

Proteins	Groups with different number of 10AgNOR			Mean value for 3 groups
	group 1 (n=63)	group 2 (n=89)	group 3 (n=63)	
α-Spectrin	113.65±4.10	123.36±3.14**	125.39±4.40**	120.8±3.88
β-Spectrin	137.61±1.90	143.32±2.61**	149.90±3.36**+	143.61±2.62
2.1-Ankyrin	25.18±2.29	24.68±1.37	31.30±1.66**++	27.05±1.77
2.2-Ankyrin	13.74±1.05	13.24±0.84	16.23±1.10*++	14.4±1.0
2.3-Ankyrin	14.98±0.89	15.94±0.81	16.27±0.79	15.73±0.83
BP 3	224.66±3.60	231.99±2.85**	227.90±3.87	228.18±3.44
BP 4.1	41.89±1.34	42.24±1.02	42.24±1.02	42.12±1.13
BP 4.2	55.63±1.36	56.58±1.01	57.40±1.01	56.54±1.13
BP 4.5	111.76±3.70	111.81±2.17	117.28±4.30	113.62±3.39
BP 4.9	28.44±1.20	31.28±1.17*	31.50±1.52*	30.41±1.30
BP 5	59.81±2.37	58.83±1.41	59.39±2.01	59.34±1.93
BP 6	19.58±1.36	23.93±1.01**	26.10±1.33**+	23.20±1.23
BP 7	84.31±3.78	87.70±3.02	91.98±4.02*	88.00±3.61
BP 8	30.90±1.94	30.99±1.39	29.62±1.67	30.50±1.67
Hemoglobin	15.87±1.26	19.13±1.14**	17.91±1.26	17.64±1.22
Total protein	818.2±12.2	818.28±11.40	841.7±12.8*++	826.06±12.13

Note. *p<0.05, **p<0.01 compared to group 1; +p<0.05, ++p<0.01 compared to group 2.

characters, thus insufficient presence of NORs with a size of 0 arb. units can be explained by isolation with distance, when the population is divided into dominant and recessive alleles. Due to the existence of a certain threshold value to 10AgNOR (below this threshold a zygote is eliminated at the early stages of embryonic fission), individuals with dominant alleles survive. This leads to an increase of NOR number in individuals and also to insufficient presence of chromosomes without silver-stained regions. Accumulation of NORs with large Ag-blocks (4 arb. units) can occur under extremely unfavorable environmental conditions [1]. This explains low number of NORs with a size of 4 arb. units in the population of Kursk region, a region with relatively favorable ecology.

Our study allows us to conclude that there is a tendency for the increase in the quantity of all studied EMP with increasing transcriptional activity of NOR.

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