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Nuclear and nucleolar indices in HEp-2 cells in norm and under the action of Oral Polio Vaccine

(Submitted by academician K. G. Karageuzyan 16/IV 2002)

The size and number of the nucleoli are the important parameters of a differentiation level and functional condition of a cell. In normal cells the nucleolar activity varies in a differentiation course, at change of a functional condition of cells and at change of the cell cycle phases [1]. The parameters of the size and nucleolar quantity serve a valuable diagnostic attribute of the proliferation speed of the transformed cells [2-5]. In interphase nucleuses nucleolar organizer regions (NOR) of the chromosomes correspond to the fibrillar centers (FC), containing rDNA surrounded by the dense fibrillar and granular components [6,7]. Decondensed DNA filaments are uniformly distributed in FCs and in transcriptionally active nucleoli they are also present in proximal portion of the dense fibrillar of component surrounding the FCs [8]. Number and the sizes of the FC vary in different cells. The number of FC does not depend on number of chromosomes with active NOR. The sizes of the FC depend on a functional condition of a cells, and from intensity of the transcription of the rDNA [9,10]. In spite of the fact that in the metaphase chromosomes transcription of the rDNA does not occur, the main part/all NORs is actively impregnated by silver [11]. Also the important parameter is the area of nucleuses and nucleoli. According to the data [12] the nucleolar areas demonstrate strict dependence from cell population doubling time and consequently from proliferative activity of cells. The dependence of a functional condition and sizes of the nucleoli from the proliferative condition of the transformed cells is most obvious [13, 14].The interrelation between number, size of the NORs and number, size, form of the nucleoli are determined by the quantity or the transcriptional activity of the rDNA [15].

Quantitation of nuclear DNA content by cytometry has come into practice for assistance in the diagnosis and grading of malignant tumors. DNA aneuploidy, that is, the deviation from the ploid content of normal cells, represents one of the features most frequently associated cell transformation and tumor progression. In general aneuploidy is directly related to poor differentiation and cell proliferation. Which can be determined by morphometric, flow and image cytometry [16-18].

The aim of our work was investigation of the relation of the cell proleferation with dynamics of the various nuclear and nucleolar parameters: nuclear and nucleolar DNA quantity, area, perimeter of HEp-2 cell line. The experiments were carried out in normal condition and under the influence of the of Oral Polio Vaccine (OPV).

In work we used the continuous cell culture of a human larynx cancer - HEp-2 sensitive to the poliomyelitis virus. Cells was cultivated in Eagle medium with glutamine and 10% bovine serum. The cell line was received from laboratory U 322 INSERM "Retrovirus et maladies associeйes" Marseilles. The choice of HEp-2 as a model is motivated by the facts from publications on

susceptibility of these cells to the Sabin poliovirus strains [19]. A monolayer of the intact cells was used in 48 hours after the passage. Cells was resowed in dose 10^5 cell/ml.

Was used the mixture of the three Sabin strains Oral Polio Vaccine (OPV) (Polio Sabin^{TM} [oral] Poliomyelitis vaccine, live attenuated SB BIOLOGICALS Rixensart - BELGIUM, $TCID_{50}$/dose I - 10^6, II - 10^5, III - $10^{5.8}$). Infected cells were incubated at 36.5-37^{o}C. The OPV was used at multiplicity of infection 0,1 TCD_{50} per cell on the 24 hour after cell resowing. After 24 hour infection influence we began the calculation of parameters. Infected and intact cells were incubated at the temperature 37^{o}C. Viral titer in TCD_{50}/ml was calculated by the method of Kärber.

The cells preparations of HEp-2 culture were fixed in 96^o ethyl alcohol for 30 minutes and painted in fresh Shiffs reactive, by Feulgen (hidroliz 5N HCL 60 minutes at 22^{o}C). The content of DNA in a nucleus and nucleolus was defined by means of computer-equipped microscope-photometer SMP 05 (OPTON). The television method was used on 575 nm wave. In each case 50 - 100 cells were measured. Quantity of DNA was defined in conventional units (C.U.). In each nuclei we determined the area and perimeter and total DNA quantity. In the same nucleuses we contoured each nucleoli with the perinucleolar and intranucleolar chromatin and then determined quantity of the DNA, area and perimeter. Then these indices of all nucleolus was calculated in "total" nucleolus in each nucleus and nucleolus/nucleus ratio at the quantity of the DNA, area and perimeter. Nucleolar DNA was determined in FC and in connected with it intranucleolar and perinucleolar chromatin. Concentration of DNA in a nucleus and nucleolus calculated under the relation of the DNA quantity to the area. Concentration of the DNA in nucleus was done with excluding the DNA quantity and area of the nucleolus. The average data in experience carried out taking into account of groups of cells with the various number of the nucleoli. For this purpose the data of percent of the cells quantity of each group multiplied on average index of the investigated parameters. After all received data were summed up and average parameters in each experimentt were determined.

After quantitative DNA-staining, the nuclear Integrated Optical Density (IOD) is the cytometric equivalent of its DNA content. For the quantitation of nuclear DNA was rescaled the IOD values by comparison with those from cells with known DNA content. Therefore the DNA content is expressed in a "c" scale in which 1c is half (haploid) the mean nuclear DNA content of cells from a normal (non-pathological) diploid population in G_0/G_1 cell cycle phase. The DNA image cytometric measurements identified cell nucleuses as aneuploid if they deviate more than 10% from the 2c, 4c, 8c, 16 c, i.e. if they are outside 2c±0,2, 4c±0,4, 8c±0,8, 16c±1,6. The number of all cells in euploid regions of the DNA histogram rescaled by the mean corrective factor of the tissue type under investigation (1.8c-2.2c; 3.6c-4.4c; 7.2c-8.8c; 14.4-17.6) also was calculated [21]

All statistical analysis were performed with two-tailed Student's t test in the SPSS version 8.0 computer software package (SPSS, Inc., Chicago, IL).

The data of DNA cytometry in a nucleus and nucleolus, in normal condition and under the action of the OPV are given in tables 1 and 2.

Table 1

Parameters of the HEp-2 cells in the control (48 hour culture)

Number of nucleolus in the nucleus	% of the cells in population*	Nucleus			Summarized nucleolus		
		quantity of DNA (in C.U.)	area	perimeter	quantity of DNA (in C.U.)	Area	perimeter
1	26,9	179,1±21	64,1±7	16,1±2	22,6±3,9	8,1±1,4	5,3±1,1
2	33,6	157,2±28	56±11	15,6±2	27,0±6,7	9,8±2	7,0±1,5
3	26,6	190,1±36	68±13	17,6±2	29,3±5,9	10,5±2	8,44±2,0
4	9,8	231,8±25	83±32	18,7±4	36,3,8±3,4 •	12,7±1,5	11,0±1,7 ••
5 and more	3,1	211,1±24	76±28	22,3±6	30,9±6,8	11,1±2,6	14,7±1,9**

*Without the account the mitosis, dead and nonnucleolar cells

**Significant in comparison with 1 nucleolar cells $t = 4,27$ $p < 0,001$, with 2 nucleolar cells $t = 3,18$ $p < 0,01$ with 3 nucleolar cells $t = 2,27$ $p < 0,05$

•Significant in comparison with 1 nucleolar cells $t = 2,64$ $p < 0,05$

••Significant in comparison with 1 nucleolar cells $t = 2,80$ $p < 0,01$

The difference between minimal and maximal meanings of the DNA quantity as in the nucleus as in nucleolus, at control and experiment was insignificant.

Under action of a virus there are no significant changes in the DNA quantity in a nucleus of HEp-2 cells (180,8±28,1 in the control, 178,8±31,4 at a virus infection). The area of nucleuses also remains without changes (64,6±9,8 - control and 64,9±10,2 - OPV infection). The difference in (among) DNA quantity into "total" nucleolus also is absent (27,46±3,4 - control, 26,26±3,2 - infection).

As it follows from the tables 1 and 2 the difference of the DNA quantity between separate populations of HEp-2 cells was often noticeably (the comparison of 4 and 2 nucleolar cells $t=1,99$ $p < 0,05$). So in cells of one line, in nucleus with different numbers of nucleoli we defined high difference in DNA quantities. It is probably can be explained by the genotipically realization of the NORs number.

Table 2

Changes of parameters of HEp-2 cells under the action of OPV

Number of ucleolus in the nucleus	% of the cells in population*	Nucleus			Summarized nucleolus		
		quantity of DNA (in C.U.)	area	perimetr	quantity of DNA (in C.U.)	area	perimetr
1	12,7	155±25	60±12	18±3,4	20,2±2,7	7,4±0,8	4,9±1,1
2	35,5	178±30	65±12	19±3	23,4±4,8	8,5±1,7	7,1±1,1
3	25,1	168±23	61±7,7	18±2,3	27,4±7,9	10±2,9	8,9±2,1
4	22,5	197±24	70±9,5	18,6±5	31,0±3,8 •	12±1,5	13,1±1,1
5 and more	4,2	224±25	81±11	20±5,1	36,1±4 ••	13±2,6	14,0±1,7**

*Without the account the mitosis, dead and nonnucleolar cells

**Significant in comparison with 1 nucleolar cells t = 4,48 p < 0,001 with 2 nucleolar cells t = 3,39 p < 0,01 with 3 nucleolar cells t = 2,15 p < 0,05

fSignificant in comparison with 1 nucleolar cells t = 5,26 p < 0,001 with 2 nucleolar cells t = 3,85 p < 0,01

•Significant in comparison with 1 nucleolar cells t = 2,31 p < 0,05

••Significant in comparison with 1 nucleolar cells t = 4,99 p < 0,001 with 2 nucleolar cells t = 1,98 p = 0,05

Our experiments showed that with the increasing of the nucleoli quantity the quantity of the DNA in individual nucleolus decreases, but the quantity of the total DNA total nucleoli has the tendency to increase in HEp-2 cells both in the control, and under the action of OPV.

From our data increasing of the nucleolus number in nucleus the DNA quantity in individual nucleoli decreases, but the quantity of DNA in "total" nucleolus has the tendency to increase in HEp-2 resowing cell line both in the control, and under the action OPV.

According to [27] informative parameter of the cells differentiation is the area of a nucleus. From our experiments were received the same area of a nucleus both in control 65,6±7,8, and during infection of OPV 64,9±8,1. Also there were not the changes of the nuclear perimeter (16,8±2,8 - control 18,3±3,3 - infection).

We investigate possible changes in nucleolar indices. They are almost the same - nucleolus area (9,85±1,1 in control, 9,79±1,8 - virus action) and perimeter 7,57±1,6 - control 8,91±2,1 - action of OPV).

Also we investigated the concentration of the DNA in a nucleus. This index was characterized by constancy with very few deviations in control (2,79±0,02). The concentration of the DNA wasn't changed under the influence of OPV but the deviation was increased (2,75±0,11). Also the DNA concentration in the nucleolus wasn't changed (2,79±0,04 - intact cells, 2,69±0,08 experience).

Table 3.

Nucleolus/nucleus ratio in the control of the HEp-2 cells

Number of nucleolus in the nucleus	Nucleolus/nucleus		
	DNA	area	Perimeter
1	0,13±0,03	0,13±0,02	0,31±0,05
2	0,17±0,02	0,17±0,03	0,45±0,04
3	0,16±0,03	0,16±0,03	0,48±0,07
4	0,16±0,04	0,16±0,04	0,63±0,09*
5 and more	0,15±0,03	0,15±0,03	0,64±0,04*

*Significant in comparison with 1 and 2 nucleolar cells t = 5,15, t = 3,3, p < 0,001, in comparison with 3 nucleolar cells t = 1,98, p < 0,05

**Significant in comparison with 1 nucleolar cells t = 3,1 p < 0,01

Table 4.

Nucleolus/nucleus ratio in the HEp-2 cells under the action of the OPV

Number of nucleolus in the nucleus	Nucleolus/nucleus		
	DNA	area	Perimeter
1	0,13±0,03	0,13±0,03	0,27±0,07
2	0,13±0,02	0,13±0,02	0,39±0,07
3	0,16±0,04	0,17±0,04	0,5±0,1
4	0,16±0,03	0,17±0,03	0,73±0,1*
5 and more	0,16±0,04	0,16±0,02	0,71±0,1**

*Significant in comparison with 1 nucleolar cells $t = 3,6$ $p < 0,001$, with 2 nucleolar $t = 2,6$, $p < 0,01$

**Significant in comparison with 1 nucleolar cells $t = 3,77$, $p < 0,001$, with 2 nucleolar $t = 2,79$, $p < 0,01$

From tables 3 and 4 we see that the DNA quantities in the nucleolus/nucleus ratio do not depend from the nucleolus number in the nucleus in the control (average meaning in population 0,156±0,014) and in infection condition (average meaning in population 0,151±0,021). These data were received with taking account of the percent of each type of cells in the population (tables 1 and 2). The significant difference in the nucleolus/nucleus ratio was absent not only in population, but also in individual cells.

According to the data of tables 3 and 4 the essential difference in HEp-2 cells with various quantity of nucleolus in nucleus is the sums of perimeters of the "total" nucleolus in the nucleus. These data demonstrate almost direct linear dependence on the increasing of the nucleolus quantity both in experience and in the control.

Our data demonstrate the absence of changes of the total nucleolar DNA with the increasing of the number of nucleolus in the nucleus while quantity individual nucleolar DNA was appreciably decreased in process of the increasing of nucleolus in the nucleus.

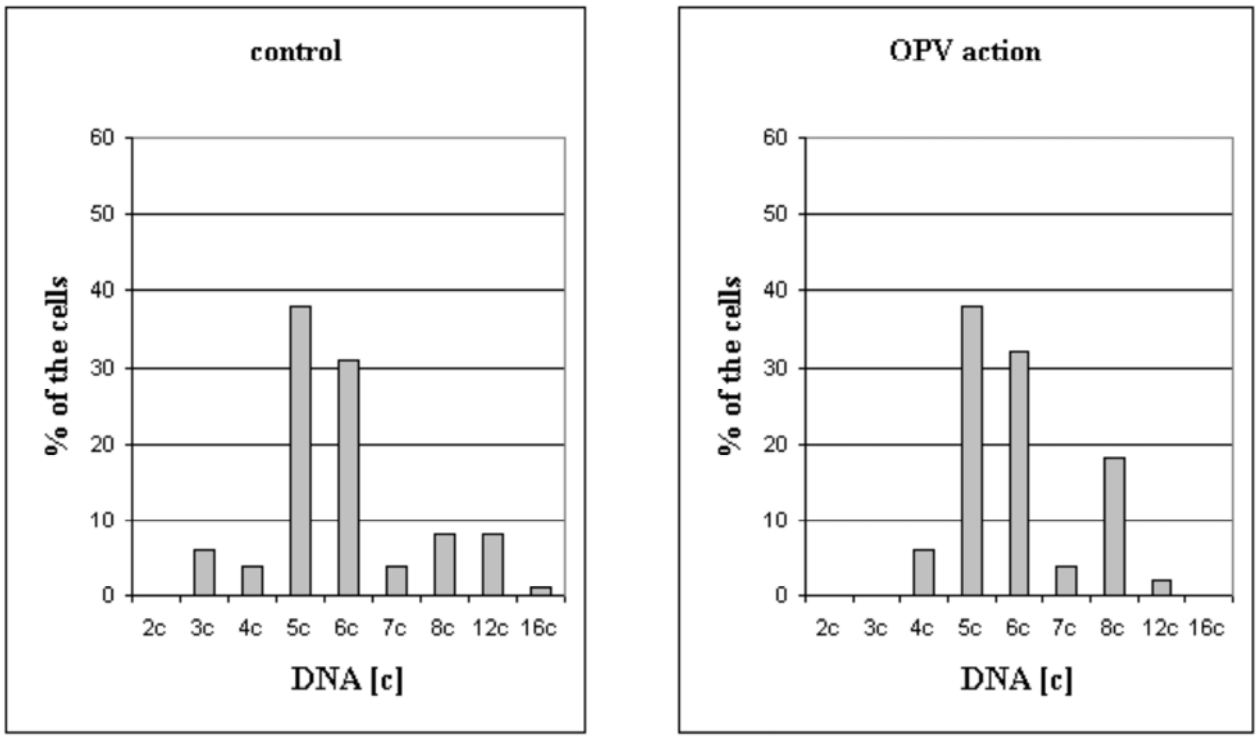

Figure 1. Distribution of the nucleus by the DNA ploidy (in "c" units) in HEp-2 cells

Fig 1 summarizes changes in DNA ploidy indices in normal condition and under the OPV action. The present results indicate that under the action of OPV significant changes in ploidy of HEp-2 cells were absent (6,03 "c" in control 5,96 "c" - OPV infection). Percentage of aneuploid cells was decreased, and percentage of euploid cells was increase (13±4,1 in control 24±3,2 OPV action, $t=2,11$, $p < 0,1$).

We have shown that in HEp-2 cell line quantity of the nucleolus does not depend on the quantity of the DNA in a nucleus, both at action of a virus, and in intact cells HEp-2. Quantity of the DNA in the nucleolus in direct proportion with the quantity of the DNA in a nucleus, both in experience, and in the intact HEp-2 cells. Conducting the nucleoli cytometry in the intact cells and under influence of the OPV, the relation of the sums of the nucleolar perimeters in a nucleus is the significant factor. It increases/decreases linearly while number of nucleoluses increases/decreases in a nucleus. Was shown the increase of the summarized nucleolar DNA at the increase of the nucleolus number in a nucleus of HEp-2 cells more expressed at action of a virus. Also was shown the reduction of the DNA quantity individual nucleoluses in process of growth of the number of nucleolus in HEp-2 cells.

There was tendency to the increasing of the cells number with euploid DNA quantity in infected cell line in comparison with control.

The infected cell line had the tendency increases the number of cells with euploid quantity of the DNA in nucleus in comparison with control. These data allow to assume, that under the influence of OPV there was the a decrease of a proliferation activity of HEp-2 cells.

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Զ. Ա. Կարալյան

Կորիզային և կորիզակային ցուցանիշները HEp-2 բջջային կուլտուրայում նորմալ պայմաններում և պոլիոմիելիտի եռավալենտ պատվաստանյութի ազդեցությամբ

Հետազոտվել են կորիզների և կորիզակների որոշ ցիտոմետրիկ և քանակական ցուցանիշներ HEp-2 բջջային կուլտուրայում պոլիոմիելիտի ստանդարտ (Սէբին) եռավալենտ պատվաստանյութի ազդեցության տակ և նորմալ պայմաններում: Հայտնաբերվել են հետևյալ արդյունքներ՝ կորիզակների քանակը կախված չէ կորիզում ԴՆԹ-ի քանակից; կորիզակներում ԴՆԹ-ի քանակը (հարկորիզակային և ներկորիզակային քրոմատին) ուղիղ համեմատական է կորիզում ԴՆԹ-ի քանակին ինչպես պոլիոմիելիտի եռավալենտ պատվաստանյութի ազդեցությամբ, այնպես էլ նորմալ պայմաններում: Մեր տվյալները ցույց են տալիս, որ ԴՆԹ-ի քանակի կորելյացիան յուրաքանչյուր կորիզակ/կորիզ համակարգում կայուն է ինչպես նորմալ պայմաններում (0.156 ± 0.014), այնպես էլ վիրուսի ազդեցության տակ (0.151 ± 0.021): Կորիզակային պարագծերի գումարը կորիզում էական գործոն է տարբեր քանակի կորիզակներով կորիզների միջև տարբերությունը որոշելու համար: Այն աճում/նվազում է գծայնորեն, երբ կորիզակների քանակը աճում/նվազում է կորիզում: Ընդհանուր կորիզակային ԴՆԹ-ն ավելանում է, երբ ավելանում է կորիզակների քանակը: ԴՆԹ-ի քանակը յուրաքանչյուր կորիզակում նվազում է, երբ կորիզակների քանակը կորիզում բարձրանում է:

З.А. Каралян

Ядерные и ядрышковые показатели в клетках линии HEp-2 в норме и под действием оральной тривалентной полиовакцины

Исследованы некоторые цитометрические и количественные показатели в ядрах и ядрышках клеток линии HEp-2, в норме и под действием стандартной трехвалентной полиовакцины (Сэбин) (ОПВ). Установлено, что количество ядрышек не связано с количеством ДНК в ядре; количество ДНК в ядрышках (интра- и перинуклеолярный хроматин) прямо пропорционально количеству ДНК в ядре, как в норме, так и под действием ОПВ. Наши данные показывают, что количество ДНК в каждой системе ядрышко/ядро стабильно как в норме (0.156 ± 0.014), так и под действием вируса (0.151 ± 0.021). Сумма периметров ядрышек в ядре - важный показатель при сравнении ядер с различным числом ядрышек - линейно снижается/повышается со снижением/повышением числа ядрышек в ядре. Суммарное количество ДНК в ядрышках увеличивается с ростом числа ядрышек в ядре. Однако количество ДНК в индивидуальном ядрышке снижается при увеличении числа ядрышек в ядре.