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On the nucleolar and cytoplasmic RNA density during "cell dedifferentiation" represented by blastic transformation of human mature T lymphocytes – a cytochemical study

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Abstract: The present study was undertaken to provide information on the nucleolar and cytoplasmic density in specimens stained for RNA during "cell dedifferentiation" represented by blastic transformation of mature T lymphocytes. Nucleolar and cytoplasmic RNA´s were visualized using a simple cytochemical method followed by computer assisted densitometry and size measurements of digitised images. An increased nucleolar and cytoplasmic RNA density accompanying the blastic transformation was significant after 48 hours of cultivation with phytohemaglutinin (PHA) when stimulated cells were characterized the largest nucleolar size reflecting S or G2 phase of the cell cycle. On the other hand, significantly larger ratio of the nucleolar to cytoplasmic density was noted only after a shorter cultivation when stimulated cells were presumably in the G1 phase. Thus the increased nucleolar and cytoplasmic RNA density together represented an accompanying phenomenon of the cell proliferation and cycling state. From the methodical point of view, the nucleolar and cytoplasmic RNA densitometry appeared as a simple as well as useful additional method to study "dedifferentiation" or various cell states at the single cell level. In addition, it was also interesting that the increase of the nucleolar diameter in stimulated cells was much larger than that of the nucleolar density. Such difference suggested that the RNA content in nucleoli was related mainly to their size.

Key words: nucleolar and cytoplasmic density, lymphocytes, blastic transformation

Introduction

It is generally known that human mature resting T lymphocytes stimulated with phytohemaglutinin (PHA) "dedifferentiate" and transform back to proliferating blastic stage [1,3]. Such transformation of small mature resting to large cycling blastic cells is accompanied by nucleolar enlargement, which is related to the increased nucleolar biosynthetic activities of stimulated cells [1,3-5,24]. In addition, ring shaped nucleoli transform to large nucleoli with a relatively uniform distribution of RNA [1,15,18]. On the other hand, the information on the nucleolar and cytoplasmic RNA density in the course of blastic transformation is missing although it seems to be likely that it might be different.

The present study was undertaken to provide more information on the nucleolar and cytoplasmic density in specimens stained for RNA during "cell dedifferentiation" represented by blastic transformation of mature T lymphocytes. Nucleolar and cytoplasmic RNA were visualized using a simple cytochemical method followed by computer assisted densitometry and nucleolar diameter measurements of digitised images. The results indicated that an increasing density accompanying the blastic transformation was significant after 48 hours of cultivation with PHA when the cells were in S/G2 phase of the cell cycle. On the other hand, significantly larger ratio of the nucleolar to cytoplasmic RNA density was noted only after a shorter cultivation when stimulated cells were presumably in the late G1 phase.

Materials and methods

Human lymphocytes were isolated from the peripheral blood of healthy volunteers using a discontinuous density gradient [2] of

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Table 1. Nucleolar and cytoplasmic density in specimens stained for RNA.

Time of cultivation (hrs)	Nucleoli (arbitrary density units)	Cytoplasm (arbitrary density units)	No/Cy	No D (μm)##
0	77.80 ± 22.43[a]	75.12 ± 22.90	1.07 ± 0.29	1.0 ± 0.2
24	82.15 ± 12.96	66.90 ± 19.56[§]	1.25 ± 0.31[*]	2.1 ± 0.4[*]
48	107.70 ± 19.96[*⊕]	95.5 ± 24.79[*⊕]	1.15 ± 0.24	2.8 ± 0.8[*]

Legend: # – based on more than 40 measurements for each group; ## – see ref. [22]; *– statistically different from 0 hrs using t-test ($p<0.009$); ⊕ – statistically different from 24 hours using t- test ($p<0.001$); § – statistically different from nucleoli using t-test ($p<0.001$); a – mean and standard deviation; No/Cy – ratio of nucleolar to cytoplasmic RNA density; NoD – mean nucleolar diameter.

Histopaque (Sigma, St. Louis, MO USA). Cells, 1×10^6/ml, were suspended in medium RPMI 1640 supplemented with 10% heat-inactivated FBS and stimulated with 10 μg/ml PHA-P from Sigma at 37°C in a 5% CO_2 humidified atmosphere for time frames up to 48 hrs [8,12]. Then, harvested cells were prepared for the light microscopic observations using a Shandon Cytospin 2 cytocentrifuge (Shandon Southern Products, UK) – 800 rpm for 10 min. and unfixed cytospins were stained for RNA with acidified methylene blue at pH=5.3 [13,21].

Micrographs were captured with a Camedia digital photo camera C.4040 ZOOM (Olympus, Japan) placed on Jenalumar microscope (Zeiss, Germany) equipped with the double adapter to provide a larger magnification of resulting images. Then the images were processed and nucleolar size was measured with Quick Photoprogram (Olympus, Japan). Mean nucleolar diameters were determined on the screen at magnification ×4300 and were based on two measurements of the long and minor axis for each nucleolus [10,14,17]. It should be mentioned that the nucleolar size and distribution of RNA facilitated to estimate the cell cycle phase of studied cells [6,9,11,20,23,24].

The nucleolar and cytoplasmic density in specimens stained for RNA was measured after image conversion to grey scale using the NIH Image Program – Scion for Windows (Scion Corp., USA). The density was expressed in relative arbitrary units, which were calculated by subtracting measured density of nucleoli or cytoplasm from the density surrounding the measured cell. Such calculation and standardisation of arbitrary density units facilitated the comparison of results in various portions of cytospins, which occasionally exhibited various artificial densities due to both preparation and staining techniques. This approach decreased artificial measurements and thus provided better results than the background adjusted to zero, which depended on the investigator. In contrast to the nucleolar size measurements, the nucleolar and cytoplasmic densities were measured at lower magnification using dry 50x objective to decrease the resolving power of the microscope. Such approach facilitated to cover a larger and more uniform density measurements of nucleolar and cytoplasmic regions stained for RNA.

Results

Nucleolar size and types

Quantitative data are in the Table 1. The nucleolar size during blastic transformation gradually increased. Maximal values of mean nucleolar diameter in stimulated lymphocytes were noted after 48 hrs of cultivation and apparently reflected S and G2 phase [6,9,11, 16,20,23,24]. In resting mature lymphocytes representing cells in G0 or /early G1 phase, nucleoli were small and mostly ring shaped (Fig. 1) [see also 3,6,18, 23,24], After 24 hours of cultivation in the presence of PHA, stimulated cells were characterised by enlarged nucleoli with more or less distinct nucleolonemas which were described in late G1 and especially S or G2 phase of the cell cycle (Fig. 2) [see also 3,6,15,23, 24].

Nucleolar and cytoplasmic density in specimens stained for RNA

For quantitative data see the Table 1. In control resting lymphocytes the nucleolar and cytoplasmic density was almost the same (Fig. 3). The nucleolar to cytoplasmic density ratio was about one. In transforming lymphocytes to blastic state (Figs. 4,5), the nucleolar density increased but such increase was significant only after 48 hours of cultivation with PHA. On the other hand, the cytoplasmic density was apparently smaller at first but then significantly increased. Therefore, the increased nucleolar to cytoplasmic density ration in stimulated cells significantly increased only after 24 hours of cultivation in the presence of PHA. It was also interesting that the nucleolar density in stimulated cells increased less dramatically than the nucleolar diameter (Fig. 6).

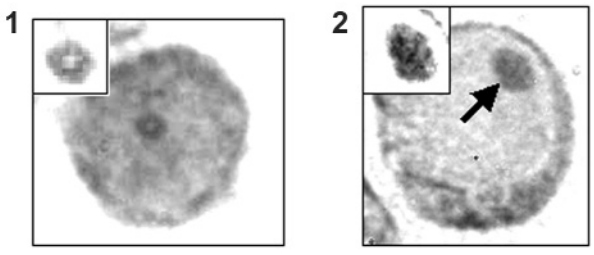

Fig. 1. Resting lymphocyte with a ring shaped nucleolus (arrow and insert), (magnification approx. ×3500). **Fig. 2.** Stimulated transformed and "dedifferentiated" blastic cell with a large nucleolus with a more uniform distribution of RNA (arrow), which shows nucleolonemata at larger magnification (insert), (magnification approx. ×2100).

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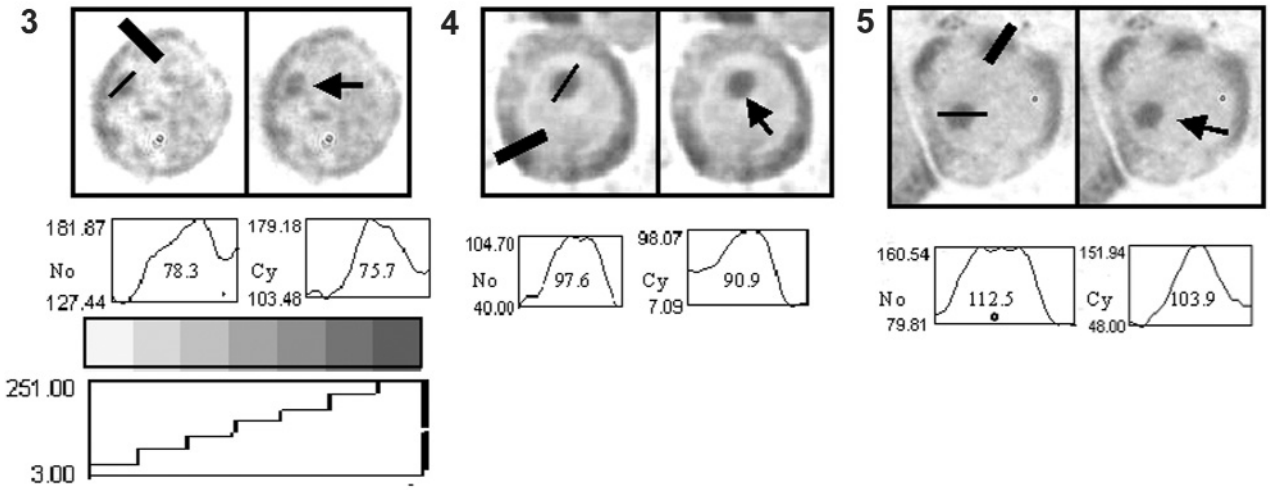

Fig. 3. Nucleolar (No – thin black line) and cytoplasmic (Cy – thick black line) RNA density in a small resting lymphocyte. Nucleolus – arrow. The lower part of the Figure contains nucleolar (No) and cytoplasmic (Cy) densitograms. Numbers in densitograms indicate density values calculated by subtracting measured values from the background surrounding the investigated cell. The density scale is below densitogram (magnification approx. ×2200). **Fig. 4.** Nucleolar and cytoplasmic RNA density in a stimulated and transformed blastic cell after 24 hours of cultivation in the presence of PHA. For other legend see Fig. 3 (magnification approx. ×1700). **Fig. 5.** Nucleolar and cytoplasmic RNA density in a stimulated and transformed blastic cell after 48 hours of cultivation in the presence of PHA. For other legend see Fig. 3 (magnification approx. ×1300).

Discussion

The relatively increased nucleolar RNA density during blastic transformation (present study) is not surprising and represents a further phenomenon, which is related to the generally known increased nucleolar RNA transcription [3-5,7,15,16,23]. Moreover, the known transformation of ring shaped nucleoli in resting lymphocytes to enlarged nucleoli with less or more distinct nucleolonemas in transformed blastic cells is also in harmony with the increased nucleolar biosynthetic activity and return to the cell cycle [3,4,6,7,15,20,23, 24]. It should be added that the increase of the nucleolar diameter in stimulated cells was much larger than that of the nucleolar density. Such difference suggests that the RNA content in nucleoli depends mainly on their size. A similar RNA density of small and large nucleoli was also noted previously in leukaemia granulocytic progenitors [19].

The delayed increase of the RNA cytoplasmic density in stimulated cells seems to be interesting and resulted in an increased nucleolar to cytoplasmic RNA density ratio. Such delay might be easily explained by the preceding increased nucleolar RNA transcription and assembly of ribosomes before their transport to cytoplasm [3,5,7,23]. On the other hand, when biosynthetic activities of nucleoli in resting cells are repressed [4,6,7,15,24], the nucleolar and cytoplasmic RNA densities are similar but smaller (present results). At this occasion it should be mentioned that in stimulated and cycling blastic cells the nucleolar and cytoplasmic RNA densities were also similar. However, they were significantly higher than in resting lymphocytes. In that case, both the nucleolar and cytoplasmic biosynthetic activities are known to substantially increase in comparison with resting cells [3-5,7,9,15].

From the methodical point of view the present study, regardless of the interpretation, clearly demonstrated that the cytochemical RNA densitometry was an useful procedure that might be complementary to

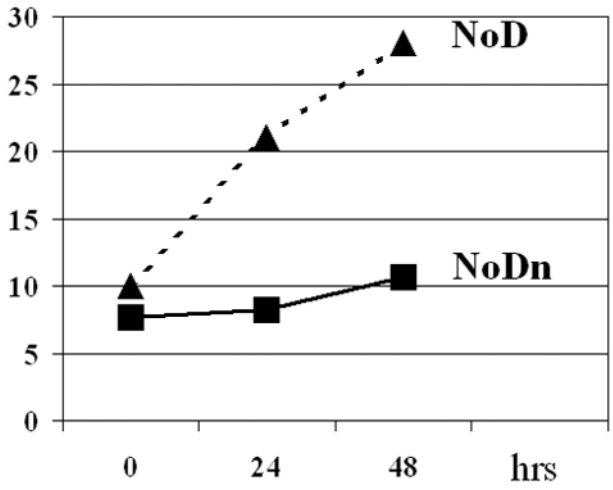

Fig. 6. Nucleolar diameter (NoD) and density (NoDn) in stimulated lymphocytes. Mean values of the nucleolar diameter are multiplied by 10 and mean values of the nucleolar density are divided by 10. Standard deviations are in the Table 1. Note that the increase of the nucleolar diameter was larger than that of the nucleolar density.

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other methodical approaches facilitating to distinguish resting and stimulated – "dedifferentiated" cycling cells at the single cell level. In addition, such approach appeared to be useful in smear or cytospins specimens, in which the number of investigated cells might be very limited.

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