To the ring-shaped nucleolus seen by microscopy using human lymphocytes of blood donors and chronic lymphocytic leukemia patients

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ABSTRACT

The present study was undertaken to provide more information on the peripheral RNA containing ring of ring-shaped nucleoli (RSNo). Human lymphocytes of blood donors and patients suffering from B chronic lymphocytic leukemia mostly characterized by RSNo represented very convenient cell models for such study. According to the light microscopy the peripheral RNA ring possessed several highly condensed foci. Such regions represented accumulated dense RNA fibrillar components (DFCs) seen by the electron microscopy. In contrary, the incidence of dense granular RNA-containing components (GCs) in surrounding portions of the RNA ring was small. Thus, the structural and morphological organization of the peripheral RNA ring of RSNo apparently reflects sites of micro-segregated foci of DFCs and a small incidence of GCs. That structural organization of the peripheral RNA ring of RSNo appeared to be a prerequisite for further regressive nucleolar changes resulting in the development of micronucleoli in terminal lymphocytes.

Key words: ring-shaped nucleolus; human lymphocytes.

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Conflict of interest: the authors declare that they have no competing interests, and all authors confirm accuracy.

Ethics approval: the Institute Supervising Authorities approved the use of cell blood samples of leukemia patients and blood donors originally examined for the routine laboratory diagnostic checks for the present study of nucleoli of lymphocytes, on the basis of the Certificate of Accreditation for Laboratory Hematology 178/2024 – CIA.

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Introduction

Ring-shaped nucleoli (RSNo) represent a very unique nucleolar type in addition to other nucleolar types classified according to the structural components identified by both light and electron microscopy.[1-4] They consist of a single large fibrillar center (FC) surrounded by RNA containing ring with dense RNA-containing fibrillar and granular components (DFCs and GCs). These nucleolar regions may be also detected by the silver reaction for silver-stained proteins depending on the modified procedure for their visualization.[5,6] The presence of RSNo was reported in a variety of cells in advanced differentiation steps or after the reduction of the RNA transcription.[2,3,4] At this occasion it should be also added that RSNo may be present in the cell nucleus either as a dominant nucleolus or as an additional nucleolus to other nucleolar types.[7]

The present study was undertaken to provide more information on the peripheral RNA-containing ring of dominant RSNo. "Mature lymphocytes" of the T lymphocytic as well as B lymphocytic lineages appeared to be very convenient models for such study because they mostly possess such nucleoli.[8] It is also generally known that mature lymphocytes are present in the peripheral blood of blood donors or leukemic persons with B chronic lymphocytic leukemia (CLL) in a satisfactory number and are currently used for necessary diagnostic checks before blood donation or therapeutical treatments.

The results of the present study demonstrated that RSNo possess highly condensed foci in the peripheral RNA ring. Such regions apparently correspond to the accumulated and segregated DFCs seen by the electron microscopy. In contrary, the incidence of GCs in the peripheral RNA ring appeared to be small. Thus, the structural and morphological organization of the peripheral RNA ring of RSNo seems to reflect the decreased RNA transcription and pre-ribosomal assembly due to the micro-segregation of DFCs and small incidence of GCs.

Materials and Methods

RSNo were studied in lymphocytes of the peripheral blood of 5 blood donors and 5 patients suffering from B CLL who did not receive antileukemic therapy at the time of taking samples for the present study. All these samples were originally used for the routine laboratory diagnostic checks under conditions approved by the supervising authorities of the Institute on the basis of the Certificate of Accreditation for Laboratory Hematology 178/2024 - CIA.

For the present study, unfixed (not older than 24 h) blood smears were stained for RNA by acidic methylene blue buffered with Mc Ilvain's buffer at pH 5.3. Silver-stained proteins adjacent to fibrillar centers of RSNo were stained by modified silver reaction for smear preparations.[5,6,9,10]

For the electron microscopy, specimens were fixed 1 h at room temperature in 2% osmium tetroxide and/or 1.5% glutaraldehyde in phosphate buffer at pH 7.4, post-fixed in 0.5-0.1% uranyl acetate during dehydration in ethanol and embedded in the epoxy resin. Ultrathin sections were stained with uranyl acetate and/or lead citrate.[11] For the visualization of main silver-stained proteins in adjacent regions of the nucleolar RNA peripheral ring to fibrillar centers the specimens were fixed in 4% formaldehyde in phosphate buffered saline pH 7.2 for 10 min, treated with 0.1-1N HCl or ribonuclease (2 mg in 1 mL of redistilled water) and exposed to silver nitrate mixture (1 part of 1 g/1 mL silver nitrate in redistilled water and 1 part of 40% formaldehyde) at 60°C for 10 min. After such treatments specimens were dehydrated in ethanol and embedded in the epoxy resin.[5,11] The microscopic image density of the peripheral RNA ring with dense foci of DFCs and the background with GCs was measured on the computer screen after the conversion of captured color (light microscopy) or gray (electron microscopy) images to the gray scale using the red channel of the NIH Image Program Scion for Windows (Scion Corp., Chicago, IL, USA). The measured density was expressed in optical image arbitrary density units (ADnU) using the Scion Image Program. The ratio of dense foci to the background of the RNA peripheral ring of RSNo per cell was calculated by dividing measured ADnU of 3 dense RNA foci by ADnU of the surrounding RNA ring background (Figures 1 and 2). The number ratio of DFCs in dense foci to surrounding RNA region with GCs in electron micrographs was calculated by dividing the number of DFCs by the number of GCs in 1 μm^2 on the computer screen regardless of the image magnification.

The results of all measurements and calculations were evaluated using Primer of Biostatistic Program, version 1 developed by SA Glantz (McGraw-Hill, Canada, 1968).

Results

In blood smear specimens stained for RNA the peripheral RNA ring of RSNo in mature lymphocytes possessed several dense foci (Figure 1, Table 1). The density of these foci was apparently larger than that of other portions of the RNA ring (Table 1). Expressed by optical image density measurements the density of these foci was 1.6 and 1.5 larger than the rest of the RNA peripheral ring. Generally, no substantial differences of the incidence of dense foci in the nucleolar peripheral RNA ring were noted between RSNo of

Table 1. The peripheral RNA ring of ring-shaped nucleoli (width, dense foci, optical image density, light and electron microscopy): mean and standard deviation of at least 50 measurements.

DnFo (number)	LM DFCs/RDn (ADnU)	EM DFCs/RDn (ADnU)	EM DFCs/GCs (estimated number per 0.1 μm^2)	Donors
3.5±0.6	1.6±0.3	1.8±0.1*#	1.5±0.5°	CLL patients
		2.0±0.3§		
		1.9±0.1^		
3.6±1.4	1.5±0.1	-----	-----	Blood donors

DnFo, dense foci of the RNA ring; LM, light microscopy; DFCs, dense fibrillar components; RDn, RNA ring density; EM, electron microscopy; GCs, granular components; ADnU, arbitrary density units; *density measurements in the original electron micrograph after image processing increasing the contrast; #significant difference in comparison with LM DFCs/RDn using t-test ($p<0.05$); °very large variability, as indicated by the variation coefficient x100 = 33.3%; CLL, chronic lymphocytic leukemia; §density measured after increased magnification (4x) of the same original electron micrograph; ^density measured at 2 different magnifications of original electron micrographs.

lymphocytes of blood donors and CLL patients (Table 1) although the former were mostly represented by T and the latter by B lymphocytes.[12,13] Higher magnifications, i.e. electron micrographs of CLL lymphocytes demonstrated that dense foci in the peripheral RNA ring of RSNo observed by the light microscopy (see above) possessed accumulated DFCs (Figure 2). The dense foci in the peripheral RNA ring of RSNo were also clearly seen in specimens stained for silver-stained proteins under conditions which visualized the sites of the presumed RNA transcription (Figure 3). On the other hand, the density of these segregated foci of DFCs was slightly influenced by the preparation of samples for the electron microscopy and used magnification (Table 1). Nevertheless, the density of dense foci with DFCs was larger than the rest of the peripheral nucleolar RNA ring with rare GCs (Table 1). In addition, the profile numbers of DFCs in dense foci were larger than the number of GCs in the nucleolar peripheral RNA ring. Thus, the resulting ratio of DFCs to DGCs was also large despite the larger variation coefficient (Table 1).

Discussion

Previous studies demonstrated that RSNo in mature lymphocytes were characterized by the reduced pre-ribosomal RNA transcription.[2,3] According to above presented observations the light microscopy demonstrated that the peripheral RNA ring of RSNo in mature lymphocytes of both blood donors and leukemic patients possessed several dense foci. The large number of lymphocytes with RSNo in the peripheral blood of leukemic patients facilitated to study such foci in ultra-thin sections by the simple transmission electron microscopy.

The dense foci of the RNA nucleolar ring of RSNo in the electron microscope consisted of DFCs. Such foci appeared as "microsegregation" of a large concentration of DFCs which was reported previously in a relationship to the depressed RNA transcription.[3] The large concentration of DFCs in these foci was in contrast with a small number of DGCs in the remaining portions of the nucleolar peripheral RNA ring. At this occasion it should be mentioned that DFCs reflect sites of newly transcribed pre-ribosomal RNA and are precursors of DGCs representing pre-ribosomal particles.[3,14-20] Thus, it might be presumed that the dense foci of the peripheral ring of RSNo with accumulated and segregated DFCs with the small incidence of DGCs reflected the decreased pre-ribosomal RNA transcription and the decreased assembly of pre-ribosomal RNA particles. In addition, previous studies of chromatin and DNA demonstrated that aggregates of DFCs in the peripheral ring of RSNo in mature non-leukemic lymphocytes appeared to be devoid of DNA.[21] Such events were also previously suggested by biochemical studies of leukemic lymphocytes in patients suffering from CLL.[22]

Summarizing the above presented observations, the structural organization of the peripheral RNA ring of RSNo indicated that these nucleoli represent a specific nucleolar type in addition to other nucleoli classified according to the light and electron microscopy.[1,3,4] The structural organization of the peripheral RNA ring of RSNo appeared to be a prerequisite for further regressive changes resulting in the development of some micronucleoli. Such micronucleoli in terminal stages of the lymphocytic cell lineage mainly composed of DFCs might represent fragments of the original structural organization of the peripheral RNA ring. [23]

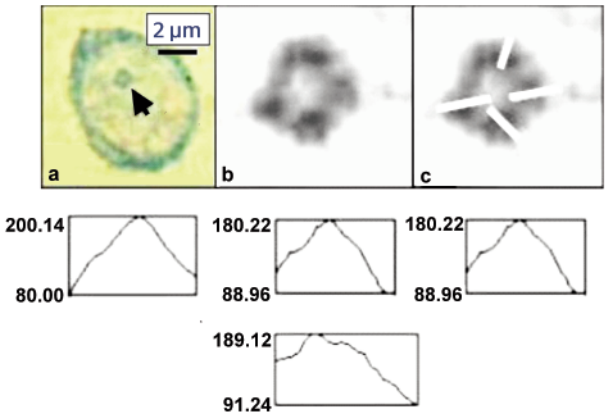

Figure 1. RSNo in a leukemic lymphocyte stained for RNA. **a**) Captured image. **b**) Magnified image processing facilitated to see foci of the large density. **c**) White lines of measurement. Corresponding density graphs are below. The RNA density ratio of dense foci (upper values of density graphs) to the density of the RNA ring (lower values of density graphs) = 2.1±0.2 (mean and standard deviation).

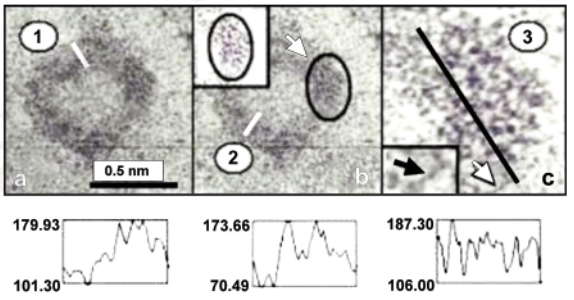

Figure 2. RSNo of a mature CLL lymphocyte. **a**) Captured electron micrograph. **b**) Processed image with the insert of a large dense focus (arrow) of the peripheral RNA ring. **c**) Magnified large dense focus facilitates to see dense fibrillar components. Arrows and insert, selected dense fibrillar components; arrows, dense fibrillar component. Black and white lines (1, 2, 3) of density measurements (**a-c**). Measured foci with corresponding density graphs are below under micrographs. The thin black line (**a,b**) facilitates the orientation and comparison of captured and processed images (**a,b**). The RNA density ratio of dense foci (upper values of density graphs) to the density of the RNA ring (lower values of density graphs): 1.9±0.3 (mean + SD).

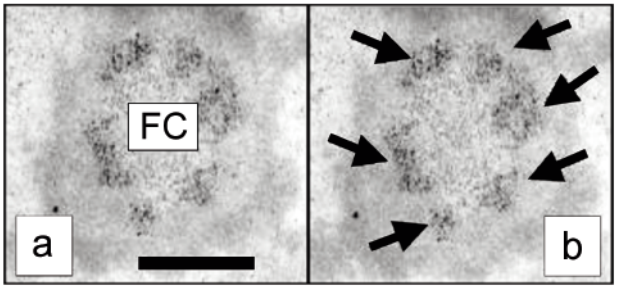

Figure 3. RSNo of a mature CLL lymphocyte. **a**) Captured electron micrograph; FC, fibrillar center; scale bar: 0.5 µm. **b**) Processed image with the increased density of silver-stained dense foci (arrows) of the peripheral RNA ring adjacent to the FC.

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