Short Communication

Dominant Nucleolus in the Progenitor Cell Using Human Bone Marrow Erythroid and Granulocytic Cell Lineages as a Model. A Morphological and Cytochemical Note

(dominant nucleoli / progenitor cells)

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Abstract. Progenitor cells of the human erythroid and granulocytic cell lineages are characterized by the presence of several nucleoli. One of these nucleoli is larger and possesses more fibrillar centres than others. Such nucleolus is apparently dominant in respect of both size and main nucleolar function such as nucleolar-ribosomal RNA transcription. Such nucleolus is also visible in specimens using conventional visualization procedures, in contrast to smaller nucleoli. In the terminal differentiation nucleated stages of the erythroid and granulocytic development, dominant nucleoli apparently disappeared, since these cells mostly contained very small nucleoli of a similar size with one fibrillar centre. Thus, the easily visible dominant nucleoli appear to be useful markers of the progenitor cell state, such as proliferation, and differentiation potential.

Introduction

Progenitors of the human erythroid or granulocytic lineages are characterized by the presence of several nucleoli, some of which are visible in smear preparations stained with panoptic staining procedures (Undritz, 1972; Yeo et al., 2019). In contrast, small nucleoli in these cells are frequently less visible because of the surrounding shell of the perinucleolar heterochromatin. After visualization by cytochemical methods, erythroid or granulocytic progenitors – proerythroblasts or myeloblasts – possess both large nucleoli and small nucleolar

bodies that are not masked by heterochromatin (Busch and Smetana, 1970; Smetana, 2002).

The present study was undertaken to provide more information on the large nucleoli of erythroid or granulocytic progenitors, since they were considered to be markers of the proliferation potential and were helpful in the identification of these cells (Undritz, 1972; Bessis, 1973).

The results demonstrated that the largest nucleoli in the cell nucleus of erythroid and granulocytic progenitors were dominant in respect of both, the nuclear size and the main nucleolar biosynthetic activity. The latter was expressed by the presence of a larger number of nucleolar fibrillar centres (NoFCs) in comparison with smaller nucleoli. In contrast, the reduced nucleolar size and the number of fibrillar centres in the late or terminal nucleated differentiation stages of the studied cell lineages were very similar. Thus, dominant nucleoli in these cells have disappeared.

Material and Methods

The nucleolar and nuclear diameters were measured in single proerythroblasts or myeloblasts in bone marrow smears of four selected patients with the chronic phase of Ph+ chronic myelocytic leukaemia (CML). These patients received the current "specific" anti-leukaemic therapy with imatinib mesylate (Braziel et al., 2002; Malekawa et al., 2007) and exhibited a markedly reduced granulocyte to erythroid ratio (4.8 ± 1.8/1) close to that in non-leukaemic persons (2-5/1) (Rundles, 1983; Naeim, 2008). The number of erythroid cells and myeloblasts was satisfactory for the present study. In addition, in the studied bone marrow biopsies, the erythroid cell lineage represented non-leukaemic and granulocytic leukaemic cell lineages. The studied bone marrow smears of CML patients were originally taken for diagnostic purposes, and their anonymous utilization for research purposes was approved by ethical standards set at the Institute in compliance with the ethical rules of biomedical research.

Nucleoli and nuclear outlines were visualized in unfixed bone marrow smears or cytospins by a simple but

sensitive method for the demonstration of RNA using methylene blue buffered with McIlvain's buffer to pH 5.3 (Smetana et al., 1969; Ochs, 1998). NoFCs were visualized by the silver reaction to demonstrate the main nucleolar proteins under standardized conditions, which facilitated observation of these nucleolar components as silver-stained particles (Ochs, 1998; Smetana et al., 1999).

Micrographs were captured with a Camedia digital camera C4040 ZOOM (Olympus, Tokyo, Japan) placed on a Jenalumar microscope (Karl Zeiss AG, Oberkochen, Germany). The increased magnification and contrast adjustments by image processing (Quick Computer Photoprogram, Olympus, Japan) facilitated easy measurements of the nucleolar and nuclear major axes in single cells (Politi et al., 2003). The rough estimate of the nucleolar and nuclear size was calculated using these measurements (Smetana et al., 2019). The mean number of NoFCs was calculated for each single nucleolus. The results of all measurements and calculations at the single-cell level were evaluated using Primer of Biostatistic Program, version 1 developed by S.A. Glantz (McGraw-Hill, Canada, 1968).

Results and Discussion

Early erythroid progenitors

Early erythroid progenitors – proerythroblasts (Table 1) – mostly possessed several nucleoli of various shapes and sizes. Some of these nucleoli were small and visible only in specimens stained for RNA or silver-stained NoFCs. The largest dominant nucleolus in the nucleus was usually not rounded and was characterized by a less regular shape and a large long axis. In contrast, the smaller nucleoli were usually more rounded (Fig.1). The silver reaction in the largest dominant nucleoli exhibited the presence of a very large number of NoFCs, which were occasionally interconnected. The less prominent

nucleoli possessed markedly smaller numbers of NoFCs. The largest nucleoli comprised almost one third of the nuclear space.

Terminal nucleated stages of the erythroid lineage

Terminal nucleated stages of the erythroid lineage – late erythroblasts (Table 1) – possessed only one or two small nucleoli visible only in specimens stained for RNA and silver-stained NoFCs (Fig. 2). In addition, small nucleoli in late erythroblasts mostly possessed only one NoFC (Fig. 2), i.e., less than 6 % of NoFCs present in proerythroblasts. These small nucleoli comprised less than 10 per cent of the nuclear space.

Early granulocytic progenitors

Early granulocytic progenitors – myeloblasts (Table 1) – possessed several nucleoli of various sizes. The largest
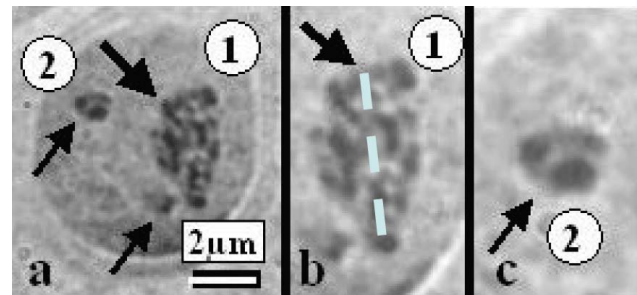

Fig. 1. **(a)** Proerythroblast stained for NoFCs with a large – dominant nucleolus (large arrow – 1) and small nucleoli (small arrows). **(b)** The enlarged and computer-processed large dominant nucleolus (1) with multiple NoFCs. **(c)** A small enlarged and computer-processed nucleolus (2) with several NoFCs.

Table 1. Human erythroid and granulocytic progenitors, terminal mature nucleated late erythroblasts and terminal mature granulocytes with nuclear segments. The long axis, nucleolar and nuclear diameter of NoFCs and the rough size estimate of the nuclear space occupied by dominant nucleoli.*

	DNo μm (M)	Nu μm (M)	No/Nu × 100 (C)	DNoFCs (N)	SNoFCs (N)	NoCo (C)
Proebl	3.6 ± 0.8	12.8 ± 1,1	28.1 %	17.0 ± 4.2	2.8 ± 0.9	2.2 ± 0.9
Late ebl	(No μm) 0.7 ± 0.1	6.0 ± 1.2	11.6 %	(No) 1.2 ± 0.7	1.4 ± 1.0	
Mybl	3.0 ± 0.8	12.4 ± 0.8	24.1 %	10.20 ± 4.4	7.1 ± 4.5	3.2 ± 0.9
Segm	(No μm) 0.8 ± 0.2	10.4 ± 2.9	7.6 %	(No) 1.3 ± 0.7	3.1 ± 1.0	

* – Based on at least 120 measurements in ~60 proerythroblasts or myeloblasts, 200 measurements in > 100 late erythroblasts or mature neutrophils with segmented nucleoli of four patients

M – measurement, N – counted number, C – calculated data, No – nucleoli, DNo – largest dominant nucleoli, SNo – smaller nucleoli, Nu – nucleus, (DNo/Nu) x 100 – the rough estimate of the nuclear space occupied by dominant nucleoli based on the nucleolar and nuclear long axis measurements, NoCo – number of nucleoli per one nucleus expressed as nucleolar coefficient, Proebl – proerythroblasts (erythroid progenitors), Late ebl – late erythroblasts, Mybl – myeloblasts (granulocytic progenitors), Segm – mature neutrophils with segmented nuclei.
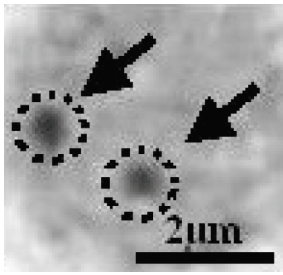
Fig. 2. Computer-processed and enlarged part of the nucleus of a late erytroblast with silver-stained single NoFCs in two nucleoli (arrows)

nucleolus was mostly rounded and contained multiple fibrillar centres (Fig. 3). The number of fibrillar centres in these nucleoli was apparently higher than in smaller nucleoli. In contrast to proerythroblasts, the sum of NoFCs in the small nucleoli of myeloblasts was occasionally large, but never larger in a single small nucleolus in comparison with the largest one. Thus, the largest nucleoli appeared to be dominant. It should also be mentioned that dominant nucleoli comprised about one quarter of the nuclear space.

Terminal differentiation and maturation stages of neutrophilic granulocytic lineage

Terminal differentiation and maturation stages of the neutrophilic granulocytic lineage – segmented neutrophils (Table 1) – mostly possessed micronucleoli of a similar size that was about 1 μm (Fig. 4). The number of fibrillar centres in such nucleoli was also markedly reduced. These small nucleoli occupied less than 10 % of the nuclear space.

Summarizing the above-described observations, it seems to be apparent that the largest nucleolus in human bone marrow erythroid or granulocytic progenitor cells was dominant not only with respect to the nucleolar size, but also to the nucleolar main function such as transcription of the nucleolar-ribosomal RNA. The latter was expressed by a markedly larger number of NoFCs in the dominant nucleoli compared with smaller nucleoli in the same nucleus. NoFCs with adjacent RNA-containing components are known to be sites of the ribosomal RNA synthesis and assembly of preribosomal particles (Smetana, 2011; Smirnov et al., 2016; Weipoltshammer and Schöfer, 2016; Penzo et al., 2019). Thus, the dominant nucleolus seems to be a very useful marker of the nucleolar biosynthetic activity in single progenitor cells. In addition, dominant nucleoli are eas-
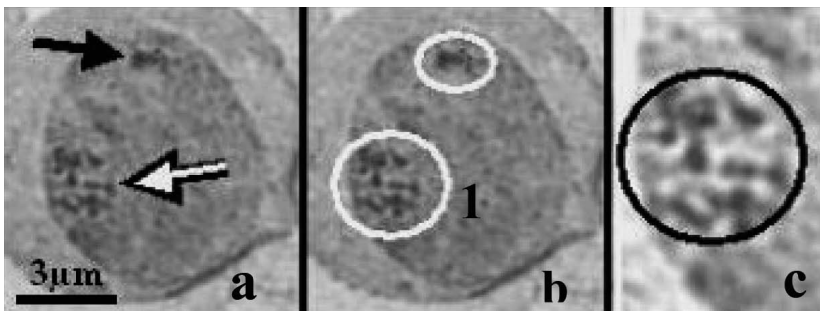

Fig. 3. **(a)** Myeloblast stained for NoFCs with a large – dominant nucleolus (white arrow) and a small nucleolus (black arrow). **(b)** The dominant nucleolus (1) with multiple NoFCs. **(c)** The enlarged and computer-processed dominant nucleolus with multiple NoFCs.
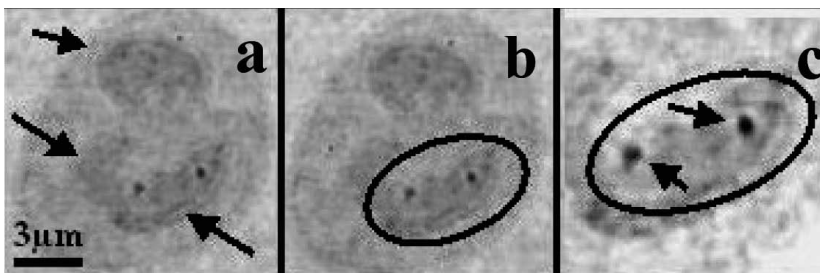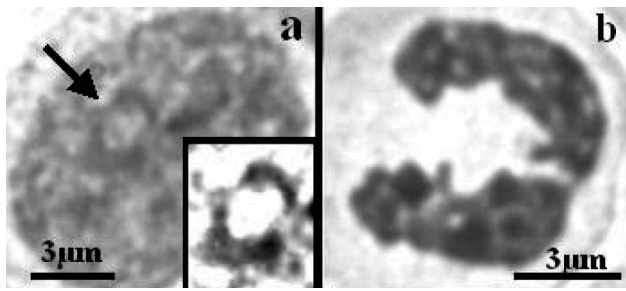

Fig. 4. **(a)** Mature differentiated neutrophilic granulocyte with three nuclear segments (arrows) stained for NoFCs. **(b)** Two distinct nucleoli in one of the three nuclear segments. **(c)** Enlarged and computer-processed nucleoli containing single NoFCs – arrows.

Fig. 5. **(a)** Granulocytic progenitor – myeloblast – with a dominant nucleolus (arrow) easily identified by a distinct shell of the perinucleolar chromatin (insert). **(b)** Mature differentiated neutrophilic granulocyte with two nuclear segments. The currently used staining with standard polychrome May-Grünwald – Giemsa-Romanowsky (MGGR) procedure did not allow identification of small nucleoli that are seen in the silver-stained specimens in Figs. 1, 2 and 4.

ily visible in current specimen preparations for the light microscopy (Fig. 5). In contrast, identification of small nucleoli in the currently stained specimens was not possible (Fig. 5), in agreement with numerous reports and haematological monographs (Whitby and Britton, 1947; Undritz, 1972; Bessis, 1973; Cline, 1975; Guigley et al., 2018; Skubitz, 2018).

The presented observations have also demonstrated that during the cell differentiation and maturation, dominant nucleoli have disappeared. The reduction in the number of NoFCs was more extensive compared with the size of the dominant nucleoli. This phenomenon is difficult to explain. It should be noted that transcription of ribosomal RNA and assembly of pre-ribosomes is only one of the nucleolar functions. Therefore, it seems to be likely that the nucleolar space must also serve for other nucleolar functions. At present, the nucleolus is considered to be a multifunctional nuclear organelle the study of which is a subject of other more sophisticated methodological approaches (Pederson, 1998; Lo et al., 2006; Boivert et al., 2007; Stepinski, 2018).

The explanation of the nucleolar dominance observed in the present study would be difficult and would require further studies using different methodological approaches. Previous studies on plants and flies suggested that the nucleolar dominance depends on the epigenetic on/off switch that controls the number of active genes (Preuss and Pikaard, 2007; Warsinga-Pepe et al., 2020). The present observation just demonstrated an "asymmetric distribution" of nucleolar active genes transcribing rRNA in the nucleus that apparently disappeared during the cell differentiation and maturation characterized by a well-documented reduced need for rRNA.

The large dominant nucleoli may be occasionally present in apoptotic cells. However, NoFCs in such nucleoli were absent or substantially reduced in number (Smetana et al., 2017).

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