Short Communication

To the Large Nucleolar Bodies in Apoptotic Leukaemic Granulocytic Progenitors without Further Differentiation. Are Large Nucleoli Always Present in Proliferating Cells?

(large nucleolar bodies / spontaneous apoptosis)

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Abstract. Large nucleoli have generally been believed to be present in less differentiated and proliferating cells including the malignant ones. Such nucleoli have also been considered to be active in the biosynthetic process and major cell developmental activities. In contrast, after cytostatic treatment, apoptotic leukaemic progenitors still containing nuclei did not exhibit substantial reduction of the nucleolar size but displayed decreased nucleolar biosynthetic activity. The present study was undertaken to provide more information on the large nucleoli in spontaneously occurring apoptotic leukaemic progenitors without further differentiation. Leukaemic progenitors of established cell lineages originating from leukaemic patients represented a very convenient model for such study. Some of them exhibit morphological signs of the spontaneously occurring apoptotic process. Since such signs are expressed by nuclear and cytoplasmic morphological variability, the present study dealt with spontaneously occurring apoptotic progenitors with preserved nuclei characterized by heavy chromatin condensation and occasional fragmentation. Based of nucleolar body and nuclear maximal diameter measurements it seems to be clear that the nucleolar size in these cells was not substantially reduced, contrary to that of the nucleus. However, large nucleolar bodies in spontaneously occurring apoptotic cells were characterized by markedly reduced biosynthetic activity, as expressed by the decreased number of nucleolar transcription markers such as nucleolar fibrillar centres. In conclusion, large nucleoli may be present not only in proliferating, but also in spontaneously occurring apoptotic cells.

Introduction

Large nucleoli have generally been believed to be present in less differentiated and proliferating cells including the malignant ones. Such nucleoli have also been considered to be active in the biosynthetic process and major cell developmental activities (MacCarty and Haumeder, 1934; Foot, 1937; McGrew, 1965; Cardozo, 1954; Busch and Smetana; 1970, Bessis, 1973; Derenzini et al., 2009). The apoptotic process in the terminal stages of cell differentiation and maturation is accompanied by decreasing nucleolar size and formation of heterogeneous nucleolar and nucleolar ribonucleoprotein aggregates (Biggiogera et al., 1997, 2004). However, leukaemic progenitor cells after induction of the apoptotic process by cytostatics also may possess large nucleoli with similar RNA concentration to proliferating cells (Smetana et al., 2004, 2008). On this occasion it should be mentioned that the nucleolar size and rRNA abundance seem to be genetically controlled (Ma et al., 2016). In addition, the high concentration of RNA in large nucleoli and the decreasing RNA content in the cytoplasm of apoptotic cells might also reflect inhibition of the "frozen" RNA transport to the cytoplasm (Smetana et al., 2008).

The present study was undertaken to provide more information on the nucleoli in spontaneously occurring apoptotic leukaemic progenitors without further differentiation. Cultured leukaemic progenitors of established HL 60, Kasumi 1 and K 562 cell lineages originating from leukaemic patients represented a very convenient model for such study. They represent leukaemic progenitors – myeloblasts, which do not differentiate without using differentiation inductors (see Dalton et al., 1988; ATCC, 2017). However, some of them may exhibit morphological signs of the apoptotic process after aging or cytostatic treatment (Smetana et al., 2004, 2007, 2008). Since such signs may be reflected by the nuclear and

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Abbreviations: NoB(s) – nucleolar body (bodies), NoFC(s) – nucleolar fibrillar centre(s).

cytoplasmic polymorphy (Plunkett, 1995; Willingham, 1999; Biggiogera et. al., 2004), the present study deals with spontaneously occurring apoptotic progenitors characterized by heavy chromatin condensation and occasional fragmentation but still with the preserved nucleus.

Material and Methods

Nucleolar bodies (NoBs), nucleolar fibrillar centres (NoFCs) and nuclear outlines were visualized in unfixed cytospins of cultured myeloblasts by simple but sensitive methods for the demonstration of RNA using buffered methylene blue and silver reaction for nucleolar proteins (Smetana et al., 1969, 1999; Ochs, 1998). The methods for RNA demonstration facilitated visualization of NoBs and nuclear outlines of single cells for the maximal diameter measurements. The silver reaction for demonstration of NoFCs was a marker of the nucleolar biosynthetic activities such as nucleolar rRNA transcription (Kacerovská et al., 1981; Smirnov et al., 2016)

Micrographs were taken with a Camedia digital photo camera C-4040 ZOOM (Olympus, Tokyo, Japan) placed on a Jenalumar microscope (Zeiss, Jena, Germany) with two special mechanical adapters to increase the maximal magnification. The captured images were processed with Quick Photoprogram (Olympus) in combination with L-view and Power Point Microsoft programs (Microsoft, Reno, NV). Maximal diameters of the largest nucleolar bodies and nuclear outlines were measured directly on the screen using Quick Photoprogram. Since NoBs and the nuclei were frequently not regularly rounded, their largest diameters were used to calculate the rough estimate of the nuclear region occupied by the dominant largest nucleolar body.

Moreover, the nuclear shape was influenced by cell preparation and spreading (Tocco et al., 2018) used in the present study. On this occasion it should also be mentioned that the measured nuclear largest diameter (axis) appeared to be mostly an informative parameter (Tseleni et al., 1997; Politi et al., 2003). The maximal nuclear and NoB diameters were calculated for each single myeloblast in all cultured cell lineages. Similarly, the number of NoFCs was counted in each largest dominant nucleolus of myeloblasts in all cultured cell lineages. All reported values such as maximal NoB to maximal nuclear diameter ratio as well as the number of NoFCs were expressed by mean values and standard deviations. The largest NoB to maximal nuclear diameter ratio multiplied by 100 was used to estimate the approximate size of the nuclear region occupied by the largest nucleolar body. All calculated data for proliferating and apoptotic cells with preserved nuclei were compared using the *t*-test at the significance level $2\alpha = 0.05$ (Primer of Biostatistic Program, version 1 developed by S.A. Glantz, McGraw-Hill, Canada, 1968).

HL 60, Kasumi 1 and K 562 myeloblasts (see ATCC, 2017) were incubated in RPMI 1640 medium supplemented with 10 (HL 60, K 562) or 20 % (Kasumi 1) foetal calf serum at 37 °C in an atmosphere containing 5 % CO_2 and harvested 24 h after feeding (for details see Smetana et al., 2004, 2007, 2008). The cultivation medium for K 562 myeloblasts also contained penicillin (100 U/ml) and streptomycin (50 µg/ml). The percentage of spontaneously occurring apoptotic myeloblasts in all studied cell lineages ranged from 3 to 6 %.

Results and Discussion

Based on NoB and nuclear diameter measurements it seems to be clear that the largest NoB occupying the nucleus in spontaneously occurring apoptotic leukaemic granulocytic progenitors with a preserved nucleus was not substantially reduced (Figs. 1, 2). Such observation is reflected by similar or increasing NoB : nuclear maximal diameter ratios (Table 1). However, the biosynthetic activity of large nucleoli in spontaneously occurring ap-

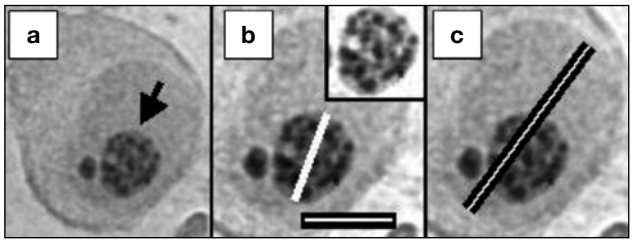

Fig. 1. Myeloblast of the HL 60 lineage. **a.** Original captured image (largest dominant nucleolus – arrow). Panels **b** and **c** represent computer-processed images. Maximal diameter measurement of the largest NoB (**b**) and nucleus (**c**) – white and black and white lines. Black and white bold line in panel b indicates 3 µm. Insert in panel b represents a magnified NoB with numerous distinct NoFCs. Calculation of the roughly estimated region occupied by the NoB: largest NoB (3.2 µm) / largest nuclear diameter (9.0 µm) = 0.35 × 100 = 33.5.

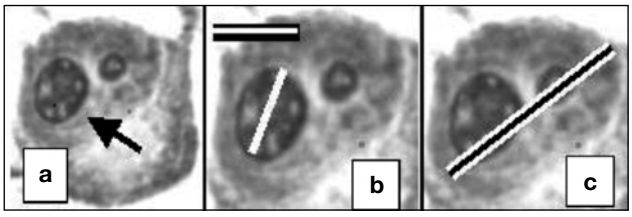

Fig. 2. Apoptotic myeloblast of the HL 60 lineage with the preserved nucleus containing heavily condensed chromatin. **a**. Original captured and computer processed phase contrast image with a few NoFCs, which appear as white dots (arrow). Panels **b** and **c** represent a magnified nucleus. Maximal diameter measurement of the largest NoB (**b**) and nucleus (**c**) - white and black and white lines. Black and white bold line in panel b indicates 3 µm. Calculation of the roughly estimated region occupied by the NoB: largest NoB (2.9 µm) / largest nuclear diameter (7.8 µm) = 0.371 × 100 = 37.1.

*Table 1. NoB : nuclear maximal diameter ratio and the number of NoFCs in leukaemic granulocytic progenitors**

Cell lineage	MxNoB/MxNu DmR	NoFCs	State
HL 60	29.7 ± 5.6•	13.4 ± 3.7•	P
	33.0 ± 6.1	5.2 ± 2.0#	Apo
Kasumi 1	28.0 ± 7.5	19.0 ± 6.9	P
	41.3 ± 2.8#	4.6 ± 0.4#	Apo
K 562	24.1 ± 5.0	14.6 ± 1.6	P
	26.0 ± 2.8	4.0 ± 2.0#	Apo

* – Based on 40 diameter measurements and 40 NoFC counts for each group of cells

• – Mean and standard deviation

– Significant difference from proliferating cells using *t*-test ($2\alpha = 0.05$)

P – proliferating cells, Apo – apoptotic cells with highly condensed or fragmented chromatin, MxNoB/MxNu DMR – maximal NoB/largest nuclear diameter ratio

optotic granulocytic progenitors was markedly decreased, as indicated by the reduced number of NoFCs (Table 1). Large nucleoli in apoptotic cells thus need not express high biosynthetic activity, but in contrast, reflect its marked decrease. Similarly as in spontaneously occurring apoptotic myeloblasts, such phenomenon was observed previously after induction of the apoptotic process by aging or cytostatic agents (Smetana et al., 2004, 2008). Thus, such phenomena should also be considered during evaluation of leukaemic progenitors without and after the anti-leukaemic cytostatic treatment. There is the possibility that myeloblasts with NoBs and altered but preserved nucleus might be considered as cells in the state of premature maturation and aging that enter the apoptotic process (Bessis, 1973; Buchwalter and Hetzer, 2017). In addition, according to the above-presented results, the nucleolar RNA transcription in the studied single apoptotic cells with preserved nuclei was markedly reduced.

The relatively similar or increased NoB : nuclear maximal diameter ratio in leukaemic apoptotic progenitors with preserved nucleus in comparison with proliferating cells apparently reflects the stability of the nucleolar size and size of the nuclear region occupied by NoB. In contrast, the nuclear size was decreased. In the present study, the maximal diameter of largest NoBs was similar in both proliferating (3.8 ± 1.0 μm) and spontaneously occurring apoptotic (3.6 ± 1.0 μm) myeloblasts of the HL 60 cell lineage. However, in comparison with the maximal nuclear diameter (11.0 ± 1.6 μm) in proliferating myeloblasts, in apoptotic myeloblasts with the preserved nucleus the maximal nuclear diameter was apparently reduced (8.0 ± 3.2 μm). Therefore, the similar or increased NoB : nuclear maximal diameter ratio was due to the nuclear size reduction (Figs. 1, 2). On this occasion it should be noted that the less apparent nuclear diameter reduction was already observed in cultured aging myeloblasts or myeloblasts after long-term treatment with anti-leukaemic therapy (Smetana et al., 2007). It should also be noted that the nucleolar RNA content rather depends on the nucleolar size than RNA concentration (Smetana et al., 2006). Thus, in apoptotic cells with the preserved nucleus, the nucleolar RNA content was not substantially reduced. In addition, large NoBs in apoptotic malignant – granulocytic leukaemic progenitors with the decreased cytoplasmic RNA content might also reflect the "frozen" RNA in the nucleolar body (Smetana et al., 2008, 2011).

In conclusion, large nucleoli may be present not only in proliferating, but also in spontaneously occurring or therapeutically induced apoptotic cells with the preserved nucleus.

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