

## Original Article

# To the Nuclear Region Occupied by Nucleolar Bodies in Human Leukaemic Myeloblasts of Kasumi 1 and K 562 Lineages

(nucleolar body / nuclear diameter ratio / cultured human leukaemic granulocytic progenitors)

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**Abstract.** Previous observation demonstrated that measured nucleolar and nuclear diameters and the resulting calculated ratio might facilitate estimation of the approximate size of the nuclear region occupied by the nucleolar bodies. The size of nuclear regions occupied by nucleolar bodies decreased during the differentiation and maturation of leukaemic lymphocytes, but was constant for each differentiation or maturation stage. The present study was undertaken to provide more information on the approximate size of the nuclear regions occupied by nucleolar bodies in leukaemic granulocytic progenitors. Myeloblasts of established Kasumi 1 and K 562 cell lineages originating from human myeloid leukaemias were convenient models for such study because they represented only one and early differentiation stage of granulocytic progenitors. According to the results, the maximal and mean nucleolar body : maximal and mean nuclear diameter ratios in myeloblasts without heavy nuclear alterations were stable and not markedly influenced by the anti-leukaemic treatment or aging. Thus, the roughly estimated size of nuclear regions occupied by nucleolar bodies in these cells appeared to be similar and stable regardless of aging or anti-leukaemic treatment. In contrast, the anti-leukaemic treatment or aging in such myeloblasts induced marked reduction of the nucleolar biosynthetic activity reflected by the decreased number of nucleolar fibrillar centres.

## Introduction

Some previous studies of the nucleolus : nucleus diameter ratio provided useful information on the malignant cell already in the past century (see Foot, 1937; McGrew, 1965; Busch and Smetana, 1970; Tseleni et al., 1997). In addition, the simple calculation based on nuclear and nucleolar diameter measurements facilitated estimation of the approximate size of the nuclear region occupied by the nucleolus during cell differentiation and maturation. According to this calculation, the size of the nuclear regions occupied by nucleolar bodies in leukaemic lymphocytes decreased during the differentiation and maturation of leukaemic cells. However, it was characteristic of each differentiation or maturation stage regardless of the anti-leukaemic therapy of the studied patients.

The present short study was undertaken to provide more information on the approximate size of the nuclear regions occupied by nucleolar bodies in granulocytic progenitors without further differentiation. Cultured myeloblasts of established Kasumi 1 and K 562 cell lineages originating from human leukaemias represented convenient models for such measurements because they represented only one early differentiation stage (American Type Cell Culture Collection, 2017). Moreover, it was possible to influence the proliferation and nucleolar biosynthetic activities of these cells by aging or anti-leukaemic drugs. On this occasion, it should be mentioned that the nucleolar biosynthetic activity in single cells was expressed by the number of nucleolar fibrillar centres (NoFCs) with adjacent dense fibrillar components representing functional units of the ribosomal RNA transcription (Kacerovská et al., 1980; Smirnov et al., 2016).

According to the results of the present study, it was apparent that the calculated approximate size of nuclear regions occupied by nucleolar bodies in surviving leukaemic myeloblasts without heavy nuclear alteration was very stable and constant regardless of aging or anti-leukaemic treatment. In contrast, the nucleolar biosyn-

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Received December 4, 2018. Accepted December 27, 2018.

The present study was supported in part by the Research Project of the Institute of Haematology and Blood Transfusion.

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Abbreviations: NoB – nucleolar body, NoFCS – nucleolar fibrillar centres, Nu – nuclear.

thetic activity in these cells was markedly reduced by aging or treatment with anti-leukaemic drugs.

## Material and Methods

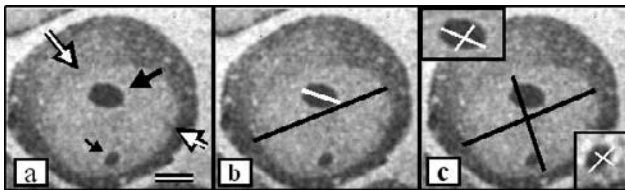
Kasumi-1 myeloblasts (Figs. 1, 2), received as a generous gift from Dr. O. Krejčí (Division of Experimental Haematology, Cincinnati Children's Hospital Medical Center, USA), were cultured in RPMI 1640 medium with 20% foetal bovine serum (Gibco, Grand Island, NY) at 37 °C in atmosphere containing 5% carbon dioxide (see also LGC, 2006). Aliquots were cultured without or with TSA (Sigma Aldrich, St. Louis, MO) at concentration 120 nM for 24 h. The used concentration of TSA was determined previously according to the efficiency on cultured leukaemic myeloblasts (Smetana et al., 2007). Cytospins of harvested cells were prepared using a Universal 16 R centrifuge, rotor 1624 (Hettich, Tuttlingen, Germany), 800 RPM for 5 min.

K 562 myeloblasts (Figs. 3, 4 – European Collection of Animal Cell Cultures, UK) were cultured in RPMI 1640 medium supplemented with 10% foetal calf serum, 100 U/ml penicillin and 50 µg streptomycin at 37 °C in 5% humidified atmosphere at 37 °C. Cultures of these cells without and with imatinib (1µM imatinib mesylate, Novartis, Switzerland) were fed by dilution in fresh medium three times a week and harvested after 72 h. Aging cells were harvested after cultivation without feeding for the same time. The used concentration of the added drug, corresponding to the dose used in patients,

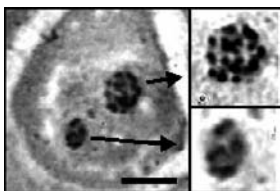
was known to induce an anti-proliferation effect that was more pronounced after 72 h of cultivation (Smetana et al., 1977). Cytospins of harvested cells were prepared using a Shandon II cytocentrifuge (Shandon Southern Products, Cheshire, UK) – 6000 RPM for 10 min.

Nucleolar bodies and nuclear outlines (Figs. 1, 3) were visualized in unfixed cytopins by acidified methylene blue for demonstration of RNA (Smetana et al., 1969; Ochs, 1998). NoFC (Figs. 2, 4) were visualized in unfixed cytopins by the silver reaction under conditions facilitating a distinct view of silver particles within nucleolar bodies (Ochs, 1998; Smetana et al., 1999).

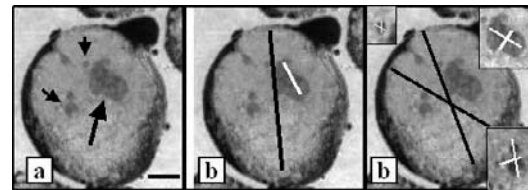
Micrographs of the studied cells in cell monolayer portions of cytopins were captured with a Camedia digital photcamera C-4040 ZOOM (Olympus, Tokyo, Japan) placed on a Jenalumar microscope (Carl Zeiss, Jena, Germany) with two special mechanical adapters. The resulting images were processed with Quick Photoprogram (Olympus) in combination with L-view and Power Point Microsoft programs (Microsoft, USA). Nuclear (Nu) and nucleolar body (NoB) diameters of Kasumi 1 and K562 myeloblasts (Figs. 1, 3) were measured directly on the screen using Quick Photoprogram. The maximal and mean nucleolar to maximal and mean nuclear diameter ratios ( $MxNoBs/MxNu R$  and  $MnNoB/MnNu R$ ) were calculated for each myeloblast. The resulting calculated NoB : nuclear diameter ratios multiplied by 100 estimated the approximate size percentage of nuclear regions occupied by NoBs in single cells. The results of all measurements and calculations at the sin-



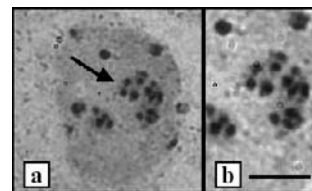
*Fig. 1.* Myeloblast of Kasumi 1 lineage. **a.** The large dominant nucleolus – large arrow, smaller nucleoli – small arrows, the nuclear outline – white arrows. **b.** Black line – nuclear maximal diameter, white line – nucleolar maximal diameter. **c.** Black lines – nuclear mean diameter measurements, white lines in inserts – nucleolar mean diameter measurements. The white bar in panel a represents 2 µm.



*Fig. 2.* Myeloblast of Kasumi 1 lineage stained with the silver reaction exhibits the presence of NoFCs in both nucleoli. In inserts (arrows), NoFCs are more distinct after the image processing and larger magnification. The black bold bar in the Figure represents 2 µm.



*Fig. 3.* Myeloblast of K 562 lineage. **a.** The large dominant nucleolus – large arrow, smaller nucleoli – small arrows. **b.** Black line – nuclear maximal diameter, white line – nucleolar maximal diameter. **c.** Black lines – nuclear mean diameter measurements, white lines in inserts – nucleolar mean diameter measurements. The black bold bar in panel a represents 2 µm.



*Fig. 4.* Myeloblast of K 562 lineage stained with the silver reaction exhibits the presence of a large number of NoFCs in the largest dominant nucleolus (arrow, panel a). In panel b, the large numbers of NoFCs are more distinct after the image processing and larger magnification. The black bold bar in panel b represents 2 µm.

gle cell level such as mean and standard deviation were evaluated using Primer of Biostatistic Program, version 1 developed by S.A. Glantz (McGraw-Hill, Canada, 1968).

## Results

### *Myeloblasts of Kasumi 1 lineage (Table 1)*

The percentage of altered cells cultured with TSA markedly increased, but approximately 55 % of cells did not show apoptotic or necrotic changes. In these cells without heavy nuclear alteration, both MxNoBs/MxNu and MnNoBs/MnNu diameter ratios did not show substantial differences in comparison with untreated myeloblasts. Thus, the approximately estimated size of nuclear regions occupied by nucleolar bodies in these myeloblasts did not exhibit substantial changes after cultivation with TSA. In contrast, the number of NoFCs representing sites of the nucleolar – rRNA transcription was significantly reduced after such treatment. The reduced number of NoFCs frequently translocated to the nucleolar periphery, as it was described for aging cells (Smetana et al., 2006). It should be mentioned that TSA has anti-leukaemic potential as a histone deacetylase inhibitor (Sasaki et al., 2008).

### *Myeloblasts of K 562 lineage (Table 1)*

As was described previously (Smetana et al., 2006), only a small percentage (up to 20 per cent) of cells exhibited a distinct apoptotic or necrotic alteration after cultivation with the anti-leukaemic drug – imatinib mesylate. Most of aging cells did not exhibit such changes. The MxNoBs/MxNu or MnNoBs/MnNu diameter ratios in aging myeloblasts did not substantially change in comparison with control cells. These ratios in myeloblasts “surviving” the treatment with imatinib also did not show substantial changes in comparison with controls. Thus, the estimated size of the nuclear regions occupied by nucleolar bodies in these myeloblasts was apparently similar to that in control cells. In contrast, the number of NoFCs in these aging myeloblasts or myeloblasts cultured with imatinib was markedly reduced. In

addition, these NoFCs frequently translocated to the nucleolar periphery (Smetana et al., 2006).

## Discussion

As it was expected, the nucleolar biosynthetic activity expressed by silver-stained NoFCs markedly decreased in “surviving” aging myeloblasts or myeloblasts treated with anti-leukaemic drugs. In contrast, the approximate size of the nuclear region occupied by nucleolar bodies in these myeloblasts without a heavy nuclear alteration did not exhibit marked changes. Thus, the approximate size of nuclear regions occupied by nucleolar bodies in the one “surviving” early differentiation stage, represented by myeloblasts, was very stable and constant regardless of the decreased nucleolar biosynthetic activity. Similar features of the early differentiation stage of the lymphocytic lineage – lymphoblasts – were also noted in patients suffering from chronic lymphocytic leukaemia. It was also interesting that the approximate nuclear size occupied by nucleolar bodies was similar in aging myeloblasts and myeloblasts “surviving” the used anti-leukaemic treatment. It should also be mentioned again that both aging myeloblasts and myeloblasts treated with the anti-leukaemic drugs were characterized by reduction of the nucleolar biosynthetic activity.

Leukaemic myeloblasts “surviving” experimental anti-leukaemic treatment in the present study might to some extent resemble myeloblasts remaining and surviving the cytostatic therapy in leukaemic patients who suffered from the “minimal residual disease”. However, in these patients, the incidence of leukaemic progenitors in blood-forming tissues is low and hidden by other cell components. Detection of these cells would therefore require a more sophisticated approach (Ommen, 2016). In the present study, the “surviving” myeloblasts of established cell cultures represented one and the same differentiation stage without any “contamination” by other cell stages or types, and thus were easily identified by simple morphology.

Concerning the methodological approach, the estimated size of nuclear regions occupied by nucleoli based

*Table 1. MxNoB/MxNu and MnNoB/MNu diameter ratio in myeloblasts “surviving” the cytostatic treatment and aging without heavy nuclear alteration\**

Cell lineage	SvC	MxNoB/MxNuR	MnNoB/MnNuR	NoFCs	Treatment
Kasumi 1	98.4 ± 1.2	22.8 ± 3.2	17.8 ± 1.4	15.1 ± 3.0	0 (control)
	<b>55.0 ± 11.1<sup>§</sup></b>	24.2 ± 4.5	16.2 ± 1.5	<b>4.7 ± 2.3<sup>§</sup></b>	TSA 120
K 562	94.1 ± 1.2	22.0 ± 4.8	13.6 ± 3.1	13.7 ± 1.4	0 (control)
	92.5 ± 2.1	20.5 ± 5.3	12.5 ± 2.1	<b>5.7 ± 2.0<sup>§</sup></b>	aging
	<b>87.0 ± 1.7<sup>§</sup></b>	20.6 ± 3.3	12.8 ± 2.4	<b>5.3 ± 0.5<sup>§</sup></b>	imatinib

\* – Based on ~200 measurements of the nucleolar and nuclear diameter and number of NoFCs+DFC in 50 cells in each group of myeloblasts

§ – **Bold numbers** express the statistical difference from controls using *t*-test ( $2\alpha = 0.05$ )

MxNo/MxNu R – nucleolar :: nuclear ratio based on maximal diameter measurements; MnNo/MnNu R – nucleolar :: nuclear ratio based on mean diameter measurements; NoFCs – nucleolar fibrillar centres reflecting the nucleolar biosynthetic activity; SvC – percentage of “surviving” cells without heavy nuclear alteration.

on maximal nucleolar and maximal nuclear diameters provided larger values than calculations based on the mean nucleolar and nuclear diameter measurements. According to the latter, the estimate of the nuclear region occupied by nucleoli was naturally smaller because of the measurement of all nucleolar bodies including the small ones. Nevertheless, both these calculations based on maximal and mean diameter measurements exhibited similar trends produced by the used experimental treatments of cultured myeloblasts (see Table 1). On this occasion, it should be mentioned that measurements of the maximal nucleolar and maximal nuclear diameters were easier and less influenced by the nucleolar or nuclear shape irregularities. Moreover, it has been previously concluded that the measurement of the maximal (“major”) nuclear diameter provided more information than other nuclear morphometric measurements (Monge et al., 1999). The volumetric calculations of the size of nuclear regions occupied by nucleoli in cytopspins would require addition of a virtual height to the nucleolar or nuclear measured diameters. However, the real nucleolar and nuclear height would be more variable because of the known various thickness of the different cytopspin portions. On this occasion, it should also be considered that the nuclear size or shape might also be influenced by the cell “spreading”.

### Acknowledgement

The authors would like to express their gratitude to the technicians of the Institute of Haematology and Blood Transfusion, Prague and the Department of Paediatric Haematology and Oncology of the Charles University Second Faculty of Medicine, Prague.

### Competing interests

The authors have declared that no competing interests exist.

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