acta histochemica 113 (2011) 58–61

Contents lists available at ScienceDirect

acta histochemica

journal homepage: www.elsevier.de/acthis

Short Communication

Nucleolar and cytoplasmic RNA density–concentration in leukemia granulocytic progenitors in human bone marrow biopsies: A short cytochemical note

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ARTICLE INFO

Article history:
Received 9 June 2009
Received in revised form
8 July 2009
Accepted 9 July 2009

Keywords:
Granulocytes
Granulocytic differentiation
Bone marrow
Myeloid leukemia
Computer-assisted RNA image densitometry

ABSTRACT

The present study was undertaken to provide more information on the differentiation and maturation of human granulocytes using computer-assisted image RNA densitometry at single-cell level. The bone marrow of patients suffering from chronic phase of chronic myeloid leukemia represents a very convenient model for such measurements because of the satisfactory number of early stages, as well as advanced stages, of the granulocytic cell lineage represented by neutrophils. In contrast to the erythroid cell lineage, similar nucleolar and cytoplasmic RNA density–concentration values were found only in early granulocytic progenitors such as myeloblasts and promyelocytes. In advanced stages of the granulocytic development starting with myelocytes, these cells were characterized by a larger decrease in the cytoplasmic RNA concentration in comparison with that of the nucleoli. Thus, the nucleolar to cytoplasmic RNA concentration ratio in these cells was above 1. On the other hand, it should be pointed out that late differentiation stages of granulocytes, starting with myelocytes, possessed nucleolar bodies (nucleoli without surrounding perinucleolar chromatin) of a markedly reduced size.

Introduction

It is generally known that the basophilic properties of nucleoli and cytoplasm decrease during the differentiation and maturation of blood cells. It has also been established that these basophilic properties of differentiating and maturing blood cells are due to the presence of RNA (Thorell, 1947; Vendrely and Vendrely, 1959; Undritz, 1972; Bessis, 1973). On the other hand, quantitative information on the RNA density reflecting the RNA concentration in nucleoli and cytoplasm in these cells is very limited. Previous studies reported the decreasing nucleolar and cytoplasmic RNA "richness" during differentiation and maturation of granulocytes related to the decreasing nucleolar and cell size (Thorell, 1947; Vendrely and Vendrely, 1959). However, the cytochemical procedures used in these studies did not facilitate clear visualization of small nucleolar bodies in advanced differentiation and maturation stages of the granulocytic lineage (Smetana, 1980, 2002).

The present study was undertaken to provide more information on the RNA density, reflecting RNA concentration, in nucleoli and cytoplasm of differentiating cells studied by computer-assisted image densitometry *in situ* at the single-cell level. It should be added that for this study, RNA in nucleoli and cytoplasm was visualized in unfixed bone marrow smears by a simple but sensitive cytochemical method using a low concentration of methylene blue, which was buffered with McIlvain's buffer to acid pH (Smetana et al., 1969; Ochs, 1998). Such a procedure facilitated seeing clearly the nucleolar bodies, not only in early progenitors but also in advanced stages of the granulocytic development represented by neutrophils (Smetana, 2002). The bone marrow of patients suffering from chronic phase of chronic myeloid leukemia represented a convenient model for RNA density measurements of individual cells because of a sufficient number of both early and advanced stages of the studied cell lineage. Moreover, the morphology of differentiating and maturing granulocytic precursors generally does not differ from that in non-leukemic persons. It should also be noted that each stage of differentiating and maturing granulocytes is well-defined and characterized by a decreasing nucleolar size (Undritz, 1972; Cline, 1975; Marmont et al., 1981; Smetana et al., 1998). In addition, any information on quantitative changes of the RNA concentration in nucleoli and cytoplasm of various differentiation stages of human granulocytes at the single-cell level might be a very useful tool for interpretation of their abnormalities in various blood disorders. The abnormality and asynchrony of nucleolar presence and cytoplasmic basophilia in granulocytic precursors have been a subject of descriptions and discussions in the cytochemical as well as hematological literature

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0065-1281/$ - see front matter © 2009 Elsevier GmbH. All rights reserved.
doi:10.1016/j.acthis.2009.07.008

since the last century, but without quantitative data on the nucleolar and cytoplasmic RNA concentration (e.g. Vendrely and Vendrely, 1959; Bessis, 1973).

The results indicated that in advanced stages of differentiating neutrophils, the density of the cytoplasm, reflecting the RNA concentration, decreased earlier than that of nucleoli. Thus, the nucleolar to cytoplasmic RNA density ratio in these stages was increased, although the nucleolar size was markedly reduced.

Materials and methods

The granulopoietic lineage was studied in bone marrow smears of 6 selected patients suffering from the chronic phase of chronic myeloid leukemia with an increased incidence of granulocytic precursors, which provided a sufficient number of early differentiation stages for RNA density measurements. The granulocytic to erythroid ratio in hyperplasic bone marrows of these patients, without signs of bone marrow fibrosis, was 8.1 ± 6.0, i.e. slightly above that in non-leukemic persons (Rundles, 1983). All patients in the study were treated with imatinib (Braziel et al., 2002) and the morphology of granulocytic precursors did not differ substantially from that in untreated patients or non-leukemic persons (Undritz, 1972; Cline, 1975; Marmont et al., 1981; Smetana et al., 1998). The bone marrow biopsies were taken for diagnostic purposes and the ethics committee of the institute approved the protocols for the present study.

RNA in nucleoli and cytoplasm was visualized without fixation in bone marrow smears by a simple but sensitive procedure using methylene blue at pH 5.3 buffered with McIlvain's buffer (Smetana et al., 1969; Ochs, 1998). The low pH and citric acid of the buffer prevented the loss of RNA from unfixed cells in dry smears, which were not older than 24 h.

Micrographs were captured with a Camedia digital photo camera (C4040Z, Olympus, Tokyo, Japan) placed on a Jenalumar microscope (Zeiss, Germany) equipped with a double adapter to provide a larger magnification of captured resulting images on the computer screen. Image processing was used to provide increased contrast of nucleolar bodies and permitted measurements of their largest diameter using specific software program (Quick Photoprogram, Olympus, Japan) to provide basic information on the size of the nucleoli.

The nucleolar and cytoplasmic densities reflecting the RNA concentration was measured after the conversion of captured colored images (predominantly blue signals) to gray scale using the red channel (NIH Image Program, Scion for Windows, Scion Corp., Frederick, MD, USA). The RNA concentration was expressed in arbitrary density units calculated by subtracting the mean background density (Bg dens) surrounding each measured cell from measured mean density of nucleolar bodies (No dens) or from measured mean density of the cytoplasm (Cy dens). The cytoplasmic density was measured in two locations, which exhibited the lowest and highest positivities. The formula used for nucleolar RNA concentration was: No RNA conc = No dens–Bg dens. The formula used for cytoplasmic density was: Cy RNA conc = Cy dens–Bg dens. Such calculations and standardization of arbitrary density units facilitated the comparison of results in monolayers of bone marrow smears, which occasionally exhibited different artificial densities due to smear preparations. This approach decreased artificial measurements and thus provided better results than the background adjusted to zero, which depended on the investigator. The results of the measurements such as mean, standard deviation and significance were evaluated using "Primer of Biostatistic Program, version 1" developed by S.A. Glantz (McGraw-Hill, Canada, 1968). The mean of nucleolar to cytoplasmic ratios for each granulocytic differentiation stage was calculated from mean values of nucleolar and cytoplasmic RNA concentrations.

Results

Nucleolar diameter

The quantitative data are presented in Table 1. Nucleolar diameter in myeloblasts was about 2 μm. In promyelocytes the nucleolar diameter was slightly, but not significantly, decreased. Marked reduction of the nucleolar diameter was noted in further differentiation stages starting with myelocytes. At this stage the mean diameter was about 1 μm. In further differentiation and maturation stages such as metamyelocytes, stab and segmented forms of neutrophils, the nucleolar diameter was mostly below that size.

Nucleolar and cytoplasmic RNA densities

The quantitative data are presented in Table 1. The nucleolar and cytoplasmic RNA densities in myeloblasts were similar (Fig. 1). Therefore, the nucleolar to cytoplasmic RNA density ratio was about 1. Similar values of nucleolar and cytoplasmic

Table 1
The nucleolar diameter, nucleolar and cytoplasmic densities in differentiating granulocytes[a]

Diff. stage	No Dm		No Den		Cy Den		No/Cy Den	
	(μm)	%	AU	%	AU	%	AU	%
Mybl	**2.00** ± 0.22[b]	100	**178.5** ± 32.5	100	**170.2** ± 23.3	100	**1.08**	100
Promyelo	**1.84** ± 0.70	92.0	**169.5** ± 32,0	94.9	**162.5** ± 17.1	95.4	**1.04**	96.2
Myelo	**1.00** ± 0.12[c]	50.0	**150.2** ± 27.7[c,d]	84.1	**121.3** ± 17.3[c,e]	71.2	**1.23**	113.8
Meta,B,Se	**0.81** ± 0.03[c]	40.50	**124.0** ± 17.3[+]	69.4	**96.2** ± 16.5[c,e]	56.5	**1.28**	118.5

AU – arbitrary units; % – percentage of values in comparison with the earliest differentiation stage myeloblast = 100%; No Dm – nucleolar diameter; No Den – nucleolar RNA density; Cy Den – cytoplasmic RNA density; Myb – myeloblast (early differentiation stage); Promyelo – promyelocyte (early differentiation stage); Myelo – myelocyte (advanced differentiation stage); Meta,B,Se – metamyelocyte; band segmented form of maturing granulocytes (advanced, non-dividing differentiation and maturation stages).

[a] Based on 30 measurements for each differentiation stage in each of 6 patients.
[b] Mean and standard deviation.
[c] Significantly different from myeloblasts and promyelocytes using *t*-test ($p < 0.001$).
[d] Significantly different from promyelocytes using *t*-test ($p < 0.02$).
[e] Significantly different from NoDn using *t*-test ($p < 0.001$).