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Original Research Article

Perinucleolar heterochromatin during the cell differentiation using human leukemic neutrophils as a convenient model

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ABSTRACT

The perinucleolar region represents a special nuclear compartment involved in the cell malignancy and the perinucleolar heterochromatin reflects the presence of silent genes. The present study was undertaken to provide complementary and missing information on the perinucleolar heterochromatin in differentiating neutrophils in the bone marrow of patients with the chronic myeloid leukemia. That lineage is a very convenient model because of the increased number of granulocytic precursors that is satisfactory for size as well as optical density measurements in single cells. Moreover, the differentiation stages of neutrophils are well defined and easily identified. According to diameter measurements the enlarged width of the perinucleolar heterochromatin shell accompanied the decreasing nucleolar size in advanced stages of the cell differentiation. Such trend was not influenced by the anti-leukemic therapy with imatinib. Thus the increasing size of the perinucleolar heterochromatin shell with silent genes might reflect the genomic stability of the perinucleolar region during the cell differentiation. On the other hand, the increased perinucleolar heterochromatin condensation after the specific anti-leukemic therapy with imatinib indicated a "premature terminal differentiation" of leukemic neutrophils.

Introduction

Some studies demonstrated that the perinucleolar region represents a special nuclear compartment that is involved in the cell malignancy (Pederson, 1998, 2011; Huang, 2000; Sludarczyk et al., 2010; Norton and Huang, 2013). However, the morphology of the perinucleolar region is less known although the perinucleolar heterochromatin is considered to be one of repressive nuclear compartments (Politz et al., 2013). The previous study on leukemic lymphocytes indicated that the width of the perinucleolar heterochromatin shell markedly increased during the cell maturation. On the other hand, this study did not provide any information on the morphology of that perinucleolar region during the cell differentiation (Smetana et al., 2013). Thus the present study was undertaken to provide complementary information on the perinucleolar heterochromatin shell during the cell differentiation.

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Since the definition of the cell differentiation and maturation in the cytological and especially hematological literature was not always rigorously respected (Astaldi and Lisiewicz, 1971; Lajtha and Schofield, 1974; Walker, 1990), in the present study the term of the cell differentiation reflects proliferating cell stages of neutrophils in the human bone marrow of leukemic patients (e.g. Smetana et al., 2011). As it is generally known, myeloblasts represent early and myelocytes are advanced precursors during the cell differentiation of the neutrophylic cell lineage. Myelocytes are actually last dividing steps of the neutrophylic differentiation and further mature to not-dividing cells. The identification of mentioned differentiation stages is very simple similarly as the identification of large nucleoli in these cells (Busch and Smetana, 1970; Undritz, 1972). It should be also added that the increased number of differentiating neutrophylic precursors in the bone marrow of patients suffering from the chronic phase of the chronic myeloid leukemia is satisfactory for diameter and optical density measurements at the single cell level. In addition, the general morphology of differentiation stages of these granulocytic precursors after staining with panchrome procedures is similar to that in not-leukemic persons (e.g. Marmont et al., 1981).

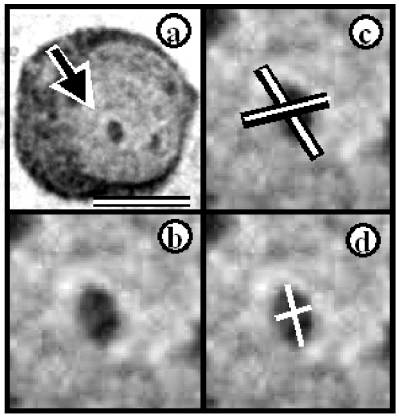
The results indicated that the decreasing size of large nucleoli in advanced stages of the cell differentiation was accompanied by the enlarged width of the perinucleolar heterochromatin shell. In addition, such trend during the differentiation process was not substantially influenced by the used anti-leukemic therapy. From the methodical point of view differences in the diameter measurements were apparently due to the differences of used methods for the visualization of the nucleolar body and surrounding perinucleolar heterochromatin shell. It seems to be interesting that the heterochromatin condensation state of the perinucleolar region was not substantially influenced by the differentiation process in patients untreated by the anti-leukemic therapy. However, after such therapy the perinucleolar heterochromatin condensation state significantly increased.

Material and methods

The differentiation of granulocytic precursors was studied in bone marrow smears of 14 patients with chronic phase of Ph1 + CML classified according to common characteristic clinical and laboratory markers including the cytology, genetics and FACS phenotyping. 7 patients were untreated and 7 patients were treated with the current anti-leukemic therapy with imatinib at the time of taking samples. The granulocytic to erythroid ratio and the percentage of myeloblasts in hyperplasic bone marrows of CML patients were slightly above that in not-leukemic persons. The bone marrow biopsies were originally taken for diagnostic purposes and the ethics committee of the Institute approved the protocols.

Bone marrow smears were stained by the May-Grünwald – Giemsa-Romanowsky (MGGR) standard polychrome staining procedure and simple cytochemical methods for the demonstration of DNA and RNA. MGG was useful for both identification of bone marrow cells and also for the chromatin visualization as a histochemical tool including heterochromatin optical density measurements (Naegeli, 1931; Undritz, 1972; Wittekind, 1983; Smetana et al., 2011). The simple method for the demonstration of DNA was useful for the additional visualization of the nucleolus associated chromatin. In this procedure DNA was stained by methylene blue at pH 5.3 after HCl hydrolysis (Smetana et al., 1967; Busch and Smetana, 1970). The cytochemical method for the demonstration of RNA was sensitive for the selective visualization of nucleolar bodies surrounded by the unstained perinucleolar heterochromatin shell. It should be mentioned that this procedure also facilitated identification of differentiation stages of granulocytic precursors according to the morphology of RNA containing cell components including nucleoli (Smetana et al., 1969; Busch and Smetana, 1970; Ochs, 1998).

Micrographs were captured with a Camedia digital camera C4040 ZOOM (Olympus, Japan) placed on Jenalumar microscope (Zeiss, Germany). The double adapter on the microscope increased the magnification of captured images transferred to the computer screen and facilitated size and density measurements at the single cell level. The nucleolar mean diameters without and with the perinucleolar heterochromatin shell were calculated from the measured large and small axis of single myeloblasts or myelocytes (Politi et al., 2003, see also Figs. 1–4). The mean perinucleolar hetrochromatin width was



PNoHChDm+NoBDm 3.5 + 2.5 = 6 : 2 = 3μm
NoBDm 3.0 + 1.7 = 4.7 : 2 = 2.3μm
MPNoHChWDm 3 – 2.3 = 0.7μm

Fig. 1 – Early differentiation stage – myeloblast stained for RNA. (a) The black and white bar represents 5 μm, nucleolar body surrounded by unstained perinucleolar heterochromatin – arrow. (b) The magnified stained nucleolar body and unstained perinucleolar heterochromatin from the previous figure. White lines within the micrograph c and d indicate sites of measurements. (c) The diameter measurements of the nucleolar body + perinucleolar heterochromatin. (d) The diameter measurements of the nucleolar body. The calculation of the mean diameter of the perinucleolar heterochromatin width (MPNoHChWDm) is in the frame. PNoHChDm – perinucleolar heterochromatin diameter, NoBDm – nucleolar body diameter.