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Annals of Clinical Pathology

Research Article

On the Heterochromatin Condensation State Diversity in Myeloblasts of Chronic Myelocytic and Acute Myeloblastic Leukemias

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Submitted: 26 October 2015

Accepted: 09 November 2015

Published: 14 November 2015

ISSN: 2373-9282

Keywords

- Acute myeloblastic and chronic myelocytic leukemias
- Heterochromatin
- Cell differentiation

Abstract

The heterochromatin condensation state (HChCS) in myeloblasts was studied using the computer assisted optical densitometry in May-Grűnwald – Giemsa-Romanowsky (MGGR). Stained bone marrow smears of patients who suffered from acute myeloblastic and chronic myelocytic leukemias. The results of the measurements demonstrated significant differences of the HChCS in peripheral nuclear regions between myeloblasts of acute myeloblastic (FAB classification M2) and the chronic phase of chronic myelocytic leukemias. In contrary to the chronic phase of chronic myeloid leukemia (CML), the HChCS in M2 acute myeloblastic leukemia (M2 AML) was significantly larger in the nuclear periphery. It should be added that the HChCS in the periphery of these M2 AML myeloblasts with the altered differentiation was similar to that in fully differentiated stages of CML granulocytes. The HChCS in myeloblasts with the differentiation ability in cultures originated from human leukemias was similar to that in bone arrow myeloblasts of CML, Thus, the large HChCS in the nuclear periphery of M2 AML myeloblasts apparently reflects an additional type of their known altered differentiation, On the other hand, it should be mentioned that some of M2 AML myeloblasts with a looser HChCS in the nuclear periphery were similar to those in CML or in growing Kasumi 1 and K 562 cell cultures with the cell differentiation ability. Such similarity suggests that a small population of such myeloblasts in M2 AML might possess the ability for further differentiation. However, it remains unknown whether such cells in patients suffering from M2 AML might represent the origin of differentiated mature leukemic or not-leukemic granulocytes. In addition, the methodical approach used in the present study did not facilitate to distinguish exactly the facultative or constitutive heterochromatin in both M2 AML and CML myeloblasts.

INTRODUCTION

It is generally accepted that the nuclear heterochromatin regions are location sites of silent genes [1,2]. According to the optical densitometry measurements heterochromatin regions consists of highly accumulated - condensed - fibrils and resemble to a great extent mitotic chromosomes with silent genes [3,4], It seems to be likely that such structural organization of the heterochromatin prevents the association of chromatin or DNA fibrils with a variety of factors necessary for the gene transcription. It should be also mentioned that a high heterochromatin condensation state (HChCS) with silent genes in both central and peripheral nuclear territories is possibly related to the genome stability in these nuclear regions [5-7]. Moreover, in hematopoietic cells, heterochromatin nuclear compartments might be involved in the regulation of cell type specific gene expression [7].

In addition, the heterochromatin is also useful for the cell identification of nucleated blood cells including the differentiation or maturation stages. On the other hand, in specimens stained by commonly used procedures, the heterochromatin appearance in leukemic myeloblasts of both acute myeloblastic and chronic myelocytic leukemias is similar regardless of the location in the nucleus. However, recent studies indicated that the heterochromatin density is different in central “gene rich” and peripheral “gene poor” nuclear regions of leukemic myeloblasts in chronic myelocytic leukemia. From the methodical point of view, these differences reflecting HChCS in single cells were apparent after computer assisted image optical density measurements in bone marrow smears stained by May-Grűnwald – Giemsa-Romanowsky (MGGR) [8].

To provide more information the HChCS in human leukemic myeloblasts with the altered differentiation, these cells were

studied in M2 acute myeloid leukemia (M2 AML). The results were compared to myeloblasts with the morphological differentiation ability [9] in the chronic phase of the Ph+ chronic myelocytic leukemia (CML). It should be mentioned that in patients suffering from these leukemias the heterochromatin morphology of these cells appears to be very similar despite the different biology. It is known that in contrary to CML, myeloblasts of AML are characterized by a disturbed differentiation capability [10,11].

In the present study, the HChCS in myeloblasts was studied using the computer assisted optical densitometry in bone marrow smears of patients suffering of M2 AML and CML or in established cell lines originated from both acute and chronic myeloid leukemias. The results of measurements demonstrated significant differences of the HChCS) in peripheral nuclear regions between myeloblasts of M2 AML and CML. In contrary to CML, the HChCS in M2 AML was significantly larger in the nuclear periphery and was similar to that in nuclear central regions. It should be added that the HChCS in most of M2 AML myeloblasts was similar to that in fully differentiated stages of CML granulocytes. The HChCS in cultured myeloblasts with the differentiation ability in cultures originated from CML was similar to that in bone arrow myeloblasts of CML, Thus, the differentiation process is accompanied by the increased HChCS in the nuclear periphery. In most of M2 AML myeloblasts the heavy HChCS in the nuclear periphery reflects an additional type of the premature cell differentiation.

MATERIAL AND METHODS

The HChCS was studied in myeloblasts of bone marrow smears of 6 patients with AML (M2 type according to FAB classification) and in 6 patients with the chronic phase of Ph+ CML. All studied leukemic patients exhibited common characteristics of the clinical state and laboratory markers including the cytology, genetics and FACS phenotyping. The incidence of myeloblasts in bone marrow smears of AML M2 patients was larger than 50 per cent of non-erythroid cells. The percentage of myeloblasts in bone marrow smears of patients suffering from the chronic phase of CML was smaller than 6 per cent of nucleated cells. The studied bone marrow biopsies were originally taken for diagnostic purposes and were approved by the ethic committee and leading authorities of the Institute.

The HChCS was also studied in cytospins of myeloblasts of growing established cell cultures that originated from patients suffering from acute myeloblastic (Kasumi 1) and chronic myeloid leukemias (K 562) [12]. These cells were cultured in RPMI medium with 20% (Kasumi 1) or 10% (K 562) bovine serum at 37°C. The cultivation medium for K 562 in addition contained penicillin and streptomycin.

Visualization and microscopic procedures

Bone marrow smears and cytospins were stained by the May-Grünwald – Giemsa-Romanowsky (MGGR) standard polychrome procedure and a simple cytochemical methods for the demonstration of DNA. MGGR was useful for both identification of bone marrow cells and for the chromatin visualization as a histochemical tool including heterochromatin optical density measurements [13-16]. The heterochromatin of myeloblasts in tissue cultures was visualized by a simple method for the demonstration of DNA because of the absence of other cell types and necessity of their identification. It should be added that no differences of the heterochromatin density and size measurements were noted after the comparison of these both visualization procedures [8]. The DNA was stained by methylene blue at pH 5.3 after HCl hydrolysis [16-18].

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 and facilitated diameter and density measurements at the single cell level on the computer screen. Mean diameters of the cell nucleus were calculated from the measured large and small nuclear diameters in each single myeloblast using the M.I.S. Quick Photo Program (Olympus, Japan).

The heterochromatin image optical density was measured after the conversion of captured color signals to gray scale using the red channel of NIH Image Program - Scion for Windows (Scion Corp., USA). The condensed heterochromatin optical image density reflecting the condensation state was measured in 3 central and 3 peripheral regions of each single myeloblastic nucleus (Figure 1). The results of density measurements were expressed in arbitrary density units. These arbitrary density units were calculated by

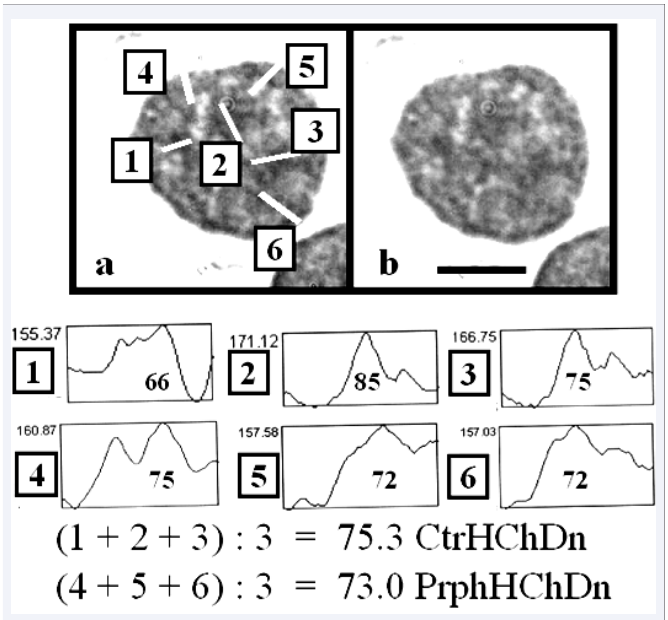

Figure 1 M2 AML myeloblast stained by MGGR procedure. Lines and numbers within micrographs indicate sites of measurements (a). The complementary and control image (b) just represents the heterochromatin regions without measurement lines. Numbers within density graphs represent calculated arbitrary density units based on measured values and subtracted density surrounding the cell. Numbers at the density graphs correspond to numbers of measurement lines. The calculation of mean density values of central and peripheral nuclear heterochromatin regions is on the bottom of the Figure. The numbers in the calculation formula correspond to the numbers of the measurement lines in micrographs. Computer assisted processing to see nuclear chromatin structure increased the contrast of the nucleus. However, the density of selected heterochromatin sites was measured in captured digitalized images without any processing. CtrHChDn – Central Heterochromatin Density, PrphHChDn – Peripheral Heterochromatn Density. The bold black bar in the Figure b represents 5 μm.

subtracting the mean background density surrounding each measured cell from the mean heterochromatin density of central and peripheral nuclear regions [8], The similarity or difference of the nuclear central and peripheral heterochromatin optical density were also expressed by calculated mean values of the central to peripheral nuclear heterochromatin density ratios for each measured cell. At this occasion it should be mentioned that the heterochromatin optical density was measured on original captured images transferred to the computer screen without any high contrast and bleaching computer processing.

The results of all measurements at the single cell level 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).

RESULTS

The quantitative data are in the Table 1.

Bone marrow myeloblasts

The mean HChCS of central nuclear regions of M2 AML in myeloblasts was mostly high and similar to that in the nuclear periphery. In most of CML myeloblasts the mean HChCS in nuclear central regions was also large. However, it was significantly larger than in the nuclear periphery. Thus, the HChCS ratio of central to peripheral nuclear regions of myeloblasts in M2 AML was smaller than in CML. It should be also noted that in some of M2 AML myeloblasts (37%) the HChCS in the nuclear periphery was smaller than in central nuclear regions. The mean nuclear diameter of myeloblasts in M2 AML was significantly smaller (11.9±0.4 μm) than in CML (12.2±4 μm).

Myeloblasts of Kasumi 1 and K 562 cell lineages

The mean HChCS in the nuclear periphery of both Kasumi 1 and K 562 myeloblasts was smaller than in central nuclear regions. However, such difference was less apparent in myeloblasts of Kasumi 1 cell lineage that originated from AML, The resulting calculated HChCS ratio of central to peripheral nuclear regions was smaller for myeloblasts of Kasumi 1 cell line originating from acute myeloid leukemia than for K 562 cell lineages that originated from chronic myeloid leukemia. The nuclear mean diameter in Kasumi 1 myeloblasts was smaller (13.7±1.5 μm) than in myeloblasts of the K 562 cell lineage (14.4±1.3 μ).

DISCUSSION

The present results demonstrated marked differences of the peripheral HChCS between leukemic bone marrow M2 AML myeloblasts with the characteristic altered differentiation and myeloblasts with the differentiation ability in CML or growing cell cultures [11,12,19]. In bone marrow myeloblasts of M2 AML the heterochromatin condensation state in the nuclear periphery was similar to that in nuclear central region. Such similarity was observed in fully mature and not-proliferating terminal stages of CML granulocytes, [8]. It should be also noted that the significantly smaller nuclear diameter in AML M2 myeloblasts than in CML is in harmony with the classical cytology as swell as. molecular biology according to which the nuclear size is small when most of chromatin is condensed and most of non-coding genome in the nucleus is tightly packed [20, 21]. All these observations apparently reflect an additional phenomenon to the known altered differentiation of M2 AML myeloblasts, which apparently mature in this early differentiation stage, The premature chromatin clumping – heterochromatin formation – even in single young leukemic cells was noted in the last century but the HChCS in various nuclear regions was not detected because of the lack of measurements methods [22]. On the other hand, some myeloblasts of AML M2 were characterized by a significantly smaller HChCS in the nuclear periphery and were similar to those in CML [8,23]. Thus such cells might still retain the capacity of a further development and differentiation similarly as myeloblasts in CML or cell cultures. The re-entering of some AML myeloblasts to the further development was also recognized in the classical hematological cytology previously [9].

These above presented suppositions are supported by differences of the HChCS between central and peripheral nuclear regions in myeloblasts of the chronic phase of CML [8] and cultured cell lineages. The further differentiation of CML myeloblasts to more differentiated cells is characterized by the increase of the HChCS state in the nuclear periphery. In addition, in fully differentiated and mature cells the HChCS in the nuclear periphery reached that in nuclear central regions [8, 23]. Moreover, the differentiation ability of myeloblasts in established cell cultures from human leukemias is also known [12].

Since the heterochromatin regions possess silent genes, the looser hetrochromatin condensation in the nuclear periphery in myeloblasts might reflect the prerequisite of the gene activation

Table 1: HChCS in central and peripheral regions of leukemic progenitors.

HChCS			
Myeloblasts	**Central**	**Peripheral**	**C/P**
(Leukemia)	nuclear regions		ratio
AML M2 BMSm	757±15.7	72.2±10.3	1,04
CML BMSm	81,1±10.4	63,8±9.23###	1.27
Kasumi 1 CSp	76.1±9.6	68.8±7.6#	1.1
K 562 CSp	77.1±9.3	55.2±3.9#	1.39

Legend
* - **Mean** and standard deviation of arbitrary density units of HChCS [Smetana et al., 2011] was based on more than 100 measurements for CML and 180 measurements for AML M2 myeloblasts and 60 measurements for cultured myeloblasts of Kasumi 1 or K 562 cell cultures.
# - Significant difference from the mean HChCS in central nuclear regions of myeloblasts using t- test
- Significant difference from the mean HChCS in nuclear periphery of M2 AML myeloblasts using t-test C/P ratio – the ratio of HChCS in central to peripheral nuclear regions, BMSm – bone marrow smears, CSp – cytospins

for the differentiation process (see above). The prerequisite of the gene activation at the heterochromatin periphery is the DNA loosening and the loop formation as it has been reported previously [6]. In addition, the heavy and looser HChCS might contribute to the genomic stability in either central or peripheral nuclear regions of leukemic myeloblasts with the different differentiation ability. It should be also mentioned that the heterochromatin stability might reflect the differentiation ability of the stem cell as it was reported previously [24]. It seems to be also interesting that similarly as in the CML, the HChCS in the nuclear periphery is smaller in Kasumi 1 myeloblasts then in K 562 cells despite differentiation ability reported for both these cell lineages. However, these myeloblastic cell lineages originated from different myeloid leukemias, the former from acute myeloblastic and the later from the chronic myelocytic leukemia [12]. Thus a possibility exists that that difference of the HChCS just reflects the different origin and is related to the genome stability with various differentiation prerequisites in the nuclear periphery. In addition, the methodical approach used in the present study did not facilitate to distinguish exactly the facultative or constitutive heterochromatin [1, 25-28] in both M2 AML and CML myeloblasts. However, more studies and different methodical approaches would be required for these directions and were not the subject of the present study,

ACKNOWLEDGEMENT

The present study was supported in part by the Research Fund of the Czech Ministry of Health**.** The authors would like to express thanks to the physicians and laboratory assistants of the Clinical Section of the Institute for the continuous support. The authors also would like to express their gratitude to Drs. P. Otevřelová and M, Pluskalová for the cell cultivation and I. Jirásková for the technical assistance.

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Cite this article

Smetana K, Mikulenková D, Hrkal Z, Klamová H (2015) On the Heterochromatin Condensation State Diversity in Myeloblasts of Chronic Myelocytic and Acute Myeloblastic Leukemias. Ann Clin Pathol 3(3): 1056.