ORIGINAL ARTICLE

The DNA chromatin condensation expressed by the image optical density of chromosomes and heterochromatin in proliferating single human leukemic granulocytic progenitors

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Summary

The appearance of heterochromatin is generally accepted as a useful tool for the evaluation of the cell state including pathology; however, information on the heterochromatin DNA condensation state expressed by the image optical density in interphase nuclear regions and mitotic chromosomes with silent genes is very limited. Since human proliferating myeloblasts are a very convenient model, they were studied in the bone marrow of leukemic patients and established cell cultures using computer assisted image densitometry at the single cell level after heterochromatin visualization by a simple but sensitive cytochemical procedure for demonstration of DNA. As was expected, a high DNA image optical density was noted in central heterochromatin regions in contrast to the nuclear periphery at the nuclear envelope. Similarly, a high nuclear DNA image optical density was also expressed in mitotic chromosomes. Thus the possibility exists that the large heterochromatin DNA condensation expressed by the large image optical density in central nuclear regions, as in mitotic chromosomes, is related to silent gene locations. The similar width of mitotic chromosomes and chromatin fibrils in the heterochromatin regions in the interphase nuclei supports that explanation. The chromatin DNA fibrils in the central heterochromatin nuclear regions of interphase cells might just represent masked silent chromosomal segments. Such a conclusion is in harmony with “classical” cytology in the first part of the last century, which suggests the chromosome continuity from the mitotic division to the interphase where each chromatin region (“Kernbezirk”) actually represents a chromosomal territory.

Key words: heterochromatin image optical density; chromosomes; central nuclear regions; proliferating cells-human leukemic myeloblasts

INTRODUCTION

Recent studies indicate that heterochromatin DNA condensation expressed by the image optical density

(IOD) is apparently different in “gene rich” central and “gene poor” peripheral nuclear regions (Cremer and Cremer 2006, Smetana et al. 2008) in the early stages of differentiation and maturation of erythroid, granulocytic and lymphocytic cell lineages. It seems to be of interest that the heterochromatin DNA IOD in these cells is larger in nuclear central regions than in the nuclear periphery at the nuclear membrane (Smetana et al. 2008, 2011a, b). However, it should be noted that in advanced differentiation or maturation stages the heterochromatin DNA IOD in the nuclear periphery also increases and is similar to

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that in central nuclear regions, including the perinucleolar chromatin (Smetana et al. 2011a, b). It should be also noted that differences in the heterochromatin DNA condensation in the nuclear central and peripheral regions might be related to different gene expression and cell differentiation (Gregoryev et al. 2006, Finlan et al. 2008, Kumaran and Spector 2008). On the other hand, the heterochromatin DNA IOD, i.e. the condensation state, was not compared with that in mitotic chromosomes with silent genes. Since the number of mitotic myeloblasts in the bone marrow of non-leukemic patients is small, the heterochromatin DNA ID in central as well as peripheral nuclear regions and mitotic chromosomes was measured in bone marrow mitotic granulocytic progenitors of patients suffering from chronic myeloid leukemia. The morphology of these cells is known and similar to that of non-leukemia sufferers (Bessis 1973, Cline 1975). In addition, the heterochromatin DNA density was also measured *in vitro*, i.e. in established cultures of leukemic early granulocytic progenitors such as HL-60, K-562 and Kasumi-1 cells (see ATCC-LGC). These cultures in the growing phase provided a satisfactory number of proliferating cells for computer assisted heterochromatin DNA IOD measurements of both interphase nuclear regions and mitotic chromosomes.

The results demonstrated that the chromatin heterochromatin IOD in central nuclear regions was similar to that of mitotic chromosomes and was significantly larger than at the nuclear membrane. Moreover, no substantial differences in the heterochromatin DNA density exist between human cultured leukemic and bone marrow granulocytic progenitors in the leukemic patients studied. It should here be mentioned that the established HL-60, K-562 and Kasumi-1 cell cultures originated from granulocytic progenitors of patients suffering from myeloid leukemia. In addition, the width measurements of DNA chromatin fibrils in heterochromatin regions were similar to those of mitotic chromosomes except for the metaphase.

MATERIAL AND METHODS

Myeloblasts of the granulopoietic lineage were studied in established and commercially available cell lines such as HL-60, K-562 and Kasumi-1 cells (see ATCC-LGC - Product informatic sheet, cell lines and hybridomas, London, GB, 2011) and bone marrow smears of selected patients suffering from the chronic phase of chronic myeloid leukemia without cytostatic

treatment at the time of taking samples for the present study. The granulocytic to erythroid ratio in the hyperplastic bone marrows of these patients was slightly above that in non-leukemic persons (Rundles 1986) and the morphology of the granulocytic progenitors did not differ substantially from that in non-leukemic persons (Bessis 1973, Cline 1975). Bone marrow biopsies were taken for diagnostic purposes and the ethics committee of the Institute approved the protocols.

Chromatin was visualized by a simple, but sensitive cytochemical method for DNA demonstration using methylene blue at pH 5.3 after HCl hydrolysis (Smetana et al. 1967, Busch and Smetana 1970). Micrographs were captured with a Camedia digital photo camera (C4040Z, Olympus, Tokyo, Japan) placed on a Jenalumar microscope (Zeiss, Germany) with a double adapter to increase the magnification of captured images on the computer screen.

The nuclear IOD reflecting the heterochromatin condensation was measured on a computer screen of focused nuclei stained for DNA after the conversion of captured colored images to gray scale using the red channel (NIH Image Program, Scion for Windows, Scion Corp., USA, see Fig. 1). The heterochromatin condensation was expressed in arbitrary IOD units calculated by subtracting the mean background IOD surrounding each measured cell from the measured mean heterochromatin IOD of mitotic chromosomes and the central or peripheral nuclear regions of interphase cells. The IOD values of the background were always determined by measurements of two different locations around the measured cell. The heterochromatin IOD was measured in two different locations of central or peripheral nuclear regions in each cell. Such measurements and standardization of arbitrary IOD units facilitated the comparison of results in bone marrow smears, which frequently exhibited different artificial background IOD due to smear preparations. Such an approach apparently decreased the artificial results of IOD measurements and provided better results than the background adjusted to zero or the comparison of measured values to the largest IOD of the perinucleolar chromatin (Smetana et al. 2011a, b). The results of measurements of each cell such as mean, standard deviation and significance were evaluated using the "Primer of Biostatistic Program, version 1" developed by S. A. Glantz (McGraw-Hill, Canada, 1968). For control of the measured heterochromatin DNA IOD in the central and peripheral nuclear regions, the captured digital images were processed by computer bleaching and increasing contrast using Windows 7XP Presentation program. It should be noted that on

Fig. 1. Chromatin DNA optical density measurements in mitotic (on the left) and interphase (on the right) Kasumi myeloblasts in the same location of the specimen. The numbers and letters in circles indicate the sites of the measurement. The numbers in rectangles indicate the mean values in the mitotic division (on the left) and in the interphase (on the right). The captured image (A) and magnified images (B, C). The larger heterochromatin DNA optical density in central nuclear regions is reflected by large density values in the Fig. C (a–d), in comparison with the nuclear periphery (1–3). The black bar in the Fig. A indicates 10 µm.

this occasion, heterochromatin ID was carried without such processing using standard conditions of staining, microscopic light illumination intensity and image capturing.

The width of chromatin fibrils in the heterochromatin of interphase nuclei and mitotic chromosomes was measured in the captured images (see above) on the PC screen using the M.I.S. QuickPHOTO Program (Olympus, Japan). The width of 4 and more chromosomes was measured in 20 mitotic cells in each cell culture or bone marrow. Chromatin fibrils were measured in at least 4 heterochromatin locations of 20 single interphase nuclei in each cell culture and bone marrow. As for the DNA image density, the results of the measurements were evaluated using the Primer of Biostatistic Program (see above).

RESULTS

The DNA IOD (condensation) in chromosomes and heterochromatin fibrils (Table 1)

The DNA IOD of chromosomes in the mitotic cells of myeloblasts in the bone marrow of leukemia patients and all cell cultures was very similar. Also, the heterochromatin DNA IOD in the central nuclear

regions of interphase myeloblasts in bone marrow and cell cultures did not exhibit substantial differences. In addition, the DNA IOD of chromosomes and heterochromatin in the central nuclear regions was also similar without substantial differences. On the other hand, the heterochromatin DNA IOD in the nuclear periphery at the nuclear membrane of both bone marrow and cultured myeloblasts was significantly smaller than in central nuclear regions (Fig. 1). The difference between heterochromatin DNA IOD in the central and peripheral nuclear regions was also more apparent after the computer bleaching and increased contrast of the captured image. It should be also noted that a less condensed heterochromatin was noted in the nuclear periphery of a limited number of bone marrow granulocytes in the more advanced maturation stages. Such cells apparently might reflect a further type of maturation asynchrony that is not rare especially in leukemic cells (Smetana et al. 2011a, b).

The diameter (width) of chromosomes and heterochromatin fibrils in cultured myeloblasts (Table 2)

The width of chromosomes in the prophase, anaphase or telophase stages of the mitotic division appeared to be similar (Fig. 2) without substantial differences (see Table 2). In contrast, the diameter of chromosomes in

Table 1. The DNA image optical density (condensation) of chromosomes and nuclear heterochromatin measured in single cells*.

Myeloblasts	Chromosomes	Heterochromatin	
		central nuclear regions	at nuclear membrane
HL-60	98.5±20.8 [§]	100.1±22.5	66.6±26.2 [#]
K 562	95.3±27.2	110.4±16.4	68.0±7.5 [#]
Kasumi	100.3±13.3	107.7±14.2	78.9±17.5 ^{#Δ}
CML bone marrow	100.2±14.2	101.1±12.6	85.2±13.8 ^{#Δ}

* based on 50 measurements in each group of cultured myeloblasts and 40 measurements of myeloblasts in bone marrow of patients suffering from CML

[§] mean and standard deviation

[#] statistically significant as compared with chromosome and heterochromatin in central nuclear regions (t-test; 2α=0.05)

^Δ statistically significant versus HL-60 and K 562 myeloblasts (t-test)

Fig. 2. Chromatin DNA fibrils in mitotic (1) and interphase K 562 (2) myeloblasts in the same location of the specimen. (A), Note the width similarity in both mitotic (B) and interphase (C) cell at higher magnification. The bold bars indicate 10 μm.

the metaphase was significantly larger and was accompanied by a slightly larger variability (8.4 %) in comparison with other phases of the cell cycle (5.1–8.1%). The diameter of fibrils of the central heterochromatin in the interphase nucleus did not

differ from that of chromosomes in the early and advanced phases of the mitotic division. It should be mentioned that the density of all measured fibrils and mitotic chromosomes also exhibited a remarkable similarity.

Table 2. The width and density of central heterochromatin fibrils and chromosomes in cultured myeloblasts*.

Phase	Width in μm	Density
Prophase	$0.74 \pm 0.06^{\S}$	97.7 ± 29.9
Metaphase	$0.83 \pm 0.07^{\#}$	101.3 ± 26.2
Ana- and telophase	0.77 ± 0.04	93.6 ± 13.9
Interphase nucleus ⁺	0.74 ± 0.05	93.8 ± 21.5

* based on 20 measurements of in each group of myeloblasts in the defined state

$\S$ mean and standard deviation

$\#$ statistically significant versus other groups (t-test)

⁺ chromatin fibrils in both central and peripheral nuclear regions

DISCUSSION

As in previous studies, the heterochromatin condensation reflected by the IOD in “gene rich” perinucleolar or extranucleolar central nuclear regions of granulocytic precursor stem cells – myeloblasts – was significantly larger than in the “gene poor” nuclear periphery at the nuclear membrane (Smetana et al. 2008, 2011a, b). Such a difference seems to be characteristic of highly immature and proliferating cells and disappears during further cell differentiation or terminal maturation. It is interesting that the large heterochromatin DNA IOD – condensation – of central nuclear regions is similar to that of mitotic chromosomes. The interpretation of this observation is difficult although some heterochromatin regions may be condensed throughout the whole cell cycle (Arrighi 1974). The heavy heterochromatin condensation in both central nuclear regions and mitotic chromosomes might reflect the suppression of the gene activity in both these cell compartments. It might prevent both small DNA segment loosening and loop formation at chromosomal or heterochromatin periphery in the central nuclear regions for the replication or transcription process (Fakan and Puvion 1980, Fakan 2004, Cremer and Cremer 2006). Actually, it is generally accepted that the heavy chromatin condensation in mitotic chromosomes is related to both DNA replication and RNA transcription arrest during the mitotic division. Moreover, according to numerous studies, the heavy heterochromatin condensation in the interphase might also reflect the gene silencing (Frenster 1974, Zhimulev and Beliaeva 2003, Grigoryev et al. 2006, Pikaard and Pontes 2007). The chromatin condensation represented by bundles of chromatin fibrils is also less accessible to the enzymatic activity

(Pederson 1972). On the other hand, it should be noted on this occasion that the present study does not facilitate the distinction between the constitutive and facultative heterochromatin or heterochromatin required for the gene activation (Grigoryev et al. 2006, Grewal and Songtao 2007).

The width measurements of chromatin DNA fibrils in the central heterochromatin of interphase cells and chromosomes in the early and advanced mitotic phases demonstrated a marked similarity. Thus the possibility exists that chromatin DNA fibrils in the central heterochromatin nuclear regions of interphase cells might just represent masked silent chromosomal segments. Such a conclusion is in harmony with “classical” cytology, suggesting that the chromosome continuity in the mitotic division and interphase in which each chromatin region – “Kernbezirk” – actually represents a chromosomal territory (see e.g. Hertwig 1929, Wassermann 1929, Pederson 2004). The increased width of chromosomes in the metaphase is not surprising because of known chromatid-splitting steps in measured cells. The comparison of the central heterochromatin fibril or chromosome width measured in the present study with various measurement data or models of chromosome or chromatin fibrils would be difficult. The width of both chromosomes and chromatin fibrils is influenced by methods used for specimen preparation and microscopic measurements including evaluation, as has been known since the first part of the last century (Hertwig 1929, Wassermann 1929). Nevertheless, in the present study the width similarity of chromosomes and central heterochromatin fibrils was noted in the same specimens using the same measurement and evaluation procedures. In addition, the width measurements of DNA fibrils were in harmony with the image optical density of

chromosomes and heterochromatin in central nuclear regions (see above and Tables).

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