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The Heterochromatin Condensation State in Peripheral "Gene Poor" and Central "Gene Rich" Nuclear Regions of Less Differentiated and Mature Human Leukemic Cells: A Mini-Review with Additional Original Observations

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Abstract

In the morphological cytology the heterochromatin is one of very useful tools for the cell identification including the differentiation and maturation stage. However, the heterochromatin condensation state was less studied although it appeared to be different in "gene rich" central and "gene poor" peripheral nuclear regions. The heavy heterochromatin condensation state in the central "gene rich" nuclear regions might reflect a marked structural stability and protect the genomic integrity. It must be also noted that the heterochromatin condensation state in these nuclear regions is more variable than in the nuclear periphery because of the presence of more as well as less condensed heterochromatin territories. On the other hand, the heterochromatin condensation state in the nuclear periphery markedly increases during the cell differentiation and maturation. In fully differentiated and mature cells the heavy heterochromatin condensation state is similar in both central and peripheral nuclear regions. The resulting ratio of the heterochromatin condensation state in central nuclear regions to the nuclear periphery is higher in less differentiated cells and then decreases during the maturation (terminal differentiation) process. That ratio facilitates to compare cells in different differentiation or maturation stages of various cell lineages because the estimated arbitrary density units are frequently variable depending on the background of the cell surrounding.

Keywords: Heterochromatin condensation state; Central and peripheral nuclear regions; Cell differentiation and maturation; Human leukemic cells

Introduction

In morphological and especially clinical cytology (hematology) the nuclear size and nuclear heterochromatin regions are very useful tools for the cell identification including the differentiation and maturation [1-4]. On the other hand, the heterochromatin condensation state was less studied although it appeared to be different in both "gene rich" and "gene poor" nuclear regions [5,6]. Numerous studies also demonstrated that the heterochromatin reflects the presence of silent genes [7-11]. It should be added that the appearance of chromatin fibrils in the heterochromatin during interphase did not show substantial differences from those in morphologically defined mitotic chromosomes [12].

Methods

It is generally recognized that leukemic blood lineages represent convenient models for the differentiation and maturation of human cells when the patients are untreated with cytostatic therapy at the time of taking diagnostic samples. Since the definitions of the cell differentiation and maturation in earlier and recent hematological or cytological literature are not rigorously respected [13-15], in the present article the term of the cell differentiation reflects proliferating developmental stages of studied cell lineages. Maturing or mature cells represent not-proliferating resting and terminal stages of their development. These stages are also frequently described as terminal differentiation [10,16,17].

The early as well as late differentiation and maturation stages of leukemic blood cells are well known and morphologically defined. As it is generally accepted, they represent very convenient models for the differentiation and maturation process [18,19]. Moreover, the number of these cells in diagnostic bone marrow biopsies or peripheral blood samples of leukemic patients is increased and satisfactory for the heterochromatin density measurements. In addition, the established and commercially accessible leukemic cell lines such as HL-60, Kasumi 1 and K-562 lineages (see American Type Culture Collection, USA, 2013, General Culture Collections, GB, 2013) provide further subjects for additional and complementary measurements.

From the methodical point of view, the heterochromatin condensation state in single cell nucleus may be easily visualized in situ by a simple image processing or by the computer assisted optical image densitometry [5,6]. Such approaches facilitate to see clearly how the heterochromatin condensation state in central and peripheral nuclear regions differs between proliferating and not-proliferating cells and develops in differentiating or maturing cells (Figure 1).

In contrast to simple image processing, the computer assisted image optical densitometry offers a possibility for the quantitative expression of the heterochromatin density, i.e. condensation state in individual cells using arbitrary density units [5,6] (Figure 1). It is possible to measure the heterochromatin density in digital images of stained cells by the polychrome procedure after the conversion of captured color images to gray scale using the red channel (NIH Image Program, Scion for Windows, Scion Corp., USA). The polychrome May-Grünwald – Giemsa-Romanowsky staining procedure is useful not only for the cell

identification including differentiation and maturation stages but also for .the heterochromatin density measurements [1,4-6,20]. The calculated arbitrary density units facilitate to estimate the ratio of the mean heterochromatin condensation state in central to peripheral nuclear regions (Figure 1). Such ratio appeared to be very useful for comparative studies of various cell lineages including their differentiation and maturation stages [5,6].

The Heterochromatin Condensation State during the Cell Differentiation and Maturation

The leukemic granulocytic cell lineage

In the chronic phase of the chronic myelocytic leukemia the morphology of early as well as late developmental stages of granulocytes do not differ from "normal" not-leukemic cells [2-4]. The early differentiation stages are characterized by a looser and less condensed heterochromatin in nuclear periphery than in nuclear central regions. The further differentiation and maturation (terminal differentiation) is accompanied by a marked and significant increase of the heterochromatin condensation at the nuclear envelope reaching maximal density values in fully mature terminal granulocytes [6]. Thus the ratio of the heterochromatin density in central to peripheral nuclear regions during the differentiation and maturation significantly decreased (Table 1).

Cell lineage	Early differentiation stage (-blast)	Maturation stage (-cyt)
CML granulocytes#	1.19 ± 0.11 (9.2%)	1.00 ± 0.05** (5%)
monocytes##	1.14 ± 0.15 (13.1%)	0.97 ± 0.08** (8.2%)
lymphocytes###	1.13 ± 0.10 (8.8%)	1.01 ± 0.04** (3.9%)
*: Mean and standard deviation based on at least 60 measurements in each group of cells		
**: Significant difference from early differentiation stage using t-test (p<0.001)		
#: See ref. 6		
##: Smetana et al., unpublished results		
###: See ref. 5		
- Percentages in brackets represent the variation coefficient: (Standard Deviation:/Mean) x 100		
- Early differentiation stage, i.e. myeloblast for the granulocytic lineage; monoblast for the		
monocytic lineage; lymphoblast for the lymphocytic lineage		
- Maturation stage, i.e. mature segmented neutrophylic granulocyte for the granulocytic lineage; Mature monocyte for the monocytic lineage; mature lymphocyte for the lymphocyticlineage		

Table 1: The ratio of the heterochromatin condensation state in central to peripheral nuclear regions in leukemic cells*

In acute myeloblastic leukemia with the altered differentiation process [21-23] the early differentiation stages were characterized by a heavy heterochromatin condensation state already in the nuclear peripheral regions (Smetana et al. unpublished results). Such heterochromatin condensation state was similar to that in fully mature terminal granulocytes in the chronic myelocytic leukemia. Thus these cells apparently are in the not-proliferating mature stage. On the other hand, in a small number of early differentiation stages – myeloblasts – the heterochromatin in nuclear peripheral regions was looser similarly as in the chronic myeloid leukemia. Such small condensation state in the nuclear periphery was also noted in proliferating myeloblasts in the growing cultures of the HL-60, Kasumi 1 and K-562 cell lineages (see below). It should be mentioned that these cell lines originated from myeloblasts of patients who suffered from acute myeloblastic and chronic myeloid leukemia (see General Cell Collection, GB, 2013, American Type Culture Collection, USA, 2013).

The leukemic lymphocytic cell lineage

In the chronic lymphocytic B cell leukemia immature cells possess less condensed heterochromatin in nuclear periphery in comparison with nuclear central regions [5]. Therefore, the heterochromatin density ratio in central to peripheral nuclear regions was relatively high. In mature lymphocytes the heterochromatin condensation state in the nuclear periphery increased and was similar to that in nuclear central regions. The resulting heterochromatin density ratio of central to peripheral nuclear regions decreased (Table 1, Smetana et al., unpublished results). At this occasion it must be mentioned that the heterochromatin condensation state was also similar in both nuclear and peripheral nuclear regions of mature not-leukemic T lymphocytes. The experimental "dedifferentiation", i. e. blastic transformation, of these cells cultured with phytohemaglutinin in short term cultures of these cells induced a significant loosening of heterochromatin in the nuclear periphery [24].

The leukemic monocytic lineage

The acute myelomonocytic leukemia variants such as monoblastic and monocytic leukemias provided a satisfactory model to study the heterochromatin condensation state in early, less differentiated and mature cells of the monocytic cell lineage. Monocytic progenitors – monoblasts were mostly characterized by a less condensed heterochromatin in the nuclear periphery. In contrary, the heterochromatin condensation state in the nuclear periphery of mature monocytes was significantly increased and was similar to that in nuclear central regions. Thus, the calculated heterochromatin density of central to peripheral nuclear regions during the differentiation and maturation decreased in comparison with early monocytic progenitors (Figure 1).

The cultured leukemic myeloblasts of HL-60, K 562 and Kasumi-1 cell lineages

The proliferating myeloblasts in growing cultures of all studied leukemic cell lineages exhibited a smaller heterochromatin condensation state in the nuclear periphery than in central nuclear regions. Thus the ratio of the heterochromatin condensation of central to peripheral nuclear regions was large, i.e. 1.14 ± 0.09 for HL-60, 1.23 ± 0,09 for Kasumi 1 and 1.22 ± 0.14 for K 562 cells. In some of few apoptotic cells in these growing cultures the heterochromatin condensation state in the nuclear periphery markedly increased and was similar to that in the nuclear central regions. The resulting heterochromatin condensation ratio of the central to peripheral nucleolar regions in these cells significantly decreased and ranged

about 1. Similar increase of the heterochromatin condensation state in the nuclear periphery was also noted in cultured myeloblasts after the induction of the apoptotic process using the histone deacetylase inhibitor sodium valproate or the photodynamic effect after sensitization with laevulinic acid [25,26]. The resulting heterochromatin density ratio of central to peripheral nuclear regions decreased .to 1.03 ± 0.09 for Kasumi 1 myeloblasts treated with histone deacetylase inhibitor valproic acid and 1.00 ± 0.03 for HL-60 myeloblasts after the photodynamic treatment, At this occasion it should be noted that the chromatin condensation need not to be always associated with the apoptotic process [27]. The chromatin condensation de novo also occurs during the mitosis and chromatin fibrils in mitotic chromosomes and are similar to those in the interphasic heterochromatin [12,27].

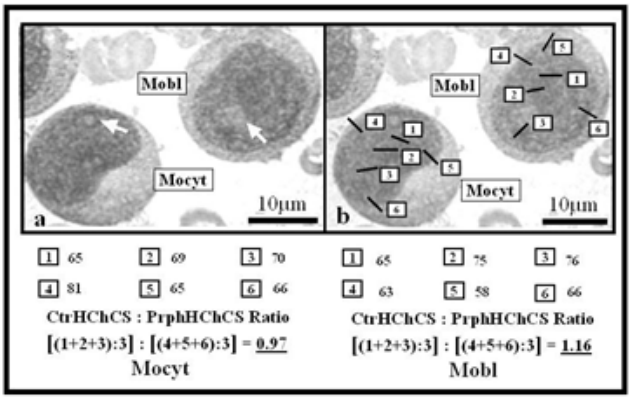

Figure 1: Mature monocyte (Mocyt) and monocytic progenitor – monoblast (Mobl) of a patiemt suffering from acute monocytic leukemia. The monoblast contains a characteristic large nucleolus (arrow) and one of small characteristic nucleoli (arrow) is visible in the monocyte (Figure a). In the Figure b, the numbered bars indicate measured lines of the heterochromatin density in both central and peripheral nuclear regions. The corresponding numbers with the heterochromatin density are below micrographs. The calculated ratios of the central (CtrHChCS) to peripheral (PrphHChCS) nuclear heterochromatin condensation state are below the measured density values. The thick black bar at the bottom of both Figures represents 10 μm.

Discussion and Conclusions

The results clearly indicated that in all studied leukemic lineages the differentiation and maturation was accompanied by a markedly increased heterochromatin condensation state in the nuclear peripheral regions. It is actually possible that these "gene poor" regions are responsible for the cell differentiation and maturation. The increased heterochromatin condensation state in nuclear periphery might be related to the gene silencing [11] in fully differentiated and mature terminal stages of the leukemic cell development [5,6]. The heavy heterochromatin condensation might prevent both small DNA segment loosening and loop formation at its periphery for the replication or transcription process [16,28]. At this occasion it should be mentioned that the peripheral heterochromatin nuclear regions - chromosomal territories - are considered to be gene poor, but might be still important because of the gene activity silencing, i.e. DNA replication as well as RNA transcription cessation [11,16,29-31].

On the other hand, the altered differentiation and maturation of early granulocytic progenitors – myeloblasts in acute myeloblastic leukemia - variant FAB M2 - is also in harmony with the above-discussed suppositions. Most of these cells mature and do not develop to further differentiation stages of the granulocytic lineage [2,21,23,31-34]. They are characterized by a marked heterochromatin condensation state in peripheral nuclear regions that is apparently similar to that in fully mature granulocytes. In contrary, few early granulocytic progenitors – myeloblasts in this leukemia exhibit a looser heterochromatin condensation state in the nuclear periphery despite the margination effect [35] and are similar to proliferating myeloblasts in growing cell cultures. Thus it seems to be likely that such myeloblasts represent the proliferating cell subpopulation in that acute myeloblastic leukemia [36-38]. It is also known that active genes are located at the heterochromatin in perichromatin regions [8,28,39]. The less condensed heterochromatin periphery might be necessary for chromatin remodeling, i.e. for loosening required for the accessibility of factors participating in the gene activation [40]. In addition, the gene activity has been also noted in the nuclear periphery at the nuclear membrane in proliferating cells [8,29], Moreover, the decreased heterochromatin condensation state in nuclear peripheral regions was observed after the de-differentiation and transformation of mature resting lymphocytes to proliferating lymphoblast like cells, which returned to the cell cycle [24].

The heavy heterochromatin condensation state in the central "gene rich" nuclear regions is difficult to interpret. However, it might reflect a marked structural stability and protect the genomic integrity in these nuclear regions [41]. On the other hand, it must be noted that the heterochromatin condensation state in these nuclear regions is more variable than in the nuclear periphery because the presence of less condensed heterochromatin territories as documented by standard deviations or variation coefficient of the nuclear central to peripheral heterochromatin density ratios (Table 1) [42]. Thus despite a relatively higher mean heterochromatin density, i.e. condensation state, there are some less compact heterochromatin territories in central nuclear regions that might be more accessible for factors necessary for the gene activation [41]. At this occasion it must be mentioned that the image optical densitometry used in the present study does not provide further information on the genes present in studied nuclear regions. It should be added that that methodical approach also does not facilitate to distinguish the constitutive and facultative heterochromatin regions [8, 10,41,43-46]. However, it might still provide useful and complementary information on the heterochromatin for further studies of chromosomal territories in different nuclear regions using other methodical approaches [45,47,48]. In addition, it might be also useful to detect pathological abnormalities of the individual cells including various differentiation and maturation asynchronies or alterations, which are common in malignant disorders including leukemias [2,49].

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