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Two subpopulations of progenitors of leukemic lineages in human myeloid leukemias exhibit different heterochromatin condensation state in central and peripheral nuclear regions (A morphological note with additional original observations)

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Abstract

The computer assisted optical image densitometry indicated that myeloid leukemias were characterized by two subpopulations of progenitor cells classified according to the heterochromatin condensation state (HChCS). The first subpopulation of these cells was characterized by a larger HChCS in the nuclear central regions than in the nuclear periphery. Such progenitors seemed to possess the potential for further differentiation and were predominantly present in patients suffering from the chronic phase of chronic myelocytic and acute monoblastic leukemias. The second subpopulation of progenitors was characterized by a marked similarity of HChCS in both central and peripheral nuclear regions. That similarity was also noted in terminally differentiated granulocytes and monocytes. Thus, these progenitors were in the state of premature terminal differentiation and reflected the altered differentiation process. The large dominant incidence of such progenitors was noted in acute myeloblastic, acute promyelocytic and acute myelomonocytic leukemias with a known alteration of the further differentiation process.

It is generally accepted that heterochromatin territories represent sites of sleeping genes and participate in the cell genome stability [1-6]. The heterochromatin condensation state (HChCS) seems to be different depending on the location in various nuclear regions and cell differentiation state [7]. Such differences may be easily visualized by computer assisted optical image density measurements at the single cell level and image processing such as computer image bleaching [7]. Quantitative data based on these measurements and calculations based on optical image density measurements are presented in the table 1.

Chronic phase of Ph1 chronic myelocytic leukemia (CML)

Granulocytic progenitors were mostly characterized by larger HChCS in central nuclear regions than in the nuclear periphery [7]. It should be mentioned that HChCS in the nuclear periphery during further differentiation increased. In terminal differentiation steps represented by neutrophilic granulocytes with the segmented nucleus, HChCS exhibited a marked similarity in nuclear as well as peripheral nuclear regions [7]. The ratio of HChCS in central nuclear to peripheral nuclear regions was larger for progenitors in comparison with terminally differentiated cells. On the other hand, some granulocytic progenitors also exhibited similar HChCS in both central and peripheral nuclear regions. Such progenitors reflected signs of premature terminal differentiation. According to "classical hematological cytology" premature terminal differentiation of leukemic cells was one of frequent abnormalities of these cells [8].

Acute myeloblastic leukemia (FAB M2) M2AML

HChCS exhibited a marked similarity in both central and peripheral nuclear regions of granulocytic progenitors, which are known for the altered differentiation [9,10]. As it was mentioned above, such similarity is characteristic for terminally differentiated granulocytes. Thus, these progenitors were in the state of premature terminal differentiation. The ratio of HChCS in central nuclear to peripheral nuclear regions calculated for progenitors and terminally differentiated cells was similar. On the hand, a small subpopulation of progenitor cells was characterized by larger HChCS in central nuclear regions similarly as progenitors with the differentiation potential in patients suffering from CML. There is a possibility that such progenitors might represent a source for further differentiation steps of granulocytes in M2AML patients [9,10].

Acute promyelocytic leukemia (FAB M3) M3PML

According to preliminary results HChCS in most of promyelocytes was similar in both central and peripheral nuclear regions. Thus these early-differentiated granulocytic precursors were in the state

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Table 1. Ctr/Prph HChCS R and the percentage of progenitors (myeloblasts, monoblasts) or precursors (promyelocytes) and terminally differentiated cells (neutrophils with segmented nucleus, monocytes) with smaller Ctr/Prph HChCS R than 1.1 in selected myeloid leukemias*

Type of leukemia	CML	M2AML	M3PML	M4AML	M5AML
Cells	Myeloblasts^{o}		Promyelocytes^{o}●		Monoblasts
Ctr/Prph HChCS R	1.2±0.1	1.0±0.1	1.0±0.1	1.0±0.1	1.1±0.1
% Cells < 1.1	29.8±17.3	76.4±0.4	70.4±12.4	66.2±10.7	25.0±0.4
Cells	Neutrophils with segmented nucleus^{o}●			Monocytes	
Ctr/Prph HChCS R	~1.0	~1.0	~1.0	~1.0	~1.0
% Cells < 1.1	~99.0	~99.0	~99.0	~99.0	~99.0

* - **Mean** and standard deviation of measured HChCS in single cells in 3 to 4 patients in each group of patients. HChCS was measured and calculated in at least 100 cells for each differentiation cell stage. • - Preliminary results, º - Data published in part [7,10], Ctr/Prph – central to peripheral nuclear regions, R – ratio, %Cells<1.1 – percentage of cells with Ctr/Prph HChCS R smaller than 1.1 of Arbitrary Units. That percentage apparently reflects the incidene of cells in the state of terminal differentiation.

of premature terminal differentiation. It should be mentioned that promyelocytes in M3PML are generally considered to lose the differentiation potential [11,12]. The ratio of HChCS in central nuclear to peripheral nuclear regions calculated for precursors and terminally differentiated cells was similar. However, some promyelocytes were characterized by higher HChCS in central and smaller HChCS in peripheral nuclear regions similarly as myeloblasts and promyelocytes in CML with the differentiation potential. Therefore, these promyelocytes might contribute to the incidence of differentiated cells of the granulocytic cell lineage in M3PML patients without the therapy with known differentiation inducers such as retinoids.

Acute myelomononocytic leukemia (FAB M4) M4AML

Monocytic progenitors – monoblasts mostly exhibited a marked similarity of HChCS in both central and peripheral nuclear regions. Thus, these monocytic progenitors were in the state of premature terminal differentiation. Almost all terminally differentiated monocytes also exhibited similar HChCS in central nuclear regins and nuclear periphery similarly as terminally differentiated granulocytes. Since a slightly smaller number of monocytic progenitors in M4AML was characterized by a larger HChCS in central nuclear regions, no wonder that such cells might further differentiate after treatment with a differentiation inducer as described previously [13]. The ratio of HChCS in central nuclear to peripheral nuclear regions in monocytic progenitors and differentiated monocytes did not show substantial differences.

Acute monoblastic leukemia (Fab M5) M5AML

In contrast to M4AML, the similarity of HChCS in central and peripheral nuclear regions in monocytic progenitors was less frequent. Thus, the incidence of monocytic progenitors in the state of premature terminal differentiation in M5AML was smaller than in M4AML. On the other hand, the similarity of HChCS in central and peripheral nuclear regions was noted in all terminally differentiated monocytes similarly as in terminally differentiated granulocytes. The predominant number of monocytic progenitors in M5AML was mostly characterized by a larger HChCS similarly as in granulocytic progenitors with the differentiation potential in CML. Such observation was strongly supported by the experimentally induced differentiation of M5AML monocytic progenitors in cell cultures [13]. In addition, the ratio of HChCS in central to peripheral nuclear regions in these cells was larger than in M4 AML due to more frequent incidence of monocytic progenitors with the differentiation potential.

Discusion and conclusions

The above-mentioned observations indicated that all studied myeloid leukemias were characterized by two subpopulations of progenitor cells classified according to the HChCS. The first subpopulation of progenitor cells was characterized by larger HChCS in the nuclear central regions than in the nuclear periphery. Such progenitors seemed to possess the potential for further differentiation and were predominantly present in patients suffering from CML and M5AML. The differentiation potential of granulocytic progenitors in CML is known and the incidence of monocytic progenitors with the differentiation potential in M5AML might be also presumed [7,13]. At this occasion it should be noted that cultured granulocytic progenitors of established leukemic cell lines with the differentiation potential are also characterized by a larger HChCS in central nuclear regions [9].

The second subpopulation of progenitors was characterized by a marked similarity of HChCS in both central and peripheral nuclear regions. Such similarity was also noted in terminally differentiated granulocytes and monocytes. Thus, progenitors with similar HChCS in nuclear central as well as peripheral regions were in the state of premature terminal differentiation and reflected the altered differentiation process. The large dominant incidence of such progenitors was noted in M2AML, M3PML and M4AML, i.e. in acute leukemias with a distinct alteration of the further differentiation process [10-14]. On the other hand, it should be noted that a small incidence of progenitors with a larger HChCS in nuclear central regions was also present in these leukemias. Such progenitors with the differentiation potential may be a source of further differentiation steps of both granulocytic and monocytic lineages in these patients.

From the methodical point of view it should be mentioned that the image optical density measurements easily facilitated to estimate HChCS in both central and peripheral nuclear regions. However, this methodical approach did not facilitate to distinguish exactly the location of constitutive and facultative heterchromatin. However, there is a possibiliy that central nuclear regions with the HChCS might correspond to the constitutive heterochromatin [6]. Actually, some studies indicated that these regions might contain genes rsponsible for the development of the cell specifity [1,15,16]. Since the facultative heterochromatin retains the potemtial to convert to euchromatin [17], there is a possibility that the HChCS in the nuclear periphery of studied leukemic cells might correspond to facultative heterochromatin.. Such speculation is supported by increasing HChCS in the nuclear periphery during the differentiation and decreasing HChCS during the de-differentiation process [7,18].

At the end of the discussion it must be noted that the relationship of the incidence of leukemic progenitors classified according to HChCS to the clinical state of the disease was not discussed because the number of studied patients was limited. Nevertheless, the present study might provide a complementary morphological information on leukemic cells that was based on heterochromatin density measurements at single cell level.

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Conflicts of interests

The authors have declared that no conflicts of interests exist.

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