Short Communication

The Morphology of Cell Differentiation, Terminal Differentiation and Ageing Seems To Reflect the Same Process: a Short Note

(differentiation and ageing / morphology / human granulocytic and erythroid cell lineages)

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Abstract. Based on simple microscopic cell morphology in blood and bone marrow smear preparations, it seems to be likely that the cell differentiation and terminal differentiation in human blood cells, and particularly in erythroid or granulocytic lineages, simultaneously reflect ageing of the lineage progenitors and terminal differentiation steps. The terminal differentiation stages of both these lineages actually appear as senescent cells. Abnormal ageing of progenitor cells may represent one of the "dysplastic" phenomena of the premature terminal differentiation state. Such state is characterized by heterochromatin condensation and nucleolar morphology similar to that in fully differentiated terminal cells of granulocytic or erythroid lineages. It should also be mentioned that in some known erythropoietic disorders, less differentiated erythroblasts may lose nuclei similarly as "normal" fully terminally differentiated cells of the erythroid cell lineage. It seems to be clear that cells in both abnormal less differentiated and terminally differentiated stages of erythroid or granulocytic lineages lose the ability to multiply similarly as senescent cells. On the other hand, the background of cell ageing and differentiation is very complicated and requires a different approach than the simple microscopic morphology at the single cell level. However, the morphology and clinical cytology at the single cell level might still contribute with complementary data to more sophisticated complex studies of that topic. In addition, the morphological approach facilitates the study of the main components of single cells in various states, including the differentiation steps or ageing.

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Abbreviations: CML – chronic myelocytic leukaemia.

Introduction

In biomedical sciences, the definition of ageing is very difficult and depends on the studied subjects (van Deursen, 2014; Ahmad, 2018; daCosta, 2018; Marques and Velarde, 2018; Smetana et al., 2013; 2018; Wang et al., 2018; Groark and Young, 2019; Zjablovskaja and Florian, 2020). Therefore, the present notes deal with human cell lineages and cells at the single cell level. The human granulocytic and erythroid cell lineages in selected patients suffering from chronic myelocytic leukaemia (CML) appear to be very convenient models, because all developmental cell stages are well known and easily identified. Moreover, in selected diagnostic bone marrow smears of CML patients, the number of cells in each of the granulocytic or erythroid cell differentiation stage is satisfactory for morphological studies. In addition, the morphology of developmental stages of the leukaemic granulocytic lineage resembles that in non-leukaemic persons (Bessis, 1973; Cline, 1975). Moreover, bone marrow smears of patients with CML also contain non-leukaemic cells of the erythroid lineage.

Generally recognized main simple morphological markers of cell differentiation and terminal differentiation or ageing, visible in smear preparations:

1, 2: The decreased nucleus : cell body and nucleolus : nuclear body size ratios express nuclear and nucleolar size reduction within the cell and the nucleus;

3: Increased heterochromatin condensation state and size of heterochromatin regions;

4: Loss of mitotic activity and mitotic division potential.

1. Generally, less differentiated cells such as granulocytic and erythroid progenitors are characterized by a large nucleus surrounded by a small cytoplasmic shell. During further differentiation of neutrophil granulocytes, the proportion of nuclear size in the cell body is usually decreasing (Fig. 1). Thus, the nucleus : cytoplasm ratio is gradually diminishing and in most of differentiated cells is smaller regardless of the smear or cytospin preparation. The calculated rough estimate

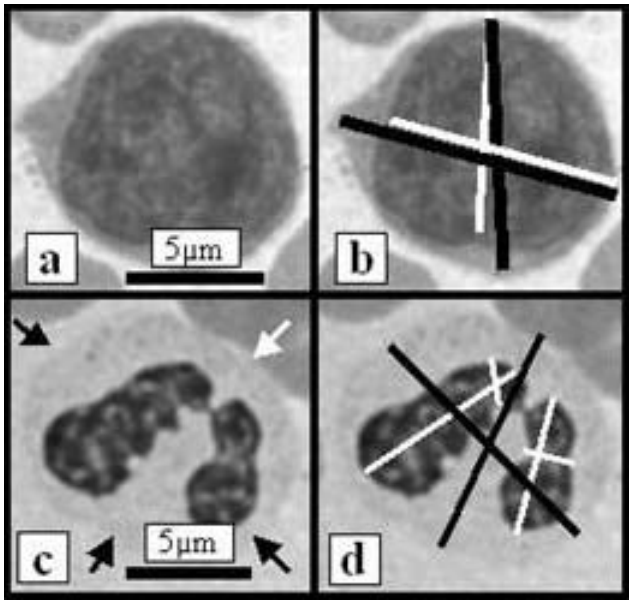

Fig. 1. Leukaemic granulocytic progenitor (**a**, **b**) and terminally differentiated leukaemic granulocyte (**c**, **d**). Lines of the largest and smallest cell body (black) and nuclear (white) diameter measurements (**b**, **d**). According to the rough estimate based on these measurements (Smetana et al., 2019), the nucleus in the progenitor occupies ~ 78 % of the cell body. For comparison, in the terminally differentiated granulocyte, the segmented nucleus occupies a reduced cell space, i.e. ~ 41 % of the cell body.

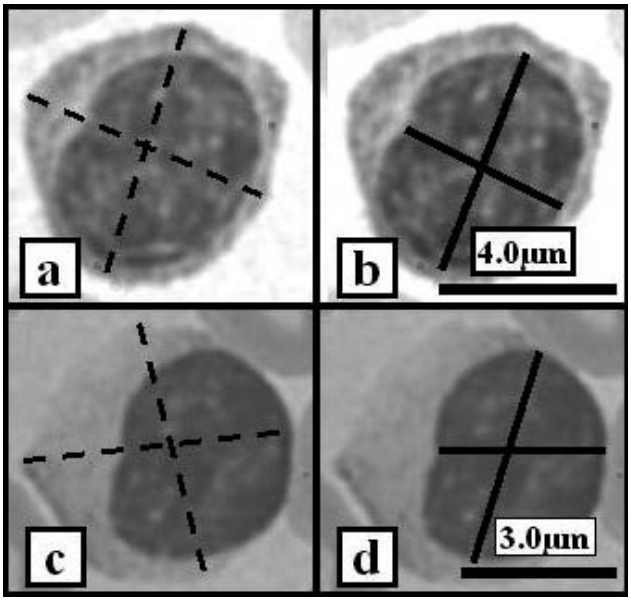

Fig 2. Leukaemic lymphocytic progenitor (**a**, **b**) and differentiated leukaemic lymphocyte (**c**, **d**). Cell body largest and smallest diameter (**a**, **c**), nuclear largest and smaller diameter (**b**, **c**). The nucleus in the progenitor occupies ~ 78 % of the cell body. The reduction of the cell body space occupied by the cell nucleus in a differentiated cell (~ 71 %) is less apparent.

of the cell space occupied by the nucleus is smaller than in progenitors (Table 1). However, in some cell lineages, for example in erythroid or lymphocytic lineages, such difference may be less apparent depending on the haematological disorder (Fig. 2).

In "anaemia with abnormal erythroblasts (sideroblasts)" of the myelodysplastic syndrome, the nucleus may occupy about 70 % of the cell space of erythroid progenitors, and in differentiated late erythroblasts, the nucleus still occupies about 60 % of the cytoplasm. In B-cell chronic lymphocytic leukaemia, the nucleus of lymphocytic progenitors occupies about 80 % of the cell space and in terminally differentiated lymphocytes, the cell space occupied by the nucleus is only slightly reduced to 75 %. On the other hand, the condensation state of enlarged heterochromatin

Table 1. Heterochromatin condensation state, nucleus : cell body, nucleolus : nucleus body ratios, number of nucleolar fibrillar centres, mitotic activity in progenitors, and terminal developmental stages of the granulocytic and erythroid cell lineages in patients suffering from CML

Cell stage	Ctr : Prph HChCS R	LNu : LCy R ×100	LNo : LNu R ×100	NoFC	Mit
Progenitors					
Myeloblasts	> 1.1*	81.8 ± 1.2%	22.4 ± 1.5%	~10	+
Proerythroblasts	> 1.1	75.2 ± 6.6%	28.1 ± 2.0%	> 10	+
Terminal differentiation stages					
Segmented granulocytes	< 1.1	33.8 ± 7.6%	6.6 ± 1.0%	< 5	0
Late erythroblasts	< 1.1	44.8 ± 6.6%	12.6 ± 0.7%	< 5	0

LCy – largest cell diameter, LNo – largest nucleolar diameter, LNo : LNu R x 100 – approximate percentage of the nuclear space, LNu – largest nuclear diameter, LNu : LCy x 100 – approximate percentage of the cell space, Mit – mitotic division, NoFC – nucleolar fibrillar centres, Nu – nuclei, R – ratio, * – in myeloblasts characterized by premature terminal differentiation, the Ctr : Prph HChCS R is smaller than 1.1

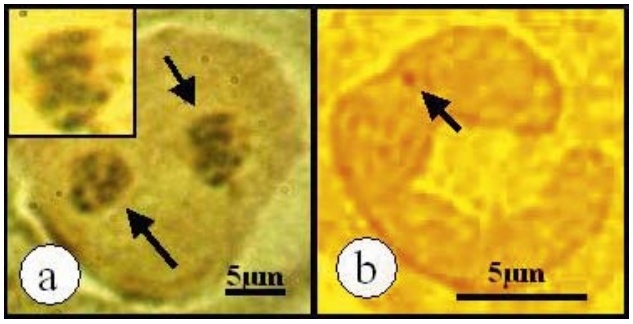

Fig. 3. Fibrillar centres in nucleoli (arrows) of a leukaemic granulocytic progenitor (**a**) and differentiated cell (**b**). (**a**) Multiple fibrillar centres in nucleoli are present in the granulocytic progenitor (insert) in comparison with the micronucleolus of the differentiated cell that contains only one fibrillar centre (**b**, arrow). Silver reaction and computer processing (Smetana et al., 1999b).

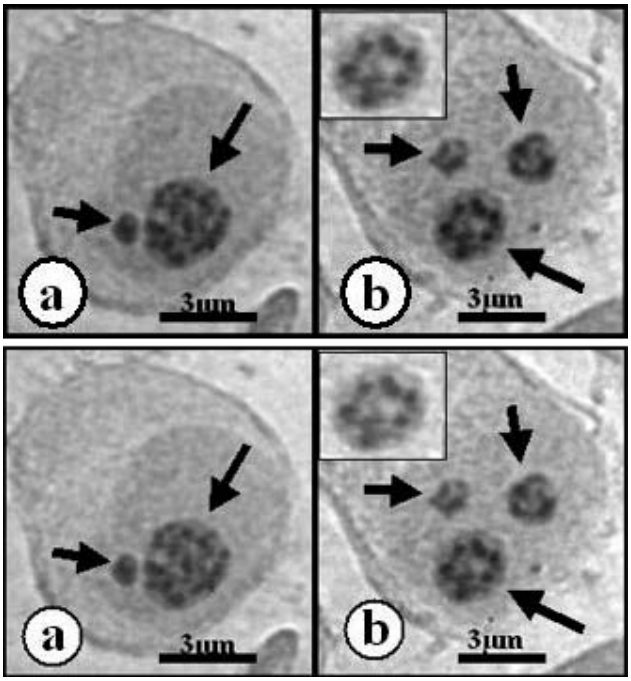

Fig. 4. Fibrillar centres in nucleoli (arrows) of granulocytic progenitors in growing (**a**) and ageing (**b**) cell cultures. Fibrillar centres in the ageing progenitor translocate to the nucleolar periphery (**b**) and are apparently reduced in number (insert). Silver reaction and computer processing (Smetana et al., 1999b).

territories in terminally differentiated cells is more distinct and the nucleolar size is also reduced.

2. The nucleolar size also decreases during differentiation and terminal differentiation (Figs. 3, 4), the nucleolus : nucleus ratio is reduced and dominant nucleoli disappear. Thus, the nuclear space occupied by nucleoli in terminally differentiated cells is reduced in comparison with progenitor cells (Table 1, Smetana et al., 2019; 2020a). It seems to be also interesting that the size reduction of the nuclear space occupied by nucleoli is smaller than the reduction of the number of nucleolar fibrillar centres. As mentioned above, fibrillar centres reflect the nucleolar transcription of ribosomal RNA and preribosomal assembly (Smetana, 2011; Smirnov, et al., 2016; Weipotshammer and Schöfer, 2016; Penzo et al., 2019). Therefore, such difference might be due to multifunctional features of nucleoli in addition to that function (Pederson, 1998; Lo et al., 2006; Boivert et al., 2007). Concerning nucleoli, it should also be noted that experimentally induced ageing of cultured granulocytic progenitors is accompanied by translocation of fibrillar centres to the nucleolar periphery (Fig. 4) and expulsion from the nucleolus (Smetana et al., 2004).

Similar translocation was also noted in leukaemic granulocytic progenitors in bone marrows of leukaemic patients with CML. However, such translocation in leukaemic patients was less frequent (Smetana et al., 2005). In addition, most of terminally differentiated cells of granulocytic or erythroid lineages possess only micronucleoli, which represent the last steps of nucleolar development and reflect cessation of nucleolar RNA transcription. In abnormal erythropoiesis, such nucleoli may also disappear even in less differentiated cells (Smetana et al., 1999a). It should be noted again that the nucleolus is generally involved in cell ageing, senescence and programmed cell death through decreasing RNA transcription and ribosome subunit assembly (Smetana, 2003; 2011; Hein et al., 2012).

3. In lineage progenitors, such as granulocytic or erythroid lineages, during further differentiation there is a marked increase of the heterochromatin condensation state, especially at the nuclear periphery (Smetana et al., 2008, 2011). In progenitors with differentiation potential, the heterochromatin condensation state in the nuclear periphery is smaller than in nuclear central or pericentric regions (Fig. 5). On this occasion it should be mentioned that heterochromatin regions are sites of silent genes and participate in cell genomic stability (Alcobia et al., 2000; Cremer and Cremer, 2005; Kosak et al., 2007; Cohen and Jia, 2014). It should also be noted that some abnormal granulocytic progenitors exhibit a marked similarity of heterochromatin condensation state in central and peripheral nuclear regions characteristic of differentiated granulocytes (see below). These cells are apparently in the state of premature terminal differentiation – senescence – and are rare in CML but frequent and dominant in acute myeloblastic leukaemia (Smetana et al., 2015, 2020b). Mature granulocytes in this type of leukaemia might represent a differentiation product of progenitors with less condensed heterochromatin at the nuclear periphery, as it was presumed previously (Smetana et al, 2020b; Zjablovskaja and Florian, 2020b).

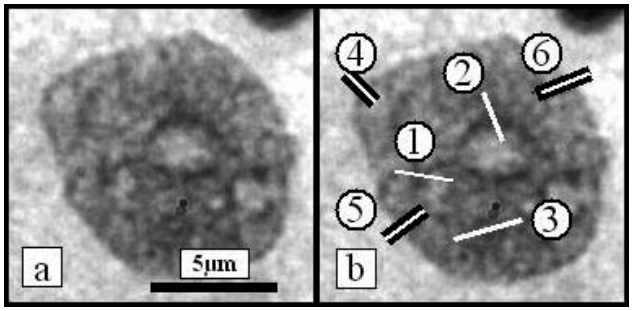

Fig. 5. The chromatin structure in a leukaemic granulocytic progenitor (**a**) with lines of the density measurement expressed in arbitrary density units (**b**). The calculated central [(1 + 2 + 3) : 3] to peripheral [(4 + 5 + 6) : 3] heterochromatin condensation ratio was 1.38, i.e. above 1.1 (see Table 1 and Smetana et al., 2011, 2020b). Cytochemical method for DNA and computer image processing.

In fully differentiated cells such as granulocytes or late erythroblasts, the heterochromatin condensation state is very high and similar in both central and peripheral regions (Fig. 6, Smetana et al., 2008, 2020b). It should also be mentioned that the cell senescence was accompanied by formation of heterochromatin regions that were designated senescence-associated heterochromatin foci (Narita et al., 2003). In the erythroid cell lineage, terminally differentiated erythroblasts lose nuclei with a highly condensed heterochromatin state. It is generally known that such cells without nuclei survive as proerythrocytes or reticulocytes (Simpson and Kling, 1967; Bessis, 1973). During such state, the remaining cytoplasmic components such as mitochondria, ribosomes, endoplasmic reticulum decrease and finally disappear. It should be mentioned that the loss of nuclei was also noted in less differentiated erythroblasts in pathological or experimentally induced erythropoietic disorders (Akebayashi, 1967). The appearance of polychromatophylic erythrocytes still containing RNA in the cytoplasm apparently reflects such phenomenon and reflects the premature nuclear loss and senile state (Bessis, 1973).

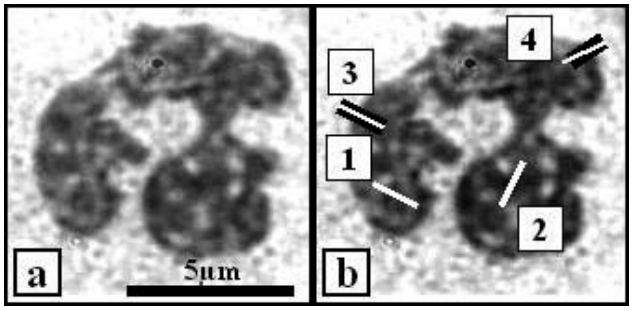

Fig. 6. The chromatin structure in a leukaemic terminally differentiated granulocyte with the segmented nucleus (**a**) and density measurement lines (**b**). The calculated central [(1 + 2) : 2] to peripheral [(3 + 4) : 2] heterochromatin condensation ratio was 0.97, i.e. below 1.1 (see Table 1 and Smetana et al., 2011, 2020b). Cytochemical method for DNA and computer image processing.

4. It is generally known that cells in terminal differentiation stages of granulocytic and erythroid lineages lose the ability to divide (Table 1, Bessis, 1973). On this occasion it should be mentioned that ageing is also terminated by reduction and loss of the division potential, possibly due to the shortening of chromosomal telomeres (Allsopp and Harley, 1995; Drummond et al., 2007; Sanders and Newman, 2013; Wang et al., 2018). However, the present notes do not deal with mitotic chromosomes. The shortening or altered expression of telomeres would need a different methodological approach, similarly as the study of other factors and cell compartments participating in the ageing process (Drummond et al., 2007; Lopez-Otin et al., 2013; Sanders and Newman, 2013; Smetana Jr. et al., 2013, 2018, Ahmad, 2018; Wang et al., 2018; Groark and Young, 2019). On the other hand, the telomere shortening may not be related to the biological age of the whole body or age-related diseases. However, it should be mentioned that the whole body ageing and related diseases depend on the cell ageing and senescence of certain cell lineages (Sanders et al., 2013; Smetana Jr. et al., 2013; van Deursen, 2014).

Conclusive remarks

Summarizing morphological notes above (see also Table 1), it seems to be likely that the cell differentiation and terminal differentiation especially in erythroid or granulocytic lineages reflect the ageing of the lineage progenitors. The terminal differentiation stages of these lineages actually represent senescent cells without the mitotic ability. The abnormal ageing of progenitor cells may appear as one of the "dysplastic" phenomena of premature terminal differentiation states (Bessis, 1973; Smetana et al., 2020b). The premature terminal differentiation of granulocytic progenitors is more frequent in patients suffering from acute myeloblastic leukaemia or myelodysplastic syndrome characterized by abnormal and altered differentiation of these cells (Cáceres-Cortés, 2013; Smetana et al., 2020b). Such progenitor cells exhibit a heterochromatin condensation state similar to that in terminally differentiated cells of granulocytic or erythroid lineages without the division ability. Most definitions of ageing (see above) also indicate that terminal senescent cell states of this process are characterized by loss of the mitotic potential. On this occasion it should be mentioned that in the generally known abnormal erythropoiesis, even less differentiated and immature erythroblasts may lose nuclei similarly as "normal" fully terminally differentiated nucleated erythroblasts of the erythroid cell lineage. Thus, it is clear that such abnormal cells miss the ability to multiply. On the other hand, the background of ageing and differentiation is very complicated and requires a different approach than

the simple microscopic morphology of single cells (Sanders and Newman, 2013; Ahmad, 2018; Groark and Young, 2019). However, the morphology and clinical cytology might still contribute, with complementary data, to more sophisticated complex studies of that topic. In addition, the morphological approach facilitates the study of the main nuclear components of a single cell in various states, including the differentiation steps or ageing.

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Competing interests

The authors declare that no competing interests exist.

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