Technical Note

Cell Dysplasia – Cell Dysplastic Features (A Morphological Note)

(cell dysplasia)

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Abstract. Cell dysplasia is a currently used term describing various cellular developmental abnormalities visible by microscopy. However, detailed description of these developmental abnormalities might provide useful information not only on the cell state but also on the abnormal developmental steps of cell lineages, tissues and organs. The frequently noted visualized cell dysplastic features reflect nuclear- or nucleolar-cytoplasmic anarchy (asynchrony), premature heterochromatin condensation state, marked aneuploidy, abnormal nucleus-cytoplasm ratio, abnormality of cell organelles including mitochondria, abnormal presence or absence of cell lineage-specific granules, and formation of peripheral buds or blebbing on the cell surface. The description of these frequently occurring cell dysplastic features might also be helpful in recognizing and studying defined specific disorders of the "whole macro-body" expressed as a disease.

Introduction

According to the definition of the Cambridge Dictionary of Biology, dysplasia reflects abnormality of development (Fantes et al., 1990). Under the microscope, this phenomenon is characterized by a different cell size (anisocytosis), shape (poikilocytosis), increased cell pigmentation or stainability, and increased or decreased incidence of cell "normal or abnormal" mitotic activity. The cell "dysplastic" appearance is a usual expression and term widely used in clinical cytology including haematology. On the other hand, the cell dysplastic appearance reflects several cell states that might reflect malignant features or future malignant transformation (Anthony et al., 1973; Bessis, 1973). Thus, it might be very useful to use more exact terms for these cell states, because they may express different cell appearances that might be characteristic of abnormal and pathological states of the examined cell. In addition, a more exact definition of the frequent incidence of cell dysplastic phenomena might be useful for the entire cell lineage or state estimate, including the examined patient.

Methodological notes

Cell dysplastic features of early and terminal differentiation steps of blood and leukaemic cells were studied in the peripheral blood or bone marrow specimens of 20 patients suffering from chronic myeloid leukaemia (CML), 10 patients with acute myeloblastic leukaemia (AMbL), 10 patients with the myelodysplastic syndrome (MDS), 6 patients with chronic lymphocytic leukaemia (CLL) and 5 patients with multiple myeloma (MM). In addition, cell dysplastic features were also studied in cultured myeloblasts of the K256 lineage originating from CML, and HL 60 lineage originating from acute promyelocytic leukaemia. All studied cells, disregarding the state and therapy of patients or mentioned cell cultures, were selected from the microscopic image archive of the cytological laboratory of the Institute. The studied cells were originally visualized in peripheral blood or bone marrow smears and cultured cell lineages using current May-Grünwald – Giemsa-Romanovsky panoptic staining, cytochemical methods for DNA and RNA, nucleolar silver-stained proteins, and Prussian blue reaction for Fe in blood cells (Smetana et al., 1967, 1969; Undritz, 1972; Bessis, 1973; Ochs, 1998). For electron micro-

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Abbreviations: AMbL – acute myeloblastic leukaemia, CLL – chronic lymphocytic leukaemia, CML – chronic myeloid leukaemia, MDS – myelodysplastic syndrome, MM – multiple myeloma.

scopy, specimens were fixed in 1.6 % glutaraldehyde and/or 2 % osmium tetroxide, with post-fixation by uranyl acetate during dehydration with ethanol. Ultrathin sections cut by LKB Ultratome of specimens embedded in Epon-Durcupan mixture were stained with uranyl-acetate and/or lead citrate (de Harven, 1967, Smetana, 1970, Bessis, 1973). Micrographs captured using a Zeiss Jenalumar light microscope (Zeiss, Jena, Germany) or Philips 200 electron microscope (Philips, Amsterdam, Netherland) were further processed by specific software program Quick Photoprogram (Olympus, Tokyo, Japan).

The nuclear-cytoplasmic asynchrony (anarchy)

The nuclear-cytoplasmic asynchrony is a very frequent phenomenon expressed in numerous cell lineages characterized by abnormal and pathological differentiation and maturation process (Bessis, 1973). There are two variants. In the first variant, the nucleus "remains young" and the cytoplasm "is gradually maturing or fully mature" (Fig. 1). In this case, the heterochromatin DNA territories remain small, and the cytoplasmic space contains a variety of components characteristic of mature differentiation steps of the studied lineage (Smetana et al., 1973). In the second variant (Fig. 2, 3), the nucleus appears "mature" and frequently smaller, with large heterochromatin territories. The RNA basophilic content with abundant ribosomes in the cytoplasm remains high and cytoplasmic components characteristic of advanced steps of the studied cell linages are usually absent (Anderson, 1966; Smetana, 1970). Both these variants are easily identified, because the heterochromatin and RNA content are easily visible after visualization by widely used simple cytochemical methods for DNA and RNA or by panchromatic procedures containing basic dyes such as May-Grünwald – Giemsa-Romanovsky (Smetana et al., 1967, 1969; Undritz, 1972; Bessis, 1973; Ochs, 1998). Similarly, their identification is also simple by electron microscopy due to the known characteristic appearance of nuclear and cytoplasmic components. The background of both these phenomena of the nuclear-cytoplasmic asynchrony is very simple. According to the general knowledge, the large, condensed heterochromatin DNA territories express gene silencing and the RNA content in the cytoplasm reflects, to some extent, the cytoplasmic protein synthetic potential (de Robertis and de Robertis, 1987; Cremer and Cremer, 2006).

The nucleus-cytoplasmic asynchrony is very frequent in various malignant cell lineages including haematological malignancies. On the other hand, this phenomenon may also be characteristic of abnormal or damaged non-neoplastic or malignant cells such as large erythroblasts – megaloblasts (Bessis, 1973). It is also frequent

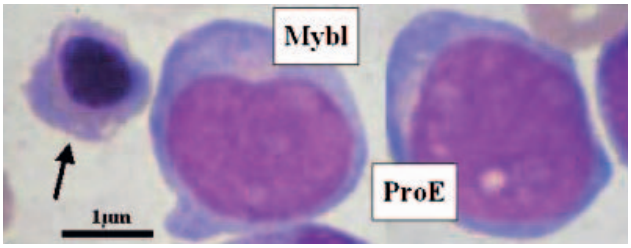

Fig. 2. Late terminal erythroblast with a highly condensed nucleus still possessed the basophilic cytoplasm similarly as early precursor cells of the erythroid cell lineage proerythroblast (ProE) and granulocytic cell lineage myeloblast (Mybl). MGGR staining procedure.

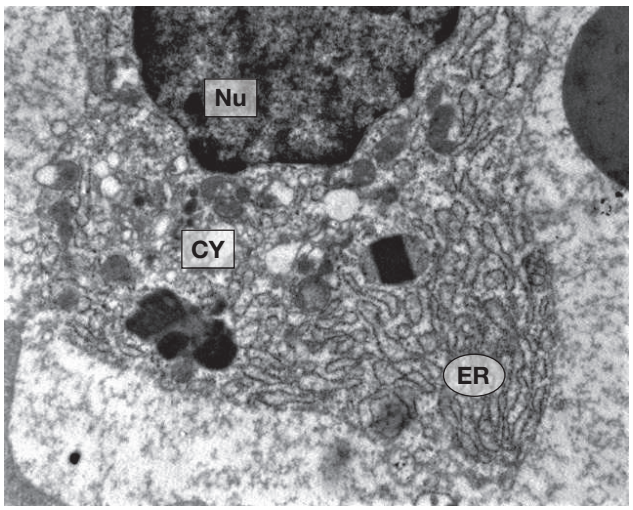

Fig. 1. Electron micrograph of a myeloma plasmacyte. The "immature nucleus" (Nu) with small heterochromatin regions at the nuclear membrane. The "mature" cytoplasm (Cy) contains characteristic abundant rough endoplasmic reticulum (ER). Ultrathin section, uranyl acetate and lead citrate staining.

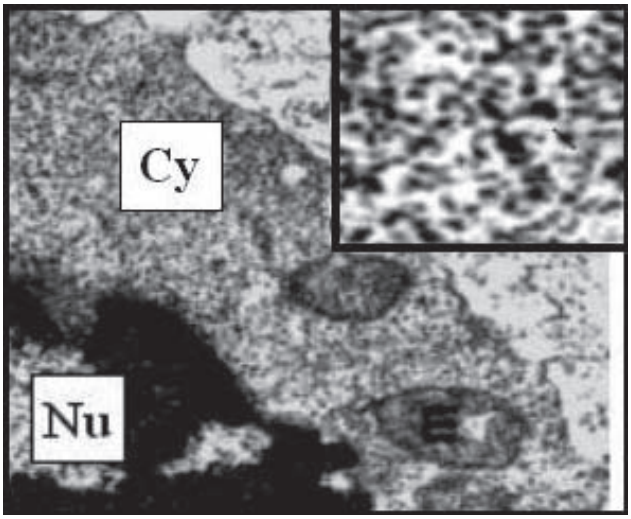

Fig. 3. Electron micrograph of a mature leukaemic lymphocyte with large, condensed heterochromatin regions of the "mature condensed cell nucleus" (Nu). The cytoplasm (Cy) still contains abundant ribosomes (large magnification in the insert). Ultrathin section, uranyl acetate and lead citrate staining.

in various somatic and haematopoietic cell lineages of patients with malignant tumours after cytostatic therapy. However, it seems to be also difficult to distinguish the nucleus-cytoplasmic asynchrony produced by the malignancy and cytostatic treatment, unless the patients did not receive such therapy before.

Other frequent types of cell dysplastic phenomena

Premature heterochromatin condensation in peripheral nuclear regions in early differentiation steps of granulocytic precursors – myeloblasts – indicates altered differentiation. This phenomenon is very characteristic and frequent in AML (Smetana et al., 2020). The marked aneuploidy seems to be a very important dysplastic phenomenon and frequently might be associated with cell malignancy (Thomas et al., 1992). On the other hand, it should be mentioned that the large size of the cell nucleus in cells with the mitotic potential should not always be considered as a cell dysplastic phenomenon, because it may just reflect the late stage of the cell cycle (Nagl, 1976). The abnormal nuclear-cytoplasmic ratio differences and changes may also be considered as a cell dysplastic phenomenon, especially when the ratio exhibits a marked variation and is different or larger in comparison with the corresponding "normal" surrounding cells. Such case is frequent in malignant cell lineages, where the incidence of this phenomenon may be helpful for identification of the malignant tumour or premalignant lesions (Watanabe et al., 1983). However, the nuclear-cytoplasmic ratio may be high even in fully differentiated cells of some cell lineages (Smetana et al., 2021, 2023).

Nuclear segmentation in the terminal differentiation steps is characteristic especially of blood granulocytes. It should be mentioned that these nuclear segments are always connected by intersegmental nuclear bridges. In contrast, the physiological nuclear segmentation in these or other nucleated blood cells is limited and absent in the Pelger-Huet anomaly. Such phenomenon as a genetically programmed or acquired dysplastic phenomenon may be expressed in haematological as well as other clinical disorders (O'Donnell et al., 1982; Liang et al., 2018). In contrast, "chaotic" segmentation reflecting nuclear fragmentation might reflect a regressive process, including the apoptotic one (Ries and Gersch, 1953; Martin and Cotter, 1994). In this case, nuclear segments, i.e., fragments, are not joined by intersegmental bridges and are characterized by heavily condensed chromatin without the characteristic structure. On the other hand, such nuclear fragmentation is morphologically different from the increased nuclear number in multinucleated cells. The latter in multinucleated cells might also be considered as a dysplastic phenomenon that apparently reflects cell polyploidy or malignancy (Undritz, 1972; Anthony et al., 1973).

The nucleolar-cytoplasmic anarchy (differentiation asynchrony) is expressed by weak nucleolar biosynthetic activity in early differentiation steps, resulting in premature differentiation or maturation of progenitors and precursors of various cell lineages. In haematological cytology, this phenomenon is generally known for leukaemic or abnormal erythroid lineages and is important from the diagnostic point of view (Smetana et al., 2010, 2022). It should be mentioned that the effect of cytostatic therapy might also be responsible for producing such dysplastic phenomena (Smetana et al., 1977). Morphologically, this phenomenon may appear as a reduction of the nucleolar size or structural abnormality of nucleolar main components. Dominant ring-shaped nucleoli present in advanced differentiated steps are characterized by a decrease in RNA transcription (Smetana, 2003). Such events may also be seen in early differentiated steps of an abnormal cell lineage, including the leukaemic one (Fig. 4). In addition, in some pathological cell lineages, nucleoli in less differentiated cells disappear, but the cytoplasmic basophilic features due to the presence of RNA – ribosomes – are still present (Norton et al., 1979; Smetana et al., 1999) (Fig. 5). On this occasion, it should also be mentioned that precursors of blood lineages may possess "active" and resting or inactive nucleoli in one and the same nucleus (Smetana et

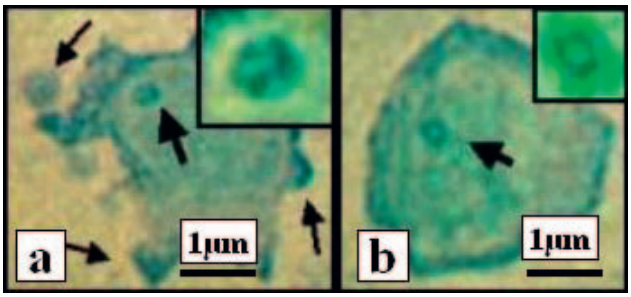

Fig. 4. Ring-shaped nucleoli in a precursor of the lymphocytic lineage – lymphoblast (**a**) and mature lymphocyte (**b**). The budding phenomenon (arrows) indicates the onset of programmed cell death of the lymphoblast (**a**). Staining for RNA with acidified methylene blue.

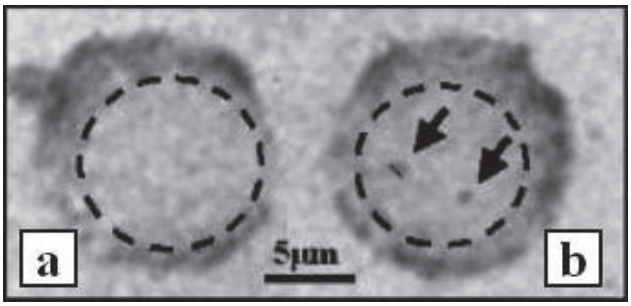

Fig. 5. Premature loss of micronucleoli in a polychromatic erythroblast (**a, b**) Micronucleoli (arrows) in a polychromatic erythroblast. The small thin line at the micronucleolus (large arrow) – < 1.0 µm. Staining for RNA with acidified methylene blue.

al., 1997, 1998). In early precursors of acute myeloid leukaemia cell lineages, such cells were apparently less influenced by the anti-leukaemic therapy (Smetana et al., 1998), presumably due to the re-activation of originally resting ring-shaped nucleoli. It should be noted that large "active nucleoli" in an "immature nucleus" may be present in cells with fully differentiated cytoplasm containing characteristic structural components. In the cytomorphological laboratory, such events were frequently noted in myeloma patients and are known for stimulated or leukaemic lymphocytes (Smetana et al., 1973, 2022).

The persistence of primary granules and/or absence of specific granules or other cytoplasmic components were observed in differentiated cells (Bessis, 1973). Such dysplasia is frequent in leukaemic cells. However, it may also be produced by a variety of chemical or physical factors including radiation. The persistence of primary granules in differentiated neutrophil granulocytes (Fig. 6) or "RNA-containing basophilic granules" in erythroblasts or erythrocytes reflects toxic alteration of the differentiation process in both these cell lineages. Concerning mitochondria, iron deposits are characteristic of refractory anaemia of patients with ringed erythroblasts suffering from the myelodysplastic syndrome (Mufti et al., 2008) (Fig. 7). Such abnormalities were already observed in the early differentiation step of erythroblasts such as proerythroblasts (Fig. 8). It should also be noted that malignant cells may exhibit shape or structural mitochondrial abnormalities, which are frequent in leukaemic lymphocytes (Smetana, 1970). However, these abnormalities may be present only in a limited number of cells. The peripheral buds and blebbing on the cell surface at the onset of the apoptotic process (Fig. 9) might also be considered as dysplastic (Plunkett, 1995; Kerr, 2002; López-Hernández, 2021). However, these known surface phenomena may be related to other cell activities such as programmed cell death, cell migration and cancer cell invasion to surrounding tissues (Fackler and Grosse, 2008). On this occasion, it should be mentioned that there is also a variety of other less distinct cell structural abnormalities that might be considered as dysplastic features. They are visible using more sophisticated visualization approaches, including electron or fluorescence microscopy. Fortunately, these phenomena are de-

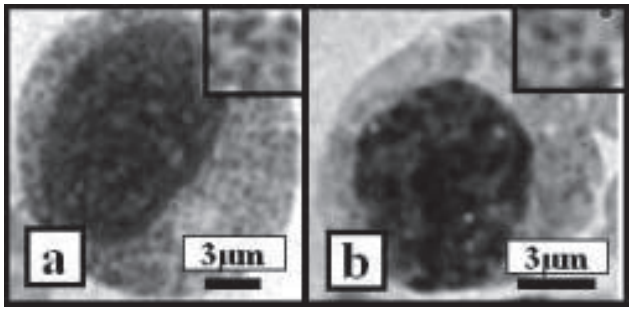

Fig. 6. Azurophilic granules (inserts) characteristic of promyelocytes (**a**) are present in the next differentiation step of the neutrophil granulocyte lineage – myelocyte (**b**). MGGR staining.

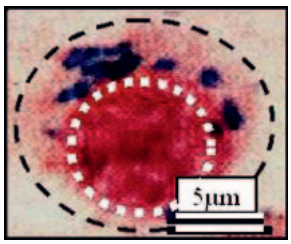

Fig. 7. Extranuclear mitochondrial Fe-containing inclusions located in mitochondrial bodies of a differentiating late erythroblast, i.e., sideroblast. Black line – cell body dashed black outline, white line – dotted nuclear white outline. Perl's reaction for Fe.

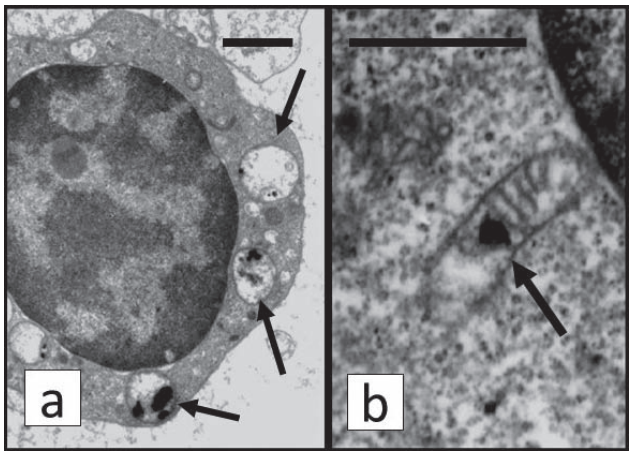

Fig. 8. Electron micrographs of ultrathin sections of a late erythroblast (**a**) and erythroid precursor – proerythroblast (**b**). In the late erythroblasts, iron deposits are found in heavily altered mitochondrial bodies (arrows). In the early erythroid precursor, the mitochondrial body is less altered despite the presence of the dense iron deposit (arrow). Uranyl acetate and lead citrate staining. Bold black bars represent 2 µm.

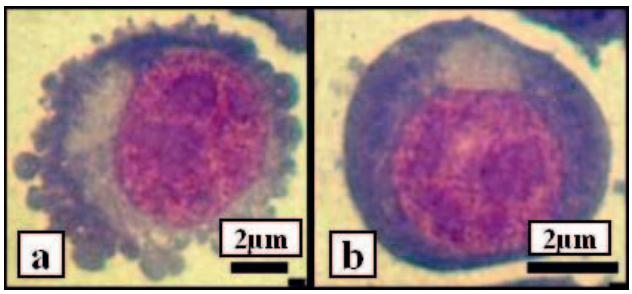

Fig. 9. The budding (blebbing) phenomenon on the surface of a leukaemic myeloblast cultured in the presence of anti-leukaemic drug – imatinib (**a**) in comparison with a control myeloblast (**b**). MGGR staining.

scribed by specific descriptive terms that facilitate easier interpretation, especially when they are influenced by possible various drug effects (Daskal, 1979). In addition, "dysplastic cells" may contain a variety of unusual structures and inclusions, which are not present in "normal and healthy cells" and are not of the intracellular origin.

Conclusion

It seems to be clear that the term "cell dysplasia" covers visible abnormality or pathology of various cell components including morphology, structure and cytochemistry. It should be mentioned that this visibility depends on the methodological approach used for visualization of the studied cell components. The incidence of dysplastic cell components in the examined cells may be very variable. However, a large incidence of various dysplastic cell components seems to be very characteristic of a specific state and alteration of developmental states of the cell lineage in the examined human and animal "macro subjects." The references to them are listed under different terms in numerous publications, including specialized journals, monographs, and even textbooks in various bio-medical fields. Actually, a more exact definition of a cell dysplastic phenomenon might be helpful in providing complementary information on the examined cell state. In addition, it might be very useful to recognize the state of the cell lineages in the examined tissues, organs and "macro subjects" with various disorders.

Competing interests

The authors declared no competing interests.

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