

A Short Note on Micronucleoli in the Course of Terminal Maturation of Human Erythroblasts

(micronucleoli / human erythroblasts / terminal differentiation / RNA staining and silver reaction for AgNOR proteins)

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Abstract. The incidence of micronucleoli in the course of terminal differentiation of human erythroblasts was studied by the cytochemical procedures for demonstration of RNA and characteristic proteins of interphase AgNORs. The last dividing stages of the erythroid lineage – polychromatophylic erythroblasts – characterized by the presence of micronucleoli exhibited significantly larger values of the nucleolar coefficient in specimens stained for AgNOR proteins than in those stained for RNA. In addition, both these and terminal non-dividing nucleated stages of the erythroid lineage – orthochromatic erythroblasts – possessed micronucleoli after staining for RNA in a much smaller percentage of cells than after staining for AgNOR proteins. Thus, both these observations indicate that micronucleoli in the course of terminal maturation of erythroblasts apparently lose the nucleolar RNA detectable by the light microscopic cytochemistry. In addition, silver-stained micronucleoli – nucleolar remnants – were also noted in erythroblasts expelling the nucleus.

As has been demonstrated in previous studies, micronucleoli represent the terminal stages of nucleolar development in maturing blood cells (see Smetana, 1980; Schwarzacher and Wachtler, 1983). In the light microscope, visualization of micronucleoli was facilitated by introduction of cytochemical procedures for selective demonstration of some main nucleolar components such as RNA and nucleolar silver-stained proteins characteristic for nucleolus organizer regions (e.g. Likovský and Smetana, 2000). According to the autoradiographic studies micronucleoli do not incorporate tritiated RNA precursors (see Smetana, 1980). Moreover, the structural organization of their components seen by the electron microscope reflects the cessation of the

main nucleolar biosynthetic function, i.e. transcription of the ribosomal RNA (Smetana et al., 1975; Zatschina et al., 1988). On the other hand, recent few studies on mature monocytes or granulocytes suggested that some micronucleoli and nucleolar remnants do not contain RNA but still possess characteristic nucleolar proteins (Smetana et al., 1997; Likovský and Smetana, 2000).

The present study was undertaken to provide more information on further development of micronucleoli in polychromatophylic and orthochromatic erythroblasts in the course of terminal maturation of these cells. Polychromatophylic erythroblasts represent the advanced maturation and last dividing stages of nucleated erythroid cells (Erslev, 1972; Lessin and Bessis, 1972). Orthochromatic (late, pycnotic) erythroblasts represent the non-dividing terminal stages of erythroblastic maturation, which lose nuclei (Erslev, 1972; Lessin and Bessis, 1972). Both polychromatophylic as well as orthochromatic erythroblasts in patients suffering of refractory anemia of the myelodysplastic syndrome (Benett et al., 1982) represent a very convenient model for such study. They either lose nucleoli or contain only micronucleoli (Smetana et al., 1999a), which in specimens stained for RNA or characteristic silver-stained nucleolus organizer region (AgNOR) proteins (Busch, 1998; Ochs, 1998) appear as small but distinct single bodies (Smetana and Likovský, 1984; Grotto et al., 1991). The results of the present study indicated that micronucleoli lose RNA, because they were visible in a larger number of polychromatophylic and orthochromatic erythroblasts after visualization with the silver reaction for AgNOR proteins than in specimens stained for RNA. In addition, the values of the nucleolar coefficient of polychromatophylic erythroblasts were larger after staining for AgNOR proteins than after staining for RNA.

Material and Methods

Micronucleoli in polychromatophylic and orthochromatic erythroblasts were studied in bone marrow smears of 5 patients suffering from refractory anemia of the myelodysplastic syndrome (Benett et al., 1982) who were not treated with any therapy that might induce nucleolar changes in these cells. On this occasion it

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Abbreviation: AgNOR(s) – silver-stained nucleolus organizer region(s).

should be mentioned that the bone marrow samples were originally taken for the diagnostic investigation at the admission to the clinical department, and the research program has been approved by the Ethic Committee of the Institute. Micronucleoli in parallel unfixed bone marrow smears were visualized by the cytochemical procedure for demonstration of RNA (Smetana et al., 1969; Ochs, 1998) and by the silver reaction for demonstration of AgNOR proteins using the colloid developer (Howell and Black, 1980; Smetana et al., 1999b). The cytochemical procedure for demonstration of RNA using acidified buffered methylene blue was carried out on smears not older than 24 h, but that for AgNOR proteins on smears older than one week. Under these conditions micronucleoli were distinct and not masked by other surrounding nuclear components. The incidence of micronucleoli and the values of the nucleolar coefficient were determined by evaluation of at least 200 nucleoli in investigated cells for each investigated person. The number of nucleoli per cell expressed by the values of the nucleolar coefficient was calculated by dividing the number of nucleoli by the number of cells in which it was counted (Gonzalez-Guzman, 1949). The polychromatic and orthochromatic erythroblasts were identified by the degree of the cytoplasmic basophilia reflecting the presence of RNA (in specimens stained for RNA) and condensed nuclear chromatin structure (in specimens stained for AgNOR proteins). The phase contrast microscopy was helpful for cell identification when it was necessary. In addition to the conventional microscopy, the microphotographs were enlarged and analyzed by image processing LUCIA® (Laboratory Imaging, Prague, Czech Republic) to distinguish the density of the specific positivity for RNA or AgNOR proteins from the unspecific background (see Figs. 1 – 3). On the other hand, on this occasion it should be mentioned that in the phase contrast the perinucleolar density was less apparent or not visible in nuclei with a highly condensed chromatin structure or when micronucleoli were masked by the condensed chromatin structure (Figs. 2, 3).

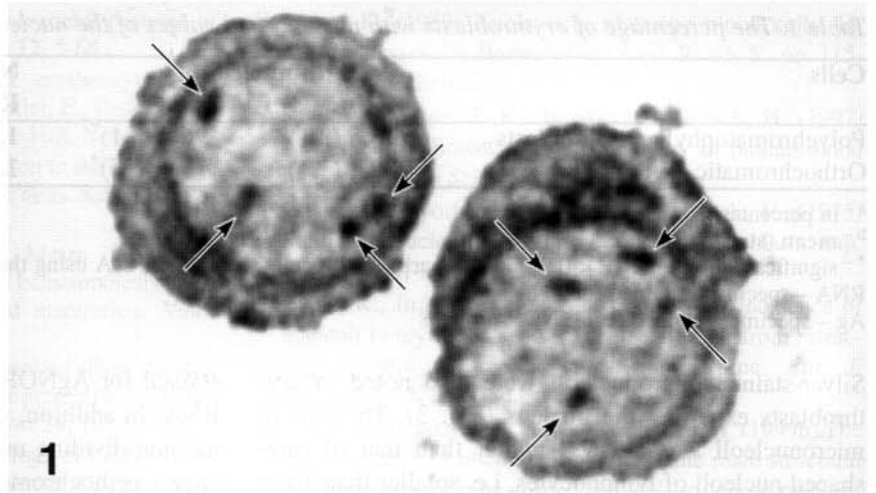


Fig. 1. Micronucleoli (arrows) in polychromatophytic erythroblasts stained for RNA. Magnification approx. 5000x. In this and following figures the background was reduced and the size increased by the image processor LUCIA®.

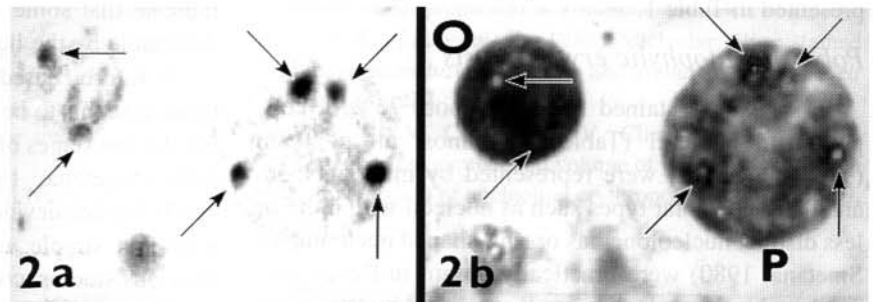


Fig. 2. Micronucleoli (arrows) in a polychromatophytic (P) and orthochromatic (O) erythroblast stained for AgNOR proteins. 2a) – conventional bright-field-light microscopy, 2b) – phase-contrast microscopy. Note the highly condensed nuclear chromatin in the orthochromatic erythroblast. The larger perinucleolar density was more apparent in the polychromatophytic erythroblast in the nuclear region with less condensed chromatin structure. Magnification approx. 4300x.

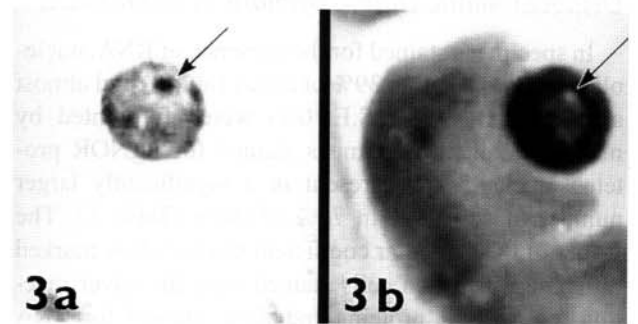


Fig. 3. A micronucleolus (arrow) in an orthochromatic erythroblast with highly condensed nuclear chromatin in the process of nuclear expulsion. 3a) – conventional bright-field-light microscopy, 3b) – phase-contrast microscopy. Magnification approx. 3800x.

Results

Micronucleoli of polychromatophytic and orthochromatic erythroblasts in specimens stained for both RNA (Fig. 1) and AgNOR proteins (Fig. 2) appeared as small but distinct and solitary bodies within the nucleus.