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Accepted after revision: November 2, 1992

Acta Haematol 1993;89:110-111

Interpretation of the AgNOR Pattern in Hematologic Cytology

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Nucleolar organizer regions (AgNORs) have been widely studied in a variety of neoplasias, but only a few investigations deal with AgNORs in blood diseases. We therefore read with interest the study by Nakamura et al. [1] on AgNORs in acute leukemias, and would like to comment on the subject of this article in the light of our own studies on AgNORs in bone marrow smears. In many investigations AgNOR spots have been counted individually, as has been done by Nakamura et al. Recent papers, however, emphasize the importance of additional criteria, such as morphologic differences between AgNORs, quantification of the silver-stained area, or separate counting of extra- and intranucleolar silver-stained spots [2-9]. It has been shown for prostatic neoplasias [2,10,11], for melanocytic skin lesions [9], as well as for other proliferating tissues [8] that a combined quantitative-qualitative study of AgNORs, or morphometry of the silver-stained areas, is superior to a simple count of AgNOR spots. According to Crocker et al. [4], in a study on hemopoietic cells, we divided AgNORs into: (1) Clusters of dots within a matrix (nucleoli of cells in proliferation); (2) small dots scattered throughout the nucleoplasm not associated with a nucleolus, and (3) condensed argyrophilic structures corresponding to a small nucleolus of non-proliferating cells. In normal hemopoiesis, each cell type had its own pattern of clusters and dots, according to the stage of maturation. Clusters were only observed in proliferating cells, while in mature non-proliferating cells only dots were seen. These results suggest physiological differences between clusters and dots and justify separate counting. We believe therefore that in leukemic cells, clusters and dots should not be counted together and the result cannot be compared with the number of dots in mature granulocytes.

The AgNOR staining technique is very sensitive to incubation time and temperature. Minor alterations of these parameters can lead to enlargement and confluence of the silver-stained spots to a large silver-stained area in the nucleolus, which artificially reduces the AgNOR count [6]. We think that this was the case in the report of Nakamura et al. [1], because in their figure 1 the nucleoli of the leukemic blasts are stained homogeneously. We are sure that with a slightly shorter incubation time the authors could have shown groups of silver-stained spots surrounded by a light matrix in the nucleoli, as has been demonstrated by us for normal [12]

Fig. 1. AgNOR – staining of bone marrow smear in acute myeloid leukemia. Blast nuclei contain at least one large cluster of silver-stained spots corresponding to a nucleolus. $\times 525$. and leukemic blasts (fig. 1) and by others for various tissues [2-9,12].

The AgNOR area is inversely related to the cell duplication time [7] and correlated to the number of Ki-67-positive cells in tumors [7, 13]. Ki-67 labels cells in the second half of the G₁, in S, G₂ and M phases [14] while bromodeoxyuridine labels only cells in the S phase. Normal myeloblasts and blasts of acute myeloid leukemia have a similar S phase time but a different cell cycle time [15]. We think therefore that in acute leukemia AgNORs should rather be compared with the percentage of Ki-67-stained blasts than with the number of bromodeoxyuridine-positive cells.

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