Nucleoli in Human Early Erythroblasts (K2, K1, K1/2 Cells)

(nucleoli / human early erythroblasts)

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Abstract. Human early erythroid precursors classified according to the nuclear size were studied to provide information on nucleoli in these cells using simple cytochemical procedures for demonstration of RNA and proteins of silver-stained nucleolar organizers. K2 cells with nuclear diameter larger than 13 µm and K1 cells with nuclear diameter larger than 9 µm corresponding to proerythroblasts and macroblasts (large basophilic erythroblasts) mostly possessed large irregularly shaped nucleoli with multiple fibrillar centres representing "active nucleoli". K1/2 cells with nuclear diameter smaller than 9 µm corresponding to small basophilic erythroblasts were usually characterized by the presence of micronucleoli representing "inactive nucleolar types". On the other hand, a few K1/2 cells contained large nucleoli with multiple fibrillar centres similar to those present in K2 cells and thus appeared as "micro-proerythroblasts". The nucleolar asynchrony expressed by the presence of large irregularly shaped nucleoli with multiple nucleoli (active nucleoli) and ring-shaped nucleoli (resting nucleoli) in one and the same nucleus of K2 or K1 cells was not exceptional and might reflect a larger resistance of these cells to negative factors influencing the erythropoiesis. The intranucleolar translocation of silver-stained nucleolus organized regions was noted in K2 cells and might indicate the premature aging of these cells without further differentiation. More studies, however, are required in this direction.

The erythroid cell lineage was frequently used for studies of cell differentiation and maturation because differentiation and maturation stages of erythroblasts are well defined. Previous „classical" studies and imaginations on the differentiation and maturation stages of this lineage called erythron were based on the nuclear size (Weicker, 1954; Stobbe, 1959). According to these studies early differentiation stages represented by precursor stem cells were characterized by a large nuclear size, which gradually diminished during further development to more mature and differentiated erythroblasts. It seems to be clear that such development is accompanied by the condensation of the nuclear chromatin structure and transformation of large nucleoli to micronucleoli with a reduced number of silver-stained nucleolar organizers (AgNORs) (Undritz, 1972; Smetana and Likovský, 1984; Grotto et al., 1991). However, limited information exists on nucleoli of human K2 cells possibly corresponding to precursor stem cells – proerythroblasts and K1 or K1/2 stages of the erythroblastic early differentiation stages corresponding to macroblasts (large basophilic erythroblasts, basophilic erythroblast I) or basophilic erythroblasts (basophilic erythroblasts II, polychromatic erythroblast I) (Rind et al., 1958; Stobbe, 1959; Bessis, 1972). On the other hand it is known that proerythroblasts and some macroblasts or basophilic erythroblasts also possess large and irregularly shaped nucleoli (Smetana, 1980). Therefore, the present study was undertaken to provide more information on nucleoli in human K2, K1 and K1/2 erythroblasts classified according to the nuclear size (see Stobbe, 1959). These differentiation stages of the erythroid cell lineage were studied in bone marrow of patients with a satisfactory number of erythroid precursors but without apparent signs of the erythropoietic alteration and abnormality. The studied patients suffered from the chronic phase of myeloid leukaemia and were previously treated with imanitib mesylate. As it is generally known, the target of this drug is represented by tyrosin kinase of leukaemic granulocytic precursors and the erythroid lineage is less altered or unaltered in comparison with other blood cell lineages including the megakaryocytic one (Braziel et al., 2002; Zonder et al., 2003).

The results indicated that large irregularly shaped nucleoli were present in K2 or K1 stages. However, these cells may occasionally contain only micronucleoli which were mostly present in K1/2 stage. On the other hand, some K1/2 cells also possessed large irregularly shaped nucleoli and resembled proerythroblasts, but of a markedly reduced size. In addition, some K2 or K1 cells possessed large nucleoli with translocation of AgNORs. The nucleolar asynchrony, i.e. the presence of both large irregularly shaped nucleoli and ring-shaped nucleoli in K2 and K1 erythroblasts, was not exceptional.

Material and Methods

Nuclei and nucleoli in bone marrow erythroblasts were studied in 10 patients suffering from the chronic phase of chronic myeloid leukaemia who were treated

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Abbreviation: AgNORs – silver-stained nucleolar organizers.

previously with imanitib mesylate and possessed a satisfactory number of erythroblasts. Nucleoli were visualized by simple cytochemical procedures for the demonstration of RNA (Smetana et al., 1969; Ochs, 1998) and AgNOR proteins (Smetana et al., 1999a) combined with phase contrast microscopy under conditions which facilitated to see clearly the nucleolar body. On this occasion it should be mentioned that measurements did not show substantial differences between the nuclear diameter of early erythroid precursors in specimens stained for RNA (11.8 μm, standard deviation 1.3) and AgNOR proteins (11.2 μm, standard deviation 1.4).

Early erythroblasts were classified according to the nuclear diameter to K2 cells (> 12 μm), K1 cells (> 9 μm) and K1/2 cells (< 9 μm). These values were close to those in the original model proposed by Weicker (1954) in which the nuclear diameter of K2 erythroblasts was 13 μm, that of K1 erythroblasts – 10.3 μm and that of K1/2 erythroblasts – 8.2 μm (see also Stobbe, 1959). The nuclear size was measured and the microscopic images were further processed using Olympus Quick Photo program (Olympus, Tokyo, Japan). Nucleoli were classified as irregularly shaped and large (> 1 μm), or micronucleoli, the size of which was about 1 μm or smaller. In addition, the incidence of the nucleolar asynchrony represented by the presence of large irregularly shaped nucleoli and ring-shaped nucleoli with RNA only in their peripheral part in one and the same nucleus (Smetana et al., 2002) was evaluated in those of early erythroblasts that possessed the former types of nucleoli. On this occasion it should be noted that large irregularly shaped nucleoli are considered to be active and ring-shaped nucleoli resting in respect of the nucleolar biosynthetic activities (Smetana et al., 2002). The number of evaluated cells for each group of early erythroblasts in individual patients was variable, ranging from 20 to 50, because of generally known small incidence of early differentiation stages of erythroblasts in human bone marrow. However, the relatively small standard deviation of the nucleolar coefficient (see Results) facilitated the evaluation of each group of early erythroblasts in all investigated patients.

Results

As expected, all three classes of early erythroid precursors, i.e. K2, K1 and K1/2 erythroblasts, possessed very basophilic cytoplasm (Figs. 1–6). K2 and K1 erythroblasts corresponding to proerythroblasts were characterized by a fine chromatin structure without large chromatin chromocentres, chromatin clusters and clumps (not shown). The values of the nucleolar coefficient, i.e. the number of nucleoli per cell, was 2.0 (standard deviation 0.2) for K2 and K1 cells (proerythroblasts and large basophilic erythroblasts) and 2.7 (standard deviation 0.2) for K1/2 cells as determined in bone marrow smears using the cytochemical procedure for demonstration of RNA.

The results are summarized in Table 1. Nucleoli in K2 and K1 cells were usually large and irregularly shaped (Figs. 1, 2). AgNORs in K2 or K1 cells were in clusters (Figs. 5, 6) which apparently corresponded to nucleoli stained for RNA (Smetana and Likovský, 1984; Grotto et al., 1991). The larger nucleolus of nucleoli was apparently dominant and possessed a higher number of AgNORs than others (Figs. 5, 6, see also Smetana et al., 1999b). On this occasion it should be noted that AgNORs in some of these nucleoli translocated to the nucleolar periphery (Fig. 6). The presence of micronucleoli in addition to large and irregularly shaped nucleoli in K2 or K1 cells, i.e. the presence of satellite nucleoli (Smetana, 2002) in these cells, was not exceptional (Fig. 2). K2 or K1 cells containing only micronucleoli were rare (not shown). The nucleolar asynchrony in K2 and K1 erythroblasts expressed by the presence of both large irregularly shaped and ring-shaped nucleoli in one and the same nucleus (Fig. 1) was also observed and its incidence was variable and ranged from 2 to 15 per cent.

K1/2 erythroblasts mostly possessed micronucleoli and apparently represented small basophilic erythroblasts (Fig. 3). However, some of K1/2 erythroblasts were characterized by the presence of large irregularly shaped nucleoli similar to those observed in K2 and K1 erythroblasts (Fig. 4). Such cells resembled proerythroblasts despite their small size and smaller diameter of their nuclei in comparison with K2 or K1 cells. The incidence of such cells usually did not exceed 10 per cent of K1/2 erythroblasts.

Table 1. Nucleoli in early erythroid precursors

Cell	Cell (class. term.)	Large irr. shaped No	Multiple AgNORs	Satellite No	AgNOR translocation	No asynchr.	Micro-nucleoli
K2	proerythrobl.	+++	+++	+	+	+	–(+)
K1	macroblast (large basoph. ebl.)	+++	+++	+	+	+	–(+)
K1/2	basoph. ebl. (small basoph. ebl.)	+(–)	+(–)	+(–)	+(–)	–	+++

K2 cells with nuclei > 12 μm, K1 cells with nuclei > 9 μm, K1/2 cells with nuclei < 9 μm.
class. term. – classical haematological terminology, irr. – irregularly, No – nucleoli, asynchr. – asynchrony
+++ – mostly present, + – present, +(–) – present but mostly absent, –(+) mostly absent but occasionally present

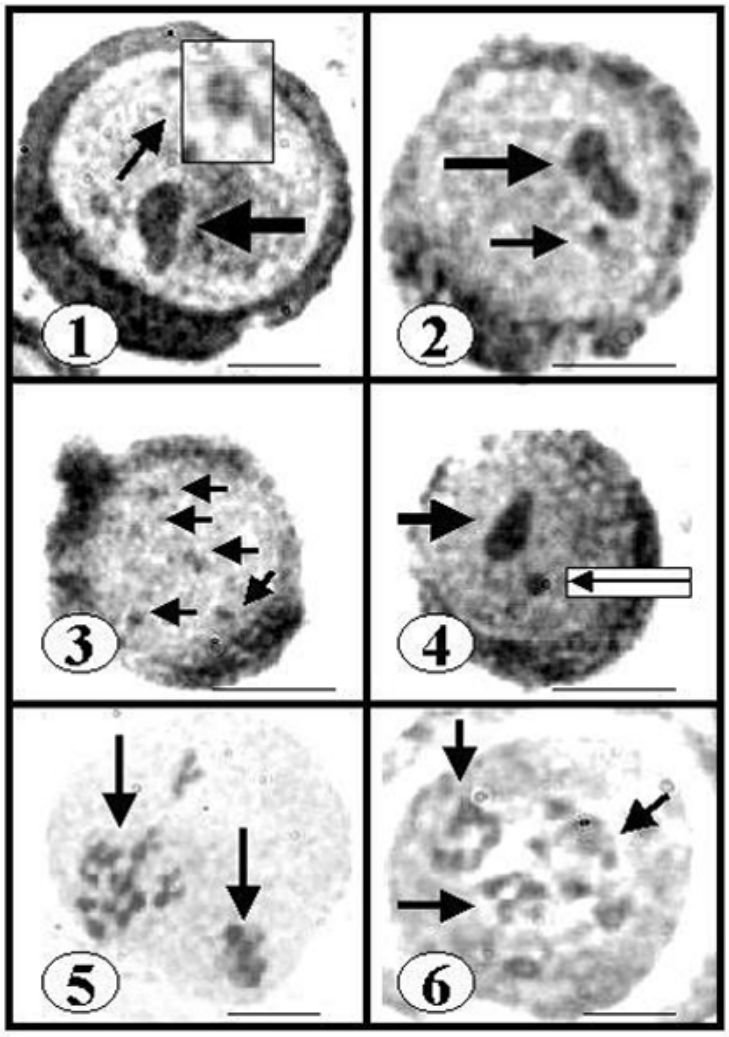

Fig. 1. K2 cell (proerythroblast) with one irregularly shaped large nucleolus (large arrow) and one ring-shaped nucleolus which is enlarged in the insert (thin arrow), i.e. with the nucleolar asynchrony. Staining for RNA. The black bar in this and following figures represents 0.5 μm. Magnification approx. 2 400×, insert 5 200×.

Fig. 2. K1 cell (large basophilic erythroblast – macroblast) with one irregularly shaped nucleolus (large arrow) and one satellite nucleolus (small arrow). Staining for RNA. Magnification approx. 3 000×.

Fig. 3. K1/2 cell (basophilic erythroblast) with multiple small micronucleoli (arrows). Staining for RNA. Magnification approx. 3 000×.

Fig. 4. K1/2 cell (basophilic erythroblast or "microproerythroblast") with one large irregularly shaped nucleolus (large arrow) and one satellite nucleolus (thin arrow in white field). Staining for RNA. Magnification approx. 3 000×.

Fig. 5. K2 cell (proerythroblast) with clusters of AgNORs (dense particles) corresponding to large irregularly shaped nucleoli (arrows). Silver reaction. Magnification approx. 2 300×.

Fig. 6. K2 cell (proerythroblast) with clusters of AgNORs corresponding to large irregularly shaped nucleoli (arrows). Note that AgNORs (dense particles) translocated to the nucleolar periphery. Silver reaction, phase contrast and image processing to see the cell nucleus as a background. Magnification approx. 2 300×.

Concerning the incidence of early erythroblasts classified according to the nuclear size in the bone marrow of studied patients, K2 and K1 cells were apparently more frequent than K1/2 erythroblasts.

Discussion

The results clearly indicated that large irregularly shaped nucleoli with multiple AgNORs representing "active nucleoli" (Smetana, 2003) are characteristic for most of K2 or K1 early developmental stages of erythroblasts. These cells would correspond to proerythroblasts and macroblasts or large basophilic erythroblasts depending on the used haematological nomenclature (Rind et al., 1958; Stobbe, 1959; Bessis, 1972; Smetana, 1980). When micronucleoli were present in these cells, they usually represented additional nucleoli, i.e. satellite nucleoli to large and irregularly shaped ones. In contrast, K1/2 erythroblasts usually possessed micronucleoli representing "inactive nucleoli" and corresponded to small basophilic erythroblasts in the usual haematological nomenclature (Rind et al., 1958; Stobbe, 1959; Bessis, 1972; Smetana, 1980; 2003). However, some K1/2 cells with intensely basophilic cytoplasm also possessed large irregularly shaped nucleoli with multiple AgNORs representing "active nucleoli" (Smetana, 2003) and resembled proerythroblasts in spite of their small size. The term microproerythroblasts would be appropriate for such cells. On this occasion it should be noted that "microproerythroblasts" were present in the very thin peripheral portion of bone marrow smears, which is suitable for morphological evaluation (Undritz, 1972). Therefore, they did not represent artificial diminished small proerythroblasts in the thick portion of bone marrow smears. Whether microproerythroblasts represent a pathological variant or deviation of the erythroid precursor stem cell – proerythroblast – remained to be clarified.

The present study also demonstrated the presence of the nucleolar asynchrony in both K2 and K1 erythroblasts, i.e. in proerythroblasts as well as large basophilic erythroblasts – macroblasts. This phenomenon was observed previously in early granulocytic precursors and apparently decreased the sensitivity of these cells to the cytostatic treatment (Smetana et al., 2002). Thus erythroid precursors with the nucleolar asynchrony might represent cells more resistant to external fac-

tors negatively influencing the erythropoietic process. On this occasion it should be noted that the incidence of cells with the nucleolar asynchrony in investigated patients was variable but did not exceed 15 per cent of K2 and K1 erythroblasts.

The intranucleolar translocation of AgNORs in some K2 and K1 cells represents a newly observed phenomenon in these cells. Preliminary studies indicated that this phenomenon is connected with cell aging because of its induction in leukaemic blasts *in vitro* (Smetana, 2004). Thus a possibility exists that some K2 or K1 cells, i.e. early erythroid precursors such as proerythroblasts or large basophilic erythroblasts – macroblasts, might age without further differentiation and maturation in this stage of the development. On the other hand, it is difficult to relate the incidence of this phenomenon to the state of investigated patients and more studies are required in this direction.

In addition, it should be mentioned that K1 cells were more frequent than K2 and especially K1/2 cells in investigated patients. The predominance of K2 and K1 cells over K1/2 cells in some of the investigated patients might indicate a partial arrest or slow differentiation and maturation process of the erythropoiesis in the chronic phase of chronic myeloid leukaemia treated with imanitib because based on presented models of the erythropoiesis, one would expect the predominance of the latter during this process (Erslev, 1972). However, it should be noted that the block of ABL kinase characteristic for chronic myeloid leukaemia used for treatment of studied patients may inhibit the proliferation without differentiation (Gambacorti-Passerini et al., 1997). However, clinically oriented future studies in respect of the state of the disease and therapy might contribute to the clarification of this observation. The present study, however, was mainly oriented to contribute to the present knowledge on nucleoli in highly immature and less differentiated cells represented by early erythroid K2, K1 and K1/2 precursors.

It should also be added from the methodical point of view that the classification of early developmental stages of erythroblasts according to the nuclear size and incidence of nucleoli is more safe and exact in bone marrow smears because nuclei of these cells are less altered by the smearing procedure than the cytoplasm. On the other hand, the cells should be evaluated only in the thin portions of smears as it has been demonstrated by Undritz (1972). In the thick portions of the bone marrow smear the observed cells are smaller and appear to be embedded in a thick surrounding plasma or are compressed or damaged by surrounding cells and tissue components.

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