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Satellite Nucleoli in the Megakaryocytic Lineage of Rats

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Summary

The present study was undertaken to provide more information on the incidence of satellite nucleoli in developmental stages of the megakaryocytic lineage. Satellite nucleoli representing solitary silver stained nucleolus organizer regions (AgNORs) present in nuclei in addition to other nucleolar types were observed in all stages of megakaryocytic development. However, the incidence of satellite nucleoli was more frequent in mature megakaryocytes than in less differentiated immature megakaryoblasts and naked megakaryocytic nuclei representing the terminal stages of megakaryocytic development after loss of the cytoplasm transformed to thrombocytes. There is a possibility that the increased incidence of satellite nucleoli in mature megakaryocytes might be due to the loss of AgNORs from active nucleoli characteristic for immature cells. The decreased incidence of satellite nucleoli in naked megakaryocytic nuclei might reflect their disintegration in the terminal stages of the megakaryocytic development.

Key words
Cell nucleolus • Megakaryoblast • Megakaryocyte • Nucleolus organizer

Introduction

The differentiation and maturation of megakaryocytes is characterized by endomitotic divisions, which result in the formation of large polyploid cells, the cytoplasm of which separates as thrombocytes (Odell *et al.* 1970, MacPherson 1971, Bessis 1973, Williams and Levine 1982, Jackson 1990). During such development, large irregularly shaped nuclei are usually partially segmented and contain numerous nucleoli. Previous studies demonstrated that during the maturation of megakaryocytes the number and size of nucleoli decrease similarly as nucleolar silver stained particles which represent interphase AgNORs (Janoutová and Likovský 1995). These changes apparently reflect the decreasing nucleolar biosynthetic activities since under certain conditions the number, size and type of nucleoli as well as the number of AgNORs are related to the nucleolar RNA transcription (Schnedl and Schnedl 1972, Hofgartner *et al*. 1979, Smetana 1980, Likovský and Smetana 1981, Wachtler and Stahl 1993). On the other hand, there is no information concerning satellite nucleoli in these cells. Satellite nucleoli are micronucleoli which are present in nuclei in addition to other nucleolar types such as large nucleoli or ring shaped nucleoli (Smetana *et al*. 1993). Cytochemical and *in situ* hybridization studies

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demonstrated that satellite nucleoli are real small nucleoli because they possess all main characteristic nucleolar components including rDNA (Smetana and Busch 1979, Smetana *et al*. 1993). Some studies also demonstrated that the number of satellite nucleoli also depends on the nucleolar biosynthetic activities since their incidence differs in cells after both stimulation and inhibition of the nucleolar RNA transcription (Smetana *et al*. 1984, 1986, 1987).

The results indicated that the incidence of satellite nucleoli is increased in mature megakaryocytes in comparison with immature megakaryoblasts. However, in naked megakaryocytic nuclei representing remnants of megakaryocytes after the loss of cytoplasm transformed to thrombocytes (Stobbe 1959, Bessis 1973), the incidence of satellite nucleoli was reduced and they were smaller than in both immature and mature megakaryocytes.

Methods

Bone marrow smears were taken from femur of 6 male Wistar rats weighing 130-150 g (conventional breed, Velaz, Prague, Czech Republic, without signs of disease, adjusted for 14 days; standard pelleted diet and drinking water were supplied *ad libitum*), which were sacrificed by cervical dislocation under ether anesthesia. Smears of marrow cells in blood serum on microscopical slides were prepared and stained panoptically by the usual hematologic procedures (Schermer 1958). Nucleoli and AgNORs were visualized by a modified two-step silver reaction for the demonstration of nucleolar silver stainable proteins with standardized silver solution (Likovský and Smetana 1981, Janoutová and Likovský 1995) and a colloid developer (Howell and Black 1980). Some of specimens were shortly poststained by 0.01 % aqueous solution of methylene blue (Geigy) to facilitate cell identification. Satellite nucleoli were investigated in 50 megakaryoblasts, megakaryocytes and naked megakaryocytic nuclei of each animal. The values of the nucleolar coefficient expressing the number of nucleoli per cell were calculated by dividing the number of nucleoli by the number of cells in which they were counted (Gonzales Guzman 1949).

The sample means and standard deviations were calculated from the results and the differences between the means were tested using the paired t-test at $p<0.05$ significance level.

Results

In silver stained specimens the nucleoli in all cells of the megakaryocytic lineage appeared as intensely stained clusters of granules representing AgNORs (Janoutová and Likovský 1995). Satellite nucleoli were represented by solitary and single intensely stained AgNORs (Fig. 1).

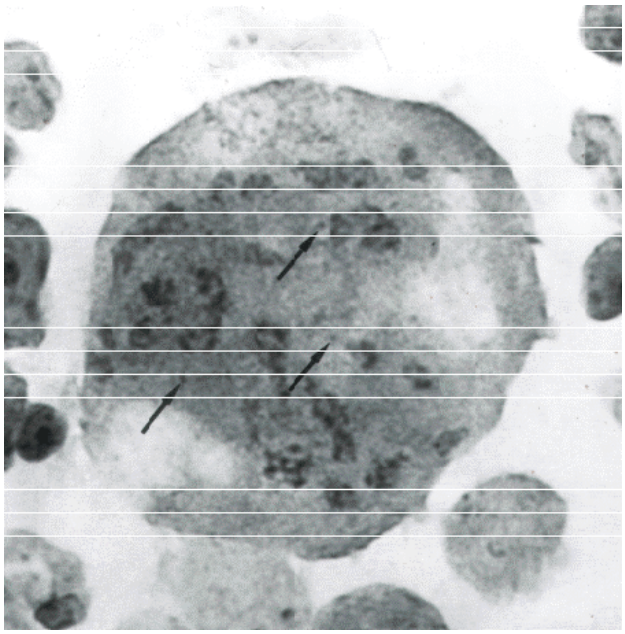

Fig. 1 *Satellite nucleoli (arrows) in megakaryocyte stained by silver for AgNORs, without poststaining (x 1500).*

Similarly as in the previous study (Janoutová and Likovský 1995), the number of nucleoli expressed by values of the nucleolar coefficient (Table 1) decreased during terminal differentiation and maturation of megakaryocytes. The smallest values of the nucleolar coefficient were noted in naked megakaryocytic nuclei, which had lost their cytoplasm. Similarly, the number of AgNORs during the terminal differentiation decreased and reached minimal values in naked nuclei (Table 1).

Satellite nucleoli were noted almost in all megakaryoblasts and all megakaryocytes (Table 2). In contrast, the percentage of naked megakaryocytic nucleoli with satellite nucleoli was significantly reduced (Table 2). In comparison with megakaryoblasts, the

incidence of satellite nucleoli was larger in mature megakaryocytes and the smallest one was noted in naked megakaryocytic nuclei (Table 2).

Table 1. The values of the nucleolar coefficient and the number of AgNORs per cell.

Cells	Nucleolar coefficient	Number of AgNORs/cell
Megakaryoblasts	18.94±3.30	50.69±6.52
Megakaryocytes	15.08±1.61	31.22±3.68*
Naked nuclei	9.17±2.17*#	26.98±5.15*

*Data are mean ± S.D., * significant difference (P<0.05) in comparison with megakaryoblasts, # significant difference (P<0.05) in comparison with megakaryocytes.*

Table 2. The number and the percentage of satellite nucleoli of all nucleoli per cell and the percentage of cells with satellite nucleoli.

Cells	Number of SNo	Percentage of SNo	Percentage of cells with SNo
Megakaryoblasts	3.87±0.66	20.4±1.1	96.0±4.9
Megakaryocytes	5.84±0.59*	38.8±1.2*	100.0±0.0
Naked nuclei	1.02±0.40*#	11.3±4.4*#	69.3±19.8*#

Number of SNo – number of satellite nucleoli per cell, percentage of SNo – percentage of satellite nucleoli of all nucleoli per cell, for further explanation see the legend of Table 1.

Discussion

The present study has provided additional information on nucleoli in the megakaryocytic lineage. Satellite nucleoli representing single AgNORs (Smetana and Busch 1979) visualized by the silver reaction (Likovský and Smetana 1981) were observed in all stages of megakaryocytic development. The increased incidence of satellite nucleoli in the nuclei of mature megakaryocytes (present observation) together with the reduction of nucleoli with mutiple AgNORs (Hofgartner *et al*. 1979, Likovský and Smetana 1981) in comparison with immature megakaryoblasts might be related to the decreased nucleolar biosynthetic activity with respect to the RNA transcription. It seems likely that satellite nucleoli might originate by separation of single AgNORs from nucleoli with decreased biosynthetic activity. The reduction of the number AgNORs in nucleoli in mature megakaryocytes observed in the present study has already been reported (Janoutová and Likovský 1995) and such reduction apparently reflects a decrease of nucleolar RNA transcription (Hofgartner *et al*. 1979, Likovský and Smetana 1981). In addition, single fibrillar centers with surrounding fibrillar regions corresponding to AgNORs (Wachtler and Stahl 1993) and thus to satellite nucleoli (Smetana and Busch 1979) were noted in nuclei of maturing blood cells characterized by the cessation of the nucleolar RNA transcription (Zatsepina *et al*. 1988). The increased incidence of satellite nucleoli was also noted in leukemic lymphocytes after cytostatic therapy (Smetana *et al*. 1995).

The decreased incidence of satellite nucleoli in naked nuclei representing remnants of megakaryocytes after the loss of cytoplasm transformed to thrombocytes (Bessis 1973) is very difficult to interpret. However, it is possible that satellite nucleoli disintegrate earlier than other nucleolar types with multiple AgNORs the number of which is also reduced. Such speculation is supported by previous studies that demonstrated the reduction of the incidence of satellite nucleoli due to the severe inhibition of the nucleolar RNA transcription (Likovský and Smetana 1981, Smetana *et al*. 1984). On the other hand, the decreased incidence of satellite nucleoli was also noted in stimulated cells with increased RNA

transcription (Smetana and Busch 1979, Smetana *et al*. 1986, 1987). In this case, however, satellite or small nucleoli fused and participated in the formation of large active nucleoli (Wachtler *et al*. 1984, Smetana *et al*. 1986). However, this is not a case of naked megakaryocytic nuclei which represent terminal stages of the megakaryocytic lineage (Bessis 1973) and are characterized by the reduction of the nucleolar number as well as nucleolar AgNORs (present study).

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