

ORIGINAL ARTICLE

The translocation of AgNORs in large nucleoli of early granulocyte progenitors in patients suffering from chronic phase of chronic myeloid leukaemia

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Summary

The present study was undertaken to provide more information of the translocation of AgNORs to the nucleolar periphery in human early granulocyte progenitors such as myeloblasts and promyelocytes. The bone marrow smears of patients untreated or treated with the cytostatic therapy appeared to be a convenient model for such study because they possessed a satisfactory number of these cells. The translocation of AgNORs to the nucleolar periphery in early granulocytic progenitors was observed in all studied patients but with different incidence. Since the translocation of AgNORs to the nucleolar periphery was induced in experimental studies *in vitro* by the cell ageing, it seems to be likely that even some granulocyte progenitors in patients suffering from chronic myeloid leukaemia might age without a further differentiation. In addition, the incidence of such cells was markedly and significantly increased by the targeted cytostatic therapy with imatinib. At this occasion it should be noted that these patients were also characterised by a significantly decreased granulopoiesis.

Key words: AgNORs; intranucleolar translocation; leukaemia early granulocyte progenitors; cytostatic therapy

INTRODUCTION

As it is generally known, nucleoli are a multifunctional cell organelle involved in cell proliferation, differentiation, maturation, ageing, cell cycle length and death due to its biosynthetic activities (Busch and Smetana 1970, Grotto et al. 1991, 1993, Hozák et al. 1994, Pederson 1998, Derenzini 2000, Olson et al. 2000, Smetana 2002,

Raška 2003, Boisvert et al. 2007). Past studies also indicated that nucleolar silver stained proteins appear to be very convenient markers of nucleolar biosynthetic activities related to the mentioned cell states (Grotto et al. 1991, 1993, Busch 1997, Derenzini 2000, Smetana 2002). These proteins are located in silver stained nucleolus organiser regions (AgNORs) that appear as intensely stained particles in microscopic specimens after using an adequate and selective cytochemical silver reaction.

It seems to be natural that proliferating early stages of the granulocyte lineage are characterised by large nucleoli with multiple AgNORs the number of which decreases during a further differentiation and maturation (Grotto et al. 1991, 1993, Smetana and Likovský 1994). In addition, previous studies of the author's laboratory also demonstrated both the decreased number and translocation of AgNORs not

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only in ageing cells but also after treatment with a cytostatic drug – imatinib mesylate. (Smetana et al. 2005a, b, 2006).

The present study was undertaken to provide more information whether such events also occurred *in vivo*, i.e. in human early granulocytic precursors in patients who were untreated as well as treated with different cytostatic therapy. At this occasion it should be noted that the bone marrows of studied patients suffering from chronic phase of chronic myeloid leukaemia possessed a satisfactory number of early granulocytic progenitors for such study. In addition, the morphology of early granulocyte progenitors is well known and these cells in bone marrow are easily recognised. Moreover, the differentiation of these cells including nucleolar changes morphologically appeared not to be different from that in not-leukaemia persons (Busch and Smetana 1970, Bessis 1972, Undritz 1972, Smetana et al. 1998).

The results indicated that the translocation of AgNORs in early leukemic granulocyte progenitors is not an unusual phenomenon and may reflect their “premature” ageing. It seems to be interesting that the incidence of the AgNOR translocation in these cells is more frequent after targeted cytostatic therapy with imatinib (Brazier et al. 2002, Maekawa et al. 2007) in patients with a decreased granulopoiesis.

MATERIAL AND METHODS

AgNORs were studied in early granulocyte progenitor – myeloblasts and promyelocytes in smears of diagnostic bone marrow thin needle biopsies taken from 30 patients suffering from the chronic phase of chronic myeloid leukaemia. 5 of these patients were untreated with cytostatic therapy, 25 patients were treated with usual presently used cytostatic therapy for this disease, i.e. 10 patients were treated with interferon α (Baccarani et al. 2003) and 12 patients were treated with imatinib directly targeted to leukaemia cells (Brazier et al. 2002, Maekawa et al. 2007). The granulocytic/erythroid ratio (Rundles 1983) was determined by evaluation cells in specimens stained with panoptic Giemsa-May-Grünwald staining (Undritz 1972). The use of the clinical material was approved by the ethical committee of the Institute.

AgNORs in studied cells were visualized in unfixed bone marrow smears under conditions described previously (Ochs 1998, Smetana et al. 1999). The distribution and number of AgNORs was determined only in large nucleoli, the size of which was larger than 1 μm and facilitated to see clearly the

distribution of silver stained particles. The nucleolar size was determined using Quick Photo Program (Olympus, Japan) in digitised images captured by Olympus C-4040 (E) Camedia digital camera (Olympus, Japan).

RESULTS

As it was expected, bone marrow samples mostly consisted of the granulocyte lineage. However, in bone marrow specimens of patients treated with the targeted cytostatic therapy, the granulocyte lineage was less predominant as expressed by the granulocytic/erythroid ratio (Table 1). In these patients, that ratio (4.8:1) was close to that in not-leukemic persons (Rundles 1963).

Early granulocyte progenitors such as myeloblasts and promyelocytes are mostly characterized by the presence of nucleoli with multiple silver stained particles corresponding to AgNORs (Fig. 1a). In patients untreated with cytostatics the translocation of AgNORs to the nucleolar periphery was detected approximately in one quarter of evaluated cells. In patients treated with interferon the incidence of such cells slightly, but insignificantly increased. In contrary, the significant and marked increase of early progenitors with the translocation of AgNORs was noted after targeted treatment with imatinib (Table 1). It should be also noted that the number of translocated AgNORs (Fig. 1b) per nucleolus was significantly reduced (4.9 ± 1.1) in comparison with nucleoli without the translocation (9.0 ± 1.1). In addition, AgNORs at the nucleolar periphery appeared to be larger and frequently fused (Fig. 1b).

DISCUSSION

The present study demonstrated that the translocation of AgNORs to the nucleolar periphery represents a more frequent and regular phenomenon in granulocyte progenitors of patients suffering from chronic phase of the chronic myeloid leukaemia. Thus even less differentiated and highly immature cells such as myeloblasts and promyelocytes might age since this phenomenon was easily induced by experimental cell ageing (Smetana et al. 2006). The possibility of ageing and apoptosis of leukaemia early granulocyte progenitors was already observed in earlier studies on acute or chronic leukaemias using various methodical approaches (Gavosto et al. 1964, Smetana 2002).

Table 1. The translocation of AgNORs in early granulocyte progenitors (myeloblasts, promyelocytes) and the granulocytic/erythroid ratio.

Treatment	AgNOR translocation	Granulocytic/Erythroid ratio	Number of patients
0	24.4 ± 8.1	$17.8 \pm 7.2 : 1$	5
Interferon	28.8 ± 4.3	$19.2 \pm 8.6 : 1$	10
Imatinib	$38.8 \pm 8.6^{* \$}$	$4.8 \pm 2.4 : 1^{**}$	12

* Statistically different from untreated patients using t-test at the significance level $2\alpha=0.05$.

§ Statistically different from patients with interferon using t-test.

** Statistically different from untreated and treated patients with interferon using t-test.

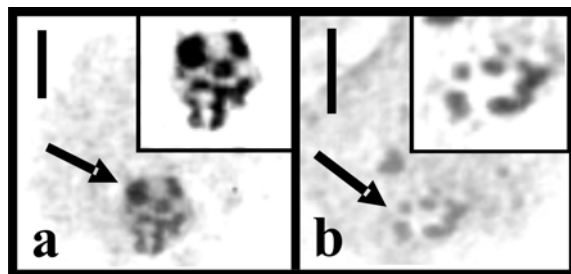


Fig. 1. Early granulocytic progenitors containing AgNORs (arrows) without (a) and with translocation (b). Bold bars represent 2 μ m.

The translocation of the reduced number of AgNORs might indicate how proteins involved in the rRNA transcription and processing after the decrease or inhibition of nucleolar biosynthetic activities migrate to the nucleolar periphery on the way out of the nucleolus.

Since the translocation of AgNORs to the nucleolar periphery is accompanied by their reduction in number, it is apparently also related to the reduced nucleolar RNA transcription and decreased proliferation (Derenzini 2000, Smetana 2002). The decrease of AgNORs in number related to the decrease of the nucleolar RNA transcription is generally known. The translocation of AgNORs to the nucleolar periphery (present results and Smetana et al. 2005a,b, 2006) and the migration of nucleolar fibrillar centers containing silver stained proteins from the nucleolus (Smetana et al. 2004) might be somehow related to the nuclear proteolytic machinery – proteasomes outside the nucleolus. Such speculation is supported by absence of the proteasomal proteolysis in the nucleolus (Scharf et al. 2007) and by the recruitment of one of main nucleolar silver

stained proteins – fibrillarin in nuclear proteasomes outside the nucleolus (Chen et al. 2002).

At the end of the discussion it should be mentioned that the incidence of leukaemia early granulocytic progenitors exhibiting the translocation of AgNORs to the nucleolar periphery may be different depending on the granulopoiesis. The present observation demonstrated the significantly larger incidence of this phenomenon after the targeted cytostatic therapy with imatinib (Brazier et al. 2002, Maekawa et al. 2007) that produced a the marked and significant reduction of the granulopoiesis expressed by the decreased granulocytic/erythroid ratio (Rundles 1983).

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