Interphase ribosomal RNA cistron staining in chronic myeloid leukaemia

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Abstract

Aim—To evaluate the haemopoietic function of bone marrow blood forming cells in human chronic myeloid leukaemia (CML) by means of silver staining of nucleolar organiser region (AgNOR).

Methods—Nucleoli were investigated in bone marrow blast cells and in erythroid, granulocytic, and megakaryocytic cells from 10 haematologically healthy subjects and from 26 patients with chronic myeloid leukemia (17 in benign phase, nine with blast crisis). The investigation was done before treatment, by means of a one step silver staining method. In every case 50 to 100 blasts, promyelocytes, myelocytes, immature (pronormoblastic and basophilic normoblastic) and mature (polychromatic normoblastic) erythroid elements, and megakaryocytes were evaluated for the mean numbers of nucleoli and for the average number of AgNORs per nucleus. Student's *t* test was used to compare the patient and control groups. Other statistical analyses were carried out by means of the computer assisted "HEMA" system.

Results—Compared with controls, activation of NORs was noticed only in CML blasts, while there was a decrease in NORs in the erythroid elements, promyelocytes, and megakaryocytes. The AgNOR score of polychromatic normoblasts and megakaryocytes started to decrease in the benign stage of CML, whereas a similar decrease in pronormoblasts, basophilic normoblasts, and promyelocytes was detected only in patients with CML blast crisis.

Conclusions—The loss of AgNOR sites in cell series in CML may be related to intrinsic defects in their proliferation.

(J Clin Pathol: Mol Pathol 1995;48:M260-M263)

Methodological approaches to understanding proliferation in haematopoiesis have recently undergone radical change. Instead of time consuming and expensive autoradiographic and cytophotometric techniques, more elegant quantitative flow cytometric, immunocytochemical, molecular biological, and other approaches have been used. The method of selective nucleolar organiser region silver staining (AgNOR) is of particular interest since it makes it possible to evaluate ribosomal cistron activity (RCA) quantitatively and directly in cytological and histological specimens.¹⁻²⁸

The first studies of nucleolar argyrophilia in haematopoietic cells were carried out by

Gonzalez-Guzman^{9,10} at a time when the exact number of chromosomes and the location of ribosomal genes were not known. He found that AgNOR scores of bone marrow erythroid and granulocytic elements from healthy subjects and from patients with chronic myeloid leukaemia (CML) was diminished with increasing cell differentiation and maturation. According to Gonzalez-Guzman, AgNOR scores were maximal in blast cells, pronormoblasts, and promyelocytes, intermediate in polychromatic normoblasts, myelocytes, and lymphocytes, and minimal in banded cells and neutrophils.

The results of our recent studies^{7,11-14} are in close agreement with the above data. However, there are intrinsic defects in the haematopoietic function of erythroid, megakaryocytic, and granulocytic elements in CML patients, which has given rise to the present study.

Methods

The investigation was performed on 26 untreated adult patients with CML (17 in "benign" phase and nine blast crisis phase) who were admitted to the Department of Haematology, Faculty Therapy Clinic, Pavlov Medical University of St Petersburg, Russia.

CML was diagnosed from typical clinical and haematological signs and from cytochemical (alkaline phosphatase) and cytogenetic findings. The typical Ph chromosome with standard translocation t(9;22) (q34;q11) was detected in 23 patients. Two cases were Ph-negative and one had no chromosome data. Additional chromosomal anomalies included variant Ph anomaly (one patient), trisomy 8 or 21 (two patients) and other chromosome abnormalities (one patient). Ten haematologically healthy subjects were used as controls.

Bone marrow smears taken by sternal puncture were air dried, fixed in methanol-glacial acetic acid mixture (3:1) and thoroughly rinsed with distilled water. After repeated air drying the preparations were put into 2N formic acid for 10 min, then rinsed again and impregnated with a mixture of 50% aqueous solution of silver nitrate with gelatin at 68°C for 4 min (after Howell and Black¹ with slight modification¹¹). The preparations were then counterstained with Giemsa (Merck) for 20 s.

In every case, 50–100 blasts, promyelocytes, myelocytes, erythroid elements of the first (Er-I) and second (Er-II) classes (Er-I, pronormoblasts and basophilic normoblasts; Er-II, polychromatic normoblasts), and megakaryocytes were evaluated for the mean numbers of nucleoli and for the numbers of

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Accepted for publication
23 May 1995

Clinical findings, laboratory tests, and nucleolar silver staining data from patients with different phases of chronic myeloid leukaemia (CML). Values are means (SEM).

CML phases	Patients (n)	Mean age (years)	Laboratory tests						Nucleolar silver staining data:					
			Hb	L	Plt	Blasts			Number of nucleoli (above); AgNORs (below)					
						pb	bm		Bl	Pr	Mc	Er I	Er II	Mg
Benign	17	52.6 (3.3)	115 (6)	173 (47)	224 (42)	2.5 (0.8)	2.5 (0.4)		2.8 (0.1) 16.4 (2.1)	3.2 (0.1) 14.8 (1.3)	3.7 (0.1) 9.1 (0.4)	3.2 (0.1) 21.4 (4.2)	3.8 (0.1) 9.6 (0.4)	17.3 (2.4) 43.9 (8.8)
Blast crisis	9	63.8 (9)	78 (7)	121 (33)	85 (26)	32 (6.9)	35 (7.1)		2.9 (0.2) 15.2 (2.3)	2.7 (0.6) 11.5 (1.4)	3.5 (0.1) 8.6 (0.7)	3.1 (0.1) 13.7 (2.0)	3.6 (0.1) 9.8 (0.8)	14.8 (2.8) 31.8 (12.6)

Hb = haemoglobin; L = leucocytes; Plt = platelets; pb = peripheral blood; bm = bone marrow; Bl = blasts; Pr = promyelocytes; Mc = myelocytes; Er I = pronormoblasts and basophilic normoblasts; Er II = polychromatic normoblasts; Mg = megakaryocytes.

AgNORs. The mean numbers of nucleoli and AgNORs per nucleus were calculated for each of the above cellular classes and groups. Student's *t* test was used to compare the patient and the control groups. Some other statistical analyses, including correlation tests, were carried out by means of the computer assisted "HEMA" system.^{27 28}

Results
Clinical findings and the results of nucleolus silver staining of bone marrow cells from controls and from CML patients are presented in the table and the figure.

NUMBERS OF NUCLEOLI
The smallest number of nucleoli was found in blasts of both controls and chronic phase CML

The mean number of nucleoli (A) and the average number of AgNORs (silver stained nucleolar organiser region) per nucleus (B) in bone marrow blasts (I), promyelocytes (II), myelocytes (III), pronormoblasts and basophilic normoblasts (IV), polychromatic normoblasts (V), and megakaryocytes (VI) from healthy controls and from patients with benign phase and blast crisis CML. * *p* < 0.05–0.001, CML v control.

patients. Numbers were intermediate in promyelocytes, myelocytes, and erythroid elements, and much greater in megakaryocytes. There was a significant difference between the number of nucleoli in leukaemic blasts and that in promyelocytes, myelocytes ($p < 0.001$), and Er-II ($p < 0.006$), respectively. The mean number of nucleoli in leukaemic blasts was higher than in controls ($p = 0.02$). On the other hand, it was markedly decreased in leukaemic promyelocytes ($p = 0.03$) and megakaryocytes ($p = 0.001$). It is noteworthy that the mean number of nucleoli in blasts from benign phase CML patients was correlated significantly with the percentage of blasts in bone marrow and with the white blood cell count ($r = 0.44$, $p < 0.006$, and $r = -0.61$, $p < 0.001$, respectively). The percentage of bone marrow blasts was also inversely associated with the numbers of nucleoli in mature erythroid (Er-II) and granulocytic (myelocyte) elements ($r = -0.64$, $p < 0.001$, and $r = -0.64$, $p < 0.005$, respectively).

In patients with CML blast crisis the mean number of nucleoli in blasts also increased, whereas in promyelocytes and megakaryocytes it was diminished compared to the controls. A highly significant correlation was found between the numbers of nucleoli in blasts and the numbers of erythrocytes ($r = 0.84$; $p < 0.001$). In contrast to the benign phase of CML, no relationship was observed between blast nucleolus numbers and bone marrow blast content or white blood cell count. The numbers of nucleoli in Er-II were closely related to the white cell count ($r = 0.83$, $p < 0.03$) and inversely related to the numbers of nucleoli in Er-I ($r = -0.81$, $p < 0.02$).

NUCLEOLAR ARGYROPHILIA OF BONE MARROW CELLS
In controls and in the patients with the benign stage of CML the maximum number of AgNORs per nucleus was found in megakaryocytes, followed by immature erythroid elements (Er-I). The count was intermediate in blasts, promyelocytes, and Er-II elements, but least in myelocytes. Compared to controls, a statistically significant decrease of AgNOR score was noticed in CML megakaryocytes ($p < 0.01$) and Er-II ($p < 0.001$). The count in blasts correlated closely with that of promyelocytes ($r = 0.94$, $p < 0.001$), myelocytes ($r = 0.83$, $p < 0.001$), and Er-I ($r = 0.98$, $p < 0.001$). Of interest is the fact that the numbers of AgNORs

in mature erythroid elements were inversely proportional to the percentage of bone marrow blasts ($r = -0.61$, $p < 0.005$) and to the white cell count ($r = -0.5$, $p < 0.03$). Furthermore, the numbers of AgNORs in nucleoli of promyelocytes ($r = 0.37$, $p < 0.049$), myelocytes ($r = 0.42$, $p < 0.01$), and Er-II ($r = 0.65$, $p < 0.002$) showed significant, but weak, correlations with the platelet count.

At CML blast crisis, nucleolar argyrophilia in blasts did not differ from that in controls and in patients in the benign phase of CML. On the other hand, the nucleolar activity of erythroid, granulocytic, and megakaryocytic elements changed greatly. For instance, the nucleolar activity of Er-II in CML patients became lower than in controls, at 8.8 (SEM 0.8) *v* 11.6 (0.4) AgNORs per nucleus ($p < 0.03$). A similar but more significant decrease in AgNOR numbers was found in CML Er-I and promyelocytes ($p < 0.001$ and $p < 0.01$, respectively).

Additional analysis showed that the AgNOR count in bone marrow blasts of patients in CML blast crisis correlated well with that in promyelocytes ($r = 0.79$, $p < 0.002$). This cellular feature of Er-I and Er-II elements was closely associated with the numbers of immature erythroid cells in bone marrow ($r = 0.89$, $p < 0.004$, and $r = 0.83$, $p < 0.001$, respectively) and with the percentage of blasts in peripheral blood ($r = 0.67$, $p < 0.03$, and $r = 0.87$, $p < 0.001$ for Er-I and for Er-II populations, respectively). However, there was an opposite interrelationship between the numbers of bone marrow blasts and nucleolar argyrophilia in erythroid precursors ($r = 0.79$, $p < 0.002$).

Discussion

These results show convincingly that, in comparison with controls, some activation of nucleolar organizer regions was noticed in CML patients only in blasts, while the NOR activity of CML erythroid elements, promyelocytes, and megakaryocytes was decreased. The AgNOR count in polychromatic normoblasts and megakaryocytes started to decrease in the benign stage of CML, whereas a similar decrease was detected in pronormoblasts, basophilic normoblasts, and promyelocytes only on patients with CML blast crisis.

The mean numbers of AgNORs in cells is well known to reflect either their proliferative capacity^{6-8,29-31} or the degree of nuclear ploidy.^{14,32} Review of published reports shows that in CML patients the proliferative potential of immature blasts and of erythroid, granulocytic, and megakaryocytic elements changes in different ways.³³⁻⁴⁰ Proliferative activity of blasts in CML patients in the benign phase does not differ markedly from controls, whereas that of CML promyelocytes, myelocytes, and erythroid precursors in all stages of the disease is slightly but definitely decreased compared to controls. It is therefore not surprising that the general level of rRNA synthesis in CML promyelocytes, myelocytes, and the basic classes of erythroid elements is also dimin-

ished. So far as CML megakaryocytes are concerned, it is well known that there is a defect in their ploidy.⁴¹

Our investigation has shown that the large numbers of nucleoli in benign stage CML blasts correlate closely with the percentage of marrow blasts and with leukocytosis. This implies that CML blasts act aggressively even early in the disease.^{38,42,43} The absence of this correlation in CML blast crisis might partly be explained by increasing accumulation of temporarily non-proliferative (quiescent) blasts in such leukemic populations.^{37,38}

The decrease in nucleolar argyrophilia in CML erythroid, granulocytic, and megakaryocytic elements in CML may be related to cellular or humoral feedback mechanisms of haematopoiesis which are triggered by greatly expanded granulocytic and megakaryocytic masses.^{44,45} The low AgNOR count in proliferating classes of erythroid, granulocytic, and megakaryocytic cells from CML patients is likely to be the result of some intrinsic defects in their proliferative potential. At present, this idea is supported by the following observations. First, megakaryocytes from CML patients are defective in their ability to produce polyploid nuclei and hence to increase their rRNA and protein synthesis. Second, intrinsic defects of CML granulocytes are reflected in a low alkaline phosphatase, low granularity of some polymorphs, and decreased phagocytosis. Third, there are numerous published reports illustrating biochemical⁴⁶ and kinetic disturbances⁴⁷⁻⁴⁹ in CML erythroid elements.

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