Predominant expression of ζ and ε globin genes in human leukemia K-562(S6) variant cell line[1]

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Summary. In the human leukemia K-562(S6) cell line (a) the accumulation of α-globin chains is low or absent, (b) ζ-globin gene expression is correlated with expression of ε-chains and (c) the genes responsible for the terminal cell division are not operated within 8–12 cell cycles, while K-562(S6) cells are fully induced to erythroid differentiation.

The human leukemia K-562 cell line, originally isolated from the pleural effusion of a patient with chronic myelogenous leukemia[2], can be induced by 50–100 μM hemin to accumulate the embryonic and fetal hemoglobins Hb Gower 1 ($\zeta_2\varepsilon_2$), Hb Portland ($\zeta_2\gamma_2$), HbF ($\alpha_2\gamma_2$)[2–6] and Hb Bart's (γ_4), as rigorously shown by Rutherford et al.[3]. Furthermore, a variant cell line, K-562(S), has been described[5] which expresses the α-like globin genes (ζ and α) at very low levels and accumulates large amounts of HbX ($\varepsilon_2\gamma_2$) and Hb Bart's[5,6].

Therefore K-562 cells represent a unique model system for studying the control of globin gene expression of the embryonic type (ζ and ε chains) and the fetal type (α and γ chains), and to elucidate possible mechanisms which operate during normal erythropoiesis in the human early embryo. The switch from the embryonic to the fetal pattern of globin gene expression is a typical feature of the first weeks of the intrauterine life in humans[7–10].

In addition, hemin induced K-562(S) cells might mimic molecular mechanisms controlling globin gene expression, similar to those which characterize some genetic disorders such as α-thalassemia and HbH disease[7–11].

In order to study both kinds of phenomena at the molecular level it is crucial to work with cell lines which maintain in vitro different patterns of globin gene expression[12].

In this paper we describe the pattern of hemoglobin accumulation of a K-562 variant cell clone after erythroid induction with hemin. This cell line appears to retain different cellular and molecular features as compared with the K-562 cell populations described by others[2–6], and it expresses the globin genes typical of the very early stages of the primitive erythropoiesis in the human embryos.

Material and methods. Cell lines and culture conditions. Standard conditions of K-562 cell growth were α-medium (Gibco), 50 mg/l streptomycin, 300 mg/l penicillin, supplemented with 10–15% fetal calf serum (FCS, Flow Laboratories) in 5% CO_2, 80% humidity. Cell growth was determined with a ZF Coulter Counter[13]. Stock solutions of 20 mM hemin (equine, Sigma) were prepared as described elsewere[5]. Measurement of cell viability was performed using the trypan-blue exclusion technique[14]. Induced erythroid differentiation was analyzed by specific reaction with benzidine[13]. K-562(S) cells were from Livia Cioè (Institute of Virology, Rome University, Italy). The subline K-562(S6) was obtained as follows: K-562(S) cells were cultured in semi-solid medium (0.33% agar, 10% FCS) for 14–21 days in the presence of 100 μM hemin and from large, hemoglobinized colonies cells were picked up and disaggregated in

Globin gene expression in K-562(S6) cells cultured for 5 days with 75 μM hemin

Hemoglobin accumulation				α-like and β-like globins (% of total globins)			
Type of hemoglobin	Chain composition	%	pg/cell	ζ	α	ε	γ
Hb Gower 1	$\zeta_2\varepsilon_2$	73.3	8.06	36.6	-	36.6	-
Hb Portland	$\zeta_2\gamma_2$	17	1.87	8.5	-	-	8.5
Hb X	$\varepsilon_2\gamma_2$	9.7	1.07	-	-	4.85	4.85
Total		100	11	45.1	-	41.5	13.3

These data were obtained from the experiments shown in figures 1 and 2.

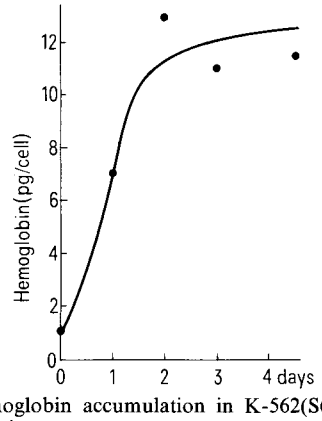

Figure 1. Hemoglobin accumulation in K-562(S6) cells cultured with 75 μM hemin.

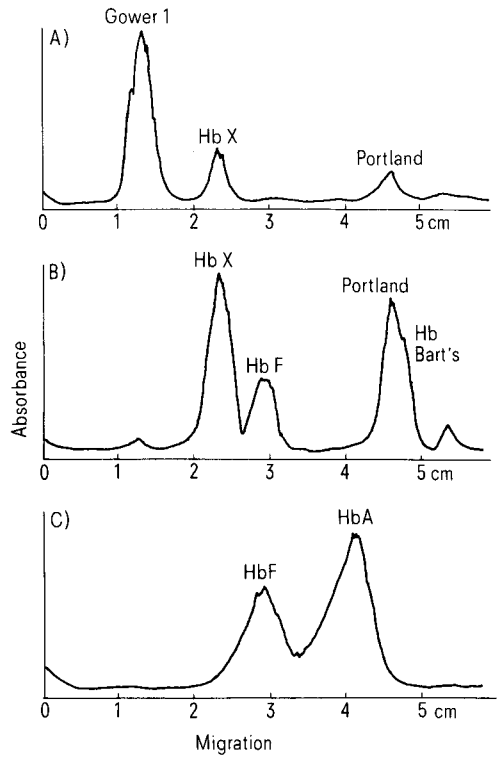

Figure 2. Densitometry scans of K-562 hemoglobins separated by cellulose acetate gel electrophoresis. *A* Lysates from K-562(S6) cells; *B* lysates from K-562(S) cells; *C* Hb markers. K-562(S) and K-562(S6) cells were cultured for 5 days with 75 μM hemin.

liquid suspension without hemin. One of the clones isolated was called K-562(S6), and further characterized. K-562(S6) cells were shown to be highly inducible by 75–100 μM hemin (90% benzidine-positive cells after 2 days cell culture) and to continue cell proliferation for at least 8–10 cell cycles in the presence of the inducer while already terminally differentiated.

Hemoglobin determination. K-562(S6) cells were cultured in the presence of 75 μM hemin at the initial cell concentration of 2×10^5 cells/ml, lysed with 3–4 cycles of freezing and thawing[5] and the quantitation of hemoglobin per cell was carried out as described elsewere[3,5,13]. In order to separate the synthesized hemoglobins, total fresh cell lysates were electrophoresed on cellulose acetate strips (Polyphor) in Tris-EDTA-borate buffer, pH 8.7[7].

Under these electrophoretic conditions the Hbs segregate to the anodal chamber with different migration rates[3–5]. A mixture of HbA ($\alpha_2\beta_2$) + HbF ($\alpha_2\gamma_2$) was always used as internal marker for each determination and the identification of the other hemoglobins was based on their relative migration[3–7]. After electrophoresis the gels were stained with benzidine[7], photographed and the relative proportion of separated hemoglobins was calculated from the areas of the relative densitometric peaks. A direct relationship between the peak area and the amount of reference Hbs was previously shown within the limits of these measurements.

Results. Human leukemia K-562(S) cell populations are heterogeneous with respect to at least 2 different parameters: a) cell proliferation in the presence of inducers such as hemin or butyric acid; b) kinetics of induced increase in the relative proportion of Hb-containing cells[5].

In order to isolate homologous sub-clones, K-562(S) cells were cultured in semi-solid α-medium as described in the methods section. Most of the cells underwent terminal cell division but 0.2% of them did not stop dividing for at least 8–12 cell cycles and were still fully induced to express globin genes.

These cells gave rise to large size colonies containing more than 500 cells which could be subcloned. One of these clones, called K-562(S6), was further characterized. Figure 1 shows the time course of Hb accumulation in K-562(S6) cells after induction with 75 μM hemin. After 1 day 80% of the cells were reactive to benzidine (data not shown) and after 2 days they reached full capacity to accumulate Hb (on the average 11 pg of Hb/cell). The types of Hbs produced are shown in figure 2 and compared to the hemoglobins of the original K-562(S) cell population. K-562(S) and K-562(S6) cells were treated with 75 μM hemin for 5 days and the cell lysates analyzed by cellulose acetate gel electrophoresis[7] and compared with appropriate amounts of the HbA and HbF markers (fig. 2). This highly reproducible technique is routinely used in studies focused on identification of normal and variant human hemoglobins, since there is a general agreement in the literature about the relative migration of embryonic, fetal and adult human hemoglobins[2–7]. We identified the benzidine positive bands of K-562(S) cells as HbX, HbF, Hb Portland and Hb Bart's according to Rutherford et al.[6] and Cioè et al.[5], who originally described the molecular features of the K-562(S) variant cell line. The lack of accumulation of adult β globin (data not shown) rules out the possibility of the presence of HbA[5].

Our results suggest that K-562(S6) cells accumulate mostly Hb Gower 1 (73%). HbX and Hb Portland were respectively 9.7% and 17%. HbF and Hb Bart's were not detected.

Therefore globin gene expression in hemin-induced K-562(S6) cells leads to the preferential accumulation of ζ (45%), ε (41%) and γ (13%) globins (table). The molar ratio ε/γ in K-562(S6) cells is strikingly different from the one determined in K-562(S) cells (3.15 vs 0.39, respectively) (fig. 2). The variant clone K-562(S6) differs also from the K-562 cell lines described by Rutherford et al.[3] and Benz et al.[4] because it does not seem to accumulate HbF.

Discussion. The experiments reported in this paper indicate that in the human leukemia K-562(S6) cell population (a) the expression of α-globin genes is low or absent and (b) ζ globin gene expression is correlated with expression of ε chains (table).

On the contrary it has recently been reported that a close relationship between ζ and ε globin gene expression is not displayed in K-562 clones which do synthesize α globin chains[12].

In addition (c) a close relationship between efficient cell proliferation in the presence of hemin and high expression of ζ and ε globin genes might take place in K-562 cells, as after long-term culture in 50 μM hemin we isolated other cell populations in which the ratios α/ζ and γ/ε are on the average 0.12 and 0.65 respectively (data not shown)[15].

Taken together our results suggest that the pattern of globin gene expression in K-562(S6) cells is different from the one reported for the original broad K-562 cell populations and it is very similar to that typical of human embryos after 1–3 weeks of gestation, ζ and ε being the predominant globins[7]. As the K-562(S6) variant cell clone does not undergo terminal cell division within 8–12 cell cycles, while being fully induced to the erythroid differentiation, it might be useful to study the first embryonic → fetal switch, when in the human embryo the expression of ζ and ε globin genes becomes repressed, and the transcription and the translation of α and γ globin mRNAs are sharply increased[7–10].

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