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HUMAN LEUKEMIA K562 CELLS: RELATIONSHIP BETWEEN HEMIN-MEDIATED ERYTHROID INDUCTION, CELL PROLIFERATION AND EXPRESSION OF c-ABL AND c-MYC ONCOGENES

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We have studied the expression of the c-myc and c-abl oncogenes in two human leukemic K562 cell lines which do express hemoglobin genes retaining a differential rate of cell proliferation. Our data indicate that in hemin-induced K562(S) cells the expression of c-abl oncogene decreases and appears to be related to a decrease in the proliferation capacity rather than to the activation of differentiated functions. The K562(hC) cell line, which produces large amounts of Hb Gower 1 retaining an efficient rate of cell proliferation, expresses indeed the c-abl oncogene at high level.

The implication of a number of cellular oncogenes in tumor cell growth has recently arisen growing interest in many laboratories (1-4). Cellular oncogenes are indeed homologous to the transforming sequences of certain retroviruses and are expressed at high level in many tumor cell lines (5,6). A decrease in the expression of some oncogenes seems to be associated with the activation of differentiated functions (7-10). This decrease has been for instance observed during the terminal differentiation of HL60 cells, which after induction with dimethylsulfoxide or retinoic acid stop dividing and accumulate at lower levels c-myc transcripts (7,8).

In the present study the relationship between the expression of oncogenes, cell proliferation and differentiation was investigated by examining the accumulation of c-abl and c-myc sequences in two human leukemia K562 cell lines which do express hemoglobin genes while retaining a differential rate of cell proliferation. K562 is a cell line which was isolated from a patient with chronic myelogeneous leukemia in blast crisis (11). These cells retain a number of tumor-associated markers but can be induced by treatment with a variety of chemicals to express erythroid differentiation with accumulation of

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embryonic and fetal hemoglobins (12-14). In addition to globin and other erythroid-type genes, these cells have been found to express some oncogenes, including c-myc (15) and c-abl (16-17).

K562 cells represent therefore a suitable "in vitro" model system to study the relationship between the activation of differentiated functions and the expression of molecular features which appears to be related to neoplastic transformation.

MATERIALS AND METHODS

Cell lines. K562(S) cells (13) were obtained from Dr.Livia Cioè (Istituto di Virologia, Università di Roma, Italy). The K562(h) cell line was developed in our laboratory by long-term (over 8 months) cultivation of K562(S) cells in 50 μM hemin (14). This cell line can be indefinitely sub-cultured in the presence of hemin and it is composed by two major cell populations, one "resistant" to hemin-mediated induction to hemoglobin expression and the other one proliferating and fully induced to express hemoglobin genes (14). These latter cells can be isolated by cloning K562(h) cells in semi-solid medium (19) or by serial dilutions (14). The K562(hC) cell line was obtained by cloning K562(h) cells by serial dilutions in 50 μM hemin. After 20 days of cell culture K562(h) clones -denominated K562(hA), K562(hB), K562(hC)...- were stained with benzidine in order to identify erythroid induced, proliferating cells. 91% of the K562(hC) cell population was found to be positive to the benzidine stain. The analysis of cell proliferation, kinetic of erythroid differentiation, hemoglobin expression, accumulation of ε- and γ-globin mRNAs of the K562(hC) and K562(S) cells are compared in Table I.

Culture conditions. Cells were maintained at 37°C in humified atmosphere in α-medium (GIBCO) supplemented with 10% fetal calf serum (FCS, Flow Laboratories) as described (19). Semi-solid cell cultures were performed in α-medium, 0.33% agar (DIFCO), 10% FCS. Hemin was prepared as described (19). Cell growth was monitored with a ZF Coulter Counter (18). K562 cells containing heme or hemoglobin were detected by specific reaction with benzidine/hydrogen peroxide solution as reported elsewhere (18).

Hemoglobin determination. In order to analyse K562 hemoglobins total fresh post-mitochondrial cell lysates were electrophoresed on cellulose acetate strips (Polyphor) in Tris-EDTA-borate buffer, pH 8.7 (19). After electrophoresis the gels were stained with benzidine, photographed and the relative proportion of separated hemoglobins was evaluated from the areas of the densitometric peaks (19).

Cytoplasmic Dot Hybridization. Dot hybridization analysis of cellular cytoplasmic preparations was performed as described by White and Bancroft (21). 50 μl of post-mitochondrial cell lysates were added to 30 μl of 20 x SSC (1 x SSC = 0.15 M NaCl, 0.015 M trisodium citrate) and 20 μl of 37% (v/v) formaldehyde (Fisher). This mixture was incubated at 60°C for 15 min and stored at -70°C. For analysis 20 μl of each sample were suitably diluted in 15 x SSC and 100 μl of each dilution were applied on a nitrocellulose sheet as described (14). The nitrocellulose filter was baked at 80°C for 90 min and pre-hybridized at 42°C in 50% formamide, 5 x SSC, 5 x Denhardt's solution (21), 0.1% sodium dodecylsulphate (SDS), 20 mM sodium phosphate pH 6.5, 100 μg/ml sonicated and denatured salmon sperm DNA (21). Hybridization with nick-translated DNA probes

specific for γ-globin (22), ε-globin (23), ribosomal (plasmid pXCR7, a generous gift by Dr. Irene Bozzoni, Centro degli Acidi Nucleici, Università di Roma, Italy), c-myc (15) and c-abl (17) RNAs was performed for 16 hours as described (14). After hybridization, the filters were extensively washed three times with 250 ml portions of 2 x SSC, 0.1% SDS (5 min) and twice with 250 ml of 0.1 x SSC, 0.1% SDS at 50°C for 30 min. Autoradiography and quantitation by scanning through a spectrophotometer were performed as described elsewhere(14).

RESULTS

In K562(S) cells cultured with 50 μM hemin a marked increase of the expression of globin genes and of accumulation of hemoglobin is observed (Table I).

This increase of erythroid differentiation-related functions is associated with a decrease of the rate of cell proliferation (Fig.1 and Fig.2).

We have isolated K562 cell lines (see method section) which express globin genes at high levels while retaining a full capacity to proliferate (Fig.2).

One of these clones, termed K562(hC), shows a predominant production of the Hb Gower 1 ($\zeta_2\varepsilon_2$) together with a rate of cell proliferation which is very similar to that of uninduced K562(S) cells (Fig.1 and Table I). The predominant expression of embryonic globin genes ζ and ε by proliferating erythroid cells is a feature typical of the primitive embryonic erythropoiesis, while the predominant expression of fetal globin genes γ by non proliferating erythroid cells would rather recall the behaviour of fetal, definitive erythropoiesis(24).

Fig.3 shows the autoradiograms of the hybridization obtained with the probes containing the c-abl and the c-myc oncogene sequences (15,17) in comparison to that obtained with the γ-globin probe (22). After the hybridizations and the relative autoradiographies, the same filters were hybridized with the plasmid

Table I. Accumulation of hemoglobins and β-like globin mRNAs in K562(S) and K562(hC) cells

Cells	Additions	Hb (pg/cell)	benzidine-positive cells (%)	Hb Gower 1 / HbF + Hb Portland	ε-globin mRNA / γ-globin mRNA
K562(S)	-	0.6	1	N.D.	1.4
K562(S)	hemin	9.2	82	0.8	1.25
K562(hC)	hemin	13.4	94	7.9	2.9

N.D. = not determined. Cells were cultured for 7 days before the indicated determinations. Hemin concentration was 50 μM. Determinations of the total Hb content and the relative accumulation of Hb Gower 1, Hb F and Hb Portland were performed by cellulose acetate gel electrophoresis as described elsewhere (14,19). ε-globin mRNA and γ-globin mRNA content was analysed by cytoplasmic dot-blot hybridization (21). The recombinant probes used in this experiment were the γ-globin specific plasmid JW151 (22) and the ε-globin specific plasmid pεO.7 (23).

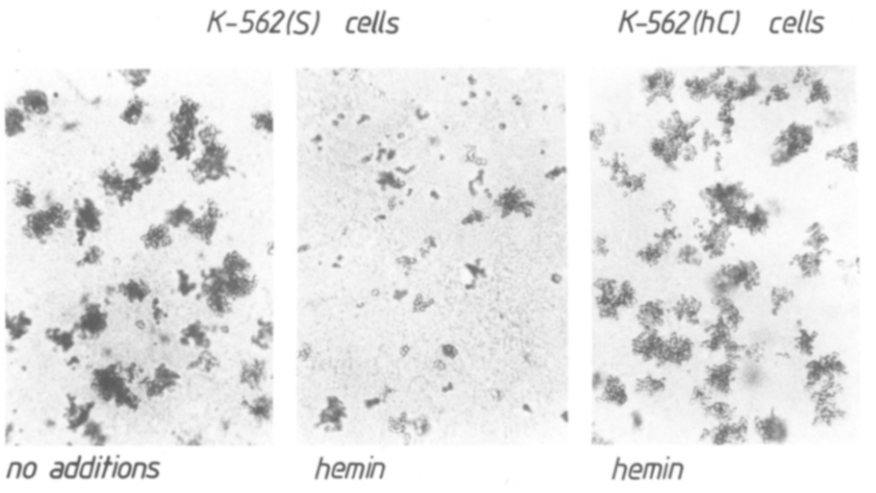


Fig.1. Rate of cell proliferation of K562(S) and K562(hC) cells. Cells were cultured for 7 days in semi-solid medium. Hemin concentration was 50 μM.

pXCR7, which contains sequences specific for the ribosomal RNA. In our experiments a final staining of the nitrocellulose filters with Coomassie Brilliant Blue was also performed as a further control of the number of cells dot-spotted onto the filters (data not shown).

The results of the densitometric scanning of the hybridization autoradiographies are shown in Fig.4 and indicate (a) an increase in γ-globin (x 2.4) specific hybridization signals of hemin-induced K562(S) cells with respect to those of uninduced K562(S) cells; (b) a significative decrease of c-abl specific hybridization and (c) no changes when the c-myc probe was used(Fig.4).

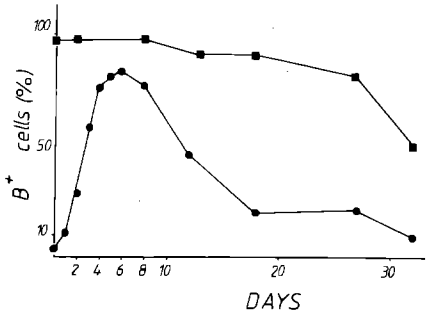
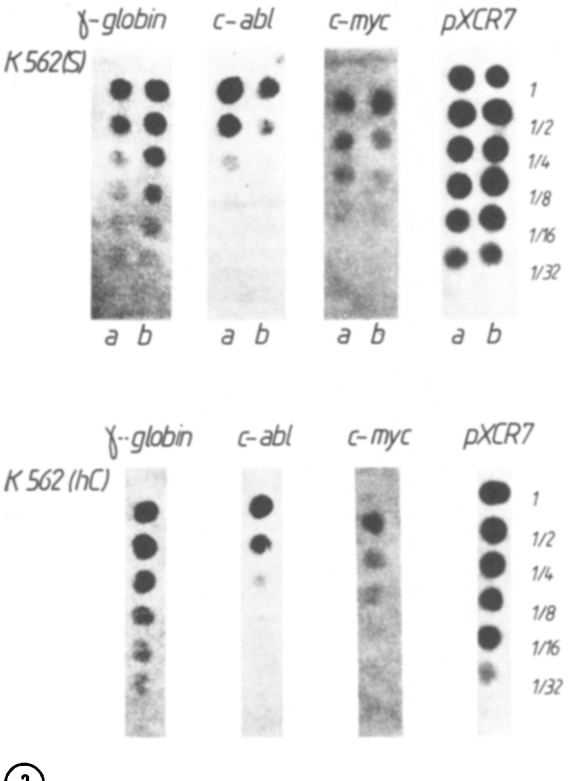


Fig.2. Effects of 50 μM hemin on the proportion of benzidine-positive cells in long-term cultured K562(S) (●) and K562(hC) (■) populations. In this experiment cell concentration was maintained between 0.2 and 0.8 x 10^6 cells/ml. The decrease in the proportion of benzidine-positive K562(S) cells after 15-20 days cell culture is due to the more efficient proliferation of undifferentiated K562(S) cells (14,20).



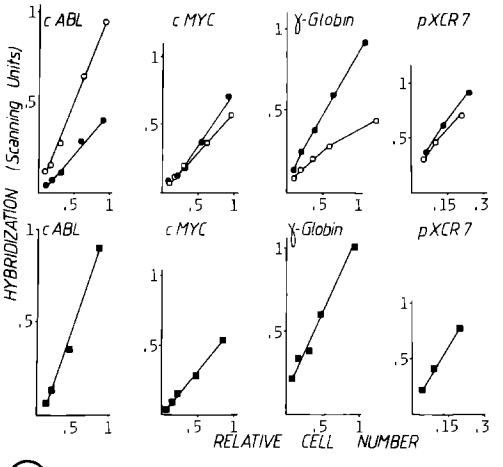


Fig.3. Dot hybridization between formaldehyde-treated cytoplasms from K562(S) cells (upper panel, a), K562(S) cells induced for 5 days with 50 μM hemin (upper panel, b), K562(hC) cells (lower panel) and the γ-globin (22), c-abl (16,17), c-myc (15) and pXCR7 (^{32}P)-DNA probes.

Fig.4. Densitometric scanning of the autoradiography shown in Fig.3. Upper panel: ◎, uninduced K562(S) cells; ●, hemin-induced K562(S) cells. Lower panel: K562(hC) cells.

The use of pXCR7 as a probe for rRNA suggests that total rRNA content does not change significantly with erythroid induction (Fig.4).

Conversely, in K562(hC) cells an high level of expression of ε-(Table I) and γ-(Fig.4) globin genes was detected and the c-abl oncogene appears to be expressed at the level of uninduced K562(S) cells (Fig.3 and Fig.4).

In addition, the level of hybridization with the c-myc and the ribosomal pXCR7 probes were very similar to those found in both uninduced and hemin-induced K562(S) cells (Fig.4).

In conclusion, c-abl sequences are expressed at high levels both in uninduced K562(S) cells which proliferate and are not differentiated and in K562(hC) cells, which proliferate and show a pattern of differentiation typical of

embryonic-type erythroid cells; by contrast c-abl sequences are expressed at distinctly lower levels in non proliferating hemin-induced K562(S) cells, which recall the fetal-type human erythroid cells.

DISCUSSION

The results presented in this paper suggest that the expression of the c-abl oncogene decreases together with a decrease in the rate of cell proliferation of hemin-induced K562 cells. The decrease in the rate of cell proliferation (Fig.1) is associated with erythroid differentiation, as indicated by hemoglobin accumulation and by a2.4 fold increase of γ-globin gene expression (Table I, Fig.3).

In the cell line K562(hC), which does retain an efficient rate of cell proliferation while fully committed to the expression of the embryonic type globin genes (Table I), c-abl transcripts were found to be present at the level of uninduced K562(S) cells (Fig.4).

We propose therefore that the decrease in c-abl production might be related to the decrease in cell proliferation of hemin-induced K562(S) cells rather than activation of hemoglobin expression.

In addition, the results obtained in this study suggest that large differences of accumulation of rRNA and c-myc specific transcripts are not present in K562(S) cells, hemin-induced K562(S) cells or K562(hC) cells.

These results are of some interest when related to the described reduction of the expression of the amplyfied c-myc oncogene in the HL60 cell line after induction by DMSO or retinoic acid to the granulocyte-macrophage differentiation (7,8).

Taken together, these data suggest that the overtranscription of amplyfied specific oncogenes might be positively correlated with the rate of cell growth. For this point of view it might be significative that other tumor-associated markers, such as the receptor for transferrin, are depressed during differentiation of both DMSO induced HL60 (25) and hemin-induced K562 cells (26).

The precise level of the molecular control of these processes (transcription, processing, stability of mRNA) remains to be determined.

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REFERENCES

1. Heldin C.H., Westermark B. (1984) Cell, 37, 9-20.
2. Downward J., Yarden Y., Mayes E., Scrace G., Totty N., Stockwell P., Ullrich A., Schlessinger J., Waterfield M.D. (1984) Nature, 307, 521-527.
3. Finkel T., Cooper G.M. (1984) Cell, 136, 1115-1121.
4. Hayman M.J., Ramsay G.M., Savin K., Kitchener G., Graf T., Beug H.(1983) Cell, 32, 579-588.
5. Bishop J.M. (1983) Ann.Rev.Biochem., 52, 301-354.
6. Bartran C.R. (1984) Eur.J.Pediatr., 141, 134-142.
7. Westin E.H., Wong-Staal F., Gelman E.P., Dalla Favera R., Papas T.S., Lautenberger J.A., Eva A., Reddy E.P., Tronick S.R., Aaronson S.A., Gallo R.C. (1982) Proc.Natl.Acad.Sci.USA, 79, 2490-2494.
8. Reitsma P.H., Rothberg P.G., Astrin S.M., Trial J., Bar-Shavit Z., Hall A., Teitelbaum S.L., Kahn A.J. (1983) Nature, 306, 492-494.
9. Grosso L.E., Pitot H.C. (1984) Biochem.Biophys.Res.Comm., 119, 473-480.
10. Jonak G.J., Knight E. (1984) Proc.Natl.Acad.Sci.USA, 81, 1747-1750.
11. Lozzio C.B., Lozzio B.B. (1975) Blood, 45, 321-334.
12. Rutherford T.R., Clegg J.B., Weatherall D.J. (1979) Nature, 280, 164-165.
13. Cioè L., McNab A., Hubbell H.R., Meo P., Curtis P., Rovera G. (1981) Cancer Res., 41, 237-243.
14. Gambari R., del Senno L., Barbieri R., Viola L., Tripodi M., Raschellà G., Fantoni A. (1984) Cell Differentiation, 14, 87-97.
15. Watt R., Stanton L.W., Marcu K.B., Gallo, R.C., Croce C.M., Rovera G., (1983) Nature, 303, 725-728.
16. Collins S.J., Groudine M.T. (1983) Proc.Natl.Acad.Sci.USA, 80, 4813-4817.
17. Collins S.J., Kubanishi I., Miyoshi I., Groudine M.T. (1984) Science 225, 72-74.
18. Fibach E., Gambari R., Shaw P.A., Maniatis G.M., Reuben R.C., Sassa S., Rifkind R.A., Marks P.A. (1979) Proc.Natl.Acad.Sci.USA, 76, 1906-1910.
19. Gambari R., Raschellà G., Biagini R., Tripodi M., Farace M.G., Romeo A., Fantoni A. (1983) Experientia, 39, 415-416.
20. Gambari R., Raschellà G., Tripodi M., Farace M.G., Fantoni A. (1983) Cell Differentiation, 12, 249-255.
21. White B.A., Bancroft F.C. (1982) J.Biol.Chem., 257, 8569-8572.
22. Wilson J.T., Wilson L.B., de Riel J.K., Villa-Komaroff L., Efstratiadis A., Forget B.G., Weissman S.M. (1978) Nucleic Acid Res., 5, 563-581.
23. Baralle F.E., Schoulders C.C., Proudfoot N.J. (1980) Cell, 21, 621-626.
24. Fantoni A., Gambari R., Farace M.G. (1981) Blood, 57, 623-633.
25. Sutherland R., Delia D., Schneider C., Newman R., Kenshead J., Greaves M. (1981) Proc.Natl.Acad.Sci.USA,78, 4515-4519.
26. Pelicci P.G., Tabilio A., Thomopoulos P., Titeux M., Vainchenker W., Rochant H., Testa U. (1982) FEBS Letters, 145, 350-354.