

Oncogenic potential of the transcription factor LYL1 in acute myeloblastic leukemia

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The LYL1 gene encodes a basic helix–loop–helix transcription factor involved in T-cell acute lymphoblastic leukemia. Using real-time quantitative RT-PCR assay, we found that the expression of LYL1 was at higher levels in the majority cases of acute myeloblastic leukemia (AML) or myelodysplastic syndrome when compared to normal bone marrow. Our study also showed that LYL1 was highly expressed in most AML cell lines and in CD34+ AML cells. To determine whether LYL1 had an affect on the phenotype and behavior of myeloid cells, we introduced full-length LYL1 cDNA into K562 cells using electroporation and U937 cells with retroviral infection. Both of the derivative cell lines with overexpression of LYL1 had an increased growth rate and clonogenicity. Forced expression of LYL1 in K562 cells enhanced spontaneous and hemin-induced erythroid differentiation but blocked spontaneous as well as PMA-induced megakaryocytic differentiation. Overexpression of LYL1 in U937 cells blocked all-trans retinoic acid-induced monocytic differentiation. The LYL1-transfected U937 cells were also more resistant to the cytotoxic drug cytarabine. These results demonstrate that LYL1 may play a role in early hematopoiesis and may be a potential oncogenic factor in AML.
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Introduction

The lymphoblastic leukemia derived sequence 1 (LYL1) gene encodes a basic helix–loop–helix (bHLH) transcription factor. It was originally identified at the breakpoint of the chromosomal translocation t(7;19)(q35;p13) found in some cases of T-cell acute lymphoblastic leukemia (T-ALL).¹ The translocation brings the LYL1 gene under the regulatory control of the T-cell antigen receptor beta gene (TCR-beta), resulting in ectopic expression of LYL1.² However, overexpression of LYL1 has been reported in cases of T-ALL without apparent chromosome aberration. T-ALL cells that express LYL1 tend to have a more immature phenotype with high-level expression of genes such as BCL2, cyclin D2 and CD34, an expression pattern found in many cases of AML.³ The involvement of LYL1 in myeloid malignancy has been suggested by studies of cell lines such as K562 in which the expression of LYL1 protein was observed.⁴ In addition to the t(11;19) found in T-ALL, other translocations involving chromosome 19p13 have been reported in AML.⁵ In this study, we show that LYL1 is expressed in the majority of patients with acute myeloblastic leukemia (AML) or high-risk myelodysplastic syndrome (MDS-CMML or RAEB). By forced expression of LYL1 in myeloid cells, we provide evidence that LYL1 may contribute to the growth behavior, drug resistance and disrupted differentiation pattern of AML cells. As such, we propose that LYL1 may be a potential oncogenic factor in AML.

Patients, materials and method

Patient samples and cell lines

Bone marrow samples from 35 patients with AML or high-risk MDS, including 21 males and 14 females, aged 22–81 years, were analyzed. The subtypes of 24 AML patients included two cases of M0, six M1, 10 M2, three M4, two M5 and a case of M6. The 11 MDS patients included two cases of CMML, four RAEB1 and five RAEB2. The diagnosis of AML and MDS were made according to the WHO classification.⁶ All patient samples were harvested with informed consent according to the Guidelines on Research Involving Human Subjects approved by the University Health Network Research Ethics Board. Six normal bone marrow samples (NBM) were studied as controls. CD34-enriched fractions of NBM cells and AML patient samples were prepared by cell sorting. Human leukemia cell lines K562, U937, HL60 and NB4 were purchased from the American Type Culture Collection (ATCC, MD, USA); OCI-AML1, 2, 3, 4 and 5 cell lines were previously established in this laboratory.⁷ All cell lines were maintained in RPMI 1640 medium (Invitrogen) supplemented with 10% heat-inactivated fetal bovine serum (FBS) and 1% antibiotic solution, and incubated at 37°C, in humidified air, with 5% CO₂.

Real-time-quantitative polymerase chain reaction assay (RT-QPCR)

The RT-QPCR was performed as described elsewhere.⁸ In brief, total RNA was prepared using the RNeasy kit (Qiagen) from freshly separated mononuclear cells. RNA samples were quantitated spectrophotometrically. The cDNA was made using the Cloned AMV First-Strand cDNA Synthesis Kit (Invitrogen) according to the manufacturer's protocol. RT-QPCR amplification was performed on an ABI Prism 7700 Sequence Detection System (Applied Biosystems). LYL1 primers were used as described.⁹ LYL1 forward primer: 5'-TCA CCC CTT CCT CAA CAG TGT-3'; LYL1 reverse primer: 5'-CGG GCC ACC TTC TGG G-3'. The glyceraldehyde-3-phosphate dehydrogenase (GAPDH) expression was analyzed for each sample as an endogenous control. GAPDH forward primer: 5'-GAA GGT GAA GGT CGG AGT C-3'; GAPDH reverse primer: 5'-GAA GAT GGT GAT GGG ATT TC-3'. The reaction mixtures, with a volume of 50 µl containing 1 × SYBR Green PCR Master Mix (Applied Biosystems), 100 nM primers and 100 ng cDNA, were preincubated for 10 min at 95°C for denaturing target cDNA and activating the AmpliTaq Gold DNA polymerase. The cDNA was amplified for 40 cycles of 15 s at 95°C (denaturation) and 1 min at 60°C (annealing and extension). The relative copy numbers of gene expression were quantitated using the comparative threshold cycle (Ct) method. Statistical differences among groups were calculated on the GraphPad Prism 3.0 software with a Mann–Whitney test.

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Creation of LYL1-expressing cell lines

The full-length cDNA of human LYL1,¹⁰ kindly provided by Dr ML Cleary, Stanford, USA, was subcloned into a neo-selectable mammalian expression vector pcDNA3 (Invitrogen) and a green fluorescence protein (GFP)-sortable retroviral vector MIEV to form constructs pcDNA3-LYL1 and MIEV-LYL1 respectively. Stable LYL1 transfectants of the erythroleukemia cell line K562 were created by electroporation on a MicroPulser Electroporator (Bio-Rad), with a capacitance of 950 μ F and a voltage of 300 V.¹¹ Neo-resistant clone were selected in growth medium containing 1 mg/ml of G418 using 96-microwell plates. Calcium phosphate precipitation was used to produce amphotropic recombinant retroviruses carrying MIEV or MIEV-LYL1 with the Phoenix AMPHO producer cell line obtained from Dr GP Nolan's lab, Stanford, USA. The myelomonoblastic leukemia cell line U937 was transfected with the 0.45- μ m-filtered supernatants of the producer cells, in the presence of 8 μ g/ml of hexadimethrine bromide (polybrene, Sigma). At 72 h after transfection, GFP-positive cells were sorted using a FACS Vantage Cell Sorter (Becton Dickson). The expression level of LYL1 in cells was assessed by Western and Northern blot analysis.

Western blotting and Northern blotting analysis

For Western blotting analysis, cells were lysed in RIPA buffer (50 mM Tris-Cl, pH 7.5, 150 mM NaCl, 1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS) with a complete protease inhibitor cocktail. An equal amount (50 μ g) of each lysate was separated on a 12% SDS polyacrylamide gel by electrophoresis and transferred onto nitrocellulose membrane (Amersham). Blots were blocked with 5% milk-PBS for 1 h and incubated with rabbit anti-human LYL1 polyclonal antibody (kindly provided by Dr ML Cleary) for another 1 h, followed by incubation with the peroxidase-conjugated mouse anti-rabbit secondary antibody. Blots were soaked in the echochemiluminescence buffer (ECL, Amersham Pharmacia) for about 1 min and exposed immediately to Kodak films. For Northern blotting analysis, equal quantities of RNA (10 μ g) were separated on a 1.2% formaldehyde agarose gel. The RNA was transferred onto nitrocellulose filter, crosslinked with UV Stratalinker 2400 (Stratagene), and hybridized at 65°C for 18 h with 50% formamide, 5 \times Denhardt's solution, 0.1% SDS and 5 \times SSC and the LYL1 probe labeled with ³²P-dCTP by random priming. Filters were washed and exposed to Kodak X-ray films at -70°C with an intensifying screen.

Cell growth, clonogenic assay, drug resistance and differentiation assays

For growth curves, cells were seeded at a density of 1×10^3 cells/ml in 10 ml of growth medium and cultured for at least 4 consecutive days. Cell numbers were counted daily with a hemocytometer.

Clonogenicity assays have been described elsewhere.¹² Cells were suspended in growth medium with 0.8% methylcellulose and plated in 96-well flat bottom plates. The plates were incubated for 7 or more days at 37°C, in humidified air, with 5% CO₂. Colonies containing more than 20 cells were counted under an inverted microscope. The clonogenic cell recovery rate (CCR) was calculated from the mean of at least 4 replicate wells. For dose response of cells to cytotoxic drug, cells were exposed to increasing concentrations of cytarabine (Ara-C) for 24 h, washed with 1% FBS-PBS, and assayed for CCR.

The K562 cells were induced towards erythroid differentiation with 30 μ M Hemin (Sigma) in growth medium for 72 h. Hemoglobin content of cells was assessed by a benzidine assay. Other lineage-specific differentiation markers including CD61 (megakaryocytic), CD11b (myeloid) and CD14 (monocytic) were detected by flow cytometry. The K562 cells were induced towards megakaryocytic differentiation with 1 nM PMA (Sigma) in growth medium for 24 h. The U937 cells were induced to myeloid and monocytic differentiation with 1 μ M of ATRA (Sigma) for 72 h. After the treatment, cells were collected and stained with fluorescein-5-isothiocyanate (FITC) or R-phycoerythrin (PE)-conjugated antibodies against CD61, CD11b and CD14 (BioSource), respectively. The antibody labeled-cells were subsequently analyzed with CellQuest software on a FACScan instrument (Becton Dickinson).

Results

Expression of LYL1 in patients with AML or high-risk MDS

Using real-time quantitative polymerase chain reaction assay we surveyed the expression of LYL1 in 35 patients with AML or high-risk MDS. The specificity of the LYL1 amplification was confirmed by the observation of a uni-molecular dissociation curve and a single band on a 3% agarose gel following electrophoresis. Our results show that LYL1 RNA is expressed at very low levels in NBM cells, but is found at high levels in the majority of patients with AML or MDS (Figure 1a). Relative levels of LYL1 expression for AML and MDS patients were 5.32 ± 3.98 and 9.30 ± 8.81 (means \pm s.d.) times the average level in NBM. There were significant differences for levels of LYL1 between NBM and AML patients ($P=0.0008$) and between NBM and MDS cases ($P=0.0011$) (Mann-Whitney test). An increase in expression of at least two times was observed in 79.2% of AML cases (19/24) and 81.8% of MDS cases (9/11). In some patients, the level of LYL1 was very high, especially in cases of MDS-RAEB. The increased expression of LYL1 has been confirmed by Northern blotting and Western blotting analysis for some patients (data not shown). In addition, our experiments showed that LYL1 RNA was highly expressed in most myeloid leukemic cell lines (Figure 1b).

As bone marrow is a mixture of immature and maturing cells, we assessed the expression of LYL1 in the CD34 enriched fractions of NBM and from AML patient samples. As shown in Figure 1c, the level of LYL1 in normal CD34+ cells was 3.6 ± 0.8 times of the level in bulk NBM cells, indicating that LYL1 is expressed in early hematopoietic progenitors, and may be important in early hematopoiesis. As AML cells are also organized as a hierarchy,¹³ we wanted to determine the level of expression in the stem cell compartment of the AML population. As can be seen in Figure 1c, CD34 enriched AML cells had a slightly higher level of LYL1 than the bulk population. This result indicates that in the AML population that LYL1 is highly expressed in the stem cell fraction, as well as the bulk maturing population.

Overexpression of LYL1 promotes cell growth and clonogenicity

Having found high-level expression of LYL1 in AML and MDS samples, we were interested in determining the effect this expression might have on the behavior of myeloid cells. To

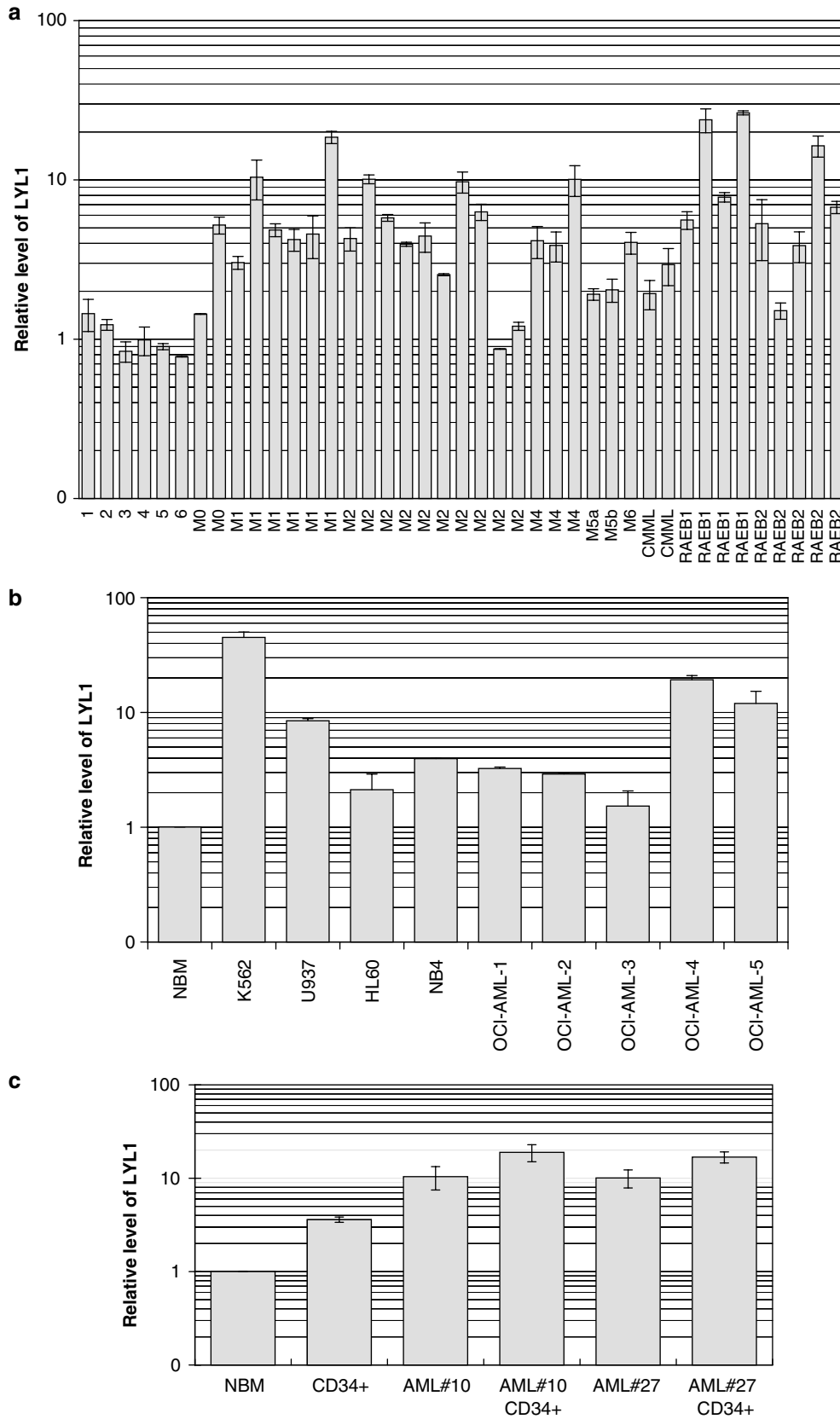


Figure 1 The expression level of LYL1 was determined using RT-QPCR. Compared to NBM cells, the levels of LYL1 were increased in majority of patients with AML or MDS (a) and in AML cell lines (b). The level of LYL1 in normal CD34+ cells was higher than that in bulk NBM cells but lower than in CD34+ cells from AML patients (c). Abbreviations: NBM: normal bone marrow; AML: acute myeloblastic leukemia; MDS: myelodysplastic syndrome.

accomplish this, we used electroporation to create stable K562 cell lines that express increased-levels of LYL1. The cell lines B11 and B21 are clones of K562 cells selected in G418 after electroporation with pcDNA3-LYL1; the cell lines A1 and A2 are clones of K562 cells selected in G418 after electroporation with vector pcDNA3. Increased expression of LYL1 in the transfectants (B11 and B21) was confirmed with Western blotting and Northern blotting analysis (Figure 2a, b). Due to the possible clonal variations of the transfected cells, we also made bulk transfectants of U937 cells in which the expression of endogenous LYL1 is very low. The U937 cells were infected with MIEV or MIEV-LYL1

amphotropic retrovirus, resulting in cell populations U937/MIEV and U937/MIEV-LYL1. Prior to each experiment, the expression of LYL1 in these cells was confirmed by Western blot analysis (Figure 2c) and the coexpression of GFP in more than 95% of the cell populations (Figure 2d). As can be seen in Figure 2, low-level expression of LYL1 RNA and protein was evident in the K562 cells but not the U937 cells, while the transfectants B11, B21 and U937/MIEV-LYL1 had higher levels of LYL1 expression compared to corresponding cells containing empty vector.

For both the K562 and U937 cells, the growth rates were significantly greater for the LYL1 expressing variants B11, B21

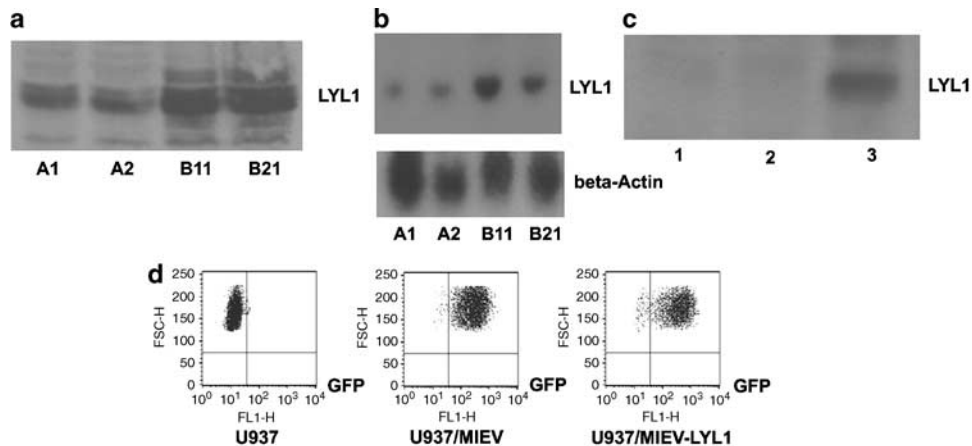


Figure 2 Forced expression of LYL1 in K562 and U937 cells. Western blot analysis (a) and Northern blot analysis (b) showed that endogenous LYL1 was present in K562 cells transfected with vector pcDNA3 (A1 and A2) while the LYL1 transfectants (B11 and B21) had higher levels of expression. Western blot analysis showed that LYL1 protein was positive in U937/MIEV-LYL1 cells but not in U937/MIEV or wild-type U937 cells (c). The expression of LYL1 was also confirmed by coexpression of GFP carried by MIEV vector in more than 95% of U937 cell pools sorted using flow cytometry (d).

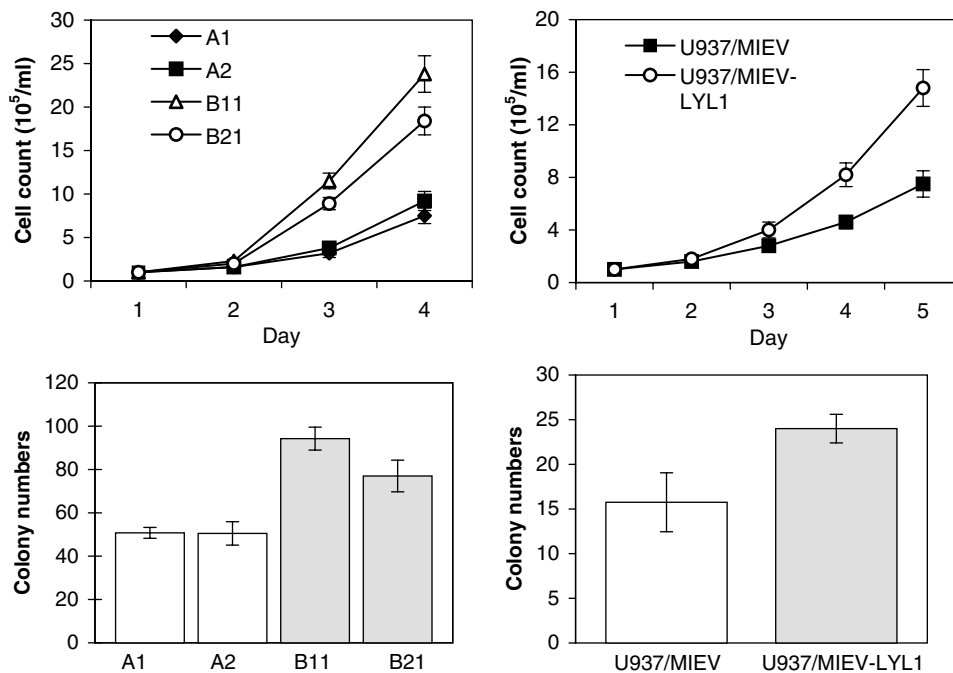


Figure 3 The growth rate and clonogenicity of cells with overexpression of LYL1. The LYL1-transfected K562 cell clones (B11 and B21) were shown to have increased growth rates as compared with control cells carrying an empty vector only (A1 and A2) ($P < 0.01$) (a). The U937/MIEV-LYL1 cells were also growing faster than the U937/MIEV cells (b). Growth curves shown here were representatives of at least three independent experiments that gave similar results. Clonogenic assay demonstrated that the clonogenic cell recovery rate (CCR) was increased remarkably in K562 (c) and U937 cells (d) with overexpression of LYL1, as compared with control clones transfected with vectors only ($P < 0.01$).

and U937/MIEV-LYL1 than control cells transfected with vector only ($P < 0.01$, ANOV test) (Figure 3a, b). Similarly, there was an increase in CCR rates for the LYL1 overexpressing cells ($P < 0.01$) (Figure 3c, d). These results suggest that increased expression of LYL1 promotes growth and colony forming ability of these cell lines.

Effect of LYL1 on drug resistance and differentiation of myeloid leukemia cells

To determine whether LYL1 have an effect on the survival of myeloid cells, we exposed the LYL1-transfected cells to varying concentrations of Ara-C for 24 h, then washed and plated the

cells for CCR. No significant change in drug sensitivity was observed for K562 cells transfected with pcDNA3-LYL1 compared with controls ($P > 0.05$, ANOV test) (Figure 4a). However, the increased expression of LYL1 in U937 cells resulted in increased resistance to Ara-C ($P < 0.01$, ANOV test) (Figure 4b).

K562 cells are capable of erythroid and megakaryocytic differentiation when treated with inducers. The K562 cells overexpressing LYL1 showed an increased degree of spontaneous erythroid differentiation, as evidenced by high level benzidine positivity compared to the nontransfected and empty vector transfected cells. This difference was further increased by the addition of hemin (Figure 4c). In contrast to the enhanced erythroid differentiation of these cells, the spontaneous and

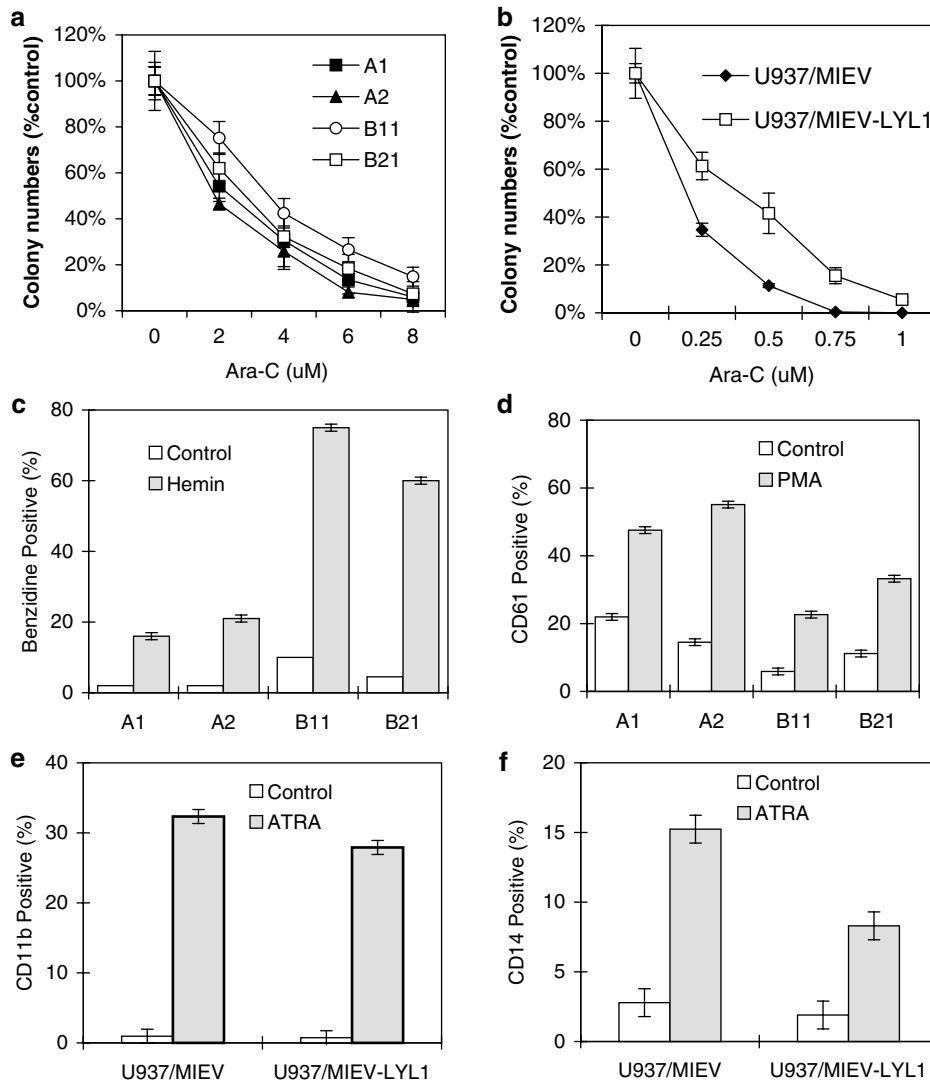


Figure 4 Chemoresistance and differentiation of the LYL1-expressing cells. For drug resistance assay, the transfectants were exposed to increasing concentrations of cytarabine (Ara-C) for 24 h and tested for CCR. The curves of CCR were plotted for means \pm s.d. of four replicate wells. The difference was not significant for k562 cells with or without overexpression of LYL1 (A1 and A2 versus B11 and B21) (a). However, increased resistance to Ara-C was observed for U937/MIEV-LYL1 cells when compared with U937/MIEV cells ($P < 0.01$, ANOV test) (b). For differentiation assay, K562 cells were induced for erythroid differentiation with 30 μ M Hemin for 72 h and stained with Benzidine. The LYL1 transfectants (B11 and B21) showed increased spontaneous as well as hemin-induced erythroid differentiation as compared with controls (A1 and A2) ($P < 0.01$) (c). The expression of the megakaryocytic marker CD61, induced by PMA or not, was inhibited in LYL1-overexpressing cells (B11 and B21) when compared with A1 and A2 ($P < 0.01$) (d). The U937 cells were induced for myeloid and monocytic differentiation with 1 μ M of ATRA for 72 h. No statistical difference was observed for the expression of CD11b between U937/MIEV and U937/MIEV-LYL1 cells ($P > 0.05$) (e). However, the percentage of CD14 positivity was lower in U937/MIEV-LYL1 cells than in U937/MIEV cells ($P < 0.01$) (f), suggesting that LYL1 expression blocked monocytic differentiation of the U937 cells.

PMA-induced megakaryocytic differentiation, as determined by cell surface CD61 expression, was reduced in the LYL1 overexpressing K562 cells ($P < 0.01$) (Figure 4d). Two different lineage markers were used to assess differentiation of U937 cells. No statistical difference was observed for the ATRA-induced expression of the myeloid marker CD11b between the U937/MIEV-LYL1 and the U937/MIEV cells (Figure 4e). However, there was a marked inhibition of the ATRA-induced expression of the monocytic marker CD14 in the U937/MIEV-LYL1 cells as compared to the U937/MIEV cells ($P < 0.01$) (Figure 4f). These results reveal a disrupted differentiation pattern in the myeloid cells expressing high levels of LYL1.

Discussion

Aberrant gene expression is a major mechanism of oncogenesis in hematological malignancies. Despite the observation of LYL gene expression in most myeloid, erythroid and B lymphocyte cell lines,¹⁴ its expression in primary acute leukemia has only been reported in a subset of T-ALL.^{3,15} In the current work, we have assessed the expression of LYL1 in NBM cells, and cells from patients with AML or MDS. LYL1 is expressed at very low levels in bulk NBM cells. By cell fractionation of NBM we show that LYL1 is more highly expressed in the CD34+ fraction. In comparison to NBM and CD34+ normal cells, we found that LYL1 is expressed at high levels in a high proportion of AML and MDS patient samples. In some cases the level of expression is similar to that found in CD34+ cells, while in others, the level of expression of LYL1 is much higher. We, and others, have demonstrated that AML cells are organized in a hierarchy with some cells having the stem cell property of self-renewal, while the majority of the cells are proliferatively inert. To determine whether LYL1 is expressed in the CD34+ stem cell fraction of AML samples, the levels of LYL1 in CD34+ blasts cells and the bulk population were compared. There was a modest enrichment of LYL1 in the CD34+ cells. This indicates that LYL1 is highly expressed in the stem cell fraction, but is also expressed in the cells that are 'more differentiated' in nature. Together these findings indicate aberrant expression of LYL1 in AML and MDS, first by increased expression in the stem population, and then by the persistent expression in the downstream progeny.

The mechanism by which LYL1 is highly expressed in AML is not known. For the cases reported here, none of the patients had a chromosomal abnormality involving 19p13, the site at which LYL1 resides. In T-ALL, it has been found that LYL1 can be aberrantly expressed as a result of chromosome translocation, or in the face of a cytogenetically normal chromosome 19. It is not known if the aberrant expression of LYL1 in cytogenetically normal cases is the result of mono- or bi-allelic expression. Regardless of the mechanism of activation, the high level of LYL1 expression is likely relevant to the malignant behavior of the primary AML cells, as in the work presented here we have shown that enforced expression of LYL1 has a marked effect on the behavior of K562 and U937 cells. In the first place, LYL1 expression increased both the growth rate and plating efficiency of the cell lines. Secondly, we found that increased expression of LYL1 altered the differentiating potential of the leukemia cells. In the case of K562 cells, a cell line with inherent erythroid differentiation potential, the expression of LYL1 on its own, weakly enhanced the basal erythroid differentiation; while hemin induced differentiation and loss of proliferative capacity were markedly enhanced by overexpression of LYL1. In contrast

to the effects on erythroid differentiation, the PMA induced megakaryocytic differentiation of K562 cells was blocked. The U937 cells expressing high levels of LYL1 were blocked in their ability to differentiate along the macrophage lineage.

We also assessed the effect of effect of LYL1 expression on the sensitivity of cells to the chemotherapeutic agent cytarabine, which is a mainstay of AML therapy. We found that the increased expression of LYL1 was associated with a reduced killing of the cells. This is likely due to changes in gene expression brought about by LYL1 and cannot be explained by the increased growth rate of the cells, which would result in increased sensitivity to cytarabine. A similar effect on cell death has been observed for TAL1.¹⁶

From the above, we postulate that the aberrant expression of LYL1 in MDS and AML plays a role in the development and phenotype of those diseases, by altering the differentiation potential of the cells, increasing the growth rate and plating efficiency of AML cells, and reducing the drug sensitivity. These observations indicate that LYL1 or its downstream molecules are potential targets in the treatment of AML or MDS. Further work will be aimed at exploring this possibility.

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References

- 1 Cleary ML, Mellentin JD, Spies J, Smith SD. Chromosomal translocation involving the beta T cell receptor gene in acute leukemia. *J Exp Med* 1988; **167**: 682–687.
- 2 Mellentin JD, Smith SD, Cleary ML. *lyl-1*, a novel gene altered by chromosomal translocation in T cell leukemia, codes for a protein with a helix-loop-helix DNA binding motif. *Cell* 1989; **58**: 77–83.
- 3 Ferrando AA, Neuberger DS, Staunton J, Loh ML, Huard C, Raimondi SC *et al*. Gene expression signatures define novel oncogenic pathways in T cell acute lymphoblastic leukemia. *Cancer Cell* 2002; **1**: 75–87.
- 4 Ferrier R, Nougarede R, Doucet S, Kahn-Perles B, Imbert J, Mathieu-Mahul D. Physical interaction of the bHLH LYL1 protein and NF-kappaB1 p105. *Oncogene* 1999; **18**: 995–1005.
- 5 Bruckner R, Jentsch-Ullrich K, Franke A, Wieacker P, Stumm M. A novel translocation (17;19)(p13;p13) in a patient with acute myelomonocytic leukemia. *Cancer Genet Cytogenet* 2000; **119**: 77–79.
- 6 Vardiman JW, Harris NL, Brunning RD. The World Health Organization (WHO) classification of the myeloid neoplasms. *Blood* 2002; **100**: 2292–2302.
- 7 McCulloch EA, Minden MD, Curtis JE. The influence of growth regulation on treatment strategy for acute myeloblastic leukaemia. *Cancer Surv* 1990; **9**: 169–198.
- 8 Han Y, San Marina S, Liu J, Minden MD. Transcriptional activation of *c-myc* proto-oncogene by WT1 protein. *Oncogene* 2004; **23**: 6933–6941.
- 9 Asnafi V, Beldjord K, Libura M, Villaresse P, Millien C, Ballerini P *et al*. Age-related phenotypic and oncogenic differences in T-cell acute lymphoblastic leukemias may reflect thymic atrophy. *Blood* 2004; **104**: 4173–4180.
- 10 Miyamoto A, Cui X, Naumovski L, Cleary ML. Helix-loop-helix proteins LYL1 and E2a form heterodimeric complexes with distinctive DNA-binding properties in hematolymphoid cells. *Mol Cell Biol* 1996; **16**: 2394–2401.

- 11 Keating A, Horsfall W, Hawley RG, Toneguzzo F. Effect of different promoters on expression of genes introduced into hematopoietic and marrow stromal cells by electroporation. *Exp Hematol* 1990; **18**: 99–102.
- 12 Hu ZB, Minden MD, McCulloch EA, Stahl J. Regulation of drug sensitivity by ribosomal protein S3a. *Blood* 2000; **95**: 1047–1055.
- 13 Bonnet D, Dick JE. Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. *Nat Med* 1997; **3**: 730–737.
- 14 Visvader J, Begley CG, Adams JM. Differential expression of the LYL, SCL and E2A helix–loop–helix genes within the hemopoietic system. *Oncogene* 1991; **6**: 187–194.
- 15 Ballerini P, Blaise A, Busson-Le Coniat M, Su XY, Zucman-Rossi J, Adam M *et al*. HOX11L2 expression defines a clinical subtype of pediatric T-ALL associated with poor prognosis. *Blood* 2002; **100**: 991–997.
- 16 Bernard M, Delabesse E, Novault S, Hermine O, Macintyre EA. Antiapoptotic effect of ectopic TAL1/SCL expression in a human leukemic T-cell line. *Cancer Res* 1998; **58**: 2680–2687.