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FLI-1 is a suppressor of erythroid differentiation in human hematopoietic cells

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The FLI-1 oncogene, a member of the ETS family of transcription factors, is associated with both normal and abnormal hematopoietic cell growth and lineage-specific differentiation. We have previously shown that overexpression of FLI-1 in pluripotent human hematopoietic cells leads to the induction of a megakaryocytic phenotype. In this report we show that FLI-1 also acts as an inhibitor of erythroid differentiation. Following the induction of erythroid differentiation, pluripotent cells express reduced levels of FLI-1. In contrast, when FLI-1 is overexpressed in these cells, the levels of erythroid markers are reduced. The ability of FLI-1 overexpressing cells to respond to erythroid-specific inducers such as hemin and Ara-C is also inhibited, and the uninduced cells show a reduced level of the erythroid-associated GATA-1 transcription factor mRNA. Furthermore, expression of a GATA-1 promoter-driven reporter construct in K562 cells is inhibited by co-transfection with a construct expressing FLI-1. Our results support the hypothesis that FLI-1 can act both positively and negatively in the regulation of hematopoietic cell differentiation, and that inhibition of GATA-1 expression may contribute to FLI-1-mediated inhibition of erythroid differentiation. *Leukemia* (2000) **14,** 439–445.

Keywords: ETS transcription factors; FLI-1; erythroid differentiation; GATA-1; GATA-1 promoter; K562

Introduction

The ETS family of transcription factors has been linked to both normal differentiation and neoplastic diseases in animal models and man.[1–3] Members of this family share a conserved DNA binding domain that recognizes a core . . . GGAA/T . . . binding sequence found in a wide range of eukaryotic promoters. ETS-1 was the first ETS gene to be identified and is responsible for the erythroleukemia-inducing capacity of the acute avian leukemia virus E26 p135$^{gag\text{-}myb\text{-}ets}$ fusion protein.[4,5] We have shown that the erythroleukemic potential of a homologous murine virus construct was also ETS-dependent in mice.[6]

Other members of the ETS family have been identified through their association with hematopoietic disease. In particular, FLI-1 is the target of insertional activation by Friend murine leukemia virus (F-MuLV) and is activated in at least 75% of the proliferating erythroblasts isolated from F-MuLV infected newborn mice.[7,8] FLI-1 is activated in murine leukemias induced by other viruses,[9] and it forms part of the Ewing's sarcoma (EWS) fusion protein generated by chromosomal rearrangements associated with Ewing's sarcoma in man.[10,11] FLI-1 can be up-regulated by the related ETS gene Spi-1/PU.1,[12,13] the primary target for insertional activation in SFFV-induced murine erythroleukemias,[14] suggesting that FLI-1 could play a role in erythroleukemia induction by SFFV as well. The fact that FLI-1 is preferentially expressed in multiple hematopoietic lineages,[11,15] and is associated with hematopoietic disease, strongly suggests that this gene is involved in the lineage-specific differentiation of hematopoietic cells. Consistent with this hypothesis, we have previously shown that FLI-1 overexpression induced changes in human pluripotent hematopoietic cell morphology and surface marker expression characteristic of megakaryocytic cells,[16] suggesting that FLI-1 plays a role in megakaryocytic differentiation. The erythroid and megakaryocytic lineages share common progenitors, and several growth and transcription factors, including GATA-1, are known to play roles in both lineages.[17–21] Since the role of FLI-1 in human hematopoietic cells is not well understood, we undertook to analyze the effects of FLI-1 expression on erythroid differentiation of human pluripotent cells, in order to identify the possible role of the FLI-1 transcription factor in the normal erythroid phenotype. Our results demonstrate that induction of the erythroid phenotype reduces FLI-1 mRNA levels. They also show that FLI-1 overexpression down-regulates the levels of erythroid-specific surface markers and increases resistance of these cells to inducers of erythroid differentiation. In addition, expression of GATA-1 mRNA is significantly reduced in FLI-1-overexpressing cells. Consistent with this observation, cotransfection of K562 cells with a FLI-1 expression construct is able to inhibit expression of a GATA-1 promoter-driven reporter. These data support the hypothesis that FLI-1 can act both positively and negatively during hematopoietic differentiation to regulate the expression of erythroid and megakaryocytic genes, and that FLI-1 may play a critical role in the divergence of erythroid and megakaryocytic cell differentiation.

Materials and methods

Cells, viruses and induction of differentiation

K562 and HEL cells were purchased from the American Type Culture Collection and maintained in RPMI-1640 supplemented with 10% fetal bovine serum. Differentiation/induction experiments were performed by culturing K562 and HEL cells in the presence of cytosine arabinofuranoside (Ara-C) (0.1 μM) or hemin (40 μM) (Sigma, St Louis, MO, USA) for 1–4 days. Differentiation was assessed by benzidine staining for hemoglobin and surface markers analysis. Virus stocks from the drug-selectable retroviral construct expressing FLI-1 were prepared as described previously.[16] Cells were infected and selected by growth in media containing 800 μg/ml Geneticin (G418, Life Technologies, Gaithersburg, MD, USA) 24–48 h after infection.

Surface markers

Cell surface antigens were examined by indirect immunofluorescence and flow cytometry. FITC or PE (phycoerythrin)-con-

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jugated monoclonal antibodies specific for the cell surface marker Glycophorin A were obtained from Pharmingen (San Diego, CA, USA) and CD71 from DAKO (Carpinteria, CA, USA). 10^6 Cells, either uninduced, induced with Ara-C or hemin, or virus-infected, were harvested, washed with PBS + 2% calf serum, and incubated in the dark with the antibodies for 45 min at 4°C. After washing three times with PBS + serum they were analyzed using flow cytometry.

Cytochemical assays

Hemoglobin expression was detected using acid benzidine staining.[22] Three μl of 30% hydrogen peroxide mixed with 500 μl of benzidine solution were added to an equal volume of cell suspension. The mixture was incubated at room temperature for 3 to 5 min and the percentage of benzidine-positive cells was determined by microscopic examination of a minimum of 300 cells.

RNA preparation and analysis

Total RNA was purified using RNAzol (TelTest, Friendswood, TX, USA) according to the manufacturer's specifications. RNAs (10 μg/lane) were separated electrophoretically on 1.2% agarose gel containing 2.2 formaldehyde, transferred to nylon membranes (New England Nuclear, Boston, MA, USA), fixed by UV crosslinking (Hoefer, San Francisco, CA, USA) and analyzed by hybridization using specific probes prepared by random priming (Life Technologies) using ^{32}P dCTP (New England Nuclear). Hybridizations were performed as described previously.[23] Probes were prepared from fragments from recombinant clones containing sequences specific for: GATA-1 – 1.5 kb *Sst*1–*Hin*dIII fragment from the GATA-1 expression vector pSEXGATA, as previously described;[16] GATA-2 – 700 bp *Eco*RI fragment of the murine GATA-2 cDNA in pUC19; PU.1 – 1.18 kb *Eco*RI fragment from p25.1;[13] FLI-1 – 1.1 kb *Ava*I–*Hin*dIII fragment of the human cDNA clone;[15] Tal-1 – 1.18 kb *Eco*RI fragment containing TAL-1 homologous cDNA fragment from lambda CZ1, ATCC No. 61493.

Transient transfection assays

K562 cells (2×10^6) were transfected with 4.5 μg total DNA using DMRIE (Life Technologies) according to the manufacturer's instructions. The control plasmids pGL2 basic expression vector, which lacks an eukaryotic promoter, and pGL2 control an SV40 promoter driven luciferase construct were utilized as negative and positive controls, respectively (Promega, Madison, WI, USA). All plates also received 0.5 μg of RSVgal, as a control for transfection efficiency. Forty-eight hours after transfection, cells were lysed and luciferase and β-galactosidase activities were analyzed using the Promega Luciferase Assay System and Tropex Galacto-Light Plus Kit (Tropix, Bedford, MA, USA), respectively. Relative luciferase activities were determined for duplicate samples in five to seven separate experiments, and were normalized by setting all β-gal levels to be equal. NIH3T3 cells (4×10^5/60 mm dish) were transfected with 18 μg total DNA using CellPhect (Pharmacia, Uppsala, Sweden) according to the manufacturer's instructions.

Results

FLI-1 expression is down-regulated during induction of erythroid differentiation of pluripotent human erythroleukemia lines

The human erythroleukemia-derived cell lines HEL and K562 can be induced to differentiate either towards erythroid or megakaryocytic phenotypes in response to specific chemical inducers.[24,25] While K562 cells express barely detectable levels of FLI-1,[16] HEL cells express high levels of FLI-1 and other ETS family members.[26] We therefore examined the levels of FLI-1 mRNA in HEL cells treated with the erythroid inducers Ara-C and hemin to determine if there was a correlation between FLI-1 expression and induction of the erythroid phenotype. The level of FLI-1 seen in HEL cells was reduced in a time-dependent manner following induction (Figure 1a, b), and relative to the level of 18S RNA in each lane we determined that there was an 85% reduction in FLI-1 expression after 72 h of Ara-C treatment (Figure 1, lane 4). A similar reduction (94%) was seen following hemin treatment

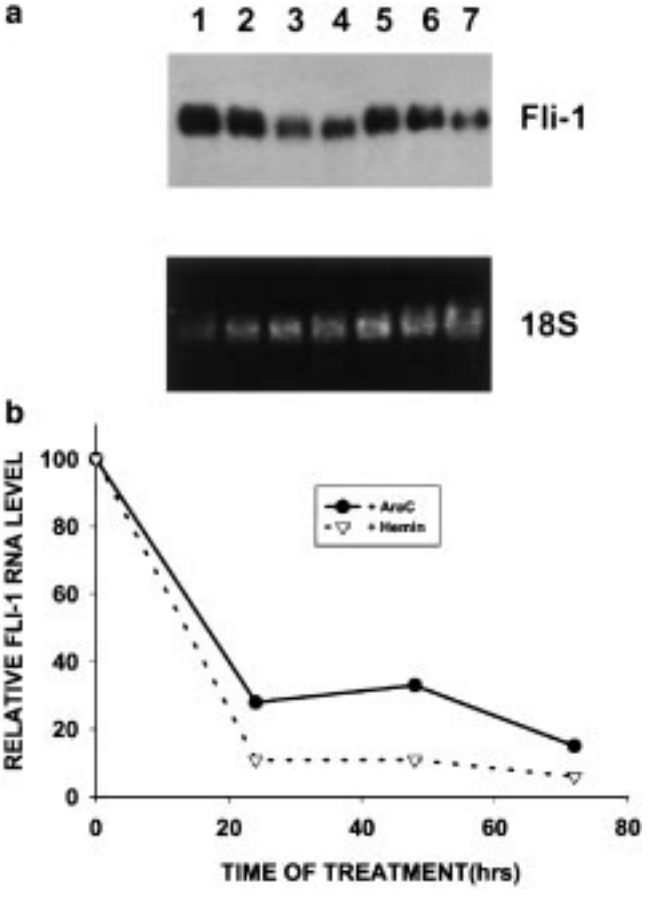

Figure 1 Treatment of HEL cells with erythroid specific inducers Ara-C or hemin down-regulates FLI-1 mRNA expression. (a) Cells were treated with Ara-C (0.1 μM) or hemin (40 μM,) and total RNA was prepared at the indicated times after initiation of treatment. RNA was separated, blotted and probed with a FLI-1-specific probe. The 18S band was used to normalize for transfer variations. Lane 1, untreated; lanes 2–4, Ara-C; lanes 5–7, hemin; lanes 2, 5, 24 h; lanes 3, 6, 48 h; lanes 4, 7, 72 h. (b) Bands shown in (a) were scanned and quantitated (IP Lab Spectrum, Signal Analytics Corporation, Vienna, VA, USA). The levels of FLI-1 were normalized using the 18S ribosomal RNA levels.

Table 1 Level of benzidine induction in HEL cells treated with erythroid inducer

Treatment	*Length of treatment (h)*	*% Benzidine positive*
Untreated		0.5
+ hemin	24	24
	48	57
	72	74
	96	76

(Figure 1, lane 7). Consistent with its ability to induce erythroid differentiation,[24,27] treatment with hemin increased the fraction of benzidine-positive cells, indicating increased hemoglobin levels (Table 1). As previously reported,[28–31] hemoglobin levels in K562 cells were also responsive to hemin, but the endogenous level of FLI-1 in these cells was too low to determine if they were reduced in cells treated with these inducers (data not shown).

The overexpression of FLI-1 correlates with reduced levels of erythroid markers

The reduction in FLI-1 mRNA levels seen in response to erythroid inducers suggested an association between expression of the FLI-1 transcription factor and the induction of an erythroid phenotype in pluripotent human cells. To test this possibility, we infected K562 cells with a retrovirus expressing the FLI-1 gene[16] and isolated G418-resistant cells stably expressing high levels of FLI-1. These FLI-1-overexpressing cells expressed significantly reduced levels of two erythroid markers, glycophorin A and CD71, the transferrin receptor (Figure 2). The mean level of CD71 fluorescence was reduced from 80.6 in vector-infected cells, to 32.3 in FLI-1-infected cells. Glycophorin A also showed an overall reduction in mean fluorescence intensity, and Figure 2 shows that a population of cells expressing little or no detectable glycophorin A was seen in cells overexpressing FLI-1. These results indicated that elevation of FLI-1 levels in K562 cells leads to the inhibition of erythroid marker expression.

FLI-1 overexpression inhibits the response of both K562 and HEL cells to erythroid inducers

The reduced level of FLI-1 in cells induced to differentiate along the erythroid pathway, together with the evidence that FLI-1-overexpressing K562 cells expressed lower levels of erythroid-specific markers, suggested that constitutive overexpression of FLI-1 might affect the response of these cells to erythroid inducers. We therefore tested the ability of FLI-1-expressing K562 and HEL cells to respond to the inducers Ara-C and hemin. As expected, treatment of both cell populations resulted in a dramatic reduction in cell proliferation. While FLI-1-expressing cells grew more slowly than control cells, we observed no apparent differences in their ability to proliferate after treatment (Table 2).

However, there were differences between the populations

Table 2 Cell proliferation of K562 cells following treatment with erythroid inducers

	t = 72 h[a]	
	Cells	*Generations*
K562 (Vector)	$46.4 \times 10^4 \pm 1.8 \times 10^4$	3.5
+ Ara-C	$15.0 \times 10^4 \pm 1.0 \times 10^4$	1.9
+ Hemin	$8.8 \times 10^4 \pm 0.3 \times 10^4$	1.1
K562 (FLI-1)	$35.2 \times 10^4 \pm 1.1 \times 10^4$	3.2
+ Ara-C	$13.4 \times 10^4 \pm 0.8 \times 10^4$	1.7
+ Hemin	$6.4 \times 10^4 \pm 0.9 \times 10^4$	0.7

[a]Cells were seeded at 4×10^4 in media ± inducers. Cell numbers were measured in duplicate, and the number of cell divisions calculated. The data is from a representative experiment.

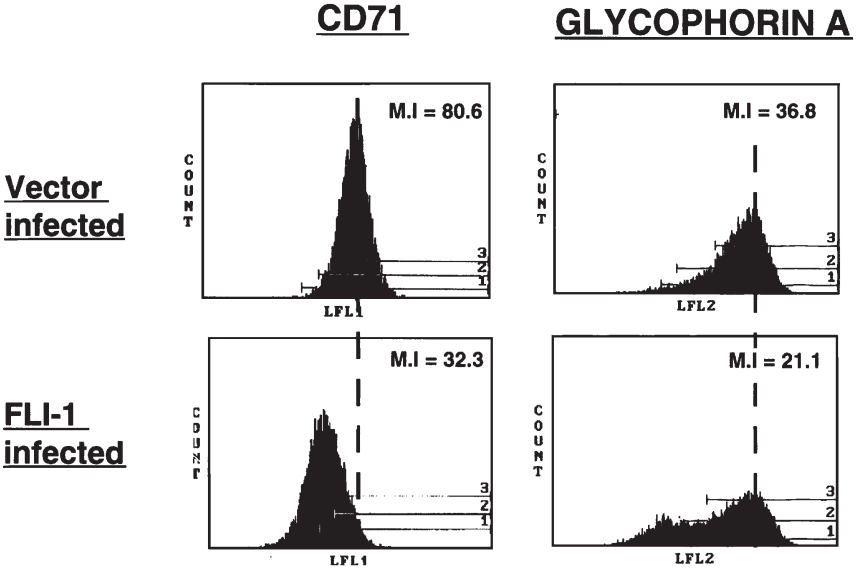

Figure 2 FLI-1 inhibits the expression of erythroid-specific cell surface markers. K562 cells were infected with FLI-1-expressing or vector virus, selected for drug resistance, and analyzed by flow cytometry using either a CD71-specific or glycophorin A-specific antibody. The mean intensity of the population of cells is given as an inset in each panel.

in their expression of erythroid markers. As shown in Table 3, FLI-1-expressing K562 populations show reduced levels of benzidine-positive cells in response to treatment with either erythroid inducer. HEL cells, which in our experiments are not responsive to Ara-C,[28] are also less responsive to hemin if they are infected with the FLI-1-expressing virus. Flow cytometry measurement of the erythroid marker CD71 level showed a 28% reduction (compared to vector-infected controls) in the response of K562 to Ara-C, while the response of HEL cells to this inducer was completely blocked (Table 3). Thus, increased FLI-1-expression inhibited both cell lines in their ability to express erythroid markers in response to inducers of the erythroid pathway.

FLI-1 overexpression reduces the levels of erythroid lineage-associated transcription factors

To investigate possible mechanisms responsible for the reduction in erythroid marker expression in FLI-1 virus-infected cells, we examined the levels of GATA-1, GATA-2 and TAL-1,[32–34] transcription factors whose expression has been associated with the erythroid phenotype. As our previous studies had shown, FLI-1-expressing K562 cells could be separated into adherent and non-adherent cell populations, both expressing high levels of FLI-1 mRNA and protein.[16] We analyzed these two populations of infected cells and found that mRNA expression levels of the three factors were substantially reduced in both non-adherent (Figure 3a, lane 2) and adherent (Figure 3a, lane 3) FLI-1-expressing cells. Both control, vector infected K562 cells (Figure 3a, lane 1) and uninfected parental cells (data not shown) expressed similar levels of all three factors. As shown here, GATA-1 was essentially completely inhibited in FLI-1-expressing cells, while GATA-2 and TAL-1 also show significant inhibition of mRNA expression. Consistent with the effects seen in K562 cells, we also observed a reduction in the level of GATA-1 mRNA in HEL cells infected with the FLI-1 expression construct. Densitometric analysis of the results shown in Figure 3b indicated that relative to the 18S RNA levels, GATA-1 levels in FLI-1-infected cells (Figure 3b, lane 2) were two-fold lower than that seen in vector control cells (Figure 3b, lane 1). Unlike K562, HEL cells express high levels of endogenous FLI-1, and the smaller reduction seen here may be due to the fact that FLI-1 infected HEL cells only expressed a two-fold higher level of FLI-1 relative to control cells (data not shown).

Table 3 Effect of FLI-1 expression on induction of erythroid markers by Ara-C and hemin

Cells[a]	*Treatment*	*% benzidine positive infected by*		*Relative CD71 level ± s.d.*[b] *infected by*	
		Vector	*FLI-1*	*Vector*	*FLI-1*
HEL	Untreated	<1	<1	1.0	1.0
	+ Ara C	<1	<1	2.23 ± 0.03	0.98 ± 0.01
	Hemin	62.5 ± 2.9	46.3 ± 2.1	*	*
K562	Untreated	1 ± 1	2 ± 1	1.0	1.0
	+ Ara C	24.7 ± 1.0	10.7 ± 0.9	2.70 ± 0.03	1.94 ± 0.07
	+ Hemin	79.5 ± 1.4	64.5 ± 0.7	*	*

*Could not be analyzed because hemin interfered with flow cytometry analysis of fluorescent markers.
[a]These results represent the effects seen in three independent analyses.
[b]The mean fluorescence intensity was determined for each sample. The level relative to that observed in either untreated vector-infected or FLI-1-infected cells (each set to 1.0) was then calculated and is reported, together with the standard deviations (s.d.) for three independent determinations.

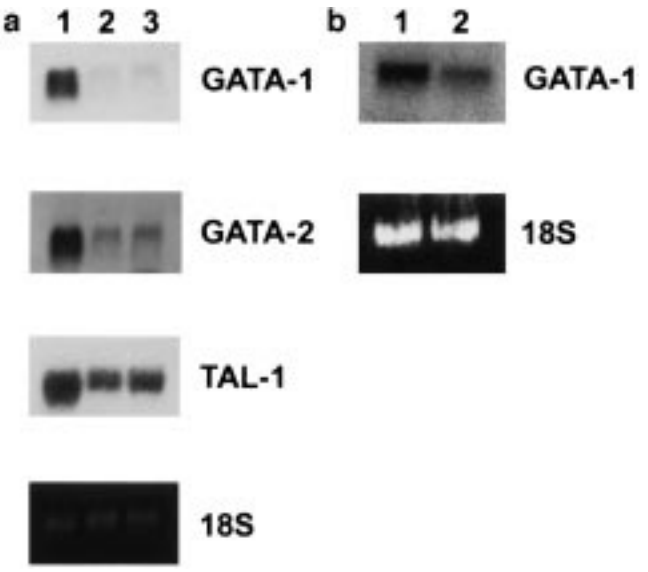

Figure 3 FLI-1 reduces the expression of erythroid-associated transcription factor RNA. (a) Total RNA from K562 cells infected with vector virus (lane 1) and the non-adherent (lane 2) and adherent (lane 3) populations of cells infected with a FLI-1-expressing virus[16] was analyzed. Northern blots were hybridized sequentially with probes specific for GATA-1, GATA-2 and TAL-1. The ethidium bromide stained level of 18S ribosomal RNA transferred to the filter was used to normalize RNA levels between lanes. (b) Total RNA from HEL cells infected with vector virus (lane 1) or FLI-1-expressed virus (lane 2) were analyzed as described in (a) with a GATA-1-specific probe.

FLI-1 inhibits GATA-1 promoter-driven expression

The critical role of the GATA-1 transcription factor in hematopoietic differentiation in general, and erythroid differentiation in particular, led us to examine the effects of FLI-1 on the expression of this gene in more detail. We utilized a FLI-1 expression construct together with a luciferase reporter gene linked to the –873 to –1 region of the murine GATA-1 promoter.[35] The GATA-1 promoter is regulated in part by an autoregulatory loop,[36] and contains consensus and non-consensus GATA-1 binding motifs,[37] as well as potential ETS binding sequences (EBS). K562 cells express high levels of endogenous GATA-1 protein, and the GATA-1 promoter-driven reporter construct was efficiently expressed when transfected into these cells (Figure 4a). Expression of the pGL2 GATA-1 reporter construct was 110-fold higher than the pGL2 basic negative control construct. Luciferase levels expressed by the pGL2 GATA-1 (–873) construct were only 23% lower than those observed when an identical amount of SV40-driven reporter construct was used (data not shown), indicating that the GATA-1 promoter functions efficiently in K562 cells.

While this pGL2 GATA-1 construct was efficiently expressed in K562 cells, expression could be reduced approximately three-fold when the reporter was co-transfected with a one- to three-fold excess of a construct expressing FLI-1 (Figure 4a), and the inhibition was dependent on the relative FLI-1/reporter construct ratios. Furthermore, when K562 cells

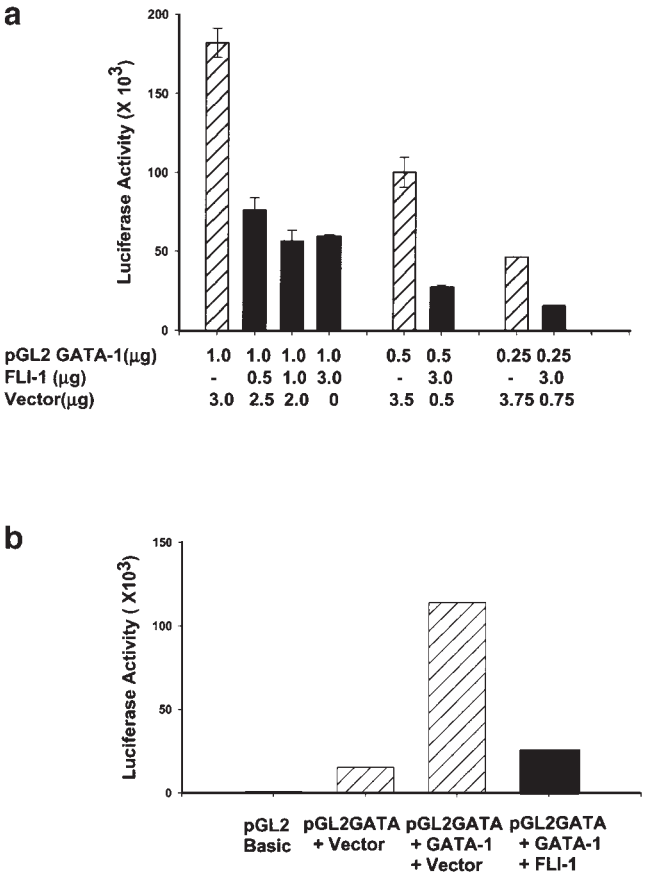

Figure 4 FLI-1 inhibits expression of a GATA-1 promoter-driven reporter construct. (a) Concentration-dependent inhibition of the pGL2 GATA-1 reporter construct in K562 cells. Error bars show standard deviations for duplicate determinations. Cells were transfected with the pGL2 GATA-1 reporter, vector plasmid, and FLI-1-expressing plasmid, as indicated. A total of 4.5 μg of DNA, including 0.5 μg of RSV β-gal, was used in each case. The level of luciferase expression was measured as described in Materials and methods and normalized relative to the RSV β-gal level. The absolute level of β-gal in the different transfections varied by less than 20%. (b) Inhibition of pGL2 GATA-1 expression in NIH3T3 cells. Cells were transfected with the following plasmid combinations: 2 μg of reporter construct (either pGL2 Basic or pGL2 GATA) and 16 μg of vector plasmid; 8 μg of vector, 8 μg of a GATA-1 expression construct and 2 μg of pGL2 GATA; or 8 μg of FLI-1 expression construct, 8 μg of GATA expression construct and 2 μg of pGL2 GATA. Relative expression of luciferase was measured as described in Materials and methods.

constitutively expressing FLI-1 were transfected with the GATA-1 reporter, they expressed less luciferase than similarly transfected, vector-infected control cells (data not shown). Expression of the β-galactosidase control vector, included in all transfections to correct for transfection efficiencies, was not affected by the presence or absence of FLI-1, indicating that the effect on the GATA-1 promoter was not due to some non-specific sequestration of essential basic transcription factors.

We also performed similar experiments in NIH3T3 cells, which lack endogenous GATA-1. As seen in Figure 4b, cotransfection of a GATA-1 expression construct, together with the reporter construct, induces a more than seven-fold increase in the GATA-1 promoter-dependent luciferase activity in NIH3T3 cells. The addition of FLI-1 to the cotransfection, however, inhibits the GATA-1-driven reporter expression more than 80%, reducing it to the level seen in the absence of exogenous GATA-1 (Figure 4b). Both these results and the results in K562 cells (Figure 4a) are consistent with the hypothesis that FLI-1 inhibits the GATA-1 promoter by interfering with the GATA-1 autoregulatory loop.

Discussion

The results we have reported here provide direct evidence for the involvement of FLI-1 in the regulation of erythroid-associated genes and erythroid differentiation in human hematopoietic cells, and indicate that FLI-1 overexpression can suppress erythroid differentiation. We have shown that FLI-1 expression in pluripotent human cells is inhibited when cells are treated with erythroid inducers, and that cells over-expressing retrovirally introduced FLI-1 express lower levels of the erythroid markers CD71 and glycophorin A. These overexpressing cells are also less responsive to the erythroid inducers Ara-C and hemin. Thus, our results indicate that FLI-1 can act as an inhibitor of the erythroid pathway in human pluripotent cells. This is consistent with the role of insertionally activated FLI-1 in F-MuLV-induced murine leukemia,[8,38,39] where the erythroleukemic cells exhibited increased proliferation and were blocked in their ability to undergo terminal erythroid differentiation.[8] Starck *et al*[12] also demonstrated that in SFFV-transformed mouse cell lines, erythroid differentiation in response to hexamethylenebisacetamide (HMBA) is inhibited if FLI-1 is overexpressed, a result consistent with our data in human pluripotent cell lines.

We had previously reported that FLI-1 overexpression acted positively to induce megakaryocytic differentiation of HEL and K562 cells,[16] while ETS-1 overexpression could induce an erythroid response.[28] Together with the data we have present here, our results suggest a model in which FLI-1 acts in a reciprocal fashion to promote megakaryocytic and inhibit erythroid differentiation. They indicate that ETS transcription factors can act coordinately and in opposition to one another, and suggest that the balance of these factors may play a crucial role in determining the fate of cells along the erythroid/megakaryocytic pathway. The involvement of ETS family members in reciprocal opposing roles in development has also been implied for FLI-1 and TEL,[40] and a similar model involving PU.1 and GATA-1 in erythroid cells has also been proposed.[41] These observations suggest such a mechanism may be frequently utilized during hematopoiesis. In addition, this model and our results in human cells are consistent with recent studies in murine cell lines indicating that constitutive FLI-1 over-expression induces proliferation and prevents the induction of a program of erythroid differentiation.[42,43]

Our data also indicate that FLI-1 may exert at least some of its effects through inhibition of the GATA-1 promoter. The GATA-1 transcription factor has been shown to play a major role in controlling expression of both erythroid and megakaryocytic genes,[37,44–46] and is critical for erythroid cell maturation and the differentiation of early precursors of both lineages.[32,47] Our results show that GATA-1 mRNA levels are reduced in both K562 and HEL cells when they constitutively express elevated levels of FLI-1. The effect was much greater in K562 than in HEL cells, but this could reflect the fact that the change in FLI-1 levels following introduction of the FLI-1-expressing retrovirus was greater in K562 cells as well. FLI-1 levels in HEL cells could only be increased about two-fold due to the high level of endogenous FLI-1 expression, and this could result in a smaller effect on GATA-1 levels. GATA-1 is required for the expression of many erythroid-specific genes, and the inhibitory effects of FLI-1 on the expression of

erythroid markers may be due in part to a reduction in GATA-1 levels.

Our analysis of the GATA-1 promoter in both K562 and NIH3T3 cells suggests that FLI-1 inhibits the promoter by blocking the GATA-1-dependent autostimulatory loop. The GATA-1 promoter has been shown to contain multiple ETS consensus binding sequences (EBS),[35,37] but preliminary analysis of a panel of promoter deletion mutants failed to clearly implicate a specific region of the promoter in the FLI-dependent inhibition. It remains to be determined whether inhibition involves direct FLI-1 binding to one or more EBS targets in the promoter. FLI-1 could also act indirectly to activate suppressors or inhibit other components necessary for expression of the promoter, or could interact directly with GATA-1 itself to reduce its ability to induce its own promoter. Rekhtman *et al*[41] have reported that both PU.1 and FLI-1 can interact directly with GATA-1, although these authors indicate that the FLI-1/GATA-1 interaction appeared to be weaker. Cooperation between ETS and GATA-1 has also been described in the regulation of EOS47, an eosinophil-specific gene in chickens,[48] as well as in the regulation of the megakaryocyte/platelet marker PF4 in rats.[49] In addition, PU.1 and GATA-1 may interact both in MEL cells and Xenopus embryos, and like FLI-1 can repress GATA-1-mediated transactivation.[41] Thus, ETS family members and GATA-1 appear to cooperate in regulating the expression of several hematopoietic genes.

It is interesting that overexpression of Spi-1/PU.1, an ETS family member whose expression is also activated by retroviral insertion in murine erythroleukemias,[50] has been reported to block the ability of K562 cells to differentiate along this pathway.[51,52] We found that FLI-1 overexpression did not alter the level of PU.1 expression in K562 cells (data not shown), indicating the inhibition we observed was not due to changes in PU.1 expression levels. Furthermore, it has been reported that PU.1 may be a positive regulator of FLI-1 expression in SFFV-transformed murine cells,[12] suggesting that both could act on the same pathway. These results suggest the possibility that FLI-1 could contribute to the downstream effects of PU.1-induced activation or inhibition.

In summary, our results indicate that FLI-1 is likely to play a significant role in human hematopoietic cells in controlling the expression of lineage-specific genes and the progression along the erythroid pathway. When considered along with our previous report that FLI-1 could positively induce progression along the megakaryocytic pathway,[16] it suggests that FLI-1 can act both positively and negatively in regulating hematopoietic differentiation. In view of the close relationship between the erythroid and megakaryocytic pathways, it may be advantageous for cells to both promote progression down one pathway while utilizing the same elements to inhibit a competing response. Our data and that of other investigators indicate that FLI-1 can exhibit these functions in both mice and man.

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