The Proto-Oncogene ERG in Megakaryoblastic Leukemias

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Abstract

Aneuploidy is one of the hallmarks of cancer. Acquired additions of chromosome 21 are a common finding in leukemias, suggesting a contributory role to leukemogenesis. About 10% of patients with a germ line trisomy 21 (Down syndrome) are born with transient megakaryoblastic leukemia. We and others have shown acquired mutations in the X chromosome gene *GATA1* in all these cases. The gene or genes on chromosome 21 whose overexpression promote the megakaryoblastic phenotype are presently unknown. We propose that ERG, an Ets transcription factor situated on chromosome 21, is one such candidate. We show that ERG is expressed in hematopoietic stem cells, megakaryoblastic cell lines, and in primary leukemic cells from Down syndrome patients. ERG expression is induced upon megakaryocytic differentiation of the erythroleukemia cell lines K562 and UT-7, and forced expression of ERG in K562 cells induces erythroid to megakaryoblastic phenotypic switch. We also show that ERG activates the *gpIb* megakaryocytic promoter and binds the *gpIIb* promoter *in vivo*. Furthermore, both ERG and ETS2 bind *in vivo* the hematopoietic enhancer of *SCL/TAL1*, a key regulator of hematopoietic stem cell and megakaryocytic development. We propose that trisomy 21 facilitates the occurrence of megakaryoblastic leukemias through a shift toward the megakaryoblastic lineage caused by the excess expression of *ERG*, and possibly by other chromosome 21 genes, such as *RUNX1* and *ETS2*, in hematopoietic progenitor cells, coupled with a differentiation arrest caused by the acquisition of mutations in *GATA1*. (Cancer Res 2005; 65(17): 7596-602)

Introduction

Numerical chromosomal aberrations (aneuploidy) are common in cancer (1). Their significance for oncogenesis is revealed by genetic syndromes of aneuploidy and cancer (2). The classic constitutional aneuploidy that shows predisposition to certain kinds of cancer is trisomy 21, also called Down syndrome. Children with Down syndrome have a marked risk for childhood leukemia (reviewed in ref. 3). Thus, additional copies of chromosome 21 are leukemogenic.

At least 10% of children with Down syndrome are born with a transient megakaryoblastic leukemia (transient myeloproliferative disorder) that resolves spontaneously. In about one fifth of these patients, it recurs as acute megakaryoblastic leukemia (3). Acquired mutations in *GATA1* in the leukemic blasts are detected in virtually all these cases (refs. 4–6; reviewed in ref. 7). GATA1 is a transcription factor that regulates megakaryocytic differentiation and the mutations observed are believed to cause accumulation of poorly differentiated megakaryocytic precursors (4).

Strikingly, *GATA1* is located on chromosome X; therefore, genetic interaction of *GATA1* with one or more genes on chromosome 21 presumably contributes to the development of Down syndrome megakaryoblastic leukemia. However, the gene(s) on chromosome 21 that promote the megakaryoblastic leukemias of Down syndrome are currently unknown. Healthy infants with Down syndrome have a significantly higher platelet count compared with normal infants (8). This observation suggests that trisomy 21 may cause developmental skewing toward the megakaryocytic lineage during hematopoietic development. This "promegakaryocytic" pressure caused by trisomy 21 may in turn enhance the selection and proliferation of cells carrying an acquired "differentiation arresting" mutation in GATA1 (9).

One gene, *RUNX1*, located in the "critical Down syndrome region" on chromosome 21, is a known key regulator of hematopoiesis and megakaryopoiesis and is commonly mutated and translocated in leukemias (10, 11). Haploinsufficiency of *RUNX1* causes thrombocytopenia in human and in mouse models (12, 13) and overexpression facilitates megakaryocytic differentiation (10). However, unlike other types of leukemias, acute megakaryoblastic leukemia has not been associated with *RUNX1* abnormalities. Moreover, the leukemogenic properties of *RUNX1* have been generally associated with loss of function (11), whereas there is an excess of an additional copy of *RUNX1* in trisomy 21.

The *ERG* gene, located on chromosome 21q22, encodes a transforming proto-oncogene (14) that is also expressed in hematopoietic stem cells and endothelial cells. Its close family member, *FLI1* (located at chromosome 11q24), is required for normal megakaryopoiesis (15). In patients with Ewing sarcoma, either *FLI1* or *ERG* fuses with the *EWS* gene to create a chimeric oncogenic protein (16). Thus, at least in the context of this particular chromosomal translocation, FLI1 and ERG are interchangeable. *ERG* has also been associated with rare cases of leukemias, such as the *TLS(FUS)-ERG* fusion gene in megakaryoblastic leukemias (17, 18). *ERG* was also recently reported to be overexpressed in myeloid leukemias with complex karyotypes (19).

We have, therefore, hypothesized that increased expression of ERG in hematopoietic progenitors caused by excess copies of chromosome 21 may contribute to the development of megakaryoblastic leukemias.

Note: Supplementary data for this article are available at Cancer Research Online (http://cancerres.aacrjournals.org/).

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Materials and Methods

Cell lines. CMK, Dami, Meg-01, and 416B cells were grown in RPMI containing 10% fetal bovine serum (FBS) with additional antibiotics and 2 mmol/L L-glutamine. UT-7/EPO (20) and UT-7/TPO (21) cell lines were maintained in Iscove's modified Dulbecco's medium (Invitrogen Life Technologies, Carlsbad, CA) supplemented with 10% FBS (Invitrogen Life Technologies) and 1 unit/mL of human recombinant erythropoietin (Kirin, Gunma, Japan) or 10 g/mL of human recombinant thrombopoietin (Kirin), respectively. Isolation of cord blood $CD34^+$ cells and *in vitro* megakaryocytic differentiation was done as described (22).

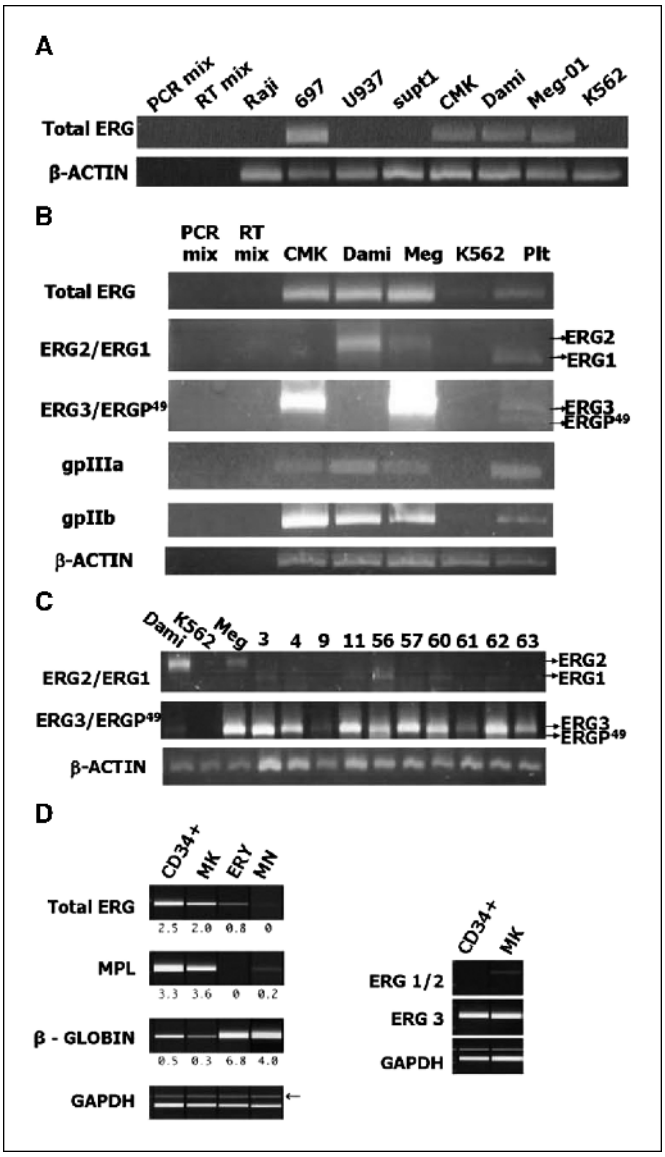

Figure 1. ERG expression. *A,* RT-PCR analysis of ERG expression in leukemia cell lines. β-actin is used as a control for the amount of cDNA. *Raji,* Burkit lymphoma; *697,* pre-B lymphoblastic leukemia; *U937,* myeloid leukemia; *CMK* and *Meg-01,* megakaryoblastic leukemia with trisomy 21; *Dami,* megakaryoblastic leukemia with two copies of chromosome 21; *K562,* erythroleukemia (both Meg-01 and K562 were derived from blast crisis of CML; *B,* ERG isoforms in megakaryocytic leukemias and platelets. gpIIIa and gpIIb are megakaryocytic markers. See Supplementary Data for nomenclature of ERG isoforms. *C,* expression of ERG isoforms in primary samples: RT-PCR analysis of three cell lines, three Down syndrome–acute megakaryoblastic leukemia patients (patients 3, 4, and 9) and seven Down syndrome–transient myeloproliferative disorder patients (patients 11, 56, 57, 60, 61, 62, and 63). *D,* ERG isoforms, MPL, and β-globin expression in cultured hematopoietic cells: $CD34^+$ lineage-negative cells purified from umbilical cord blood and their derived megakaryoblasts (*MK*) by 7 days treatment with thrombopoietin and erythroblasts (*ERY*) by 7-day exposure to erythropoietin; *MN,* mononuclear cells isolated from peripheral blood. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is used as a control for the amount of cDNA. *Arrow,* 1,000 bp marker DNA. The numbers indicate the relative concentration of PCR products against the each GAPDH amplicon. Amplification of β-globin in peripheral blood mononuclear cells was probably caused by the cDNA derived from contaminating erythrocytes in fractionated cells.

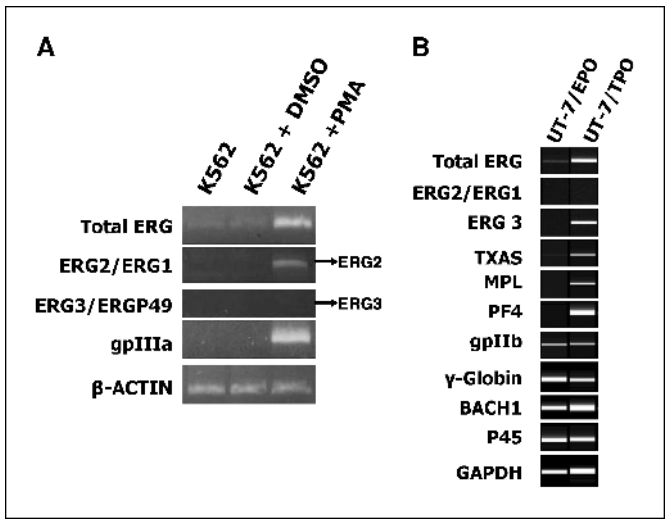

Figure 2. Induction of ERG expression during megakaryocytic differentiation of leukemia cells. *A,* RT-PCR analysis of free-growing K562 cells, K562 cells treated with the solvent DMSO and K562 cells differentiated to megakaryocytes by treatment treated with the phorbol ester PMA. β-actin is used as a control for the amount of cDNA. gpIIIa is a marker of megakaryocytes. *B,* expression of ERG isoforms in UT-7 cells treated either with erythropoietin (*EPO*) or thrombopoietin (*TPO*). RT-PCR products were electrophoresed by using a Bioanalyzer 2100 and the DNA 1000 LabChip kit (Agilent Technologies, Palo Alto, CA). GAPDH is used as a control for the amount of cDNA. ERG3 and megakaryocytic genes (e.g., *MPL, PF4, TXAS*) were expressed in UT-7/TPO, whereas these genes were not amplified in UT-7/EPO.

Patient samples. The patients were described by us previously (5). The study was approved by the institutional review board.

Platelet extraction. Ten milliliters of fresh blood extracted into EDTA were centrifuged at 120 × *g* for 10 minutes. The supernatant was transferred into 50 mL of 5 mmol/L EDTA in PBS and centrifuged again at 600 × *g* for 5 minutes. The supernatant was discarded and total RNA was extracted from the pelleted platelets with TRIzol Reagent (Life Technologies).

Reverse transcription-PCR analysis. Reverse transcription-PCR (RT-PCR) was done as previously described (5). (Primer sequences and PCR conditions are available upon request.)

Megakaryocytic induction of K562 cells. K562 cells (2×10^5 cells/mL) were induced with 15 nmol/L phorbol 12-myristate 13-acetate (PMA, Sigma Chemical, St. Louis, MO) for 77 hours as described (10).

Forced expression of ERG in K562. K562 cells (7×10^6) were coelectroporated with 10 μg of pMSCV-IRES hCD2 and 40 μg of each of the indicated plasmids: pCEFL-HA-ERG3, pCEFL-HA-ERG3ΔETS, and pKC3-FLI1. Seventy-two hours after electroporation, the cells were analyzed by flow cytometry by a standard protocol.

Plasmids and constructs. pKC3-FLI-1 was provided by Dr. Olivier Delattre (Institut Curie, Paris, France). pXM-GATA1 was provided by Dr. Peter Aplan (NIH, Bethesda, MD). pCEFL-GATA1s was generated by inserting an GATA1s cDNA fragment *Bam*HI-*Not*I into pCEFL. pCEFL-HA-ERG-3 was generated by inserting an ERG-3 cDNA fragment, HA tagged, and flanked by *Mfe*I, sites into the *Eco*RI site of pCEFL. pCEFL-HA-ERG3ΔETS was generated by cutting pCEFL-HA-ERG3 with *Nde*I and religating to create an ERG3 mutant that lacks the ETS DNA-binding domain. The gp1bα5′-567luc reporter plasmid was provided by Dr. G. Roth.

Transient transfection and reporter assays. Transient transfection of HeLa cells was carried out in 24-well plates by using JetPEI (Polyplus-Transfection, Illkirch, France) reagent according to the instructions of the

manufacturer. HeLa cells were transfected with 170 ng of the gp1bα5′-567luc reporter plasmid (23) and 335 ng of the expression plasmids encoding ERG3, ERG3ΔETS, GATA1, and GATA1s as indicated. The total amount of DNA in each transfection was kept constant at 1.34 μg by adding the pcDNA3 backbone. Cells were harvested 24 hours after transfection, and 10 μL of lysate was assayed for luciferase activity by using the Luciferase Reporter Assay System kit (Promega, Madison, WI).

Antibodies and protein analysis. Ten micrograms of nuclear extracts from the transfected HeLa cells were analyzed by 10% SDS-PAGE and transferred to nitrocellulose membrane. Following transfer, the membrane was blocked overnight in 10% skim milk–TBS–0.05% Tween 20 and incubated with anti–GATA-1 antibody M20 (1:1,000 dilution, Santa Cruz Biotechnology, Santa Cruz, CA) for 1 hour. The membrane was washed thrice in TBS–Tween 20 and incubated with the appropriate horseradish peroxidase–conjugated secondary antibody (1:20,000 dilution; The Jackson Laboratory, Cambridgeshire, United Kingdom) for a further hour, washed thrice with PBS–Tween 20, and subjected to chemiluminescence detection (Pierce, Rockford, IL). The membrane was then striped and reprobed with anti-ERG antibody C17 (1:1,000 dilution, Santa Cruz Biotechnology) and anti-Emerin antibody (1:5,000 dilution, Santa Cruz Biotechnology) for loading control.

Chromatin immunoprecipitation assays. Real-time PCR-based quantitative chromatin immunoprecipitation analysis was done as previously described (24, 25) using the murine myeloid progenitor cell line, 416B (2×10^7 cells per immunoprecipitation). Normal rabbit IgG, anti-Fli1, anti-Erg, and anti-Ets 2 antibodies were purchased from Santa Cruz Biotechnology and the anti–acetyl histone H3 antibody from Upstate Biotechnology (Lake Placid, NY). One microliter aliquots of each immunoprecipitate sample were used for SYBR green quantitative real-time PCR analysis (using a Stratagene MX3000 thermocycler). PCR products were quantified relative to a standard curve generated by titrating input chromatin. All reactions were validated by postamplification denaturation curves to confirm single product yield (data not shown). Forward and reverse primers (5′ to 3′) used for real-time PCR were as follows. Stem cell leukemia (SCL) +19, CCATACTCTTGCCAAGGCTACC and AGCAGTCCTACATGGGCCTAAA; GPIIb promoter, CTTCCAGCCATCAGCATCC and TTTCCCTTTCCCTGAAGTTCC; Endoglin untranslated region (UTR), GTCCCAGGAACCAAACAC and GGGCAGTTCTGTAAAGTGG.

Results

ERG expression in megakaryocytic and erythroid leukemias. To establish the pattern of ERG expression in leukemic cells, we surveyed several leukemic cell lines for its expression by RT-PCR (Fig. 1*A*). ERG was expressed in the three megakaryoblastic leukemia cell lines (CMK, Meg-01, and Dami) and the pre-B ALL cell line 697. CMK (derived from Down syndrome) and Meg-01 [derived from a blast crisis of chronic myelogenous leukemia (CML)] contain trisomy 21, whereas Dami is reported to have two copies of chromosome 21. ERG was low to absent in myeloid leukemia cell lines U937 and HL-60 (not shown), in K562 (a cell line derived from erythroleukemia blast crisis of CML), in a T-cell ALL cell line SUPT1, and in the mature B-cell leukemia cell lines Rajii and Daudi (not shown).

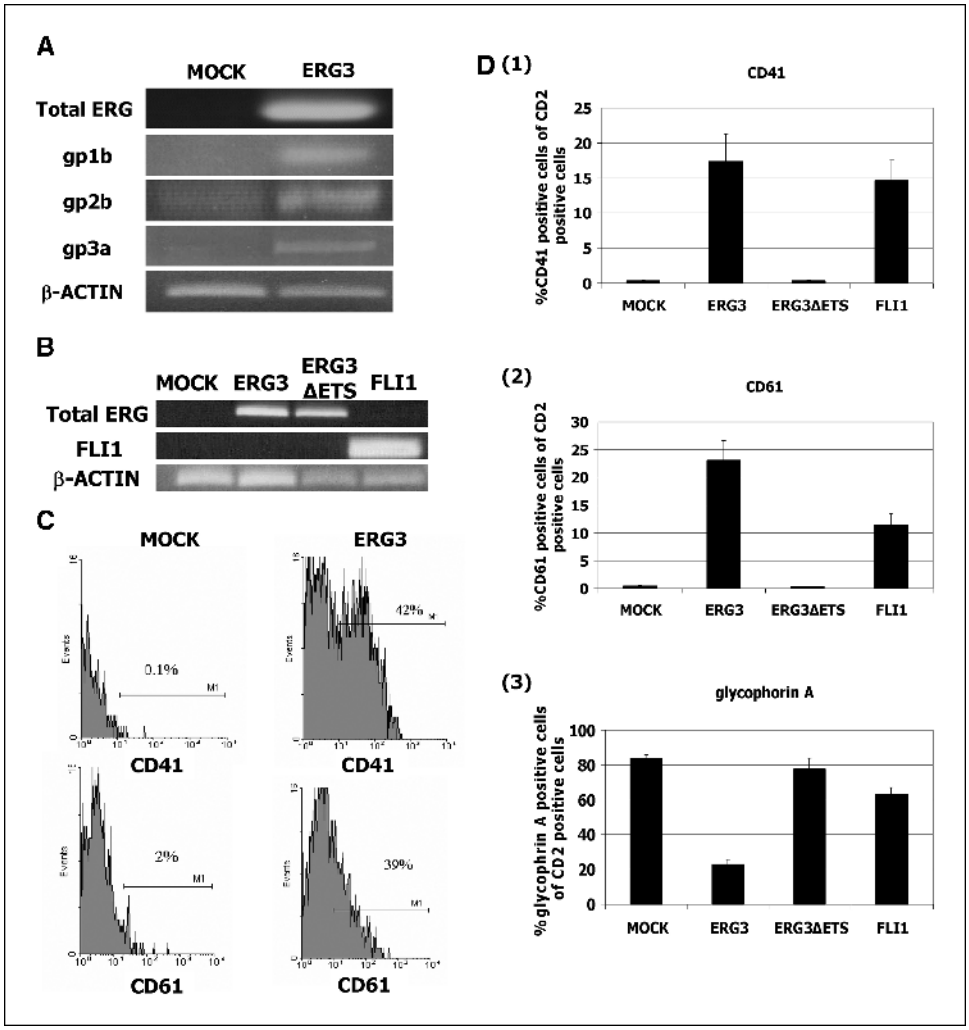

Figure 3. Forced expression of ERG in K562 cells. *A,* RT-PCR analysis of K562 cells transfected with an empty pMSCV-IRES-CD2 vector or with a vector encoding ERG-3. gpIb, gpIIb, and gpIIIa are megakaryocytic markers. β-actin is used as a control for the amount of cDNA. *B,* RT-PCR of K562 cells transfected with mammalian expression vectors encoding ERG3. A mutant ERG3 lacking the Ets domain and FLI-1, demonstrating expression of the three genes in the transfected K562 cells. *C,* flow cytometry analysis of the transfected K562 cells. The histograms show the percentage of CD41- and CD61-positive cells out of the transfected (CD2-positive) cells. Forced expression of ERG3 induced the expression of both CD41 and CD61. *D,* flow cytometry of K562 cells cotransfected with an empty pMSCV-IRES-CD2 vector and each of the three indicated plasmids. *Columns,* percentage of CD2-positive cells expressing CD41, CD61, and glycophorin A, respectively. ERG3 induces the expression of CD61 and CD41 and dramatically reduce the expression of the erythroid marker glycophorin A in a way dependent on the presence of an intact Ets domain.

There are at least five isoforms of ERG generated by alternative splicing and translation initiation sites (see Supplementary Data; refs. 14, 26). ERG isoforms showed differential expression between samples. In the CMK cell line (Fig. 1*B*) and in primary samples of Down syndrome–acute megakaryoblastic leukemia (Fig. 1*C*), mostly expression of ERG-3 was detected. ERG-2 was the only isoform detected in Dami, whereas both isoforms were expressed in the Meg-01 cell line. ERG-3 was also detected in normal platelets (Fig. 1*B*), in $CD34^+$ hematopoietic cells purified from umbilical cord blood, and in megakaryoblasts generated *in vitro* (Fig. 1*D*). ERG3 expression was low to absent in erythroblast differentiated *in vitro* from $CD34^+$ cells and in mononuclear cells purified from peripheral blood (Fig. 1*D*). These findings implicate ERG-3 as the major hematopoietic isoform. ERG expression in platelets and in megakaryoblasts suggests that it may have a role in normal megakaryopoiesis.

The erythroid and the megakaryocytic lineages are closely related and originate from a common megakaryoblastic erythroid progenitor (MEP; ref. 27). K562 cells can be induced to undergo megakaryocytic differentiation by treatment with phorbol esters. This assay is commonly used to study megakaryocytic differentiation (10). As shown in Fig. 2*A*, ERG expression (interestingly ERG-2 and not ERG-3) was induced in differentiated K562 cells. *gpIIIa* levels were also increased in treated K562 cells, indicating that they underwent megakaryocytic differentiation. To further study the differential expression of *ERG* between megakaryoblastic and erythroid leukemia, we analyzed two sublines of the multipotential MEP leukemic cell line UT-7. *ERG3* and typical megakaryocytic genes (e.g., *MPL, PF4, TXAS*) were expressed in UT-7/TPO, whereas these genes were not amplified in UT-7/EPO (Fig. 2*B*). Thus, ERG is induced upon megakaryocytic differentiation of leukemic progenitor cells.

Overexpression of ERG induces phenotypic shift of erythroleukemia cells toward the megakaryocytic lineage. To examine whether ERG plays a direct role in induction of the leukemic megakaryoblastic phenotype, we cotransfected K562 cells with expression vectors of *ERG3* and CD2 (as a marker for transfected cells). As shown in Fig. 3, overexpression of ERG-3 resulted in induction of the megakaryocytic genes *gpIb, gpIIb*, and *gpIIIa* and expression of CD41 and CD61 antigens (representing the protein products of the *gpIIb* and *gpIIIa* genes, respectively) on the surface of transfected cells (Fig. 3*A* and *C*). The extent of induction of megakaryocytic markers was higher than the positive control (FLI-1) and completely dependent on the presence of the Ets domain in ERG-3 (Fig. 3*B* and *D*). Strikingly, the induction of megakaryocytic markers by ERG-3 was associated with down-regulation of the erythroid marker glycophorin A (Fig. 3*D*). Thus, ectopic expression of ERG-3 in K562 cells caused a phenotypic shift from the erythroid to the megakaryocytic lineage.

ERG activates and occupies megakaryocytic gene promoters. We next examined the role of ERG in activating megakaryocytic gene promoters. ERG3 activated a *gpIb* reporter in HeLa cells in a manner dependent on the presence of the Ets domain. This activation was synergistic with GATA1 but only additive with the mutated GATA1 (GATA1s; Fig. 4). To establish whether endogenous ERG binds a megakaryocytic promoter *in vivo*, we did chromatin immunoprecipitation assays with anti-ERG antibody in the progenitor cell line 416B (25). The *gpIIb* promoter, which contains several evolutionarily conserved Ets binding sites, had previously been shown to be bound *in vivo* by Fli1 (28). We now show that the *gpIIb* promoter was significantly

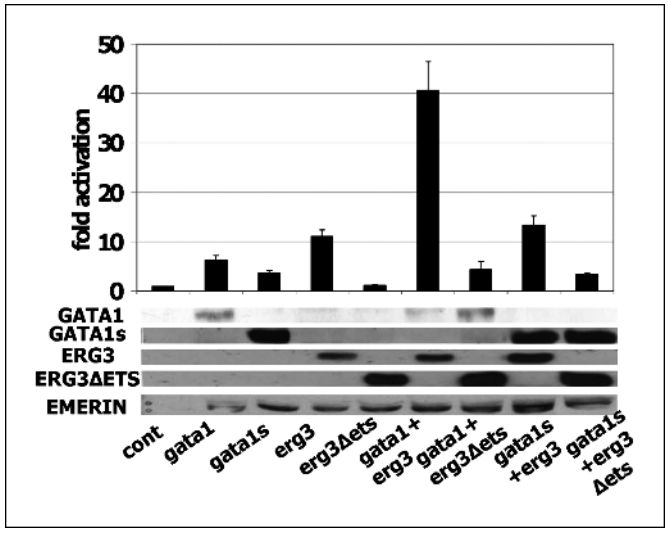

Figure 4. ERG3 activates a gpIbα5′-567luc luciferase reporter plasmid. HeLa cells were transfected with 170 ng of the gpIbα5′-567luc reporter plasmid and 335 ng of the expression plasmids encoding ERG3, ERG3ΔETS, GATA1, and GATA1s as indicated. The total amount of DNA in each transfection was kept constant at 1.34 μg by adding the pcDNA3 backbone. Cells were harvested 24 hours after transfection and assayed for luciferase activity. The results shown are a summary of three experiments done in triplicate. *Columns,* average fold increase in firefly luciferase relative to a value of 1 for the reporter alone; *bars,* SE. ERG3 activates the reporter plasmid and synergizes with GATA1. Its activity depends on the presence of the Ets motif. All the transcription factors added transactivate the reporter plasmid in a statistically significant manner ($P < 0.05$), except ERG3ΔETS. The bottom part shows Western blot analysis using nuclear extracts and the antibodies indicated on the left.

enriched in immunoprecipitates with both ERG and FLI-1 but not with ETS-2, another Ets transcription factor from the critical Down syndrome region on chromosome 21 (Fig. 5*A* and *B*). Thus, the induction of megakaryocytic genes by ERG results from direct binding to their promoters.

ERG binds the stem cell leukemia hematopoietic enhancer. The current study is driven by the hypothesis that increased expression of ERG in hematopoietic progenitors of Down syndrome patients enhances the formation of megakaryocytic precursors. The *SCL/TAL1* gene encodes a bHLH transcription factor required for the development of hematopoietic stem cells (29, 30). Both gain- and loss-of-function experiments have established that *SCL/TAL1* directs megakaryocytic development (31–33). Göttgens et al. (25) have previously shown that expression of *SCL/TAL1* in hematopoietic stem and progenitor cells is controlled by a 3′ enhancer element (+19 enhancer). The activity of the +19 enhancer was critically dependent on conserved Ets and GATA consensus binding sites that we had previously shown to be bound *in vivo* by GATA2, FLI-1, and ELF1 (but not PU.1) in hematopoietic progenitor cells (25). By performing additional chromatin immunoprecipitation experiments, we have now shown that both ERG and ETS2 are bound to this enhancer *in vivo* at a similar level as FLI1 (Fig. 5*A* and *B*). Thus, our data implicate ERG in controlling the expression of *SCL/TAL1*, a gene with a major role in directing megakaryocytic development from hematopoietic stem cells.

Discussion

This is the first study associating *ERG* expression with megakaryopoiesis and megakaryoblastic leukemias. We found *ERG* to be expressed in hematopoietic stem cells, megakaryoblasts

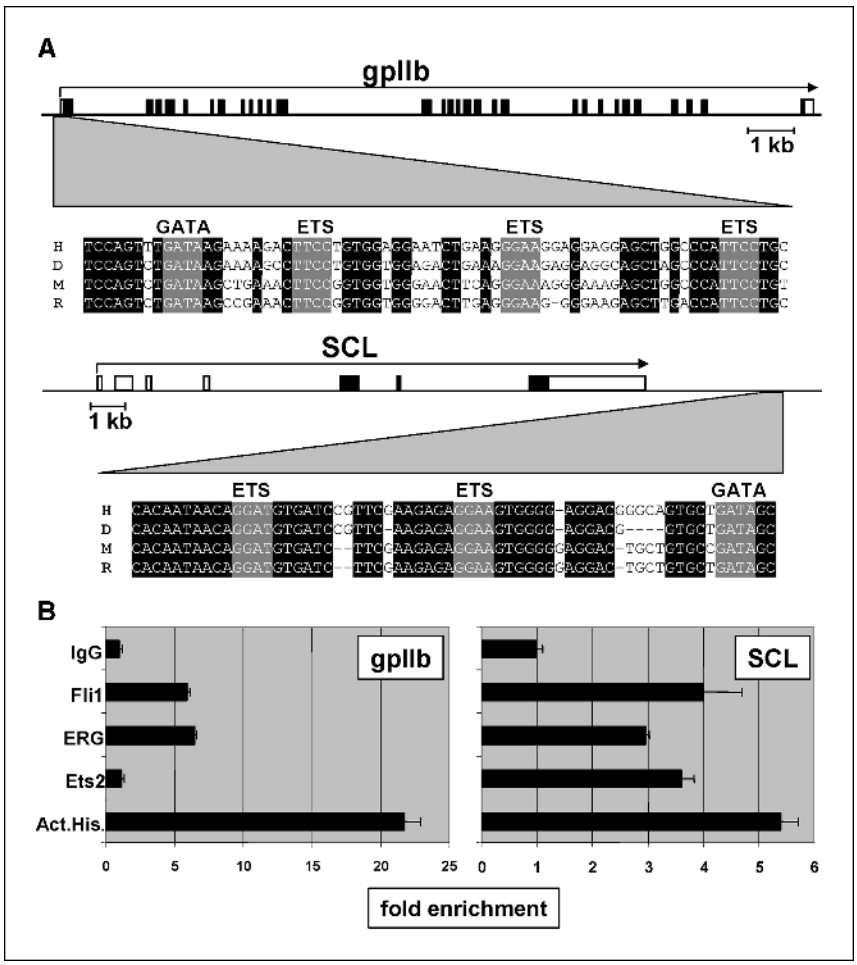

Figure 5. *In vivo* binding of chromosome 21 Ets family transcription factors to the gpIIb promoter and SCL +19 enhancer in the hematopoietic progenitor cell line 416B. *A,* diagram of the murine gpIIb and SCL loci indicating the location of the gpIIb and SCL clusters of conserved Ets and GATA sites. *Open boxes,* noncoding exons; *filled boxes,* coding exons. The triangles mark the position of the sequences of the gpIIb promoter and the SCL enhancer, respectively. *B,* the gpIIb promoter is bound *in vivo* by Fli1 and ERG, and the SCL +19 enhancer by Fli1, ERG, and Ets2. Chromatin immunoprecipitates were analyzed by quantitative real-time PCR. Rabbit IgG served as a negative control and antibodies against K9 acetylated histones as a positive control. The fold enrichment shown is normalized against the negative control IgG samples and corrected for the enrichment seen when performing quantitative PCR for a neutral region of the genome (see Materials and Methods).

and platelets, and in megakaryoblastic leukemia cells including primary transient leukemia cells of Down syndrome. The expression of *ERG* was induced upon megakaryocytic differentiation of erythroleukemia cells. Forced expression of ERG in erythroleukemia cells caused a phenotypic shift toward the megakaryocytic lineage. The induction of megakaryocytic markers was most likely the consequence of direct binding and activation as shown by luciferase reporter assays and chromatin immunoprecipitation. Finally, we have shown that ERG is bound *in vivo* to the enhancer of the stem cell and megakaryocytic transcription factor *SCL/TAL1*. Together, these findings are consistent with the hypothesis that excess copies of the *ERG* gene in hematopoietic progenitors push the hematopoietic development into the megakaryocytic lineage and, in collaboration with mutated GATA1, contribute to the clonal megakaryoblastic proliferation observed in at least 10% of infants with Down syndrome.

ERG has been reported to regulate genes involved in chondrogenesis and angiogenesis and functions as a modulator of endothelial cell differentiation (26, 34–37). It has not been previously associated with hematopoiesis, although it was detected in DNA-microarray gene expression analysis of the slow-dividing CD133-positive hematopoietic stem cells (38). Different isoforms of ERG are expressed by alternative splicing and the utilization of different 5′ UTRs. We have observed that ERG3 is the dominant isoform in normal and malignant hematopoietic cells. ERG3 differs from the nonhematopoietic isoform ERG2 by an additional exon and a different 5′ UTR and the first few amino acids. Both ERG2 and ERG3 were shown to activate Ets binding sites *in vitro* to a similar degree (14), and we have observed similar results with ERG2 in luciferase reporter and K562 transfection experiments (not shown). Interestingly, ERG-3 has been previously reported to be the major isoform expressed in endothelial cells (26). Thus, it seems that ERG3 is the major hematoendothelial ERG isoform.

We have confirmed the absence of *ERG* expression in mature lymphoid or myeloid cells (26) as well as in myeloid and mature B-cell leukemias. However, we have observed expression of *ERG3* in a pre-B leukemia cell line. This is consistent with the data obtained by microarray gene expression analysis of primary ALL samples (39, 40). Interestingly, the highest levels of *ERG* expression were observed in hyperdiploid ALL, uniformly harboring three to four copies of chromosome 21. The possible role of ERG in ALL needs further investigation.

The erythroid and megakaryocytic lineages are closely related. Both originate in a common MEP progenitor cell. The mechanisms directing the choice of development from the MEP stage are unclear because many of the critical transcription factors, such as GATA1 and FOG1, are expressed in both lineages (27, 41). We have observed striking differential expression of ERG between these two lineages. Moreover, forced expression of ERG in erythroleukemia cells caused up-regulation of megakaryocytic markers coupled with down-regulation of glycophorin A, an erythroid marker. Thus, ERG may regulate the differentiation of MEP along the megakaryocytic pathway. This hypothesis needs to be studied further in appropriate nonleukemic models.

In the current study, we have observed ERG binding *in vivo* to the *SCL* +19 enhancer that regulates SCL expression in hematopoietic stem and progenitor cells (25). Both gain- and loss-of-function studies have shown that *SCL/TAL1* is a key regulator of megakaryocytic development (31–33, 42). In particular, previous studies have shown that overexpression of *SCL/TAL1* in primary multipotent myeloid progenitor cells drives their subsequent differentiation toward the megakaryocytic lineage (32, 33). Our data are, therefore, consistent with the notion that, at least in part, ERG may mediate a promegakaryopoietic effect through regulating the levels of *SCL* expression in hematopoietic progenitors. Incidentally, we also noted binding of Ets2 to the SCL +19 enhancer, suggesting that Ets2, also located in the critical region of chromosome 21, may represent an additional candidate gene involved in the development of Down syndrome megakaryoblastic leukemia.

The pattern of ERG expression is reminiscent of the expression pattern of the chromosome 21 gene *RUNX1*. Both are expressed in hematopoietic stem cells and in megakaryocytic and lymphoid progenitors (11, 43). Forced expression of *RUNX1* in the K562 erythroleukemia cell line accelerated its megakaryocytic differentiation in response to thrombopoietin (10). In contrast, ERG induced megakaryocytic differentiation in the absence of additional growth factors. Conditional knockout experiments in mice have shown that *RUNX1* expression in early hematopoietic progenitor regulates their differentiation toward the lymphoid and the megakaryocytic lineages (12). A similar assessment of ERG function needs to be done. Because RUNX1 is known to cooperate with Ets transcription factors (44–46), the potential functional and biochemical interactions between RUNX1 and ERG warrant further studies.

Several studies have shown a linear correlation between gene copy number and gene expression (1, 39, 47). How could trisomy 21, resulting in an average 1.5× increase in expression of multiple genes, cause a dramatic phenotype of 10% incidence of congenital leukemia? We hypothesize (Fig. 6) that the excess of several chromosome 21 genes regulating megakaryopoiesis induces these leukemias in Down syndrome. This hypothesis is consistent with the thrombocytosis observed in the majority of healthy infants with Down syndrome (8). Thus, increased expression of *RUNX1, ERG*, and perhaps other genes (e.g., *ETS2*) in fetal liver hematopoietic progenitors promotes megakaryopoiesis and increases the pool of early megakaryocytic cells, whereas the acquired *GATA1* mutations and the overexpression of the chromosome 21 transcription factor BACH-1 (48) block further differentiation, leading to marked accumulation of megakaryoblasts. This model, testable by animal experiments or by studies of human fetal liver cells from Down syndrome embryos, could serve as a general paradigm by which the carcinogenic effects of numerical chromosomal changes reflect collaborative activities of several genetic elements on the amplified or deleted chromosomes.

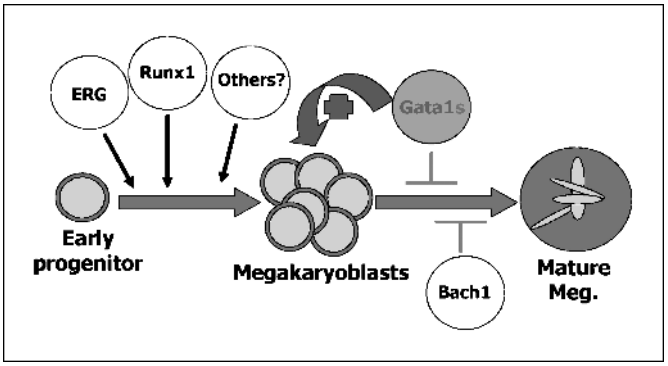

Figure 6. Proposed model for the mechanism of the megakaryoblastic leukemias of Down syndrome. Clonal megakaryoblastic proliferation in Down syndrome patients is caused by a combination of increased megakaryopoiesis caused by the excess several chromosome 21 genes (*ERG, RUNX1*, and others including *ETS2*) coupled with differentiation arrest caused by the GATA1s mutation. Recent evidence from several laboratories suggests that GATA1s itself may enhance further megakaryoblastic proliferation of the megakaryoblastic progenitors (*arrow*). BACH-1 is another chromosome 21 transcription factor recently shown to block further megakaryocytic differentiation (48).

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