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Expression of Transcription Factors during Megakaryocytic Differentiation of CD34$^+$ Cells from Human Cord Blood Induced by Thrombopoietin

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Terui, K., Takahashi, Y., Kitazawa, J., Toki, T., Yokoyama, M. and Ito, E. *Expression of Transcription Factors during Megakaryocytic Differentiation of CD34$^+$ Cells from Human Cord Blood Induced by Thrombopoietin.* Tohoku J. Exp. Med., 2000, **192** (4), 259-273 —— Although normal megakaryocytic development has been shown to require the presence of functional GATA-1 and NF-E2 transcription factors in vivo, the roles of other members of the GATA binding factors and NF-E2 family during megakaryocytic differentiation are unclear. In the present study, the expression of GATA family members, GATA-1 and GATA-2, a GATA-binding factor, EVI-1, the large subunit of NF-E2 factor, p45 and the related factors, Nrf1, Nrf2, Nrf3, BACH1, BACH2, and the small subunit of NF-E2, MAFK and MAFG has been examined in human megakaryocytic and erythroid cells by reverse transcriptase-polymerase chain reaction. CD34$^+$ cells isolated from human cord blood were induced to unilineage megakaryocytic or erythroid differentiation in liquid suspension culture in the presense of thrombopoietin or erythropoietin, respectively. Each lineage was identified by monoclonal antibody against GPIIb/IIIa or glycophorin A. In megakaryocytic culture, p45, Nrf1, Nrf2, BACH1, MAFK and MAFG mRNAs were induced similarly to erythroid culture. Nrf3 mRNA was barely detected in both cultures. BACH2 was induced only in megakaryocytic culture, although the level of expression was low. Furthermore, the profiles of transcription factors involved in hematopoiesis, EVI-1 and Ets-1 mRNAs were induced only in megakaryocytic culture. Megakaryocytic and erythroid differentiation pathways are closely related to each other, and these two lineage cells share a number of lineage-specific transcription factors. However, the results showed that the profile of the expression of these transcription factors in megakaryocytic cells is distinct from that of erythroid lineage. The dynamic changes in the levels of different transcription factors that occur during primary megakaryocytic differentiation suggest that the levels of these factors may influence the progression to specific hematopoietic pathways. ———— megakaryocytopoiesis; transcription factor; GATA; NF-E2; small Maf

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A pool of hematopoietic stem cells that can extensively self-renew and differentiate into progenitor cells sustains hematopoiesis. Differentiation of hematopoietic cells from pluripotent progenitors involves the progressive restriction of developmental potential and the acquisition of lineage-specific patterns of gene expression. The pattern of gene expression within a cell is mediated by tissue- and stage-specific transcription factors. In the megakaryocytic lineage, a number of tissue-restricted transcription factors are implicated in the development (Lemarchandel et al. 1993; Lepage et al. 1999). Among these transcription factors, GATA-1 and NF-E2 stand out for the non-redundant and essential roles that they play in megakaryocytopoiesis (Shivdasani et al. 1995, 1997).

GATA-1 was the first transcription factor shown to be necessary for erythroid-specific gene. GATA-1 is expressed in erythroid cells, megakaryocytes, and mast cells (Martin et al. 1990; Romeo et al. 1990), and also expressed in Sertoli cells in the testis (Ito et al. 1993; Yomogida et al. 1994). GATA-1 is essential for megakaryocyte growth and platelet development as well as red cell maturation (Pevny et al. 1991; Weiss et al. 1994; Shivdasani et al. 1997). In contrast, the related GATA family member GATA-2 is expressed in broad distribution among hematopoietic cells, with particularly prominent expression in early progenitors, as well as megakaryocytes and mast cell lineages (Dorfman et al. 1992; Visvader and Adams 1993). GATA-2 is required for the expansion of multipotential hematopoietic progenitors and formation of mast cells, but not for the terminal differentiation of erythroid cells and macrophages (Tsai and Orkin 1997). The functional roles of GATA-2 on megakaryocyte development remain unknown. Although GATA-2 is expressed in human megakaryocytic cell lines (Dorfman et al. 1992), analysis of primary human megakaryocytic cells has not been reported.

The transcription factor NF-E2 is expressed in erythroid cells, megakaryocytes and mast cells and has been shown to be a heterodimer formed between the large subunit (p45) and amall subunit (p18) (Andrew et al. 1993; Igarashi et al. 1994). p45 belongs to a family of basic leucine-zipper (bZip) protein that is closely related to the Drosophila Cap'n'colar (the CNC family) and is highly expressed in the erythroid and megakaryocytic lineages (Romeo et al. 1990). The CNC family members include Nrf1, Nrf2, Nrf3, Bach1 and Bach2 in addition to p45, all of which form heterodimers with small Maf proteins (Chan et al. 1993; Igarashi et al. 1994; Moi et al. 1994; Oyake et al. 1996; Kobayashi et al. 1999) and recognize the NF-E2 binding site. p18 is one of the small Maf family proteins, MafF, MafG or MafK and its expression is not restricted to hematopoietic cells (Andrew et al. 1993; Igarashi et al. 1994). Gene targetting experiment demonstrated that p45 NF-E2 is essential for proper differentiation of megakaryocytes and erythrocytes (Shivdasani et al. 1995; Levin et al. 1999). However, the roles of the other members of CNC family on megakaryocytopoiesis remain unknown.

EVI-1 is a nuclear protein containing a seven-zinc-finger domain at the N-terminal end and a three-zinc-finger domain at the C-terminal end. The

N-terminal zinc finger domains recognize GATA-binding consensus (Delwel et al. 1993). Human EVI-1 gene is located at 3q26 and can be activated in myeloid leukemias and myelodysplastic syndrome by chromosomal rearrangement (Morishita et al. 1992; Russell et al. 1994). The clinical features of these patients include elevated platelet counts, marked hyperplasia with dysplasia of megakaryocytes (Jenkins et al. 1989). Although these observations implicated the involvement of EVI-1 in abnormal megakaryocytopoiesis, the role of EVI-1 in normal megakaryocytopoiesis remains unknown.

Ets binding sites associated with GATA binding sites are present in all regions that have been described as important for megakarycyte-specific expression, e.g. GPIIb (Lemarchandel et al. 1993), GPIbα (Hashimoto and Ware 1995), thrombopoietin receptor (MPL) (Deveaux et al. 1996), GPV (Lepage et al. 1999). The Ets family contains approximately 30 members described in various animal species. Among this family, Ets-1 is of particular interest in megakaryocytopoiesis, since Ets-1 is able to bind and transactivate the megakaryocyte-specific gene, GPIIb and MPL promoters (Lemarchandel et al. 1993; Deveaux et al. 1996). In addition, the expression of Ets-1 mRNA increases during megakaryocytic differentiation with phorbol ester treatment of erythrocytic/megakaryocytic cell lines (Lemarchandel et al. 1993). However, the expression of Ets-1 in normal hematopoiesis is poorly understood.

A challenging question in hematopoiesis is how lineage-specific patterns of gene expression can arise from a common precursor cell. The problem appears to be even more complex in the case of megakaryocyte differentiation, because erythroid and megakaryocytic lineage cells share several lineage-specific transcription factors, including GATA-1 and NF-E2. In the present study, we have investigated the expression of the family members of GATA and NF-E2, and other lineage-specific transcription factors in purified human hematopoietic progenitor cells induced to unilineage megakaryocytic or erythroid differentiation. The expression pattern was monitored at mRNA level by reverse transcriptase-polymerase chain reaction (RT-PCR). The results showed that the expression profile of several transcription factors in megakaryocytic cells is distinct from that of erythroid lineage. These differences may contribute to establishment of lineage-specific gene expression between the two lineages.

MATERIALS AND METHODS

Cells

Cord blood cells (CB) were collected with informed maternal consent. Cord blood samples were diluted 1 : 1 with phosphate-buffered saline (PBS) containing 2 mM EDTA and centrifuged over Ficoll-metrizoate (HISTOPAQUE-1077, Sigma Chemical Co., St. Louis, MO, USA) gradient for 30 minutes at $400 \times g$ at room temperature. Interface cells were collected and washed twice in PBS containing 2 mM EDTA. CD34^{+} progenitor cells were isolated from these cells using a magnetic cell sorting system, Mini-MACS and CD34$^+$ progenitor cell isolation kit (Miltenyi Biotec GmbH., Bergisch Gladbach, Germany) in accordance with the manufacturer's recommendations.

Liquid suspension cultures

CD34$^+$ progenitor cells were cultured in the conditions that induce the cells to unilineage megakaryocytic or erythroid differentiation. In megakaryocytic differentiation culture, the cells were cultured in Iscove's modified Dulbecco's medium (IMDM) supplemented with 10% human AB blood type-plasma and 100 ng/ml recombinant human thrombopoietin (TPO) (Kirin Brewery Co. Ltd., Tokyo) for 14 days. In erythroid differentiation culture, the cells were cultured in IMDM supplemented with 30% fetal bovine serum, 1% bovine serum albumin, 5×10^{-5} M 2-mercaptoethanol, 2 U/ml recombinant human erythropoietin (EPO) (Kirin Brewery) for 7 days. Cultures were incubated at a concentration of 2×10^4 cells/ml at 37°C in a humidified atmosphere of 5% CO_2 and periodically analyzed for cell morphology, membrane phenotype and gene expression.

Fluorescent antibodies and phenotype analysis

Cytofluorometric studies were performed using fluorescent isothiocyanate (FITC)-conjugated monoclonal antibodies (mAbs). FITC-CD34 (Anti-HPCS-2), GPIIb/IIIa (P2) and glycophorin A (JC159) mAbs were purchased from Becton Dickinson (San Jose, CA, USA), IMMUNOTECH (Marseille, France) and DAKO A/S (Glostrup, Denmark), respectively. For all antibodies, negative controls were provided by FITC-conjugated immunoglobulin of the same isotype.

Culture cells were stained with fluorescence-conjugated mAbs for 15 minutes at room temperature. Fluorescent analysis was performed using a ORTHO Flow cytometer (CYTORON ABSOLUTE; ORTHO, Raritan, NJ, USA), with 5000 cells for each antibody.

RT-PCR

Total RNA was extracted from CD34$^+$ cells and cultured cells by an acid guanidium thiocyanate-phenol-chloroform method. One μg of total RNA was reverse transcribed by Moloney murine leukemia virus reverse transcriptase (Takara Shuzo Co., Ltd., Otsu) with random primers. The cDNAs were amplified with each set of primers shown in Table 1 using Premix Taq (Takara Shuzo) within the linear phase of amplification (24 to 30 cycles of 94°C for 30 seconds, 55°C or 58°C for 30 seconds, and 72°C for 60 seconds). RT-PCR was normalized for S14 ribosomal protein. Each sample was electrophoresed in a 3% agarose gel and stained with ethidium bromide. To exclude the contamination of genomic DNA, we designed most sets of primers to span at least one intron, or in the other cases, we confirmed negative amplification on PCR using an aliquot of total RNA as a template.

TABLE 1. *Primers for RT-PCR analysis*

Target	5' sequence	3' sequence	Size of amplicon (nt)	Reference (No.)
GATA-1	5'-GATCCTGCTCTGGTGTCCTCC-3'	5'-ACAGTTGAGCAATGGGTACACC-3'	219	44
GATA-2	5'-CCCTAAGCAGCGCAGCAAGAC-3'	5'-GATGAGTGGTCGGTTCTGGCC-3'	163	21
EVI-1	5'-AGCAACGTCGAATCACGACCTGCTTCAGAC-3'	5'-TCAGACTGTAAGAGCTCACTGGCCTCAGGT-3'	280, 250	26
Ets-1	5'-TCCAGACAGACACCTTGCAGAATG-3'	5'-GTTTGAATTCCCAGCCATCTCCTG-3'	454, 192	41
p45NF-E2	5'-TAGAGATGTACCCAGTGGAGT-3'	5'-TTAAAGTCATCTACCGGCAAG-3'	235	29
Nrf-1	5'-GCAGTGCCTAGTGAGAGTGAG-3'	5'-CACCTGATGCAGGCTGACATTC-3'	255	4
Nrf-2	5'-CCAGCTATGGAGACACACTAC-3'	5'-TGTGAGATGAGCCTCCAAGCG-3'	275	25
Nrf-3	5'-GAGCGAGGAGAATGGGGTACTAAG-3'	5'-GAAGGGTTTGCTCAGGATTGGTGG-3'	506	16
BACH1	5'-TTCATGG CACAACG GATAATTTCACTG-3'	5'-GTAACGCCAGTTCACCATCAGGAGTACT-3'	418	15
BACH2	5'-TCCTTGCCACAGAACATCAGGAAC-3'	5'-TGGATGTCTCGGCAAACTTCCTGG-3'	487	34
MAFK	5'-caggatccTTAAAGGTCAAGAAGGAGGC-3'	5'-caaagcttCTAGGATGCAGCCGAGAAGG-3'	457	38
MAFG	5'-aaggatccTTGAAGGTGAAGCGGGAGCC-3'	5'-acaagcttCCTACGATCGGGCATCCGTC-3'	476	38
GPIIb	5'-CAAGAACAGCCAGAATCCAAACAG-3'	5'-TACGAGAACTGGATCCTGAAGCCT-3'	459	10
$\varepsilon\gamma$-Globin	5'-GCAAGATGAATGTGGAAGA-3'	5'-CCCAGGAGCTTGAAGTTC-3'	274	8
EPOR	5'-TGAGACACCCATGACGTCTCA-3'	5'-TGTCCAGCACCAGATAGGTA-3'	EPOR-F: 608 EPOR-T/S: 700	27
S-14	5'-GGCAGACCGAGATGAATCCTCA-3'	5'-CAGGTCCAGGGGTCTTGGTCC-3'	145	21

MAFK and MAFG, Lower case indicates nucleotides not present in the MAFK or MAFG gene that introduce restriction site; $\varepsilon\gamma$-Globin, a primer pair for both ε and γ globin amplification; EPOR-F, a full length form of erythropoietin receptor; EPOR-T/S, a truncated or soluble form of erythropoietin receptor.

RESULTS

Unilineage megakaryocytic and erythroid culture

$CD34^+$ cells were isolated from human CB. Flow cytometry analysis showed that $CD34^+$ was expressed in 86.6% of the cells isolated with immunomagnetic beads (Table 2). These cells were cultured in suspension either in the presence of TPO or EPO for megakaryocytic or erythroid unilineage-differentiation culture, respectively. Cell morphology analysis showed a gradual maturation along megakaryocytic or erythroid pathway to terminal cells (Fig. 1). Contaminating myeloperoxydase positive cells were less than 10% in both cultures.

Expression of lineage specific genes in unilineage megakaryocytic and erythroid cultures

Surface marker analysis showed the progressive expression of specific markers for differentiated megakaryocytic or erythroid precursors, e.g., in megakaryopoietic system, GPIIb/IIIa was 60.5% and 88.2% positive at day 7 and day 14, respectively, and in erythropoietic system, glycophorin A was 70.1% positive at day 7 (Table 2).

To further study the pattern of lineage-specific genes, we performed RT-PCR analysis of lineage specific markers in hematopoietic progenitor cells differentiating along the megakaryocytic or erythroid pathway (Fig. 2). Unexpectedly, ε and/or γ-globin ($\varepsilon\gamma$-globin) gene expression was detected in megakaryocytic cells at day 14, although the level of expression was very low compared to that of erythroid cells. In contrast, megakaryocyte-specific gene GPIIb was exclusively expressed in differentiated megakaryocytic cells. Human bone marrow cells express three different isoforms of EPO receptor (EPOR), a soluble, truncated and full-length form of EPOR (EPOR-S, EPOR-T and EPOR-F, respectively), with the truncated form predominating in immature progenitors and the full-length form predominating in late-stage progenitors (Nakamura et al. 1992). EPOR-T cannot transduce mitogenic signals and acts as a dominant negative regulator over EPOR-F for cell growth and prevention of apoptosis (Nakamura and Nakauchi 1994). In megakaryocytic lineage, EPOR-F mRNA

TABLE 2. *Cell surface antigen expression in $CD34^+$ cells and cultured cells*

Cells	Cell surface antigen expression (%)		
	CD34	GPIIb/IIIa	Glycophorin A
$CD34^+$ cells	86.6	ND	ND
Day 7 cells cultured with TPO	35.9	60.5	0.5
Day 14 cells cultured with TPO	1.9	88.2	1.9
Day 7 cells cultured with EPO	12.6	11.3	70.1

ND, not done.

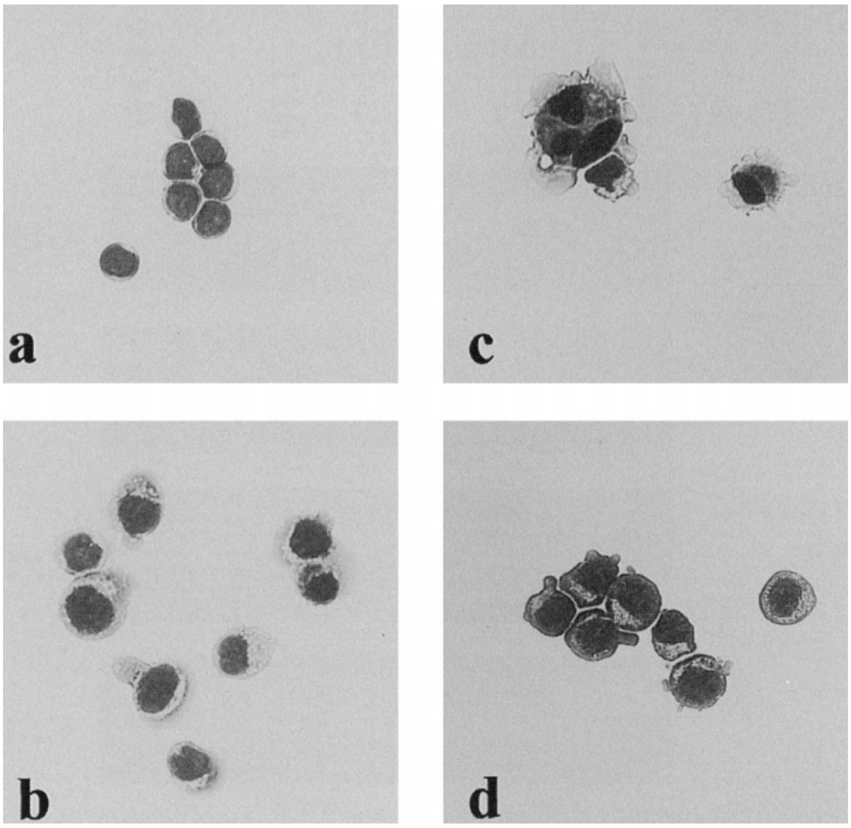

Fig. 1. Cell morphology in unilineage liquid cultures. Morphological appearance of megakaryocytic cells and erythroid cells in unilineage cultures. (a) CD34$^+$ cells purified from cord blood cells. (b) Megakaryocytic cells present in TPO-only cultures at 7 days. (c) Day 14 large polynucleated megakaryocytic cells. (d) Erythroid cells present in EPO-only cultures at 7 days.

was dominantly induced and gradually increased to a high level, although the level of expression was lower than that of erythroid cells. EPOR-T and EPOR-S mRNAs were expressed at a very low level in megakaryocytic cells.

Expression of GATA and NF-E2 factors in unilineage megakaryocytic and erythroid cultures by RT-PCR analysis

We have performed three independent experiments to evaluate the expression of the members of the GATA and NF-E2 family during megakaryocytic and erythroid differentiation. Among the GATA family, GATA-1 and GATA-2 were expressed in megakaryocytic and erythroid cells (Fig. 3). GATA-1 mRNA was barely detected in progenitor cells enriched from CB cells. GATA-1 mRNA was induced and maintained at a high level along megakaryocytic pathway as well as erythroid pathway. GATA-2 mRNA, already expressed in the uninduced progenitors at a low level, was induced and maintained at a high level in the megakaryocytic differentiation system. In contrast, GATA-2 expression level in erythroid culture at day 7 was as low as that of uninduced progenitor cells. These results were consistent with the previous report (Leonard et al. 1993).

NF-E2 related factors composed of one of the CNC family members and the

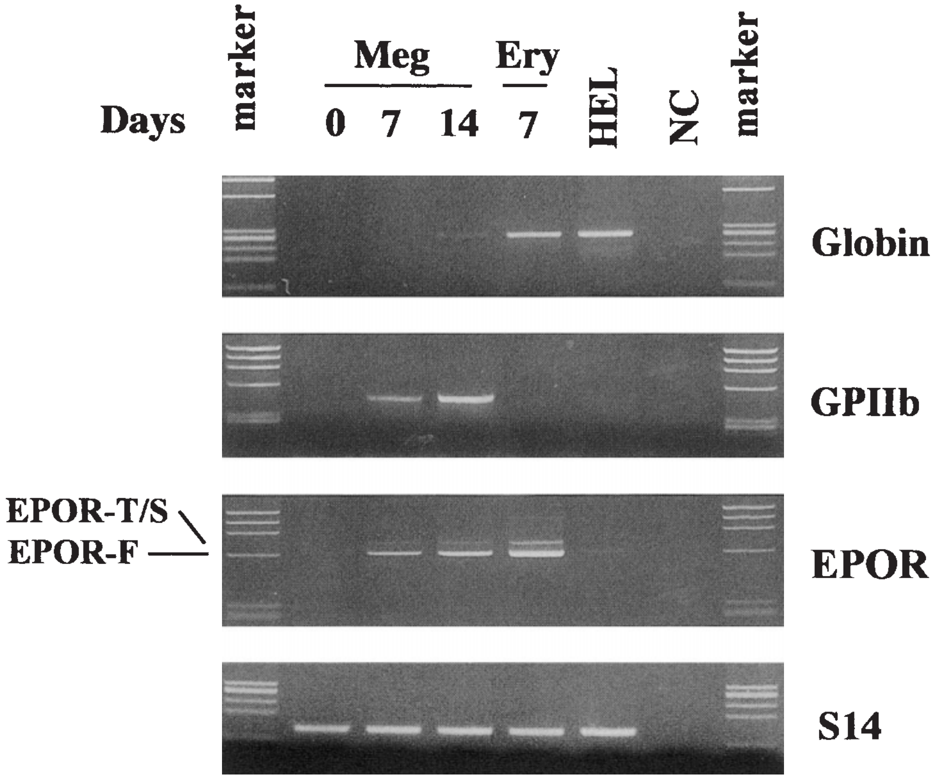

Fig. 2. Ethidium bromide-stained gels of the PCR amplification using marker genes. CD34$^+$ cells purified from cord blood cells were induced to megakaryocytic differentiation or erythroid differentiation at the indicated days. After amplification, samples were electrophoresed and photographed. First and last lanes are DNA size markers (HaeIII-digested $\phi \times 176$). As a positive control, human erythroleukemic HEL cells were used. *NC*, a negative control (no DNA).

small Mafs. We previously cloned human cDNAs encoding small Maf proteins MAFG and MAFK (Toki et al. 1997), but failed to isolate human cDNA for MAFF from several human cDNA libraries derived from hematopoietic cell lines. We analysed expression of all members of the CNC family and small Mafs, MAFK and MAFG (Fig. 4). These factors were barely detected in the uninduced progenitors. In megakaryocytic culture, p45, Nrf1, Nrf2, BACH1, MAFK and MAFG mRNAs were induced similarly to erythroid culture. Nrf3 mRNA was barely detected in both cultures. BACH2 was induced only in megakaryocytic culture, although the level of expression was very low.

Expression of EVI-1 and Ets-1 mRNAs in unilineage megakaryocytic and erythroid cultures

A GATA binding factor, EVI-1 can inhibit GATA-1-mediated transactivation and block growth of EPO-responsive cells, presumably by repressing the transcription of a subset of GATA-1 target genes (Kreider et al. 1993). EVI-1 mRNA, though barely detected in the uninduced progenitors, was induced with sustained expression in megakaryocytic culture, whereas it remained low level in

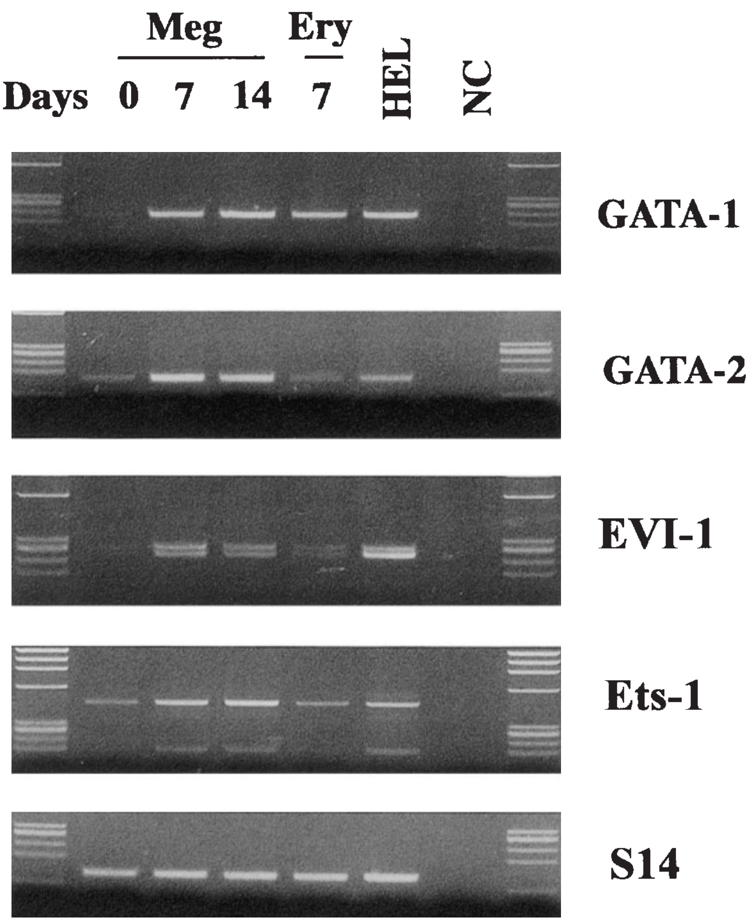

Fig. 3. GATA factors, EVI-1 and Ets-1expression in unilineage cultured cells. Same samples as Fig. 2 were analyzed. Note that GATA-2, EVI-1 and Ets-1 mRNAs were induced only in megakaryocytic cells. First and last lanes are DNA size markers (HaeIII-digested $\phi\times176$). As a positive control, human erythroleukemic HEL cells were used. *NC*, a negative control (no DNA).

erythroid culture (Fig. 3).

Although Ets-1 is not a GATA binding protein, the tandem structure of GATA/Ets binding sites is a hallmark of megakaryocytic genes and seems to be important for their tissue-specific expression. One of Ets family member, Ets-1 mRNA was already expressed in the uninduced progenitors at a low level and induced and maintained at a high level in unilineage megakaryocytic culture (Fig. 3). In contrast, the Ets-1 expression level in erythroid culture at day 7 was as low as that of uninduced progenitor cells.

Interestingly, the profile of GATA-2, EVI-1 and Ets-1 expression was very similar. These factors may play roles in megakaryocytic differentiation by affecting the transcriptional activity of GATA-1.

Discussion

The lineage-restricted transcription factors have central roles to establish or maintain lineage-specific programs of gene expression. The study of regulation of these transcription factors within individual cell lineages thus greatly improves

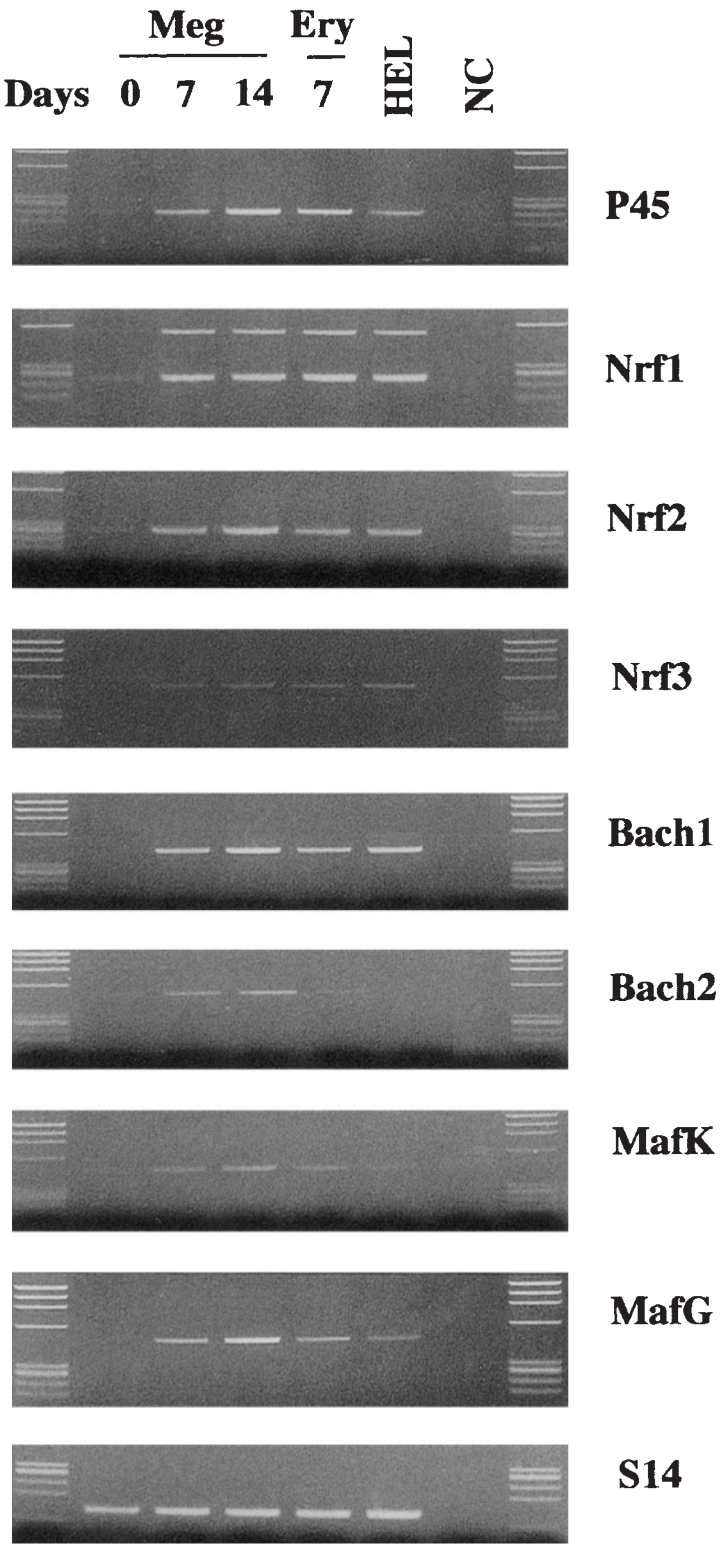

Fig. 4. NF-E2 family expression in unilineage cultured cells. Same samples as Fig. 2 were analyzed. p45, Nrf-1, Nrf-2, BACH1, MAFK and MAFG mRNAs were induced in both megakaryocytic and erythroid culture. Nrf-3 mRNA was barely detected in both cultures. BACH2 was induced only in megakaryocytic culture, although the level of expression was very low. First and last lanes are DNA size markers (HaeIII-digested $\phi \times 176$). As a positive control, human erythroleukemic HEL cells were used. *NC*, a negative control (no DNA).

our understanding of cell differentiation. However, human megakaryocytes represent 0.03% of total bone marrow cells and 0.001% of peripheral blood cells (Lee et al. 1993). Studies on megakaryocytopoiesis have been hampered by lack of relatively pure and abundant megakaryocyte population. TPO is a major cytokine that regulates megakaryocyte proliferation and differentiation. Recombinant TPO has facilitated in vitro culture of a sufficient number of primary megakaryocytes to permit studies on gene expression in primary megakaryocytes. The present study of unilineage culture demonstrated that the expression profiles of transcription factors during megakaryocytic differentiation are distinct from the closely associated erythroid pathway.

Because GATA-1 and GATA-2 are coexpressed in the megakaryocytic and erythroid lineages, it is presumed that each of these factors contributes primarily to the control of maturation along these lineages rather than committment to any one of them. A crucial role of GATA-1 in megakaryocytic and erythroid differentiation was clearly shown by gene disruption (Pevney et al. 1991; Weiss et al. 1994; Shivdasani et al. 1997). However, GATA-1 cannot be the sole determinant of differentiation in any of these lineages, because megakaryocytic markers such as GPIIb genes that are known to be regulated by GATA-1 are not expressed in erythroid cells. In both lineages, GATA-1 expression was maintained at high levels during the maturation. However, the profiles of GATA-2 expression were different between these lineages. GATA-2 expression declines as erythroid maturation progresses (Leonard et al. 1993), whereas it remained at a high level in megakaryocytic cells. Given the fact the GATA factors are highly homologous in their DNA-binding domains but very different in their primary structure, it is possible that GATA-1 and GATA-2 might bind to the same target sequence but engender different activities in hematopoietic cells. Because activation of exogenous GATA-2/estrogen receptor (ER) inhibits erythroid differentiation (Briegel et al. 1993), GATA-2 might repress the expression of the erythroid marker in maturing megakaryocytic cells. Our present results are consistent with this hypothesis.

The expression of the p45-related factors on normal megakaryocytopoiesis has not been described. In the present study, at least five members (p45, Nrf1, Nrf2, BACH1 and BACH2) of the CNC family were found to be coexpressed in primary megakaryocytic cells. These factors apparently share similar DNA-binding characteristics to p45 NF-E2 in vitro (Toki et al. 1997; Kobayashi et al. 1999). However, p45 NF-E2 was demonstrated to be the only large subunit that dimerizes with small Mafs (MafG or MafF) to constitute a heterogeneous NF-E2 complex in primary murine megakaryocytes (Lecine et al. 1998). In contrast, in mouse erythroleukemic (MEL) cells the slower migrating NF-E2 binding complex was detected (Lecine et al. 1998; Igarashi et al. 1998). Although the undefined NF-E2 binding activities have been assumed to be AP-1, it was demonstrated that Bach1 and small Maf constitute the presumed AP-1 band in MEL cells. Our present results suggest the possibility that the other members of CNC family also involve the transcriptional regulation of the target genes in megakaryocytic lineage.

The consensus binding sites for GATA-1 are contained within the consensus binding sequence for the first domain of zinc fingers of EVI-1 (Delwel et al. 1993). Inappropriate expression of the EVI-1 gene blocks erythropoiesis by suppressing the transactivation of GATA-1 target gene (Kreider et al. 1993). Furthermore, forced expression of EVI-1 resulted in the differentiation of abnormally high numbers of megakaryocytic colonies (Sitailo et al. 1999). However, since EVI-1 is not normally found in any of the hematopoietic lineages, it has remained unclear whether EVI-1 is involved in normal hematopoiesis. The present study clearly showed that EVI-1 expression is induced exclusively during megakaryocytopoiesis. This pattern is similar to GATA-2. Taken together with previous results, EVI-1 is likely to play roles in normal megakaryocytopoiesis by suppressing the transcription of a subset of GATA-1 target genes. To clarify the hypothesis, it is important to identify the target genes of EVI-1 in megakaryocytes.

The GATA-Ets association constitutes an essential difference between megakaryocytic and erythrocytic promoters and is a major determinant of megakaryocytic gene expression. Furthermore, the binding of Ets proteins is more important than that of GATA-1 for efficient expression of the MPL promoter (Deveaux et al. 1996). It should be noted that deletion of the 11q23-11qter, which bears the Ets-1 and Fli-1 genes, leads to severe thrombocytopenia (Breton-Gorius et al. 1995). The present results showed that Ets-1 is induced exclusively during megakaryocytic differentiation of primary hematopoietic cells. Erythroid/megakaryocytic cell lines were induced to megakaryocytic differentiation with induction of Ets-1 expression by phorbol ester (Lemarchandel et al. 1993). Our present results suggest that Ets-1 is a key regulator in primary megakaryopoiesis.

In summary, our data suggest that differentially regulated expression of the GATA binding factors, NF-E2 family and Ets-1 is associated with lineage selection and maintenance during megakaryocytic or erythroid differentiation. Because all megakaryocytic- and erythroid-specific genes described previously have functional GATA binding sites and GATA-1 is expressed in both lineages, additional factors like GATA-2, EVI-1 and Ets-1 in addition to GATA-1 may be necessary for modifying the target gene expression in a lineage specific manner.

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