Available online at www.sciencedirect.com
SCIENCE DIRECT
Biochemical and Biophysical Research Communications 345 (2006) 78–84
BBRC
www.elsevier.com/locate/ybbrc

RUNX1 suppression induces megakaryocytic differentiation of UT-7/GM cells

Ryohei Nagai [a], Eri Matsuura [a], Yusuke Hoshika [a], Emi Nakata [a], Hironori Nagura [a], Ayako Watanabe [a], Norio Komatsu [b], Yoshiaki Okada [a], Takefumi Doi [a,*]

[a] Graduate School of Pharmaceutical Sciences, Osaka University, 1-6 Yamadaoka, Suita, Osaka 565-0871, Japan
[b] Faculty of Medicine, University of Yamanashi, 1110 Shimokato, Chuo, Yamanashi 409-3898, Japan

Received 11 April 2006
Available online 25 April 2006

Abstract

The transcription factor RUNX1 plays a crucial role in hematopoiesis. RUNX1 regulates both differentiation and proliferation of hematopoietic cells. Several reports have shown that RUNX1 participates in megakaryopoiesis, which is a process that leads to formation of platelets. However, to date, the mechanisms by which this occurs have not been fully elucidated. In the present study, we investigated whether siRNA-mediated depletion of RUNX1 affected megakaryopoiesis of UT-7/GM cells. The depletion of RUNX1 in UT-7/GM cells resulted in up-regulation of the expression of megakaryocytic markers and polyploidization, while cell proliferation was down-regulated. Furthermore, the overexpression of RUNX1 decreased the activity of megakaryocytic gene promoters. These results suggest that RUNX1 down-regulates terminal differentiation of megakaryocytes and promotes proliferation of megakaryocytic progenitors.

Keywords: RUNX1; Megakaryocyte; UT7/GM cells; siRNA; CD41a; PF4; GPIIb

The Runt domain transcription factor RUNX1, also known as AML1, CBFA2, or PEBP2αB, belongs to a family of heterodimeric transcription factors named core binding factors (CBFs). RUNX1 contains a conserved region termed the Runt domain, with a high homology to the *Drosophila* RUNT protein. The Runt domain of RUNX1 mediates both its binding to DNA and dimerization with CBFβ, the β subunit of CBFs [1]. RUNX1 plays an important role in definitive hematopoiesis, as demonstrated from a study showing that its homozygous knockout mice displayed no definitive hematopoiesis [2,3]. Moreover, *RUNX1* is the most common target for chromosomal translocations in human leukemia, consistent with its critical role in hematopoiesis [4]. While controlling differentiation of hematopoietic cells, RUNX1 also regulates the cell cycle by shortening the G_1/S phase in hematopoietic cells [5,6]. Thus, RUNX1 is thought to function in both cell proliferation and differentiation [7].

Megakaryocytes are the hematopoietic precursors of platelets, which play an essential role in thrombosis and hemostasis. A recent study using conditional *RUNX1* knockout mice has shown that RUNX1 participates in the regulation of megakaryopoiesis [8], but its detailed role in human megakaryopoiesis has not been fully demonstrated due to the difficulty with purifying human megakaryocytes. UT-7/GM is a cell line that can be differentiated into erythroid and megakaryocytic lineages by erythropoietin and thrombopoietin (TPO) treatment, respectively [9]. As there are few cell lines that can be differentiated into the megakaryocytic lineage by TPO treatment, the UT-7/GM cell line is a useful tool for analyzing the molecular mechanisms in megakaryopoiesis.

In this study, we found that siRNA-mediated depletion of RUNX1 in megakaryocytic-induced UT-7/GM cells resulted in up-regulation of the expression of megakaryocytic markers and polyploidization, while cell proliferation

* Corresponding author. Fax: +81 6 6879 8158.
E-mail address: doi@phs.osaka-u.ac.jp (T. Doi).

0006-291X/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2006.04.057

was down-regulated. Furthermore, we showed that the overexpression of RUNX1 decreased the activity of megakaryocytic gene promoters in TPO-treated UT-7/GM cells. These results indicate that RUNX1 up-regulates cell proliferation and down-regulates terminal differentiation of UT-7/GM cells along the megakaryocytic lineage.

Materials and methods

Hematopoietic growth factors. Recombinant human granulocyte-macrophage colony-stimulating factor (GM-CSF) was provided by Sumitomo Pharmaceutical Co. Ltd. (Osaka, Japan). Recombinant human TPO was provided by the Kirin Brewery Co. Ltd. (Tokyo, Japan).

Plasmids and siRNA oligonucleotides. *RUNX1* and *CBFβ* coding sequences were obtained from a cDNA pool derived from HEL cells by PCR, and these PCR products were inserted into the *Eco*RV site of pcDNA3 (Invitrogen, Carlsbad, CA).

To make the human *GPIIb* promoter-reporter construct (GPIIb luc), we amplified 0.7-kb promoter fragments by PCR using the following primers: 5′-GCCACCTAGACCAAGGTCCATTCAG-3′ and 5′-CTTCCTTCTTCCACAACCTCCCAGG-3′. Using this fragment and the following primers, we performed a second PCR: 5′-AGGAGGAGTGAGGTACCTGCTGCACCAATA-3′ and 5′-CTTCCTTCTTCCACAACCTCCCAGG-3′. The resulting 0.6-kb PCR products were digested with *Kpn*I, and this fragment was inserted into the *Kpn*I/*Eco*RV site of pGL4.10[luc2] (Promega, Madison, WI). Preparation of the rat *PF4* promoter-reporter construct (PF4 luc) was as previously described [10]. siRNA cocktail targeting human RUNX1 (siRUNX1) was purchased from B-Bridge International Inc. (Sunnyvale, CA; Cat. No. THF27A-275), which contains three siRNAs: first sequence; sense 5′-UCGAAGUGGAAGAGGGAAATT-3′, antisense 5′-UUUCCCUCUUCCACUUCGATT-3′, second sequence; sense 5′-CCGAGAACCUCGAAGACAUTT-3′, antisense 5′-AUGUCUUCGAGGUUCUCGGTT-3′, third sequence; sense 5′-GCUAAAUACUGUAGGGAAATT-3′, antisense 5′-UUUCCCUACAGUAUUUAGCTT-3′. Negative control cocktail (siNC) was also purchased from B-Bridge International Inc. (Cat. No. C6A-0126).

Cell culture and megakaryocytic differentiation. UT-7/GM cells were maintained in Iscove's modified Dulbecco's medium (IMDM; Invitrogen) containing 10% fetal bovine serum and 1 ng/mL GM-CSF. For the induction of megakaryocytic differentiation, UT-7/GM cells were cultured with 10 ng/mL TPO or 1 nM 12-O-tetradecanoylphorbol-13-acetate (TPA) in place of GM-CSF.

siRNA transfection. Cells were suspended in Opti-MEM (Invitrogen) at 1×10^6 cells/100 μL. One hundred microliters of this cell suspension was then mixed with siRNA (175 pmol/1×10^6 cells) and transferred into an electroporation cuvette. Electroporation was performed with Gene Pulser Xcell™ (Bio-Rad, Hercules, CA) under the condition of a square-wave pulse (10 ms, 150 V). After electroporation, the cells were immediately diluted with 2 mL culture medium supplemented with 1 ng/mL GM-CSF and incubated at 37 °C in a humidified atmosphere containing 5% CO_2.

DNA transfection and reporter gene assay. PF4 luc (2 μg) and GPIIb luc (0.1 μg) were used as the reporter plasmids, and phRL-TK (0.1 μg; Promega) was used as an internal control plasmid. For the overexpression of the transcription factors, 1 μg of each expression plasmid was used for the electroporation. After the electroporation, the cells were immediately diluted with 2 mL culture medium containing 10 ng/mL TPO, and incubated at 37 °C in a humidified atmosphere containing 5% CO_2. After 48 h, cells were harvested and the reporter gene activities were determined with a Dual-Luciferase® Reporter Assay System (Promega), according to the manufacturer's instructions.

RT-PCR. Total RNA was isolated using an RNA preparation kit (Isogen; Nippon Gene, Tokyo, Japan). First strand cDNA was synthesized from 2 μg of total RNA of each cell sample using the SuperScript™ First-Strand Synthesis System for RT-PCR (Invitrogen) and oligo$(dT)_{12–18}$ primer. The cDNAs were then used as templates for individual PCRs with the specific primers (Table 1). PCRs were initiated by a denaturation step for 2 min at 96 °C and repeated by the following steps: 30 s at 96 °C for denaturation, 30 s at 60 °C (*PF4*, *GPIbα*, and *GPIIIa*) or 55 °C (*GPIIb* and *hypoxanthine-guanine phosphoribosyl transferase, HPRT*) for annealing, and 30 s at 72 °C for extension. The gene expression of *HPRT* was measured as a control gene expression.

Quantitative real-time PCR. RNA preparation and cDNA synthesis were performed as described above, except that the SuperScript™ III First-Strand Synthesis System for RT-PCR (Invitrogen) and oligo$(dT)_{20}$ primer were used. PCRs were carried out using a QuantiTect™ SYBR® Green PCR Kit (Qiagen, Tokyo, Japan) and the primers shown in Table 1 or the following *RUNX1* primers: 5′-GTGGTCCTATTTAAGCCAGCCCCCA-3′ and 5′-CTGAAGACACCAGCTTGACAGTTCC-3′. The quantitative PCR analyses were performed with the DNA Engine Opticon™ System (Bio-Rad). *HPRT* was used for the relative quantification of the gene expression.

Western blot. Harvested cells were lysed in 50 mM Tris–HCl (pH 6.8), 2% sodium dodecyl sulfate (SDS), and 10% glycerol. After sonication, the samples were centrifuged at 9000*g* for 15 min and the supernatants collected. Protein concentration was determined by the BCA™ Protein Assay (PIERCE, Rockford, IL), according to the manufacturer's instructions. After the addition of 4% 2-mercaptoethanol, 20 μg of protein samples was boiled at 95 °C for 3 min, separated by SDS–polyacrylamide gel electrophoresis, and blotted onto nitrocellulose membranes. Membranes were incubated with either anti-human RUNX1 (N-20) goat polyclonal IgG (Santa Cruz Biotechnology, Santa Cruz, CA) or anti-human glyceraldehyde phosphate dehydrogenase (GAPDH) mouse monoclonal IgG (MAB374; Chemicon International, Temecula, CA). The membranes were then treated with horseradish peroxidase-conjugated secondary antibodies. Signal detection was performed with a SuperSignal® West Dura Trial Kit (PIERCE) and X-ray film (FUJIFILM, Tokyo, Japan).

Ploidy analyses. Harvested cells were fixed with 70% ethanol at −20 °C for in excess of 12 h and then suspended in phosphate-buffered saline (PBS). After incubation with 100 μg/mL RNase A at 37 °C for 30 min, the cells were stained with 50 μg/mL propidium iodide (PI). Ploidy analyses were performed with a FACSCalibur™ flow cytometer and CellQuest (Becton–Dickinson, Franklin Lakes, New Jersey).

Analyses of CD41a expression. Approximately 5×10^5 cells were suspended in PBS containing 0.5% bovine serum albumin (PBS-B). The cells were incubated with fluorescein isothiocyanate (FITC)-conjugated mouse anti-human CD41a or FITC-conjugated mouse IgG1 Isotype Control for nonspecific labeling (eBioscience, San Diego, CA) in 50 μL PBS-B for 30 min. After the incubation, the cells were washed twice with PBS-B and resuspended in 0.5 mL PBS-B. The cells were analyzed using a FACSCalibur™ flow cytometer and CellQuest.

Table 1
Primers used for PCR amplifications

Gene	Fw. primer (5′–3′)	Rv. primer (5′–3′)
PF4	AGCATGAGCTCCGCAGCCGGGTTCT	CTTCCATTCTTCAGCGTGGCTATCA
GPIbα	CTCTGTGCCTTCGGAGGTCTTTCTG	GAGTGAGGCGAGTGTAAGGCATCAG
GPIIb	TCGATGACAACGGATACCCAGACCT	GCTGCAGCTCGGCATTTAGGGATAG
GPIIIa	TACTCTGCCTCCACTACCATGGATT	CTGGAGGACATTGCTGGAATCCATG
HPRT	ACCCGCAGCCCTGGCGTCGTGATTA	GTCCCCTGTTGACTGGTCATTACAA

Measurement of cell growth. Twenty-four hours after siRNA transfection, the cells were suspended in 1.7 mL of culture medium. Eight microliters of the suspended cells was cultured in 96-well plates containing 100 μL of culture medium with 10 ng/mL TPO in each well. On day 0, 1, 2, and 4 after TPO treatment, the cell growth was determined using a Cell Counting Kit-8 (Dojindo, Kumamoto, Japan). Briefly, 10 μL of WST-8 reagent was added to each well, and the plates were incubated at 37 °C for 1 h. After the incubation, absorbance was measured at 450 nm using a microplate reader (Bio-Rad).

Results

RUNX1 suppression by RNAi

We investigated whether the RUNX1 siRNA cocktail (siRUNX1) could effectively suppress the expression of RUNX1 in UT-7/GM cells. The immunoblot analyses of UT-7/GM cells treated with siRUNX1 for 24 h revealed that the amount of RUNX1 protein was reduced by 60–80% compared with the negative control siRNA (siNC) (Fig. 1). Furthermore, we also found that the amount of *RUNX1* mRNA was reduced by less than 50% upon treatment of UT-7/GM cells with siRUNX1 (Fig. 2A). These results indicate that siRUNX1 effectively suppresses the expression of endogenous RUNX1.

RUNX1 suppression increases the expression of megakaryocytic markers

We investigated whether UT-7/GM cells could be differentiated into the megakaryocytic lineage by TPO treatment. RT-PCR analyses revealed that the expression of megakaryocytic markers, *PF4*, *GPIbα*, *GPIIb*, and *GPIIIa*, was up-regulated in TPO-treated UT-7/GM cells (Fig. 2B). These results indicate that these cells are differentiated into the megakaryocytic lineage by TPO treatment.

Next, we investigated whether the depletion of RUNX1 had an influence on the expression of megakaryocytic markers in UT-7/GM cells. Twenty-four hours after siRNA transfection, UT-7/GM cells were induced to differentiate into the megakaryocytic lineage by TPO treatment. We defined day 0 as the day when TPO was added. The expressions of *PF4*, *GPIbα*, *GPIIb*, and *GPIIIa* on day 0, 2, and 4 were monitored via real-time RT-PCR analyses

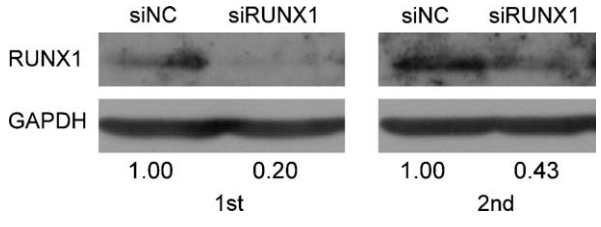

Fig. 1. The depletion of RUNX1 protein by RNAi. Twenty-four hours after siRNA transfection into UT-7/GM cells, whole cell lysates were prepared, and RUNX1 expression was determined by Western blot analysis. Two independent experiments were performed (1st, 2nd). GAPDH was used as an internal control, and the expression ratio of RUNX1 to GAPDH is indicated below the blot. The intensity of each band was evaluated by Scion Image.

(Fig. 2A). In siNC-transfected cells, the expression levels of the markers increased with time. In siRUNX1-transfected cells, the expression levels of the markers were elevated further, except for the expression level of *GPIIb* on day 4. As shown in Fig. 2B, although the level of *GPIIb* temporarily increased on day 3, it decreased on day 6 and thereafter. Therefore, these findings suggest that siRUNX1 transfection induces UT-7/GM cells to differentiate further along the megakaryocytic lineage.

We also investigated the expression of CD41a (GPIIb/IIIa) in siRNA-transfected UT-7/GM cells on day 2, 4, and 6 after TPO treatment using flow cytometry analyses (Fig. 2C). In siNC-transfected cells, the expression level of CD41a increased with time. In siRUNX1-transfected cells, the expression level of CD41a was elevated further. On the other hand, when GM-CSF was added to the media instead of TPO, this increase in CD41a expression was not observed. Taken together, these results indicate that the depletion of RUNX1 promotes the expression of megakaryocytic markers in TPO-treated UT-7/GM cells.

RUNX1 suppression up-regulates polyploidization

Polyploidization is due to endomitosis, a process that consists of repeated nuclear replications in the absence of cytokinesis and karyokinesis. As polyploidization is a phenomenon unique to megakaryocytes among the hematopoietic cells, we investigated whether the depletion of RUNX1 had an influence on polyploidization in UT-7/GM cells. It is known that polyploidization can be induced in UT-7/GM cells by TPO or TPA treatment [11,12]. We used TPA to induce polyploidization in UT-7/GM cells as TPA increases their ploidies after a few days, while TPO requires a month. Twenty-four hours after siRNA transfection, UT-7/GM cells were induced to differentiate into the megakaryocytic lineage by TPA treatment. Three days after TPA treatment, the ploidy distribution of the cells was evaluated by flow cytometry analyses after DNA staining with PI. A small peak of 8N cells was observed by TPA treatment of siNC-transfected cells. Furthermore, in siRUNX1-transfected cells, the size of the population of 8N cells increased slightly as compared with siNC-transfected cells. These results are summarized in Table 2. The distribution of 8N cells significantly increased from 3.7% ± 0.1% in siNC-transfected cells to 5.6% ± 0.2% in siRUNX1-transfected cells. Furthermore, in response to TPA treatment as well as TPO treatment, the depletion of RUNX1 promoted the expression of *PF4* and *GPIIIa* in UT-7/GM cells (Fig. 3). These results indicate that the depletion of RUNX1 tends to promote polyploidization in TPA-treated UT-7/GM cells.

RUNX1 suppression down-regulates cell proliferation

It is known that RUNX1 participates not only in differentiation, but also in proliferation of hematopoietic cells. We, therefore, investigated whether the depletion of

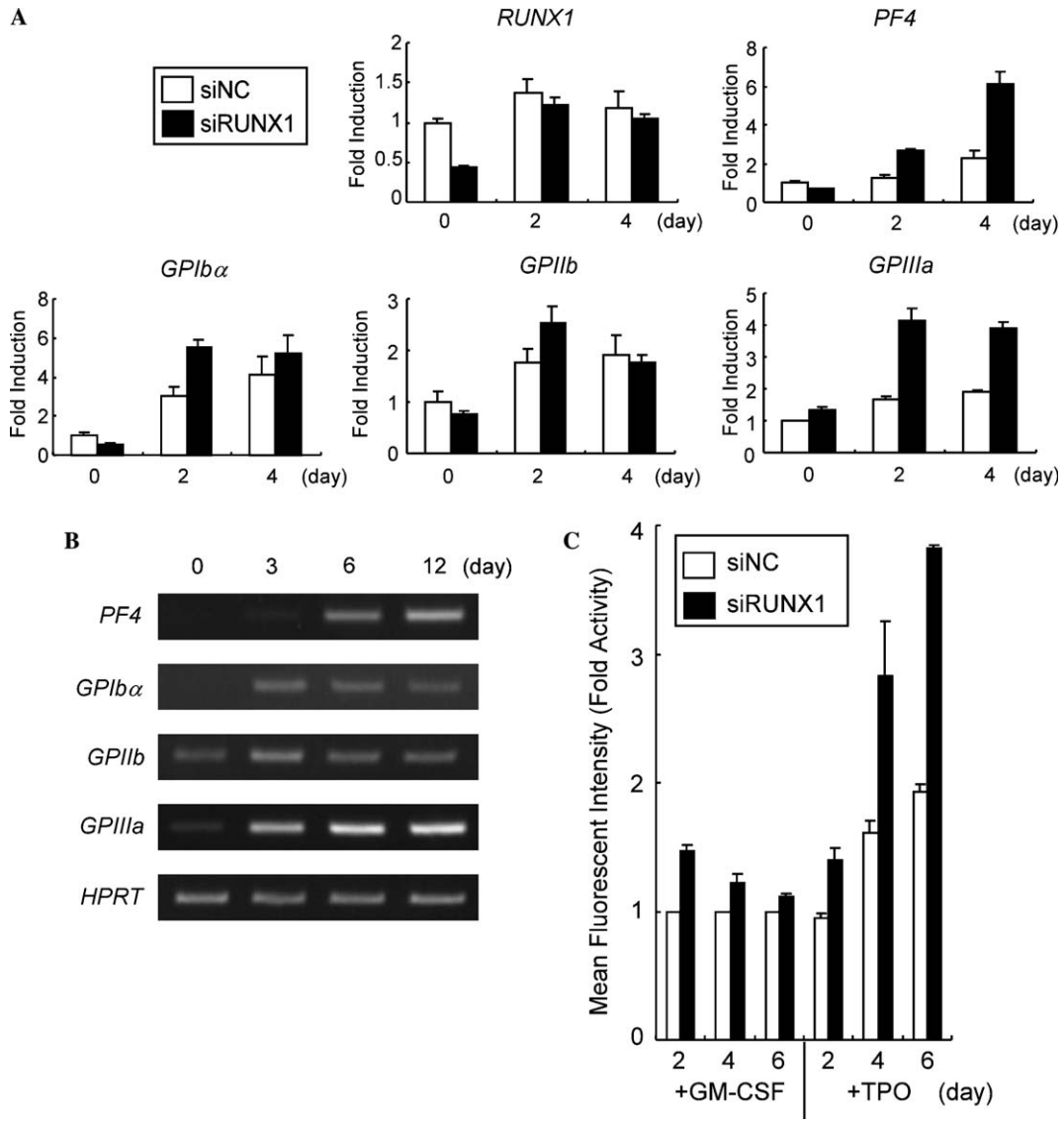

Fig. 2. RUNX1 suppression increases the expression of megakaryocytic markers. (A) Up-regulation of the mRNA levels of megakaryocytic markers by the depletion of RUNX1 in TPO-treated UT-7/GM cells. Twenty-four hours after siRNA transfection, UT-7/GM cells were induced to differentiate into the megakaryocytic lineage by TPO treatment. These cells were harvested on day 0, 2, and 4, and analyzed by real-time RT-PCR. Results represent means ± SEM of three independent experiments. (B) RT-PCR analysis of megakaryocytic markers in TPO-treated UT-7/GM cells. UT-7/GM cells cultured with TPO were harvested on day 0, 3, 6, and 12, and RT-PCR was performed. The number of PCR cycles for each gene was as follows: *PF4* (33 cycles), *GPIbα* (33 cycles), *GPIIb* (29 cycles), *GPIIIa* (33 cycles), and *HPRT* (27 cycles). (C) Up-regulation of the protein level of CD41a by the depletion of RUNX1 in TPO-treated UT-7/GM cells. Twenty-four hours after siRNA transfection, UT-7/GM cells were cultured with GM-CSF or TPO. These cells were harvested on day 2, 4, and 6. The cells were stained with anti-human CD41a mAb and analyzed by flow cytometry. The fluorescence intensity of siNC-transfected cells cultured with GM-CSF on each day is individually regarded as 1.0, and the *y*-axis shows the relative intensity. Results represent means ± SEM of three independent experiments.

Table 2
Distribution of ploidy of siRNA-transfected UT-7/GM cells after TPA treatment

	UT-7/GM	siNC + TPA	siRUNX1 + TPA
2N	57.7 ± 3.1	54.0 ± 12	50.3 ± 1.3
4N	38.8 ± 3.0	35.4 ± 1.0	38.1 ± 1.1
8N	0.9 ± 0.1	3.7 ± 0.1	5.6 ± 0.2*
≧16N	0.1 ± 0.0	0.3 ± 0.0	0.4 ± 0.1

Results represent means ± SEM of five independent experiments. Differences between siNC-transfected cells and siRUNX1-transfected cells were evaluated by Student's *t*-test.
* $P < 0.01$.

RUNX1 had an influence on proliferation of TPO-treated UT-7/GM cells. Twenty-four hours after siRNA transfection, UT-7/GM cells were induced to differentiate into the megakaryocytic lineage by TPO treatment, and WST-8 assays were performed on day 0, 1, 2, and 4 after TPO treatment. As shown in Fig. 4, the number of siRUNX1-transfected cells was ~50% of the number of siNC-transfected cells on day 2, although there was no difference in cell number between them on day 0. These results indicate that the depletion of RUNX1 inhibits proliferation of TPO-treated UT-7/GM cells.

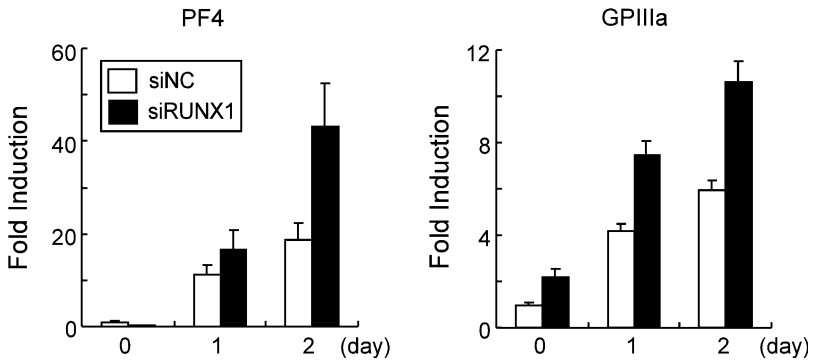

Fig. 3. RUNX1 suppression up-regulates megakaryocytic markers. Up-regulation of megakaryocytic markers by the depletion of RUNX1 in TPA-treated UT-7/GM cells. Twenty-four hours after siRNA transfection, UT-7/GM cells were induced to differentiate into the megakaryocytic lineage by TPA treatment, harvested on day 0, 1, and 2, and then analyzed by real-time RT-PCR. Results represent means ± SEM of three independent experiments.

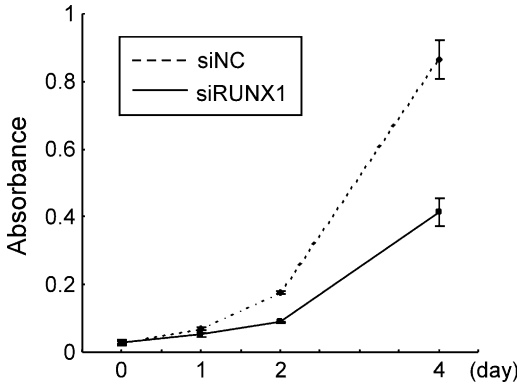

Fig. 4. RUNX1 suppression down-regulates cell proliferation. Twenty-four hours after siRNA transfection, UT-7/GM cells were induced to differentiate into the megakaryocytic lineage by TPO treatment, and WST-8 assays were performed on day 0, 1, 2, and 4. Results represent means ± SEM of three independent experiments.

The overexpression of RUNX1 down-regulates the activity of the PF4 and GPIIb promoters

In order to confirm that RUNX1 down-regulates the expression of megakaryocytic markers in UT-7/GM cells by another approach, we investigated whether the overexpression of RUNX1 had an influence on the activity of the *PF4* and *GPIIb* promoters in UT-7/GM cells. RUNX1 normally heterodimerizes with CBFβ through its Runt domain and enhances its DNA-binding ability. Therefore, we overexpressed RUNX1 and CBFβ together with the *PF4* promoter-reporter plasmid (PF4 luc) in UT-7/GM cells. Forty-eight hours after culturing with TPO, we collected the cells and measured reporter gene activity (Fig. 5A). The overexpression of RUNX1 decreased *PF4* promoter activity to ~60% of the control expression level. Furthermore, co-expression of RUNX1 and CBFβ

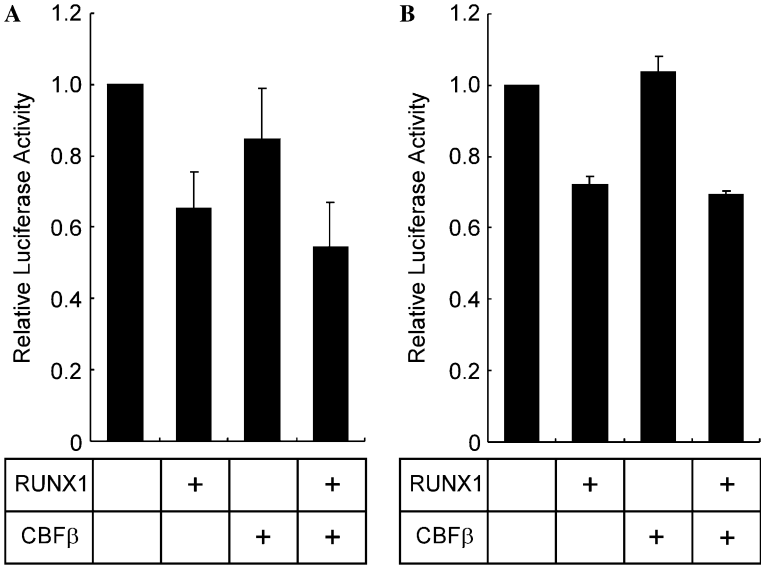

Fig. 5. The overexpression of RUNX1 down-regulates the activity of the *PF4* and *GPIIb* promoters. (A) Down-regulation of the *PF4* promoter by the overexpression of RUNX1. UT-7/GM cells were transiently co-transfected with the reporter plasmid and expression plasmids. Results were normalized to the expression of *Renilla* luciferase and are shown as fold activation relative to empty expression vector (pcDNA3). Results represent means ± SEM of four independent experiments. (B) Down-regulation of the *GPIIb* promoter by the overexpression of RUNX1. Co-transfections were performed in an identical manner to (A). Results represent means ± SEM of four independent experiments.

decreased the activity to ~50% of the control. We then investigated whether the overexpression of these transcription factors had an influence on the activity of the *GPIIb* promoter. The overexpression of RUNX1 decreased *GPIIb* promoter activity to ~70% of the control, and co-expression of RUNX1 and CBFβ also decreased the activity to ~70% of the control (Fig. 5B). Taken together, these results indicate that the overexpression of RUNX1 decreases the activity of the *PF4* and *GPIIb* promoters in TPO-treated UT-7/GM cells.

Discussion

In this study, we combined RNAi technology and an in vitro megakaryopoiesis system using UT-7/GM cells to examine in detail the mechanisms by which RUNX1 participates in megakaryopoiesis. First, we found that the depletion of RUNX1 enhanced the expression of megakaryocytic markers. Second, the depletion of RUNX1 promoted polyploidization. Third, the depletion of RUNX1 inhibited cell proliferation. Finally, the overexpression of RUNX1 decreased the activity of the *PF4* and *GPIIb* promoters. Taken together, these results demonstrate that RUNX1 down-regulates terminal differentiation of UT-7/GM cells along the megakaryocytic lineage and promotes their proliferation.

Although there are many transcription factors that promote megakaryopoiesis, there are only a few that suppress megakaryopoiesis and promote cell proliferation [13]. Presumably, these factors are required to develop megakaryocytic progenitors and maintain well-balanced thrombopoiesis. RUNX1 may be one such factor, and may play a major role, being a central regulator in hematopoiesis.

Heterozygous mutations in the *RUNX1* gene have been reported to be associated with familial platelet disorder, with predisposition to acute myeloid leukemia (FPD/AML) [14]. Analyses of bone marrow or peripheral blood cells from FPD/AML individuals showed a decrease in megakaryocyte colony formation, suggesting that RUNX1 dosage affects megakaryopoiesis [15]. However, these findings require further investigation. Here, we found that the depletion of RUNX1 triggers a significant reduction in the growth rate of TPO-treated UT-7/GM cells. This function of RUNX1 may contribute to the decrease in megakaryocyte colony formation of cells from FPD/AML individuals. Additionally, overexpression of RUNX1 in BXH2 mice has been reported to result in the development of megakaryoblastic leukemia [16]. The effects of RUNX1 on proliferation and megakaryocytic differentiation of UT-7/GM cells reported here may contribute to the megakaryoblastic leukemogenesis in these mice.

There was little influence on the activity of the *PF4* and *GPIIb* promoters by co-expression of CBFβ (Fig. 5). This may be because CBFβ was sufficiently expressed in UT-7/GM cells, or because RUNX1 failed to interact with CBFβ due to its interaction with other transcription factors. For instance, RUNX1 is known to interact with co-repressors such as mSin3A and SMRT, and down-regulates the expression of target genes [17,18]. Therefore, these interactions may also occur in UT-7/GM cells, leading to an interruption in the interaction of RUNX1 with CBFβ, and down-regulation of the activity of the *PF4* and *GPIIb* promoters.

To our knowledge, this is the first report to show that RUNX1 up-regulates cell proliferation and down-regulates differentiation of megakaryocytic cells. These findings may contribute to the elucidation of megakaryopoiesis and thrombopoiesis.

Acknowledgments

We thank Kirin Brewery for the gift of thrombopoietin. This work was supported by the Grants-in-Aid for scientific research from the Ministry of Education, Science, Sports and Culture of Japan.

References

[1] Y. Ito, Oncogenic potential of the RUNX gene family: 'overview', Oncogene 23 (2004) 4198–4208.
[2] T. Okuda, J. van Deursen, S.W. Hiebert, G. Grosveld, J.R. Downing, AML1, the target of multiple chromosomal translocations in human leukemia, is essential for normal fetal liver hematopoiesis, Cell 84 (1996) 321–330.
[3] Q. Wang, T. Stacy, M. Binder, M. Marin-Padilla, A.H. Sharpe, N.A. Speck, Disruption of the Cbfa2 gene causes necrosis and hemorrhaging in the central nervous system and blocks definitive hematopoiesis, Proc. Natl. Acad. Sci. USA 93 (1996) 3444–3449.
[4] N. Asou, The role of a Runt domain transcription factor AML1/RUNX1 in leukemogenesis and its clinical implications, Crit. Rev. Oncol. Hematol. 45 (2003) 129–150.
[5] D.K. Strom, J. Nip, J.J. Westendorf, B. Linggi, B. Lutterbach, J.R. Downing, N. Lenny, S.W. Hiebert, Expression of the AML-1 oncogene shortens the G(1) phase of the cell cycle, J. Biol. Chem. 275 (2000) 3438–3445.
[6] F. Bernardin, A.D. Friedman, AML1 stimulates G1 to S progression via its transactivation domain, Oncogene 21 (2002) 3247–3252.
[7] J.A. Coffman, Runx transcription factors and the developmental balance between cell proliferation and differentiation, Cell Biol. Int. 27 (2003) 315–324.
[8] M. Ichikawa, T. Asai, T. Saito, S. Seo, I. Yamazaki, T. Yamagata, K. Mitani, S. Chiba, S. Ogawa, M. Kurokawa, H. Hirai, AML-1 is required for megakaryocytic maturation and lymphocytic differentiation, but not for maintenance of hematopoietic stem cells in adult hematopoiesis, Nat. Med. 10 (2004) 299–304.
[9] N. Komatsu, K. Kirito, R. Shimizu, M. Kunitama, M. Yamada, M. Uchida, M. Takatoku, M. Eguchi, Y. Miura, In vitro development of erythroid and megakaryocytic cells from a UT-7 subline, UT-7/GM, Blood 89 (1997) 4021–4033.
[10] T. Minami, K. Tachibana, T. Imanishi, T. Doi, Both Ets-1 and GATA-1 are essential for positive regulation of platelet factor 4 gene expression, Eur. J. Biochem. 258 (1998) 879–889.
[11] S. Shimizu, T. Nagasawa, O. Katoh, N. Komatsu, J. Yokota, K. Morishita, EVI1 is expressed in megakaryocyte cell lineage and enforced expression of EVI1 in UT-7/GM cells induces megakaryocyte differentiation, Biochem. Biophys. Res. Commun. 292 (2002) 609–616.
[12] J. Kikuchi, Y. Furukawa, S. Iwase, Y. Terui, M. Nakamura, S. Kitagawa, M. Kitagawa, N. Komatsu, Y. Miura, Polyploidization

and functional maturation are two distinct processes during megakaryocytic differentiation: involvement of cyclin-dependent kinase inhibitor p21 in polyploidization, Blood 89 (1997) 3980–3990.
[13] H. Schulze, R.A. Shivdasani, Mechanisms of thrombopoiesis, J. Thromb. Haemost. 3 (2005) 1717–1724.
[14] M. Osato, M. Yanagida, K. Shigesada, Y. Ito, Point mutations of the RUNx1/AML1 gene in sporadic and familial myeloid leukemias, Int. J. Hematol. 74 (2001) 245–251.
[15] W.J. Song, M.G. Sullivan, R.D. Legare, S. Hutchings, X. Tan, D. Kufrin, J. Ratajczak, I.C. Resende, C. Haworth, R. Hock, M. Loh, C. Felix, D.C. Roy, L. Busque, D. Kurnit, C. Willman, A.M. Gewirtz, N.A. Speck, J.H. Bushweller, F.P. Li, K. Gardiner, M. Poncz, J.M. Maris, D.G. Gilliland, Haploinsufficiency of CBFA2 causes familial thrombocytopenia with propensity to develop acute myelogenous leukaemia, Nat. Genet. 23 (1999) 166–175.
[16] M. Yanagida, M. Osato, N. Yamashita, H. Liqun, B. Jacob, F. Wu, X. Cao, T. Nakamura, T. Yokomizo, S. Takahashi, M. Yamamoto, K. Shigesada, Y. Ito, Increased dosage of Runx1/AML1 acts as a positive modulator of myeloid leukemogenesis in BXH2 mice, Oncogene 24 (2005) 4477–4485.
[17] B. Lutterbach, S.W. Hiebert, Role of the transcription factor AML-1 in acute leukemia and hematopoietic differentiation, Gene 245 (2000) 223–235.
[18] S. Takahashi, H. Harigae, J. Kameoka, T. Sasaki, M. Kaku, AML1B transcriptional repressor function is impaired by the Flt3-internal tandem duplication, Br. J. Haematol. 130 (2005) 428–436.