Biochemical and Biophysical Research Communications 485 (2017) 380–387

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Forced FOG1 expression in erythroleukemia cells: Induction of erythroid genes and repression of myelo-lymphoid transcription factor PU.1

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ARTICLE INFO

Article history:
Received 7 February 2017
Accepted 13 February 2017
Available online 16 February 2017

Keywords:
Transcription factor
GATA-1
FOG1
Erythroid differentiation
PU.1
Histone modification

ABSTRACT

The transcription factor GATA-1-interacting protein Friend of GATA-1 (FOG1) is essential for proper transcriptional activation and repression of GATA-1 target genes; yet, the mechanisms by which FOG1 exerts its activating and repressing functions remain unknown. Forced FOG1 expression in human K562 erythroleukemia cells induced the expression of erythroid genes (*SLC4A1*, globins) but repressed that of *GATA-2* and *PU.1*. A quantitative chromatin immunoprecipitation (ChIP) analysis demonstrated increased GATA-1 chromatin occupancy at both FOG1-activated as well as FOG1-repressed gene loci. However, while TAL1 chromatin occupancy was significantly increased at FOG1-activated gene loci, it was significantly decreased at FOG1-repressed gene loci. When FOG1 was overexpressed in TAL1-knocked down K562 cells, FOG1-mediated activation of *HBA*, *HBG*, and *SLC4A1* was significantly compromised by TAL1 knockdown, suggesting that FOG1 may require TAL1 to activate GATA-1 target genes. Promoter analysis and quantitative ChIP analysis demonstrated that FOG1-mediated transcriptional repression of *PU.1* would be mediated through a GATA-binding element located at its promoter, accompanied by significantly decreased H3 acetylation at lysine 4 and 9 (K4 and K9) as well as H3K4 trimethylation. Our results provide important mechanistic insight into the role of FOG1 in the regulation of GATA-1-regulated genes and suggest that FOG1 has an important role in inducing cells to differentiate toward the erythroid lineage rather than the myelo-lymphoid one by repressing the expression of *PU.1*.

1. Introduction

Hematopoietic lineage specification is highly regulated at the transcriptional level, and for many lineages, specific transcription factors and their coregulators have been identified as critical for their specification. GATA-1 is a hematopoietic zinc-finger transcription factor, which activates the expression of most erythroid-expressed genes [1–3]. GATA-1 interacts with another master regulator of hematopoiesis, the basic-helix-loop-helix transcription factor SCL/TAL1 (TAL1) [1,4–6]. TAL1 assembles a complex containing LMO2, LDB1, and ETO2 and regulates erythroid differentiation in association with GATA-1 [5–8]. Besides TAL1, numerous proteins have been reported to associate with GATA-1 to alter its activity. Among them, FOG1 (Friend of GATA-1), a nine-zinc-finger protein, is expressed in a tissue-specific pattern that overlaps markedly with that of GATA-1 [9]. FOG1 is an essential coregulator of GATA-1 during hematopoiesis, which mediates both transcriptional activation and repression, as represented by *β-globin* and *GATA-2*, respectively [9–12]. However, the molecular mechanisms by which the activating and repressing functions of FOG1 are selectively exerted remain to be elucidated. Herein, we describe a novel role of FOG1 during erythroid differentiation identified by forcibly expressing FOG1 in the human K562

Abbreviations: FOG1, friend of GATA-1; TAL1, T-cell acute lymphocytic leukemia protein 1; LMO2, LIM domain only 2; LDB1, LIM domain binding 1; ETO2, eight-twenty-one 2; HBA, hemoglobin alpha; HBB, hemoglobin beta; HBG, hemoglobin gamma; SLC4A1, solute carrier family 4 member 1; RPMI-1640, Roswell Park Memorial Institute-1640; DMEM, Dulbecco's Modified Eagle's medium; RT-PCR, reverse transcription polymerase chain reaction; ChIP, chromatin immunoprecipitation.

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http://dx.doi.org/10.1016/j.bbrc.2017.02.068
0006-291X/

erythroleukemia cell line.

2. Materials and methods

2.1. Cell culture

All cells were cultured in a humidified incubator at 37 °C with a 5% carbon dioxide atmosphere. Human K562 erythroleukemia cell lines (ATCC® CCL-243™) were maintained in RPMI-1640 medium containing 10% fetal bovine serum (Biowest, Miami, FL, USA) and 1% penicillin–streptomycin (Sigma, St. Louis, MO, USA). PLAT-GP Packaging Cell Line (Cell Biolabs, San Diego, CA, USA) was maintained in DMEM containing 10% fetal bovine serum (Biowest) and 1% penicillin-streptomycin (Sigma).

2.2. Real-time quantitative RT-PCR analysis

Total RNA was purified with TRIzol (Invitrogen, Carlsbad, CA, USA). cDNAs were synthesized from purified total RNA using the ReverTra Ace qPCR RT Master Mix (Toyobo, Tokyo, Japan). The Quantitect SYBR Green PCR kit (Qiagen, Hilden, Germany) was used for mRNA quantification. PCR product abundance was normalized relative to amplified *GAPDH* mRNA [7]. Primer sequences used in the analysis are shown in Supplementary Table 1.

2.3. Gene transfer and vectors

Human *FOG1* messenger RNA (mRNA) was cloned into the pFN21A vector, which contained the HaloTag® epitope (Promega, Madison, WI, USA). This expression vector (10 μg) was transfected into aliquots of 2×10^6 K562 cells by Amaxa Cell Line Nucleofector II with the program T-016 (Amaxa Biosystems, Cologne, Germany).

Retroviral overexpression of FOG1 and GATA-2 was conducted using pBABE-puro vector [7]. The retroviral vector and the *env* (envelope glycoprotein) gene from the vesicular stomatitis virus (VSV-G) were cotransfected into PLAT-GP Packaging Cell Lines with FuGene HD (Promega). Seventy-two hours after transfection, the viral supernatant was used for infection. After spin infection into K562 cells at 3400 rpm for 2 h, the cells were cultured with the medium containing 1 μg/mL Puromycin (Sigma) for selection of the transduced cells.

2.4. Promoter assay

DNA fragments of the *PU.1* gene promoter (−500/+99) were obtained from human genomic DNA of a healthy volunteer, and each fragment was cloned into the Xho I/Bgl II site of the pGL4.10 [*luc2*] vector (Promega, Madison, WI, USA). GATA mutation construct was generated using the QuickChange™ Site-Directed Mutagenesis Kit (Stratagene, La Jolla, CA, USA) using the mutagenesis primer 5′- TCACCCCAAGGGGACTTTTTCCCAGCGGCAGGCC-3′. Luciferase plasmid containing both 1S promoter and +9.9-kb intronic enhancer region of *GATA-2*, as well as its GATA mutation construct, was established as described previously [13].

To assay transcriptional activity, aliquots of 1×10^6 K562 cells were cotransfected with 1) 200 ng of the promoter construct, 2) 100 ng of a *Renilla* luciferase reporter plasmid (pGL4.74), and 3) 1 μg of either pFN21A-FOG1 or pFN21A, with FuGene HD (Promega). After 24 h of incubation in culture medium, the cells were harvested, and both firefly and *Renilla* luciferase activities in the cell extracts were determined using the Dual Luciferase Reporter Assay System (Promega).

2.5. Gene silencing by small interfering RNA (siRNA)

siGENOME SMARTpool siRNAs (Thermo Scientific Dharmacon, Lafayette, CO, USA) were used in the siRNA-mediated transient knockdown of *TAL1* in K562 cells [7].

2.6. Quantitative ChIP analysis

Quantitative chromatin immunoprecipitation (ChIP) analysis was performed as previously described [1,7]. Primer sequences used in the analysis are shown in Supplementary Table 1.

2.7. Western blotting

Western blotting analyzes were performed as previously described [1,7].

2.8. Antibodies

Antibodies against GATA-2 (H-116), TAL1 (C-21), ETO2 (C-20), FOG1 (M-20), and PU.1 (H-135) were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Antibody against HaloTag® was obtained from Promega. Antibody against GATA-1 (D52H6) was obtained from Cell Signaling Technology (Danvers, MA, USA). An anti-α-tubulin antibody (CP06) was obtained from Calbiochem (San Diego, CA, USA). A control rabbit IgG was obtained from Abcam (Cambridge, MA, USA). Antibodies against acetyl-histone H3 (Lys4), acetyl-histone H3 (Lys9), trimethyl-histone H3 (Lys4), and trimethyl-histone H3 (Lys27) were obtained from Millipore (Temecula, CA, USA).

2.9. Statistics

Statistical significance was assessed using two-sided Student's *t* tests.

3. Results and discussion

Forced FOG1 expression induces expression of erythroid genes and represses that of myelo-lymphoid transcription factor PU.1 in K562 cells.

To assess the role of FOG1 during erythroid differentiation, we transiently transfected K562 cells with a pFN21A expression vector encoding human FOG1 or an empty vector control. As shown in Fig. 1A, HaloTag epitope was fuzed to the N-terminal region of FOG1. In addition, FOG1 also harbored a translation initiation site (ATG) (Fig. 1A). Thus, western blot analysis detected both HaloTag-FOG1 fusion and FOG1 proteins following the pFN21A-FOG1 vector transfection (Fig. 1B). On the other hand, FOG1 protein could not be detected in the empty vector-transfected control condition, even upon longer exposure (Fig. 1B), indicating that endogenous FOG1 expression was weak in the K562 cells. Quantitative RT-PCR analysis demonstrated that FOG1 overexpression significantly induced the expression of erythroid-related genes, such as *HBA* (hemoglobin subunit alpha), *HBB* (hemoglobin subunit beta), *HBG* (hemoglobin subunit gamma), and *SLC4A1* (solute carrier family 4 member 1) (Fig. 1C), which have been reported to be FOG1-dependent GATA-1-activated genes [11,12]. Next, we tested whether the expression of hematopoietic transcription factors could be altered by FOG1 overexpression. As shown in Fig. 1D, FOG1 overexpression did not affect the expression of master regulators of erythropoiesis, such as *GATA-1* and *TAL1* [1,4–6]. On the other hand, we demonstrated that the expression of both *GATA-2* and *PU.1* (also known as *SPI1*) was significantly downregulated by FOG1 overexpression (Fig. 1D). We also confirmed the changes in expression

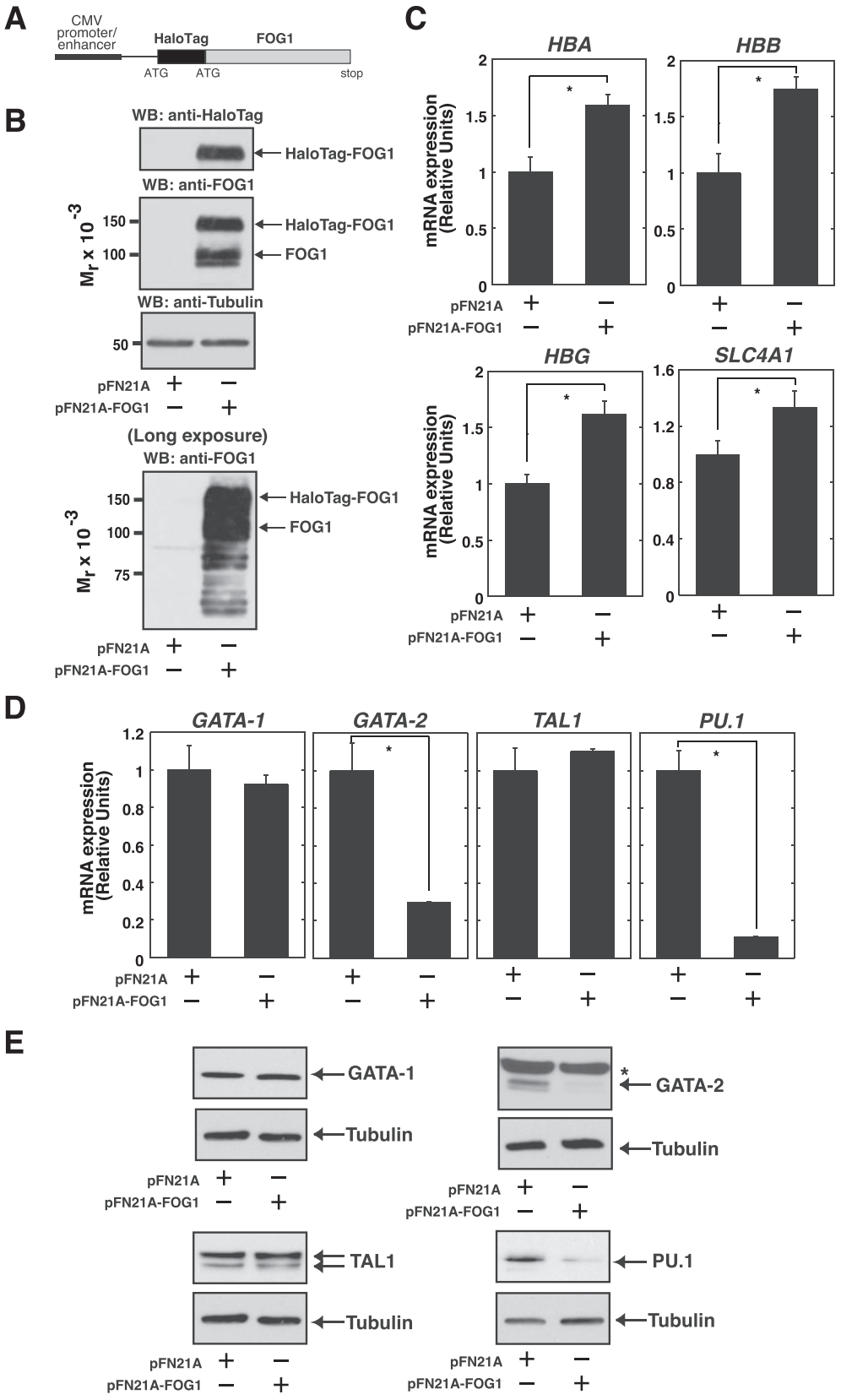

Fig. 1. Forced FOG1 expression in K562 cells.
(A) Structure of the FOG1 expression vector. Both HaloTag-FOG1 and FOG1 contain a translation initiation codon (ATG). (B) Western blotting to detect both HaloTag-FOG1 and FOG1 proteins with anti-HaloTag and anti-FOG1 antibodies. For the anti-FOG1 condition, a blot with longer exposure was also used. Alpha-tubulin was used as a loading control. (C,D) Quantitative RT-PCR analysis for erythroid genes (C) and hematopoietic transcription factors (D) in control and FOG1-overexpressing K562 cells. PCR product accumulation was normalized as expression relative to *GAPDH* mRNA (mean ± standard deviation, $n = 3$). $^{*}P < 0.05$. (E) Western blotting to detect hematopoietic transcription factors, which were analyzed as shown in Fig. 1C, in control and FOG1-overexpressing K562 cells. Alpha-tubulin was used as a loading control. An asterisk denotes a cross-reactive band.

of these transcription factors by FOG1 overexpression based on western blotting (Fig. 1E). To demonstrate the reliability of the anti-GATA-2 antibody, we conducted western blotting with the whole-cell lysate derived from GATA-2-overexpressing K562 cells (Supplementary Fig. 1). Previous reports suggested that *GATA-2*, a transcription factor expressed in hematopoietic stem cells [14], is a FOG1-dependent GATA-1-repressed gene [4,10]; during erythroid differentiation from hematopoietic stem cells, FOG1 facilitates GATA-1 chromatin occupancy at the *GATA-2* locus and represses *GATA-2* expression (termed the "GATA switch" mechanism) [4,10]. Notably, we found that *PU.1* is another FOG1-dependent GATA-1 target, which has not been described previously. Because PU.1 is known as a myelo-lymphoid-promoting transcription factor [15], our findings indicate that FOG1 may have an important role in regulating erythroid versus myelo-lymphoid lineage commitment.

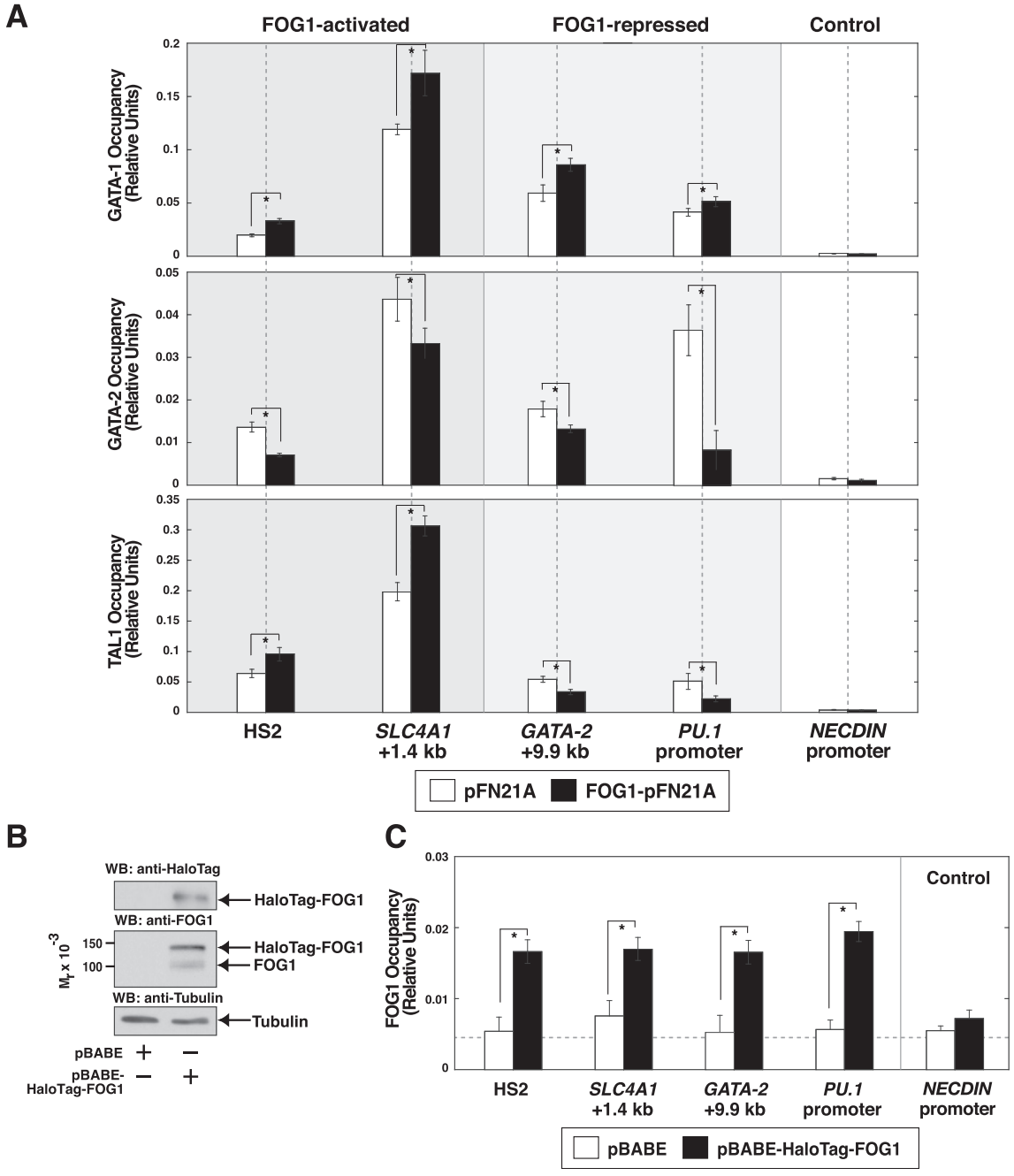

Fig. 2. FOG1-mediated changes in chromatin occupancy of hematopoietic transcription factors.
(A) Quantitative ChIP analysis of GATA-1, GATA-2, and TAL1 occupancy at FOG1-regulated GATA-1 target gene loci in control and FOG1-overexpressing K562 cells (mean ± standard deviation, $n = 3$). HS2 indicates the β-globin locus control region (LCR) (7). *NECDIN* promoter was included as a negative control region, which was not bound by GATA-1, GATA-2, and TAL1. For all analyses, control IgG signals analyzed with all primer sets did not exceed 0.00226. $^*P < 0.05$. (B) Western blotting to detect both HaloTag-FOG1 and FOG1 proteins with anti-HaloTag and anti-FOG1 antibodies, which were induced using pBABE-puro retroviral expression vector. Alpha-tubulin was used as a loading control. (C) Quantitative ChIP analysis of HaloTag-FOG1 and FOG1 occupancy at FOG1-regulated GATA-1 target gene loci in control and FOG1-overexpressing K562 cells (mean ± standard deviation, $n = 3$). *NECDIN* promoter was included as a negative control region. Control IgG signals analyzed with all primer sets did not exceed 0.0047 (as indicated by a dotted line). $^*P < 0.05$.

3.1. TAL1 chromatin occupancy is important for FOG1-mediated gene activation

Despite no obvious change in the expression of GATA-1 and TAL1, FOG1-dependent GATA-1 targets were significantly activated by FOG1 overexpression (Fig. 1). To examine the molecular mechanisms involved, we conducted quantitative ChIP analysis to assess the chromatin occupancy of GATA-1 and TAL1 at FOG1-dependent GATA-1 target gene loci. As shown in Fig. 2A, the chromatin occupancy of GATA-1 was significantly increased whereas that of GATA-

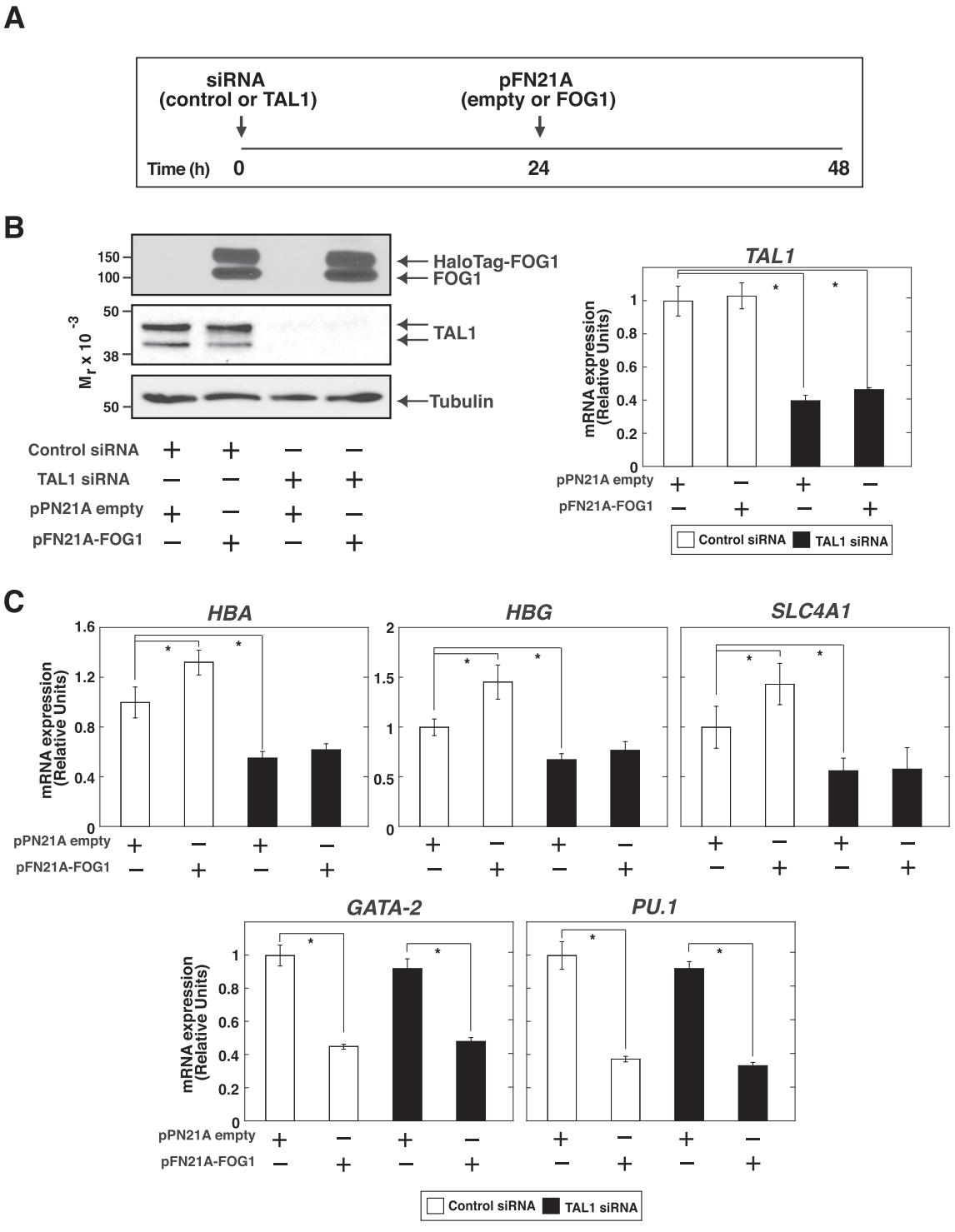

Fig. 3. TAL1 chromatin occupancy is important for FOG1-mediated gene activation.
(A) Experimental strategy for FOG1 overexpression after siRNA-mediated TAL1 knockdown in K562 cells. (B) (Left) Western blotting analysis of whole-cell extracts from each condition. Anti-TAL1 and anti-FOG1 antibodies were used. Alpha-tubulin was used as a loading control. (Right) Quantitative RT-PCR analysis for *TAL1* in control and FOG1-overexpressing K562 cells, transfected with control or anti-TAL1-siRNA. PCR product accumulation was normalized as expression relative to *GAPDH* mRNA (mean ± standard deviation, $n = 3$). $^*P < 0.05$. (C) Quantitative RT-PCR analysis for *HBA*, *HBG*, *SLC4A1*, *GATA-2*, and *PU.1* in control and FOG1-overexpressing K562 cells, transfected with control or anti-TAL1-siRNA. PCR product accumulation was normalized as expression relative to *GAPDH* mRNA (mean ± standard deviation, $n = 3$). $^*P < 0.05$.

2 was significantly decreased by FOG1 overexpression. Increased levels of GATA-1 chromatin occupancy by FOG1 were in consistent with a previous study, demonstrating that FOG1 could facilitate GATA-1 chromatin occupancy [10,12]. On the other hand, we found that TAL1 chromatin occupancy was significantly increased at FOG1-dependent GATA-1-activated gene loci, whereas it was significantly decreased at FOG1-dependent GATA-1-repressed gene locus (Fig. 2C). Presumably as a result of low endogenous FOG1 expression in K562 cells (Fig. 1B), we could not demonstrate endogenous FOG1 chromatin occupancy at FOG1-regulated gene loci based on empty vector (pBABE-puro)-transfected K562 cells (Fig. 2B and C), as well as parental K562 cells (data not shown). Instead, we confirmed FOG1 chromatin occupancy at these loci based on retroviral-mediated FOG1-overexpressing K562 cells (Fig. 2B and C). Taken together, our data suggest that FOG1 may require TAL1 to activate GATA-1 target genes. To test this possibility,

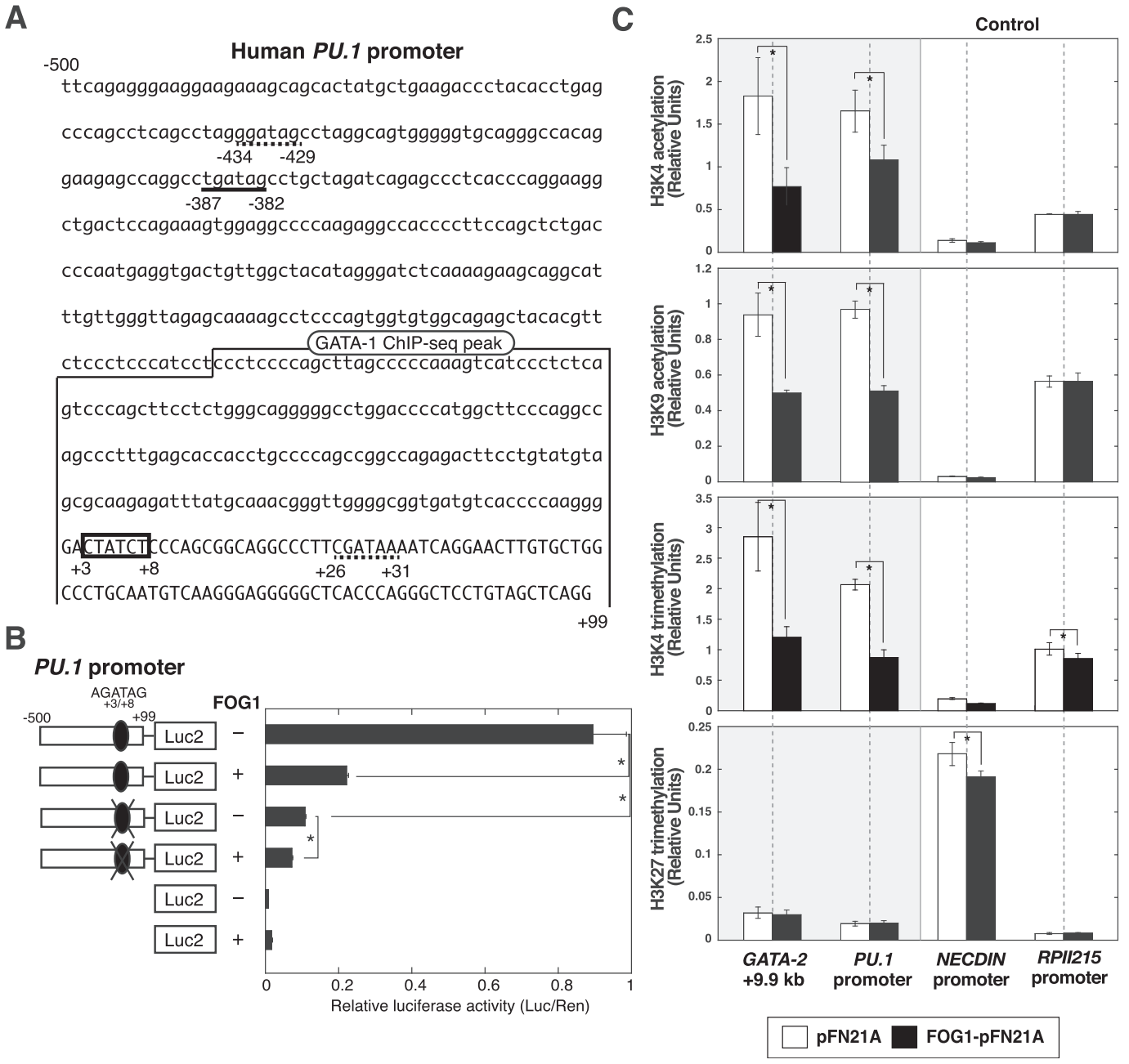

Fig. 4. FOG1 represses PU.1 transcriptional activity via a GATA-binding site located at the promoter, accompanied by a significant decrease in activating histone marks. (A) The 5′ upstream region of the human *PU.1* promoter sequence, which was obtained from the UCSC genome browser (http://genome.ucsc.edu). The exon 1 region is indicated in uppercase. GATA-1 ChIP-seq peak region (obtained from Ref. [1]) is also indicated. Evolutionally conserved consensus GATA-binding motif (+3/+8) within ChIP-seq peaks is indicated in a box. Other non-consensus GATA-binding motifs (CGATAA at +26/+31, GGATAG at −429/−434), and consensus GATA-binding motifs located downstream of the ChIP-seq peak region (TGATAG at −382/−387) are indicated as a dotted line and a solid line, respectively. (B) FOG1 expression vector was cotransfected with the *PU.1* promoter, or its GATA-mutant construct, fuzed to a luciferase reporter gene, and subjected to a transient transfection assay in K562 cells (mean ± standard deviation, n = 3). *$P < 0.05$. (C) Quantitative ChIP analysis to examine the levels of H3K4 acetylation, H3K9 acetylation, H3K4 trimethylation, and H3K27 trimethylation at FOG1-repressed gene loci (mean ± standard deviation, $n = 3$) in control and FOG1-overexpressing K562 cells. *NECDIN* and *RPII215* promoters were included as inactive and active promoter regions, respectively (also shown in Fig. 4C). IgG control signal did not exceed 0.01. *: $P < 0.05$.

FOG1 was overexpressed in TAL1-knocked down K562 cells (Fig. 3A). TAL1 knockdown and FOG1 overexpression were confirmed by both western blotting and quantitative RT-PCR (Fig. 3B). Under these conditions, FOG1-mediated activation of *HBA*, *HBG*, and *SLC4A1* was significantly compromised by TAL1 knockdown, whereas the expression of FOG1-dependent GATA-1-repressed genes (*GATA-2* and *PU.1*) was not affected by TAL1 knockdown (Fig. 3C). Our findings are in line with a previous study demonstrating that the TAL1 complex is a critical determinant of positive GATA-1 activity in erythroid cells, although the previous study did not demonstrate the role of FOG1 in recruitment of the TAL1 complex [16]. However, the molecular mechanisms by which TAL1 occupancy was decreased at GATA-1-repressed gene loci remain unknown. It has been reported that FOG1 interacts with the NuRD (nucleosome remodeling and deacetylation) complex, which is commonly linked to transcriptional repression [17]. We speculate that the NuRD complex might interfere with TAL1 occupancy selectively at GATA-1-repressed gene loci, contributing to decreased transcriptional activity. Alternatively, FOG1 might be post-translationally modified in a way that interferes with TAL1 chromatin occupancy. Further analyzes are required to address the question of how the activating versus repressing functions of FOG1 are selectively exerted.

3.2. FOG1 represses PU.1 transcriptional activity via a GATA-binding site located at the promoter, accompanied by a significant decrease in activating histone marks

We demonstrated that FOG1 overexpression strongly down-regulated *PU.1* mRNA and protein expression in K562 cells (Fig. 1D and E). Recent genome-wide analysis of GATA-1 chromatin occupancy based on the coupling of next-generation DNA sequencing technology with chromatin immunoprecipitation sequencing showed a GATA-1 peak in the *PU.1* promoter [1]. We focused on the consensus GATA-binding motif (AGATAG) within the GATA-1 ChIP-seq peak at the promoter of the human *PU.1* gene (Fig. 4A, box, +3/+8), which was shown to be evolutionally conserved across species (according to the UCSC genome browser, http://genome.ucsc.edu). We conducted a transient luciferase promoter analysis to test whether FOG1-mediated transcriptional repression of *PU.1* could be regulated at the GATA site located at its promoter. As shown in Fig. 4B, cotransfection of the FOG1 expression vector significantly reduced the promoter activity of *PU.1*, and this effect was clearly diminished by disruption of the GATA motif (from GATA to AAAA), suggesting that this +3/+8 GATA-binding motif has an important role in FOG1-mediated transcriptional repression of *PU.1*. However, we noticed a weak, but significant, decrease in the promoter activity of GATA-mutated *PU.1* promoter (Fig. 4B). A previous report suggested that GATA-1 recognizes the DNA binding consensus (A/T)GATA(A/G) [18], but many of the non-consensus sequences could be recognized by GATA-1 based on an electrophoretic mobility shift assay [19]. Thus, one possibility is that the mutation from ACTATCTC to ACTTTTTC (from GAGATAGT to GAAAAAGT in the reverse strand) created a non-consensus GAAA sequence, which might be weakly recognized by GATA-1. Alternatively, other non-consensus GATA-binding motifs within the ChIP-seq peak region (CGATAA at +26/+31) or other GATA-binding motifs located downstream of the *PU.1* promoter (TGATAG at −382/−387 or GGATAG at −429/−434) (Fig. 4A) might also contribute to FOG1-mediated transcriptional repression. On the other hand, we also confirmed that FOG1-mediated transcriptional repression of *GATA-2* occurs through the GATA-binding motif at the +9.9-kb intronic enhancer region, which was responsible for the pathogenesis of *GATA-2* deficiency syndrome [20] (Supplementary Fig. 2).

Finally, we evaluated the epigenetic landscape at FOG1-repressed gene loci in control and FOG1-overexpressing K562 cells to estimate the molecular mechanisms by which FOG1 confers transcriptional repression. As shown in Fig. 4C, histone H3 acetylated at both K4 and K9 was significantly decreased by FOG1 overexpression, without influencing these marks at the inactive and active *NECDIN* and *RPII215* promoters, respectively. In addition, active H3K4 trimethylation was also significantly decreased, whereas repressive H3K27 trimethylation was not affected by FOG1 overexpression. Because the NuRD complex contains histone deacetylase (HDAC1/2), histone-binding protein RbAp46/48, ATP-dependent chromatin remodeler (CHD3/4), metastasis-associated factor (MTA1/2/3), and methyl-DNA-binding protein (MBD2/3) [21,22], the decrease in histone H3K4 and H3K9 acetylation by FOG1 overexpression was consistent with this mechanism. Regarding the significant decrease in H3K4 trimethylation by FOG1 overexpression, we speculate that an H3K4 demethylase, KDM5A (lysine demethylase 5A), which could interact with the NuRD complex [23], may contribute to the changes of this histone mark. On the other hand, our results suggest that FOG1-dependent transcriptional repression may not involve H3K27 trimethylation. It has been reported that the transcriptional corepressor ETO2, which is a component of the TAL1 complex [1,24], suppresses GATA-1-regulated genes by interacting with HDAC [25], and could also regulate H3K27 trimethylation at selected GATA-1 loci, whereas ETO2 did not affect activating H3K4 dimethylation [8]. Thus, we speculate that the mechanism of FOG1-dependent transcriptional repression might be independent of ETO2 during erythroid differentiation. On the other hand, we did not find significant changes of these histone marks at FOG1-activated gene loci (Supplementary Fig. 3), possibly due to the relatively small increases of mRNA expression by FOG1 overexpression.

It has been considered that the dominance of either GATA-1 or PU.1 appears to be the central to the decision to specify erythroid and myeloid lineage cells [26]. However, the question remains as to how the bipotential progenitor cell population shifts toward erythroid cells at the expense of the myeloid lineage. In conjunction with a previous study showing that hematopoietic stem cells and promegakaryocytic/erythroid cells, which are deficient in FOG1, could undergo reprograming to specify myeloid cells [27], we consider that FOG1 is one of the most important factors responsible for this shift. Because FOG1 has been linked to hematological disorders such as anemia and thrombocytopenia [28,29], further understanding its role should lead to novel therapeutic approaches to alter disease-induced imbalances in hematopoietic cell production.

Acknowledgements

We thank the members of the Biomedical Research Core of Tohoku University School of Medicine for their support. The authors declare no conflict of interest. This work was supported by JSPS KAKENHI Grant Number 15K09447 to T.F..

Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.bbrc.2017.02.068.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.bbrc.2017.02.068.

References

[1] T. Fujiwara, H. O'Geen, S. Keles, et al., Discovering hematopoietic mechanisms

through genome-wide analysis of GATA factor chromatin occupancy, Mol. Cell. 36 (2009) 667–681.
[2] M. Yu, L. Riva, H. Xie, et al., Insights into GATA-1-mediated gene activation versus repression via genome-wide chromatin occupancy analysis, Mol. Cell. 36 (2009) 682–695.
[3] E. Soler, C. Andrieu-Soler, E. de Boer, et al., The genome-wide dynamics of the binding of Ldb1 complexes during erythroid differentiation, Genes. Dev. 24 (2010) 277–289.
[4] E.H. Bresnick, H.Y. Lee, T. Fujiwara, et al., GATA switches as developmental drivers, J. Biol. Chem. 285 (2010) 31087–31093.
[5] R. Lahlil, E. Lécuyer, S. Herblot, et al., SCL assembles a multifactorial complex that determines glycophorin A expression, Mol. Cell. Biol. 24 (2004) 1439–1452.
[6] I.A. Wadman, H. Osada, G.G. Grütz, et al., The LIM-only protein Lmo2 is a bridging molecule assembling an erythroid, DNA-binding complex which includes the TAL1, E47, GATA-1 and Ldb1/NLI proteins, EMBO. J. 16 (1997) 3145–3157.
[7] T. Fujiwara, Y.W. Alqadi, Y. Okitsu, et al., Role of transcriptional corepressor ETO2 in erythroid cells, Exp. Hematol. 41 (2013) 303–315.
[8] T. Fujiwara, H.Y. Lee, R. Sanalkumar, et al., Building multifunctionality into a complex containing master regulators of hematopoiesis, Proc. Natl. Acad. Sci. U. S. A. 107 (2010) 20429–20434.
[9] A.P. Tsang, J.E. Visvader, C.A. Turner, et al., FOG, a multitype zinc finger protein, acts as a cofactor for transcription factor GATA-1 in erythroid and megakaryocytic differentiation, Cell 90 (1997) 109–119.
[10] S. Pal, A.B. Cantor, K.D. Johnson, et al., Coregulator-dependent facilitation of chromatin occupancy by GATA-1, Proc. Natl. Acad. Sci. U. S. A. 101 (2004) 980–985.
[11] H.Y. Lee, K.D. Johnson, T. Fujiwara, et al., Controlling hematopoiesis through sumoylation-dependent regulation of a GATA factor, Mol. Cell. 36 (2009) 984–995.
[12] S.I. Kim, E.H. Bresnick, Transcriptional control of erythropoiesis: emerging mechanisms and principles, Oncogene 26 (2007) 6777–6794.
[13] Y. Saito, T. Fujiwara, K. Ohashi, et al., High-throughput siRNA screening to reveal GATA-2 upstream transcriptional mechanisms in hematopoietic cells, PLoS One 10 (2015) e0137079.
[14] F.Y. Tsai, G. Keller, F.C. Kuo, et al., An early haematopoietic defect in mice lacking the transcription factor GATA-2, Nature 371 (1994) 221–226.
[15] E.W. Scott, M.C. Simon, J. Anastasi, et al., Requirement of transcription factor PU.1 in the development of multiple hematopoietic lineages, Science 265 (1994) 1573–1577.
[16] T. Tripic, W. Deng, Y. Cheng, et al., SCL and associated proteins distinguish active from repressive GATA transcription factor complexes, Blood 113 (2009) 2191–2201.
[17] A. Miccio, Y. Wang, W. Hong, et al., NuRD mediates activating and repressive functions of GATA-1 and FOG-1 during blood development, EMBO. J. 29 (2010) 442–456.
[18] T. Evans, M. Reitman, G. Felsenfeld, An erythrocyte-specific DNA-binding factor recognizes a regulatory sequence common to all chicken globin genes, Proc. Natl. Acad. Sci. U. S. A. 85 (1988) 5976–5980.
[19] M. Merika, S.H. Orkin, DNA-binding specificity of GATA family transcription factors, Mol. Cell. Biol. 13 (1993) 3999–4010.
[20] A.P. Hsu, K.D. Johnson, E.L. Falcone, et al., GATA2 haploinsufficiency caused by mutations in a conserved intronic element leads to MonoMAC syndrome, Blood 121 (2013) 3830–3837.
[21] S.A. Denslow, P.A. Wade, The human Mi-2/NuRD complex and gene regulation, Oncogene 26 (2007) 5433–5438.
[22] H.F. Allen, P.A. Wade, T.G. Kutateladze, The NuRD architecture, Cell. Mol. Life. Sci. 70 (2013) 3513–3524.
[23] G. Nishibuchi, Y. Shibata, T. Hayakawa, et al., Physical and functional interactions between the histone H3K4 demethylase KDM5A and the nucleosome remodeling and deacetylase (NuRD) complex, J. Biol. Chem. 289 (2014) 28956–28970.
[24] M.A. Kerenyi, S.H. Orkin, Networking erythropoiesis, J. Exp. Med. 207 (2010) 2537–2541.
[25] J.M. Amann, J. Nip, D.K. Strom, et al., ETO, a target of t(8;21) in acute leukemia, makes distinct contacts with multiple histone deacetylases and binds mSin3A through its oligomerization domain, Mol. Cell. Biol. 21 (2001) 6470–6483.
[26] P.S. Hoppe, M. Schwarzfischer, D. Loeffler, et al., Early myeloid lineage choice is not initiated by random PU.1 to GATA1 protein ratios, Nature 535 (2016) 299–302.
[27] E. Mancini, A. Sanjuan-Pla, L. Luciani, et al., FOG-1 and GATA-1 act sequentially to specify definitive megakaryocytic and erythroid progenitors, EMBO. J. 31 (2012) 351–365.
[28] K.E. Nichols, J.D. Crispino, M. Poncz, et al., Familial dyserythropoietic anaemia and thrombocytopenia due to an inherited mutation in GATA1, Nat. Genet. 24 (2000) 266–270.
[29] K. Freson, K. Devriendt, G. Matthijs, et al., Platelet characteristics in patients with X-linked macrothrombocytopenia because of a novel GATA1 mutation, Blood 98 (2001) 85–92.