Dok2 Likely Down-regulates *Klf1* in Mouse Erythroleukemia Cells

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Abstract. *Background/Aim: Docking protein 2 (Dok2) is an adapter protein which is involved in hematopoiesis. However, it still remains unclear how Dok2 functions in regulation of transcription of hematopoietic genes. To address this issue, we knocked-down Dok2 mRNA in mouse erythroleukemia cells which highly express Dok2 intrinsically. Materials and Methods: Mouse erythroleukemia cells were transfected with Dok2 siRNA for 24 h and gene expression of erythroid differentiation-related genes, such as GATA binding protein 1 (Gata1), Krüppel-like factor 1 (Klf1), α-globin and β-globin were assessed by real-time polymerase chain reaction. Results: Among the tested genes, expression of Klf1 exhibited a 1.94-fold increase when compared to the control 24 h after transfection. Immunocytochemistry and chromatin immunoprecipitation assays revealed that Dok2 protein localizes in the nucleus and binds to the promoter region of Klf1 gene. Conclusion: Dok2 is able to control Klf1 expression by transcriptional regulation through directly binding to its promoter region.*

Hematopoiesis is a process which produces mature functional blood cells that maintain homeostasis. During hematopoiesis, hematopoietic stem cells are generated from the mesoderm, and differentiate into progenitor cells, maturing into erythrocytes, granulocytes, macrophages, lymphocytes and platelets (1). This process is strictly regulated by intrinsic and extrinsic molecules and an imbalance in this regulation can give rise to various hematological diseases, such as leukemia (2, 3). Extrinsic molecules include cytokines, chemokines and extracellular matrix proteins which are derived from hematopoietic cell controlling niches (4). Previously, we demonstrated that Delta-like 1 homolog (Dlk1)-expressing hepatoblasts function as niche cells for hematopoietic stem cells by regulating homing and cell differentiation through secretion of extracellular matrix proteins and cytokines (5). In addition to these molecules, hematopoietic cells have also been reported to be regulated intrinsically by transcription factors (1, 6). GATA binding protein 1 (Gata1) and spleen focus-forming virus proviral integration oncogene (Spi1, also known as Pu.1) are well-known transcription factors which regulate the fate of hematopoietic cells. Gata1 is a key transcription factor in erythropoiesis and regulates transcription of Krüpper-like factor 1 (Klf1) and activation of β-globin transcription (7). Ectopic expression of Gata1 in myleomonocytic cells can induce them to differentiate into eosinophil, erythroid, and megakaryocytic cells (8-10). On the other hand, Pu.1 regulates the fate of myeloid cells and is involved in granulocyte, monocyte and lymphocyte development (8, 11). In erythroid cells, Pu.1 acts as an inhibitor of erythroid differentiation by antagonizing Gata1 (1, 12). Thus, proper expression of transcription factors is important to maintain homeostasis of hematopoiesis, and abnormal functioning of these factors are known causes of leukemogenesis (13).

Docking protein 2 (Dok2) (also known as DokR, p56 and Frip), a member of the Dok protein family, is an adapter protein that is a substrate for tyrosine kinase. Dok1, -2 and -3 are selectively expressed in hematopoietic cells among the seven Dok protein members (14-17). Two groups have reported that double knock-out of *Dok1* and *Dok2* in mice induces the abnormal proliferation of myeloid cells due to an increase of proliferation and reduced apoptosis, and is associated with Rat sarcoma virus oncogene (Ras)/mitogen-

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Key Words: Mouse erythroleukemia cells, docking protein 2, Dok2, KIf1, erythropoiesis.

activated protein kinase (Mapk) and Thymoma viral proto-condogene (Akt) activation (18, 19). The role of Dok2 in myeloid leukemia has also been investigated and it was found to act as a suppressor of leukemia (15, 19). Although the function of Dok2 as a member of tyrosine kinase signaling molecules and its roles in regulating myelopoiesis have been extensively investigated, it still remains unclear how Dok2 functions in hematopoietic transcriptional regulation. To address this, we knocked-down *Dok2* gene in mouse erythroleukemia (MEL) cell line, which is a kind of leukemia cell line with the potential to be induced into erythroid cells and is widely used to study erythropoiesis. Herein we present evidence that Dok2 regulates the expression of *Klf1* through directly binding to its promoter region and demonstrated a novel role of Do2 as a mediator of gene transcription in hematopoiesis.

Materials and Methods

Cell lines. Two types of cell lines, MEL cell line (kindly provided by Dr. Brand, Ottawa Health Research Institute, Ottawa, Canada) and Friend erythroleukemia cell line (F5-5. Fl, RIKEN BioResource Center, Ibaraki, Japan) were used in this study. The cells were maintained in RPMI-1640 (Wako Pure Chemical Industries, Osaka, Japan) supplemented with 10% fetal bovine serum (FBS) and 10 U/ml penicillin and 10 mg/ml streptomycin (Sigma-Aldrich, Saint Louis, MO, USA). Cells were passaged every 3-4 days.

siRNA transfection. Knock-down of Dok2 mRNA in MEL cells was performed using siRNAs (Sigma- Aldrich) with transfection reagent, Lipofectamine® 2000 (Life Technologies, Palo Alto, CA, USA). Silencer® Negative Control No.1 siRNA (Ambion, Austin, TX, USA) was used as a control siRNA. Before the transfection, 1.0×10^5 cells were plated in 24-well plate in RPMI-1640 containing 1% FBS. Two microliters of Lipofectamine and 60 pmole of single siRNAs were mixed with 50 μl of Opti-MEM®I Reduced Serum Medium (Life Technologies), respectively, and incubated at room temperature for 20 minutes to form Lipofectamine–siRNA complex. After incubation, Lipofectamine–siRNA complexes were mixed with the cells and incubated at 37oC, 5% CO_2. Cells were collected at 24 hours after transfection and kept in RNAlater (Life Technologies) until gene expression analysis.

May-Grünwald Giemsa staining. Cultured cells were attached onto glass slides (Matsunami glass, Kishiwada, Osaka, Japan) by CytoSpin4 (Thermo Fisher scientific, Waltham, MA, USA) at 450 rpm for 7 min and dried thoroughly. Cells were fixed and stained with May-Grünwald reagent (Muto Pure Chemicals, Tokyo, Japan) at room temperature for 5 min, briefly washed with tap water and incubated with PBS, pH 6.4, for 2 min. Cells were then incubated with 1:18 diluted Giemsa solution (Muto Pure Chemicals) at room temperature for 30 min. After washing with tap water, the slides were dried and were covered with glass coverslips by using MGK-S mounting solution (Matsunami glass). Slides were observed using an Olympus CKX41 microscope (Olympus, Tokyo, Japan).

Immunocytochemistry. Cultured cells were attached onto glass slides (Matsunami glass) by CytoSpin4 (Thermo Fisher scientific) at 450 rpm for 7 min and dried thoroughly. Cells were fixed in 1% paraformaldehyde at 4˚C for 30 min. After washing with PBS, cells were incubated with PBS containing 0.05% Triton-X 100 at room temperature for 15 min. After three washes with PBS, the cells were blocked with PBS containing 1% BSA at room temperature for 30 min and incubated overnight at 4˚C with goat anti-mouse Dok2 (1:100, Santa Cruz Biotechnology, Dallas, TX, USA) primary antibody. After three washes with PBS, cells were then incubated with donkey anti-goat IgG AlexaFluor488 (1:400, Invitrogen, Carlsbad, CA, USA) and TOTO-3 iodide (1:1500; Invitrogen) at room temperature for 30 min. After a further three washes with PBS, cells were mounted on coverslips with fluorescent mounting medium (Dako Corporation, Glostrup, Denmark) and assessed using a Fluo View 1000 confocal microscope (Olympus).

Flow cytometric analysis. Cells were stained with 1 μg/ml of propidium iodide (PI) (Invitrogen) to distinguish the dead cells and analyzed by BD FACS Aria (BD Bioscience, San Jose, CA, USA).

RNA extraction and real-time polymerase chain reaction (PCR). Total RNA was extracted from cultured cells using RiboPure™ Kit (Ambion), and mRNA was reverse transcribed into cDNA using a High-Capacity RNA-to-cDNA Kit (Life Technologies). Expressions of *Dok2, Gata1, Klf1, α-globin, β-globin* and *β-actin* were assessed by using StepOnePlus™ real-time PCR (Life Technologies) with TaqMan® Gene Expression Assays (Life Technologies). mRNA levels were normalized to that of β-actin and relative expression of each gene calculated using a relative standard curve method.

Chromatin immunprecipitation (ChIP) assay. ChIP assay was performed using a Chromatin Immunoprecipitation Assay Kit (Millipore, Bedford, MA, USA) according to the manufacturer's protocol. For ChIP assay, 1×10^6 cells were used. Proteins and genomic DNA were cross-linked by formaldehyde and then sheared by sonication. Immunoprecipitation was carried out overnight with 2 μg of goat anti-mouse Dok2 antibody (Santa Cruz Biotechnology). The amount of *Klf1* and *β-globin* promoter in total input DNA and immunoprecipitated DNA were measured by StepOnePlus™ real-time PCR (Life Technologies) with Fast SYBR® Green Master Mix (Life Technologies). Promoter regions of *Klf1* and *β-globin* gene were identified by the Database of Transcriptional Start Sites Release 8.0 (http://dbtss.hgc.jp/) and were located between 500-1000 bp at 5'-end of transcriptional start sites. Primer sets binding to the promoter regions were designed by Primer Express version 3.0 software (Life Technologies). The sequences of primers were: *Klf1* promoter-specific primers, forward: 5'-TCTGCTCAAGGAG GAACAGAGCTA-3', reverse: 5'-GGCTCCCTTTCAGGCATTA TCAGA-3'; and *β-globin* promoter-specific primers, forward: 5'-GACAAACATTATTCAGAGGGAGTA-3', reverse: 5'-AAGCAA ATGTGAGGAGCAACTGAT-3'.

Statistical analysis. Results are expressed as the mean±SD. Paired samples were compared using Student's *t*-test.

Results

Expression of Dok2 in mouse erythroleukemia cell lines. Gene and protein expression of Dok2 were examined by real-time PCR and immunocytochemistry. Figure 1A shows the relative expression of *Dok2* mRNA in the two types of

erythroleukemia cell lines, F5-5. fl and MEL. *Dok2* was expressed in both cell lines and MEL exhibited 1.37±0.095-fold (p=0.027) higher expression when compared to F5-5. fl. Based on the higher expression of *Dok2* mRNA in MEL cells, we further analyzed the protein expression of Dok2 in MEL cells by immunocytochemistry. We observed that Dok2 (green) is localized in both the cytoplasm and nucleus of MEL cells. There were cells weakly-expressing Dok2 and not all cells expressed Dok2. Greater intensity was detected in cytoplasm compared to in nucleus (Figure 1B).

Loss-of-function of Dok2 in MEL cells. To investigate the function of Dok2 in MEL cells, *Dok2* mRNA was knocked-down by transfection with siRNA for 24 h. Cell morphology after siRNA transfection was observed under microscopy and also assessed by May-Grünwald Giemsa staining. Most of the cells were round and uniform in size, and some cells were swollen and contained vacuoles in their cytoplasm, both in control and *Dok2* siRNA-transfected cells (Figure 2A). May-Grünwald Giemsa staining also showed that vacuoles were present in the cytoplasm (Figure 2B). No obvious morphological differences were observed between control siRNA and Dok2 siRNA-transfected cells. Efficiency of *Dok2* knock-down was assessed by real-time PCR and 61% reduction of *Dok2* mRNA were achieved compared to the control cells (Figure 2C). The effects of *Dok2* knock-down on cell proliferation and cell viability were assessed by flow cytometry. Analysis showed that the live cell numbers were 46854±13719 cells in the control sample and 49828±5734 cells in *Dok2* siRNA-transfected cells (Figure 2D). The cell viability was calculated as percentage based on the number of live PI-negative cells among the total collected cells and was 70.1±5.69% and 66.6±1.73% in control siRNA and Dok2 siRNA transfected cells, respectively (Figure 2E). There were no significant differences of cell number (p=0.76) and viability (p=0.37) between the control siRNA-and *Dok2* siRNA-transfected cells. In summary, *Dok2* knock-down did not affect the proliferation and viability of MEL cells.

Dok2 knock-down induces the expression of erythropoietic transcription factor Klf1. MEL cell line was derived from leukemia cells and its differentiation state is equivalent to that of erythroid progenitors (20). Because of their potential to follow erythroid differentiation, they have been utilized to investigate erythropoiesis. To investigate whether Dok2 is involved in erythroid differentiation, the expression of differentiation-related genes, *Gata1, Klf1, α-globin* and *β-globin* were assessed by real-time PCR. Slight decrease of *Gata1* mRNA (0.85±0.044-fold, p=0.052) and *β-globin* mRNA (0.91±0.065-fold, p=0.15) expressions were observed after knock-down of *Dok2*. *α-Globin* mRNA expression was significantly down-regulated after Dok2 siRNA transfection and 0.76±0.064-fold (p=0.0050) lower than control siRNA-transfected cells. Among the genes we investigated, only *Klf1* was significantly up-regulated in MEL cells transfected with *Dok2* siRNA when compared to control siRNA (1.94±fold, p=0.0023) (Figure 3A).

Dok2 binds to the promoter region of Klf1. Since the knock-down of *Dok2* caused significant up-regulation of *Klf1* mRNA, we further investigated whether Dok2 directly regulates the expression of *Klf1*. By performing ChIP assay, we examined the binding of Dok2 to the promoter region of *Klf1* and *β-globin*. Figure 4 shows that the expression of Klf1 promoter was enriched 2.44±0.96-fold (p=0.048) in the sample immunoprecipitated with an antibody against mouse Dok2 when compared to the control sample without antibody. On the other hand, expression of *β-globin* promoter was not enriched and was shown to be 0.0016±0.0011-fold ($p=9.4\times10^{-13}$) lower when compared to the control sample without antibody. Thus, we found that Dok2 indeed binds to the promoter region of *Klf1*, but not of *β-globin*.

Discussion

Dok2 has been reported to be localized in the cytoplasm and also the membrane of human embryonic kidney 293T (HEK293T) cell line through its Pleckstrin-homology (PH) and Phosphotyrosine binding (PTB) domains (21). Cytoplasmic Dok2 is phosphorylated under the activated endothelial-specific receptor tyrosine kinase (Tek, also known as Tie2) and further activates Ras GTPase-activating proteins and the adapter protein Non-catalytic region of tyrosine kinase (Nck), which are involved in cell motility (22). Thus, cytoplasmic Dok2 is a molecule of tyrosine kinase signaling. Dok2 comprises a nuclear export sequence, which regulates the translocation of proteins from the nucleus to the cytoplasm, implying the existence of Dok2 in both compartments (23). However, nuclear Dok2 expression and its function have not previously been reported. Herein we showed that Dok2 protein is localized in both the nucleus and cytoplasm of MEL cells by immunocytochemistry (Figure 1B). This implies that nuclear Dok2 regulates gene expression in MEL cells.

Concerning hematopoietic cells, Dok2 is primarily expressed in hematopoietic precursors (15) and is also highly expressed in T-cells of the spleen and thymus, in addition to myeloid cells in bone marrow (24). Double knock-out of *Dok1* and *Dok2* in mice induces the abnormal proliferation of myeloid cells, characterized by an increased percentage of immature granulocytic/monocytic precursors in the spleen and bone marrow, and also leads to hyperplasia of megakaryocytes and myeloid progenitors in bone marrow (18, 19). These changes were accompanied by an increases in Ras/Mapk and Akt activation, which leads to activation of

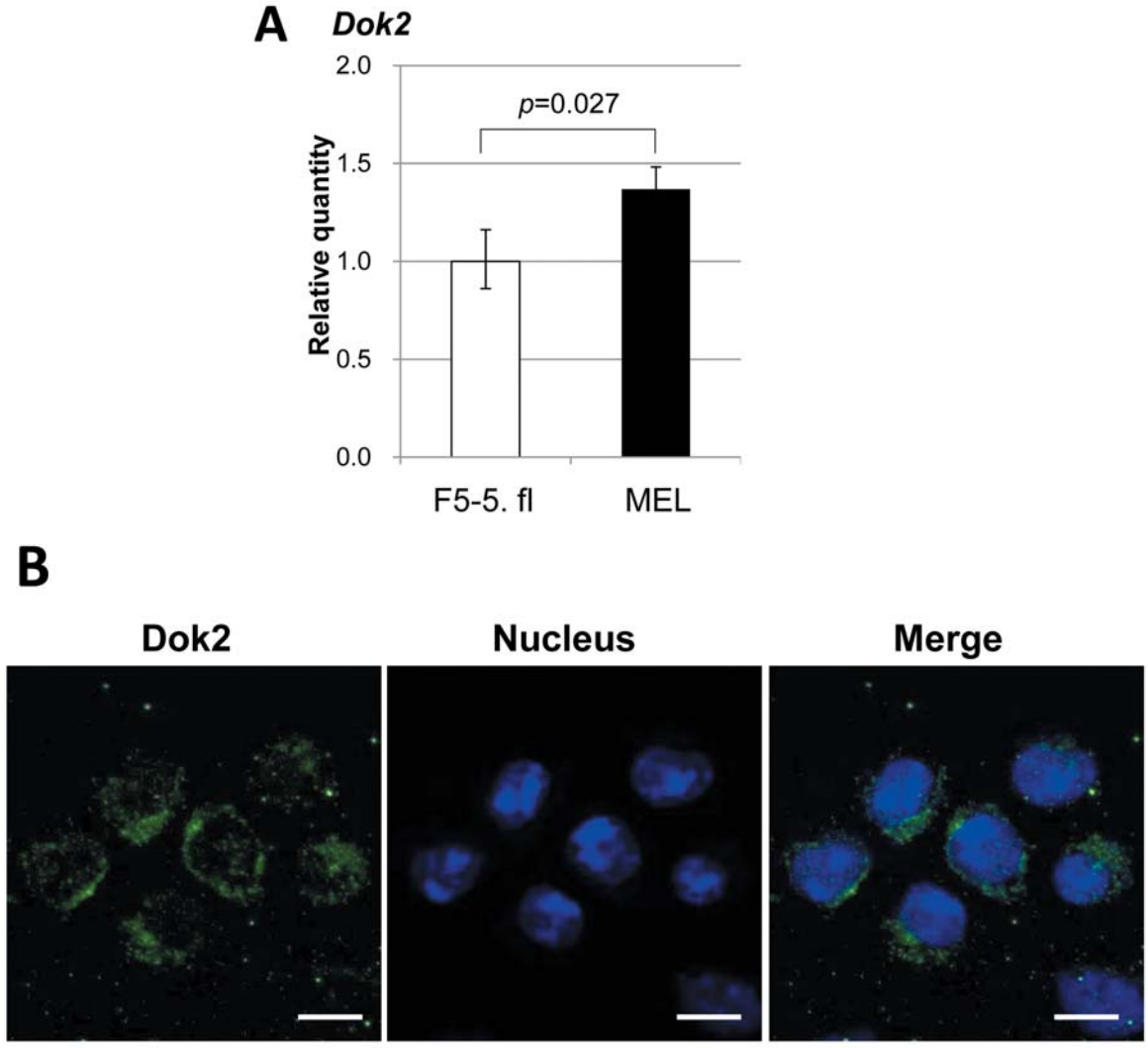

Figure 1. *Expression of Docking protein 2 (Dok2) in MEL cells. A: Relative expression of Dok2 mRNA in F5-5. fl cells and MEL cells examined by real-time polymerase chain reaction. Expression of Dok2 in MEL cells was 1.37±0.095-fold (p=0.027, Student's t-test) higher when compared to F5-5. fl cells. B: Immunocytochemical image of Dok2 protein in MEL cells. Cells were stained with goat antibody to mouse Dok2 (green) and TOTO-3 iodide (blue) for nuclear staining. Dok2 protein was localized in both cytoplasm and nucleus of MEL cells. Scale bar=10 μm.*

anti-apoptotic genes and increases survival of hematopoietic cells (25). Although Dok2 in tyrosine kinase signaling and its role in regulating myelopoiesis have been extensively investigated, the function of Dok2 in transcriptional regulation and its role in erythropoiesis has not been fully investigated. The MEL cell line is a tool widely used in the study of erythropoiesis. Based on gene and protein expression of Dok2 in MEL cells (Figure 1A and 1B), we further investigated the function of Dok2 by using siRNA transfection. Inhibition of *Dok2* expression reportedly accelerates cell proliferation of macrophage-like J774A.1 cells through affecting Rous sarcoma oncogene (Src) family kinase which mediates the induction of a cell proliferation-related gene known as Myelocytomatosis oncogene (*Myc*) (26). However, there was no significant difference in proliferation of MEL cells between control and *Dok2* siRNA-transfected cells (Figure 2D), unlike the previous report. On the other hand, we found a significant up-regulation of *Klf1* mRNA after knock-down of *Dok2* (Figure 3). Klf1 is a transcription factor which is a direct target of Gata1 and plays important roles in erythropoiesis. Three major aspects of Klf1 function have been reported: regulation of erythroid-lineage commitment, switching between γ- and β-globin, and maturation of erythrocytes during terminal

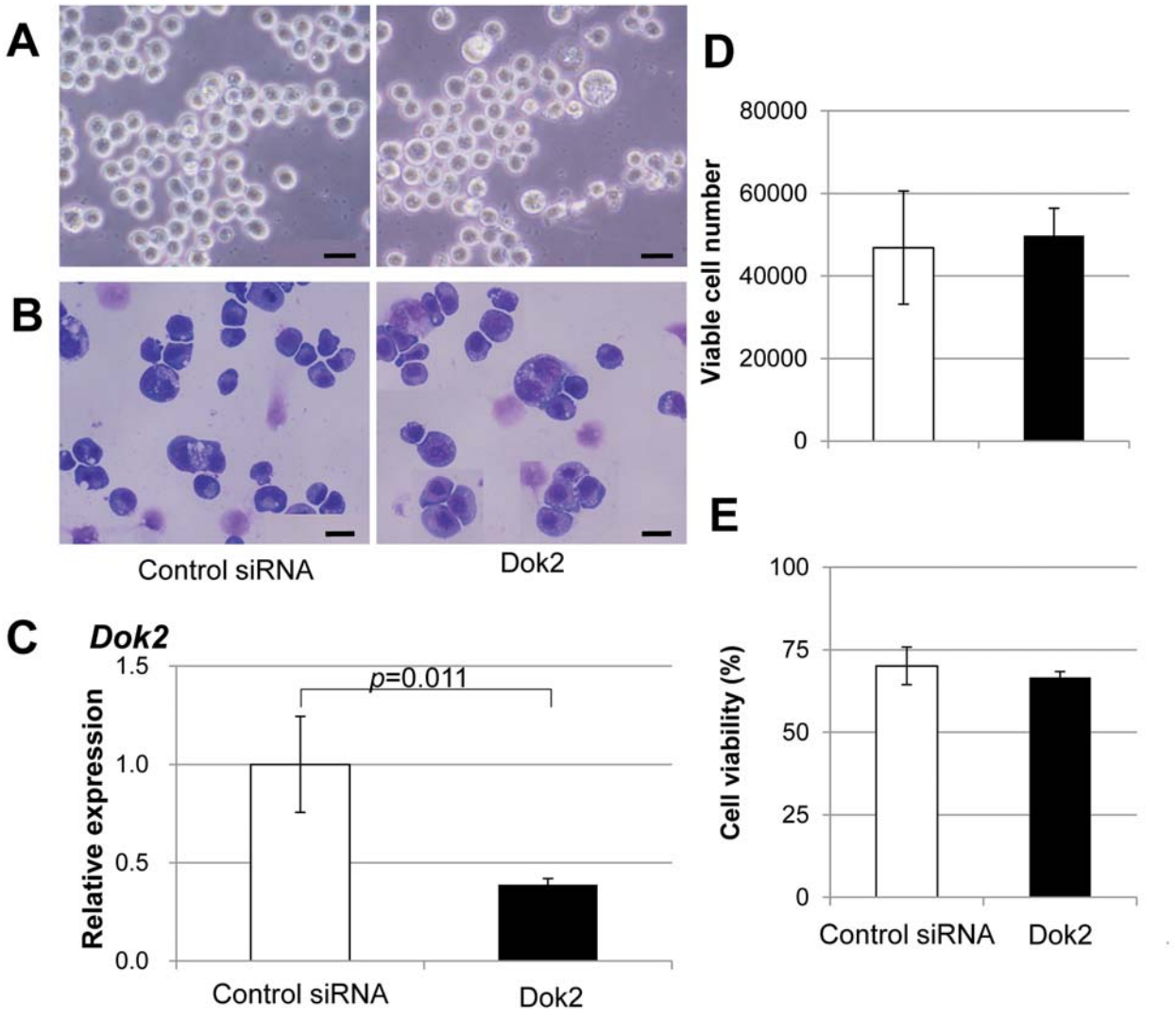

Figure 2. *Loss-of-function of Docking protein 2 (Dok2) in MEL cells. A: Microscopic images of MEL cells cultured with control siRNA (left) and Dok2 siRNA (right) for 24 h. Scale bar=20 μm. B: May-Grünwald Giemsa staining images of MEL cells cultured with control siRNA (left) and Dok2 siRNA (right) for 24 h. Scale bar=20 μm. C: Relative expression of Dok2 mRNA after 24 h of siRNA transfection. Live cell numbers (D) and viability (E) of MEL cells after 24 h of siRNA transfection.*

erythropoiesis (27). The data we presented show that Dok2 could potentially bind to the promoter region of *Klf1*, and that knock-down of *Dok2* altered *Klf1* expression. This suggests that Dok2 transcriptionally down-regulates *Klf1* in MEL cells.

Herein we provide evidence that Dok2 is able to transcriptionally regulate gene expressions in addition to its functions in downstream signaling of tyrosine kinase, and the change of *Klf1* expression after *Dok2* knock-down suggests the possibility that Dok2 is involved in regulation of erythropoiesis and not just myelopoiesis. Although the functions of nuclear Dok2 have not been elucidated, our observation suggests the novel transcription factor-like function of Dok2, and it also suggests that Dok2 is involved in the erythropoietic transcriptional system and in the maintenance of proper erythropoiesis.

Acknowledgements

We thank Ms. Chiyoko Nakamichi and Naoko Kojima for technical support, and the Ministry of Education, Culture, Sports, Science and Technology, the Ministry of Health, Labour and Welfare, and Japan Society for the Promotion of Science (JSPS) bilateral program for grant support. We thank the Mr. Anthony Swain for critical reading of this manuscript.

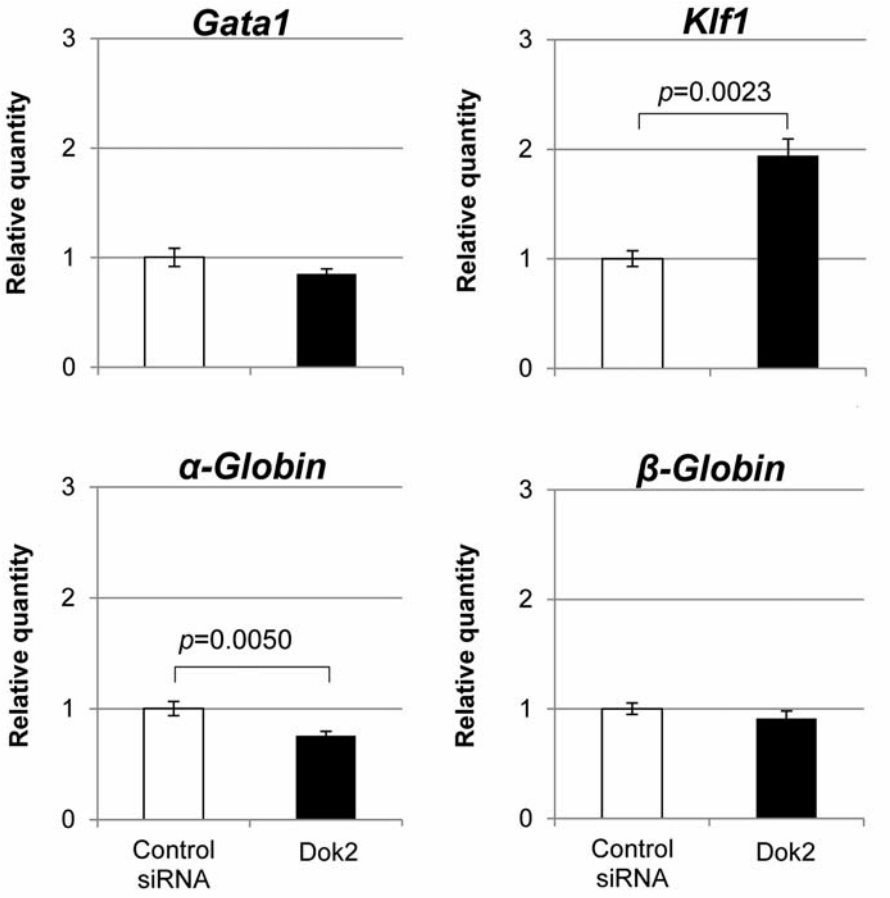

Figure 3. *Gene expression analysis of erythroid differentiation-related genes. Relative expressions of erythroid differentiation-related genes, GATA binding protein 1 (Gata1), Krüppel-like factor 1 (Klf1), α-globin and β-globin after 24 hours of siRNA transfection. mRNA levels were normalized to that of β-actin and relative expression of each gene was calculated using a relative standard curve method. Significant differences were calculated with the Student's t-test.*

References

1 Jagannathan-Bogdan M and Zon LI: Hematopoiesis. Development *140*: 2463-2467, 2013.

2 Iwasaki H: Roles of transcription factors in normal and leukemic hematopoiesis. Rinsho Ketsueki *54*: 1566-1572, 2013.

3 Tsunoda T and Shirasawa S: Roles of ZFAT in haematopoiesis, angiogenesis and cancer development. Anticancer Res *33*: 2833-2837, 2013.

4 Sugiyama D, Inoue-Yokoo T, Fraser ST, Kulkeaw K, Mizuochi C and Horio Y: Embryonic regulation of the mouse hematopoietic niche. Sci World J *11*: 1770-1780, 2011.

5 Sugiyama D, Kulkeaw K and Mizuochi C: TGF-beta-1 up-regulates extracellular matrix production in mouse hepatoblasts. Mech Dev *130*: 195-206, 2013.

6 Steinleitner K, Rampetsreiter P, Koffel R, Ramanathan G, Mannhalter C, Strobl H and Wieser R: EVI1 and MDS1/EVI1 expression during primary human hematopoietic progenitor cell differentiation into various myeloid lineages. Anticancer Res *32*: 4883-4889, 2012.

7 Dore LC and Crispino JD: Transcription factor networks in erythroid cell and megakaryocyte development. Blood *118*: 231-239, 2011.

8 Cantor AB and Orkin SH: Transcriptional regulation of erythropoiesis: an affair involving multiple partners. Oncogene *21*: 3368-3376, 2002.

9 Kulessa H, Frampton J and Graf T: GATA-1 reprograms avian myelomonocytic cell lines into eosinophils, thromboblasts and erythroblasts. Genes Dev *9*: 1250-1262, 1995.

10 Visvader JE, Elefanty AG, Strasser A and Adams JM: GATA-1 but not SCL induces megakaryocytic differentiation in an early myeloid line. EMBO J *11*: 4557-4564, 1992.

11 Scott EW, Simon MC, Anastasi J and Singh H: Requirement of transcription factor PU.1 in the development of multiple hematopoietic lineages. Science *265*: 1573-1577, 1994.

12 Voso MT, Burn TC, Wulf G, Lim B, Leone G and Tenen DG: Inhibition of hematopoiesis by competitive binding of transcription factor PU.1. Proc Natl Acad Sci USA *91*: 7932-7936, 1994.

13 Shima Y and Kitabayashi I: Deregulated transcription factors in leukemia. Int J Hematol *94*: 134-141, 2011.

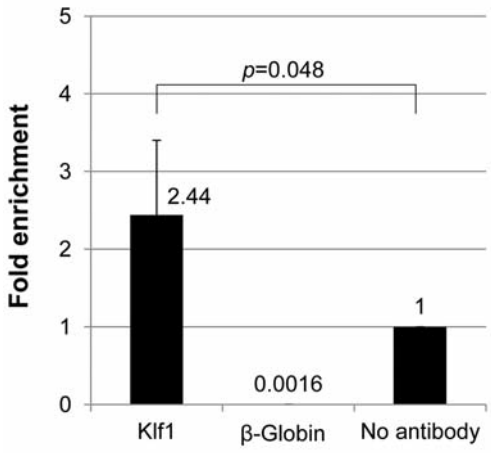

Figure 4. *Chromatin immunoprecipitation (ChIP) assay of Docking protein 2 (Dok2). The amount of Krüppel-like protein 1 (Klf1) and β-globin promoter in total input DNA and immunoprecipitated DNA were measured by real-time polymerase chain reaction. The expression of Klf1 promoter was enriched by 2.44±0.96-fold in the sample immunoprecipitated with antibody to murine Dok2 when compared to the control sample without antibody. Significant differences were calculated with the Student's t-test.*

14 Cong F, Yuan B and Goff SP: Characterization of a novel member of the DOK family that binds and modulates ABL signaling. Mol Cell Biol *19*: 8314-8325, 1999.
15 Di Cristofano A, Carpino N, Dunant N, Friedland G, Kobayashi R, Strife A, Wisniewski D, Clarkson B, Pandolfi PP and Resh MD: Molecular cloning and characterization of p56dok-2 defines a new family of RasGAP-binding proteins. J Biol Chem *273*: 4827-4830, 1998.
16 Jones N and Dumont DJ: The Tek/Tie2 receptor signals through a novel Dok-related docking protein, Dok-R. Oncogene *17*: 1097-1108, 1998.
17 Nelms K, Snow AL, Hu-Li J and Paul WE: FRIP, a hematopoietic cell-specific rasGAP-interacting protein phosphorylated in response to cytokine stimulation. Immunity *9*: 13-24, 1998.
18 Yasuda T, Shirakata M, Iwama A, Ishii A, Ebihara Y, Osawa M, Honda K, Shinohara H, Sudo K, Tsuji K, Nakauchi H, Iwakura Y, Hirai H, Oda H, Yamamoto T and Yamanashi Y: Role of Dok-1 and Dok-2 in myeloid homeostasis and suppression of leukemia. J Exp Med *200*: 1681-1687, 2004.
19 Niki M, Di Cristofano A, Zhao M, Honda H, Hirai H, Van Aelst L, Cordon-Cardo C and Pandolfi PP: Role of Dok-1 and Dok-2 in leukemia suppression. J Exp Med *200*: 1689-1695, 2004.
20 Lachman HM: c-myc protooncogene expression in mouse erythroleukemia cells. Environ Health Perspect *80*: 161-172, 1989.
21 Jones N, Chen SH, Sturk C, Master Z, Tran J, Kerbel RS and Dumont DJ: A unique autophosphorylation site on Tie2/Tek mediates Dok-R phosphotyrosine binding domain binding and function. Mol Cell Biol *23*: 2658-2668, 2003.
22 Kulkarni SV, Gish G, van der Geer P, Henkemeyer M and Pawson T: Role of p120 Ras-GAP in directed cell movement. J Cell Biol 149: 457-470, 2000.
23 Niu Y, Roy F, Saltel F andrieu-Soler C, Dong W, Chantegrel AL, Accardi R, Thepot A, Foiselle N, Tommasino M, Jurdic P and Sylla BS: A nuclear export signal and phosphorylation regulate Dok1 subcellular localization and functions. Mol Cell Biol *26*: 4288-4301, 2006.
24 Yasuda T, Bundo K, Hino A, Honda K, Inoue A, Shirakata M, Osawa M, Tamura T, Nariuchi H, Oda H, Yamamoto T and Yamanashi Y: Dok-1 and Dok-2 are negative regulators of T-cell receptor signaling. Int Immunol *19*: 487-495, 2007.
25 Yen JJ and Yang-Yen HF: Transcription factors mediating interleukin-3 survival signals. Vitam Horm 74: 147-163, 2006.
26 Suzu S, Tanaka-Douzono M, Nomaguchi K, Yamada M, Hayasawa H, Kimura F and Motoyoshi K: p56(dok-2) as a cytokine-inducible inhibitor of cell proliferation and signal transduction. EMBO J *19*: 5114-5122, 2000.
27 Siatecka M and Bieker JJ: The multifunctional role of EKLF/KLF1 during erythropoiesis. Blood *118*: 2044-2054, 2011.

Received April 4, 2014
Revised June 9, 2014
Accepted June 10, 2014