GATA Transcription Factors Inhibit Cytokine-dependent Growth and Survival of a Hematopoietic Cell Line through the Inhibition of STAT3 Activity*

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Although GATA-1 and GATA-2 were shown to be essential for the development of hematopoietic cells by gene targeting experiments, they were also reported to inhibit the growth of hematopoietic cells. Therefore, in this study, we examined the effects of GATA-1 and GATA-2 on cytokine signals. A tamoxifen-inducible form of GATA-1 (GATA-1/ERT) showed a minor inhibitory effect on interleukin-3 (IL-3)-dependent growth of an IL-3-dependent cell line Ba/F3. On the other hand, it dramatically inhibited TPO-dependent growth and gp130-mediated growth/survival of Ba/F3. Similarly, an estradiol-inducible form of GATA-2 (GATA-2/ER) disrupted thrombopoietin (TPO)-dependent growth and gp130-mediated growth/survival of Ba/F3. As for this mechanism, we found that both GATA-1 and GATA-2 directly bound to STAT3 both in vitro and in vivo and inhibited its DNA-binding activity in gel shift assays and chromatin immunoprecipitation assays, whereas they hardly affected STAT5 activity. In addition, endogenous GATA-1 was found to interact with STAT3 in normal megakaryocytes, suggesting that GATA-1 may inhibit STAT3 activity in normal hematopoietic cells. Furthermore, we found that GATA-1 suppressed STAT3 activity through its N-zinc finger domain. Together, these results suggest that, besides the roles as transcription factors, GATA family proteins modulate cytokine signals through protein-protein interactions, thereby regulating the growth and survival of hematopoietic cells.

GATA family transcription factors are composed of six members (GATA-1–6) and play indispensable roles in the development of diverse cell types according to their unique tissue distribution (1). These factors bind to a consensus DNA sequence called a GATA motif (A/T)GATA(A/G) in the regulatory region of their target genes through two highly conserved zinc finger domains, both of which include the configuration Cys-X_{7-9}-Cys-X_{2-7}-Cys. The carboxyl (C)1 finger is absolutely required for the DNA binding, whereas the amino (N) finger stabilizes the DNA-binding and confers full specificity (2). Among GATA family members, at least three GATA factors (GATA-1, GATA-2, and GATA-3) are critically involved in different aspects of hematopoiesis.

GATA-1, the first cloned member of this family, is expressed at high levels in erythroid cells, mast cells, and megakaryocytes and at lower levels in hematopoietic stem/progenitor cells. GATA-1 is considered to be essential for definitive erythropoiesis, because GATA-1-null cells did not contribute to erythropoiesis in chimeric mice generated from embryonic stem cells lacking GATA-1 (3). In addition, in vitro experiments showed that GATA-1-null embryonic stem cells are unable to differentiate into mature erythroid cells due to apoptosis (4, 5). Furthermore, lineage-selective GATA-1 knockdown mice exhibited striking thrombocytopenia as well as severe anemia (6). These results imply that GATA-1 plays an essential role not only in erythropoiesis but also in megakaryopoiesis and thrombopoiesis. However, the increased proliferation of megakaryocytes was also observed in the spleen of lineage-selective GATA-1 knock-down mice (6). Similarly, heterozygous mutant mice chimeric for GATA-1 gene displayed marked splenomegaly due to the massive accumulation of pro-erythroblasts and megakaryocytes (7). Moreover, primary megakaryocytes prepared from GATA-1-deficient mice exhibited hyperproliferation in liquid cultures (8). These lines of evidence suggest that GATA-1 might suppress the growth of erythroid and megakaryocytic cells regardless of its critical role in their development.

GATA-2 is highly expressed in pluripotent hematopoietic stem cells and moderately in early stage of erythroid cells, mast

1 The abbreviations used are: C-finger, carboxyl finger; N-finger, amino finger; WT, wild-type; ER, estrogen receptor; ED, estradiol; IL-3, interleukin-3; GM-CSF, granulocyte-macrophage colony-stimulating factor; G-CSF, granulocyte colony-stimulating factor; TPO, thrombopoietin; STAT, signal transducers and activators of transcription; Jak, Janus tyrosine kinase; MAPK, mitogen-activated protein kinase; Ab, antibody; GST, glutathione S-transferase; 4-HT, tamoxifen; HA, hemagglutinin; del, deletion mutant.

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cells, and megakaryocytes (9). GATA-2 knock-out mice die around embryonic days 9.5–10.5 due to a pan-hematopoietic defect, providing evidence that GATA-2 is a key regulator of development, maintenance, and/or function of hematopoietic stem cells (10). Furthermore, the in vitro experiment using GATA-2−/− embryonic stem cells showed that GATA-2 was necessary for proliferation/survival of early hematopoietic cells (11). However, as was the case with GATA-1, it was also reported that retrovirus-transfected wild-type (WT) GATA-2 inhibited the growth of normal hematopoietic progenitor cells (12). Moreover, we found that an estrogen-inducible chimeric form of GATA-2, GATA-2 estrogen receptor (ER), suppressed cytokine-dependent growth of normal hematopoietic progenitor cells in response to estradiol (ED) (13, 14). Therefore, at present, the functional properties of GATA-1 and GATA-2 in the growth and survival of hematopoietic cells is controversial, and, of course, its mechanism remains unknown.

Hematopoiesis is regulated by a number of hematopoietic growth factors, including IL-3, IL-5, IL-6, GM-CSF, G-CSF, erythropoietin, TPO, stem cell factor, and fms-like tyrosine kinase 3 ligand. These growth factors exert their effects through activation of various intracellular signaling cascades such as Janus family of protein tyrosine kinases (JAKs)/signal transducers and activators of transcription (STATs), Ras/mito-gen-activated protein kinase (MAPK) and phosphatidylinositol 3-kinase/Akt pathways. Among these signaling pathways, the STAT family of transcription factors plays a pivotal role in the regulation of growth and survival of hematopoietic cells (15). For example, STAT3 regulates cell growth through the transcriptional regulation of cyclin D1 and c-myc (16, 17) and mediates the anti-apoptotic signal from gp130 via the induction of Bcl-2 (18). Also, STAT5 mediates cell growth through the induction of cyclin D1 and pim-1 (19, 20) and prevents apoptosis by inducing Bcl-XL and Bcl-2 (21, 22).

In an attempt to clarify the roles of GATA transcription factors in the growth and survival of hematopoietic cells, in this study, we examined their effects on cytokine signals. Here we found that GATA-1 and GATA-2 inhibit STAT3 activity through the direct protein-protein interactions, thereby inhibiting cytokine-dependent growth and survival of hematopoietic cells. These results suggest that, in addition to the roles as transcription factors, GATA transcription factors would exert various effects through protein-protein interactions.

MATERIALS AND METHODS

Reagents and Antibodies—The antibodies (Abs) against GATA-1 (N6) and STAT3 (H190) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA), the anti-HA Ab (12CA5) was from Roche Molecular Biochemicals (Indianapolis, IN), and an anti-FLAG Ab (M2) was from Sigma.

Plasmid Constructs and cDNAs—Expression vectors for GATA-1/ER and GATA-2/ER were described previously (13). Expression vectors for WT GATA-1, GATA-2, and GATA-3 were generated by subcloning these cDNAs into pcDNA3 (Invitrogen). Various types of GATA-1 mutants were constructed by the PCR method and subcloned into pcDNA3. Expression vectors encoding FLAG-tagged WT and constitutively active STAT3 (STAT3-C) were kindly supplied by Dr. J. E. Darnell, Jr. (Rockefeller University, New York, NY) (16). An expression vector for constitutively active STAT5A (16-STAT5A) was provided by Dr. T. Kitamura (University of Tokyo, Tokyo, Japan) (23). The cDNA of SOCS1, CIS, and OSM were provided by Dr. A. Yoshimura (Kyushu University, Fukuoka, Japan).

Cell Lines and Cultures—A murine IL-3-dependent cell line Ba/F3 was cultured in RPMI (Nakarai Tesq, Kyoto, Japan) supplemented with 10% fetal bovine serum (Flow, North Ryde, Australia) and 1 ng/ml IL-3. NIH3T3, 293T, and C2C12 cells were cultured in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum.

Preparation of Stable Transfectants from Ba/F3—We stably introduced various expression vectors into Ba/F3 by electroporation (250 V and 960 microfarads) (Bio-Rad). The transfected clones were selected by the culture with G418 (12 mg/ml), puromycin (1 μg/ml), hygromycin (0.5 mg/ml), and/or blasticidin S hydrochloride (30 μg/ml) according to the transfectant plasmid.

Flow Cytometry—DNA content of the cultured cells was quantitated by staining with propidium iodide and analyzed on FACSort (BD Biosciences) (24). Cell cycle analysis was performed with the program ModFit LT2.0 (BD Biosciences).

Northern Blot Analysis—The methods for the isolation of total cellular RNA and Northern blot were described previously (24).

Comunmunoprecipitation Analysis and Immunoblotting—293T cells were transfected with various expression vectors by the calcium phosphate coprecipitation method (25). After 12 h, the cells were washed and cultured for 24 h. The isolation of total cellular lysate, immunoprecipitation, gel electrophoresis, and immunoblotting were performed according to the procedures described previously (25). Immunoreactive proteins were visualized with the enhanced chemiluminescence detection system (PerkinElmer Life Sciences, Boston, MA).

Luciferase Assays—Luciferase assays were performed with a Dual-Luciferase Reporter System (Promega, Madison, WI) as previously described (19). In short, NIH3T3 and C2C12 cells (2 × 105 cells seeded in 60-mm dish) were transfected with the indicated effector genes along with 2 μg of a reporter gene for STAT3 (4 × APER-Luc), STAT5 (3 × β-Cas-Luc) (26), or GATA-1 (pGL3Map-Luc) (25), and 10 ng of pRL-TK, an expression vector for Renilla luciferase, by the calcium phosphate coprecipitation method. After 24 h, the cells were lyzed in lysis buffer supplied by the manufacturer, following the procedure of the firefly and Renilla luciferase activities on a luminometer LB96P (Berthold, Tokyo, Japan). The relative firefly luciferase activities were calculated by normalizing transfection efficiency according to the Renilla luciferase activities. The experiments were performed in triplicate, and similar results were obtained from at least three independent experiments.

Electrophoretic Mobility Shift Assays—The isolation of nuclear extract and electrophoretic mobility shift assay were performed according to the procedures described previously (27). The sequences of the probes were as follows: 5′-GGCTGATTTCCTCCTAGATGAGGACA′3′ for STAT3 (17); 5′-CTCGTGCCCTTCTTTGAAATTCGCCCA′3′ for STAT5 (19), recognition sequences are underlined.

Chromatin Immunoprecipitation Assays—Chromatin immunoprecipitation assays were performed with a chromatin immunoprecipitation assay kit (Upstate, Lake Placid, NY) according to the manufacture’s instructions. Briefly, cells (3 × 105 per sample) were fixed with 1% formaldehyde at 37°C for 10 min. After the isolation of nuclear extract, chromatin was sonicated to shear DNA to lengths between 200 and 1000 bp by the sonifier. STAT3-DNA binding complexes were immunoprecipitated with an anti-STAT3 Ab or control rabbit IgG. The immunoprecipitated DNA was eluted and subjected to the PCR reactions using AmpliTaq Gold (PerkinElmer Life Sciences), in the following thermocycling conditions: 94°C for 10 min, 35 cycles of 94°C for 30 s, 54°C for 30 s and 72°C for 30 s, followed by 72°C for 10 min. The sequences of the primer set in the JunB promoter are as follows: sense, 5′-TCGCGTTCTTCCAGGGGAC-3′; antisense, 5′-GGCTGCTTCTCTTGGGC-3′. PCR products were electrophoresed on agarose gels and visualized by Syber Green (Cambrex, East Rutherford, NJ) staining.

GST Pull-down Assays—The production and purification of GST-STAT3 fusion proteins were performed according to the procedures as previously reported (28). 5′-Labeled GATA-1 was prepared by a TNT reticulocyte lysate system (Promega). For each binding reaction, 20 μg of GST fusion protein bound to glutathione-Sepharose beads was incubated with 50 μg of TNFα reaction solution at 4°C for 1 h. The binding complexes were separated by gel electrophoresis and subjected to autoradiography.

Isolation of Murine Bone Marrow Lin− Cells and Their Culture with TPO—Murine bone marrow mononuclear cells were isolated by density gradient centrifugation, and Lin+ cells were purified by MACS (Milteny Biotech, Bergisch Gladbach, Germany) (14). The purified Lin− cells were cultured in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum and 30 ng/ml TPO for 6 days. After this culture, about 80% of the cultured cells showed polyoid morphology compatible with megakaryocytes and were positive for CD41 (data not shown).

RESULTS

Effects of GATA-1 on Cytokine-dependent Growth and Survival of Ba/F3—To examine the effects of GATA-1 on cytokine-dependent growth of hematopoietic cells in a ligand-inducible system, in this study, we utilized GATA-1/ERT, a chimera consisting of full-length GATA-1 and the mutated ligand-bind-
Effects of GATA Transcription Factors on STAT Activity—STATs have been reported to mediate cytokine-dependent growth and survival of hematopoietic cells (15, 17–19). Particularly, STAT3 was shown to be crucial for gp130L-dependent growth and survival of hematopoietic cells (15, 17–19). Partic-
ularly, STAT3 was shown to be crucial for gp130L-dependent growth and survival of hematopoietic cells (15, 17–19). Partici-
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GATA-1 Inhibits DNA-binding Activity of STAT3 but Not of STAT5—To clarify the mechanism through which GATA-1 and GATA-2 inhibit STAT3 activity, we examined the effects of GATA-1 and GATA-2 on gp130L-induced tyrosine phosphorylation of STAT3. However, neither 4-HT-activated GATA-1 nor ED-activated GATA-2 influenced gp130L-induced tyrosine phosphorylation of STAT3 in Ba/F3/gp130/G1ERT and Ba/F3/gp130/G2ER (data not shown). So, we next examined the effects of GATA-1 and GATA-2 on DNA-binding activity of STAT3 by electrophoretic mobility shift assay. After deprivation of gp130L for 12 h, we stimulated Ba/F3/gp130/G1ERT and Ba/F3/gp130/Mock with gp130L or IL-3 for 30 min with or without the 4-HT pretreatment. Without the pretreatment, the nuclear extract isolated after the stimulation with gp130L bound to the probe for STAT3 (Fig. 3A, upper left panel, lane 2). This complex was abolished by the WT competitor but not by the mutated (MT) competitor, and was supershifted by the anti-STAT3 Ab, indicating that this complex was formed from STAT3 (Fig. 3A, upper left panel, lanes 3–5). The 4-HT pretreatment drastically suppressed DNA-binding activity of STAT3 in Ba/F3/gp130/G1ERT (Fig. 3A, upper left panel, lane 2 versus lane 7), whereas the 4-HT pretreatment by itself showed no effect on its DNA binding in Ba/F3/gp130/Mock (Fig. 3A, lower left panel). In contrast, IL-3-induced DNA-binding activity of STAT5 was scarcely influenced by the 4-HT pretreatment in Ba/F3/gp130/G1ERT (Fig. 3A, upper right panel, lane 2 versus lane 7) and Ba/F3/gp130/Mock (Fig. 3A, lower right panel). Like the 4-HT pretreatment, the ED pretreatment severely inhibited gp130L-induced DNA-binding activity of STAT3 in Ba/F3/gp130/G1ERT (Fig. 3B, upper left panel), whereas the ED pretreatment by itself did not affect its DNA binding in Ba/F3/gp130/Mock (Fig. 3B, upper left panel). Also, the ED treatment hardly influenced IL-3-induced DNA-binding activity of STAT5 in Ba/F3/gp130/G2ER (Fig. 3B, upper right panel) and Ba/F3/gp130/Mock (Fig. 3B, lower right panel). Together, these results imply that both GATA-1 and GATA-2 would inhibit DNA-binding activity of STAT3 but not of STAT5.

To further investigate the effects of GATA-1 on DNA-binding activity of STAT3 in the chromatin structure, we performed the chromatin immunoprecipitation analysis on the STAT3-binding site in the 5′ region of Jun-B gene. After deprivation of gp130L for 12 h, Ba/F3/gp130/G1ERT and Ba/F3/gp130/Mock cells were stimulated with gp130L for 30 min with or without the 4-HT pretreatment. As shown in Fig. 3C, the STAT3-binding site was amplified by PCR on all of the sheared DNA samples (top panel, indicated as “Input”). Also, this region was amplified by PCR from the anti-STAT3 immunoprecipitate ob-
tained from 4-HT-untreated, gp130L-stimulated cells (middle panel, lane 2), indicating that STAT3 activated by gp130L bound to this element. However, 4-HT pretreatment suppressed this binding severely (middle panel, lane 4). In contrast, the 4-HT pretreatment by itself did not influence this binding in Ba/F3/gp130/Mock (middle panel, lane 6 versus lane 8). In agreement with the results of the electrophoretic mobility shift assay, these findings indicate that GATA-1 also inhibits DNA-binding activity of STAT3 in the chromatin structure.

Both GATA-1 and GATA-2 Directly Bind to STAT3 in Vitro and in Vivo—To clarify the mechanism through which GATA-1 and GATA-2 inhibit DNA-binding activity of STAT3, we analyzed their bindings to STAT3. For this purpose, we transfected HA-tagged GATA-1, GATA-2, and/or FLAG-tagged STAT3 together with G-CSFR/gp130 into 293T cells and performed the coimmunoprecipitation analysis. After the stimulation with gp130L for 30 min, we prepared total cellular lysate and immunoprecipitated GATA-1 with the anti-HA Ab. As shown in Fig. 4, the immunoblot with the anti-FLAG Ab revealed that STAT3 was communoprecipitated with GATA-1 only when both molecules were cotransfected (top panel, lane 4). Also, GATA-1 was communoprecipitated with STAT3 by the anti-FLAG Ab (third panel, lane 4). Similarly, we found that GATA-2 bound to STAT3 in 293T cells (fifth and seventh panels, lane 4).

Next, we investigated whether GATA-1 and GATA-2 directly binds to STAT3 in vitro. Because we could not purify the GST fusion protein containing the full portion of STAT3 due to the formation of inclusion body, we generated several GST-STAT3 fusion proteins each consisting of the fragment as follows: GST-STAT3 (amino acids 1–154), GST-STAT3 (103–377), and GST-STAT3 (320–590). After verifying the quality and quantity of the fusion proteins by the Coomassie Brilliant Blue staining (data not shown), we analyzed their binding to in vitro
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In previous studies, we reported that GATA-2/ER suppresses the growth of normal hematopoietic stem/progenitor cells (13, 14). As for this mechanism, we found that GATA-2 down-regulates the expression of c-myc mRNA, which was assumed to be primarily responsible for GATA-2-induced growth suppression. Because the expression of c-myc mRNA was reported to be transcriptionally regulated by cytokine-induced STAT3 and Ras/MAPK activities (17, 29), in the present study, we

translated GATA-1 and GATA-2. As shown in Fig. 4B, 35S-labeled GATA-1 and GATA-2 bound to GST-STAT3 (320–590), but not to GST alone, GST-STAT3 (1–154) or GST-STAT3 (103–377). Because the DNA-binding domain of STAT3 resides in around amino acids 400–500, it was speculated that GATA-1 and GATA-2 may inhibit DNA-binding activity of STAT3 through the direct interactions.

To further confirm the relevance of this finding in normal hematopoietic cells, we examined the binding between GATA-1 and STAT3 in murine megakaryocytes that developed from bone marrow stem/progenitor cells after 5-day cultures with TPO. As shown in Fig. 4C, both STAT3 and GATA-1 were detected by immunoblot analysis on the total cell lysate prepared from normal megakaryocytes (both panels, lane 4). Also, as was seen in 293T cells, STAT3 was communoprecipitated with GATA-1 (left panel, lane 2), and vice versa (right panel, lane 3). In addition, we confirmed that neither the anti-STAT3 Ab recognizes GATA-1 (left panel, lane 2) nor the anti-GATA-1 Ab recognizes STAT3 (right panel, lane 3). Also, we verified that neither STAT3 nor GATA-1 was immunoprecipitated by the anti-actin Ab (both panels, lane 1). From these results, it was assumed that the binding between GATA-1 and STAT3 in normal megakaryocytes is not attributable to the nonspecific reactions.

GATA-1 Inhibits STAT3 Activity through Its N-zinc Finger—Next, we tried to determine which domain of GATA-1 inhibits STAT3 activity. As shown in Fig. 5A, STAT3-C-induced 4 × APRE-Luc activities were inhibited by WT, del N90, del N180, del C80, and ΔCZF but not by del NZF, del BZF, del C115, ΔNZF, or ΔCENT, indicating that the N-zinc finger domain is necessary for GATA-1 to inhibit STAT3 activity. However, because del C115 also could not inhibit STAT3 activity, the C-terminal domain around amino acids 80–115 was also supposed to play some role in this inhibition. In agreement with this finding, the in vitro binding assay showed that mutants of GATA-1 lacking the N-zinc finger did not bind to GST-STAT3 (320–590) (Fig. 5B). Also, the in vivo binding assay using 293T cells showed that GATA-1 mutants lacking the N-zinc finger did not bind to STAT3 (Fig. 5C). These results suggest that GATA-1 inhibits STAT3 activity by binding to STAT3 through its N-zinc finger domain. Furthermore, we found that del C115, which could not inhibit STAT3 activity, did not bind to STAT3 in vivo despite its in vitro binding to GST-STAT3 (Fig. 5B, lane 5 versus Fig. 5C, lane 6), suggesting that the C-terminal domain around amino acids 80–115 would be necessary for the in vivo binding between GATA-1 and STAT3.

Next, we examined the effects of deletion mutants in the in vivo system. For this purpose, we introduced WT GATA-1, ΔNZF GATA-1, and ΔNZF GATA-1 into Ba/F3/gp130, and selected several clones under the culture with IL-3, in which each transgene was effectively expressed; these clones were designated Ba/F3/gp130/WT GATA-1, Ba/F3/gp130/ΔNZF GATA-1, and Ba/F3/gp130/ΔNZF GATA-1, respectively. As shown in Fig. 5A, WT GATA-1 and ΔNZF GATA-1 can inhibit STAT3 activity, whereas ΔNZF GATA-1 cannot. These clones proliferated with the similar growth curves under the culture with IL-3 (data not shown). When cultured with gp130L, Ba/F3/gp130/WT GATA-1, and Ba/F3/gp130/ΔNZF GATA-1 cells scarcely proliferated (Fig. 5D), and a part of the cultured cells led to apoptosis (data not shown). In contrast, Ba/F3/gp130/ΔNZF GATA-1 was able to grow in response to gp130L, whereas its growth was reduced by 40% compared with that of Ba/F3/gp130/Mock (Fig. 5D). In addition, we found that the induction of JunB by gp130L was apparently inhibited in Ba/F3/gp130/WT GATA-1 and Ba/F3/gp130/ΔNZF GATA-1 cells, whereas it was retained in Ba/F3/gp130/ΔNZF GATA-1 (Fig. 5E). The similar growth characteristics and JunB induction pattern were observed at least in three other clones of each transfectant. Together, these results suggest that GATA-1 would inhibit STAT3 activity through the N-zinc finger domain, thereby impeding gp130L-dependent growth/survival of Ba/F3 cells. However, it was to be noted that ΔNZF GATA still inhibited gp130L-dependent growth of Ba/F3 cells about 40%, whereas it did not inhibit STAT3 activity. This result implied that GATA-1 would disrupt an additional gp130-mediated growth signaling pathway besides STAT3.

Discussion

In previous studies, we reported that GATA-2/ER suppresses the growth of normal hematopoietic stem/progenitor cells (13, 14). As for this mechanism, we found that GATA-2 down-regulates the expression of c-myc mRNA, which was assumed to be primarily responsible for GATA-2-induced growth suppression. Because the expression of c-myc mRNA was reported to be transcriptionally regulated by cytokine-induced STAT3 and Ras/MAPK activities (17, 29), in the present study, we
examined the effects of GATA transcription factors on STAT3 activity. As a result, we found that GATA transcription factors (at least GATA-1, GATA-2, and GATA-3) inhibit STAT3 activity through the direct protein-protein interaction. In addition, we found that GATA-1 utilizes its N-zinc finger to suppress STAT3 activity. Considering the fact that GATA transcription factors utilize both zinc fingers as DNA-binding domains (2), these domains seem to harbor a number of functions, because they also associate with various nuclear factors besides STAT3. For example, the N-zinc finger of GATA-1 interacts with FOG, a critical cofactor for GATA-1 (30, 31) and c-Myb that inhibits its activity (32). The C-zinc finger of GATA-1 forms complexes with p300/CREB-binding protein and the Sp1/EKLF heterodimer, both of which augment GATA-1 activity (33–35). Moreover, GATA-1 binds to PU.1 through the C-zinc finger (25, 36–38). In this interaction, GATA-1 and PU.1 were shown to antagonize each other. These lines of evidence together with our data suggest that, in addition to the roles as transcription factors, GATA transcription factors would exhibit various biological effects by modulating the function of nuclear factors through the direct protein-protein interactions. Also, it was supposed that the activities and roles of GATA transcription factors in cell growth, differentiation, and survival would be considerably different according to the expression profiles of these interacting factors.

In the current study, we showed that ectopically expressed GATA-1/ERT severely suppressed TPO- and gp130-dependent growth of Ba/F3 cells. In addition, because hyperproliferation of megakaryocytes and proerythroblasts were observed in GATA-1 knock-down mice (6–8), it was assumed that endogenous GATA-1 also suppresses the growth of GATA-1-positive cells, at least partly by inhibiting STAT3 activity. Supporting this hypothesis, the binding between GATA-1 and STAT3 was detected in normal megakaryocytes. Then, the next question is whether STAT3 activities are completely suppressed (or to what degree they are suppressed) in GATA-1-positive cells. As for this problem, STAT3 activated by erythropoietin or TPO was shown to still function in GATA-1-positive erythroid and megakaryocytic cells (39, 40). Taken together, these results indicate that appropriately regulated (i.e., suppressed by GATA-1) STAT3 activities would be required for normal development of GATA-1-positive erythroid and megakaryocytic cells. As was the case with GATA-1 knock-down mice that revealed the myelodysplastic syndrome-like phenotype (6, 7), the germ line mutation of GATA-1 in the N-zinc finger was reported to cause X-linked dyserythropoietic anemia and macrothrombocytopenia (41, 42). In affected individuals, erythrocytes in the peripheral blood were abnormal in size and shape (poikilocytosis and anisocytosis). The bone marrow was packed with excessive large, multinucleated erythroid precursors and with nu-
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merous small, dysplastic megakaryocytes. Although these phenotypes were believed to be caused by the defect in transcriptional activities of GATA-1, our result raises a possibility that some phenotype such as hyperproliferation of erythrocytes and megakaryocytes might be caused by unrestricted STAT3 activities due to the GATA-1 mutation in the N-zinc finger.

Among GATA and STAT family members, in the present study, we focused on the effects of GATA-1 and GATA-2 on STAT3 activity. However, it is possible that other members of GATA and STAT interact with and regulate each other. Especially, it will be interesting to examine the functional relationship between STAT3 and GATA-4/GATA-6 in cardiomyocytes, because both molecules play key roles in their development (43, 44). Also, it is attractive to explore the interaction between STAT4/STAT6 and GATA-3 in CD4+ T lymphocytes, because the functional balance among these molecules was supposed to determine the differentiation toward Th1 or Th2 lymphocytes (45–49).

In summary, we demonstrated here that, in addition to the roles as transcription factors, GATA family proteins modulate cytokine signals through protein-protein interactions, thereby regulating the growth and survival of hematopoietic cells.

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