Biochemical and Biophysical Research Communications 417 (2012) 594–600

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

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

TRIM32 promotes retinoic acid receptor α-mediated differentiation in human promyelogenous leukemic cell line HL60

Tomonobu Sato [a,b], Fumihiko Okumura [a], Akihiro Iguchi [b], Tadashi Ariga [b], Shigetsugu Hatakeyama [a,*]

[a] Department of Biochemistry, Hokkaido University Graduate School of Medicine, Sapporo, Hokkaido 060-8638, Japan
[b] Department of Pediatrics, Hokkaido University Graduate School of Medicine, Sapporo 060-8638, Japan

ARTICLE INFO

Article history:
Received 1 December 2011
Available online 11 December 2011

Keywords:
Promyelogenous leukemia
HL60
RARα
TRIM32
Ubiquitin

ABSTRACT

Ubiquitination, one of the posttranslational modifications, appears to be involved in the transcriptional activity of nuclear receptors including retinoic acid receptor α (RARα). We previously reported that an E3 ubiquitin ligase, TRIM32, interacts with several important proteins including RARα and enhances transcriptional activity of RARα in mouse neuroblastoma cells and embryonal carcinoma cells. Retinoic acid (RA), which acts as a ligand to nuclear receptors including RARα, plays crucial roles in development, differentiation, cell cycles and apoptosis. In this study, we found that TRIM32 enhances RARα-mediated transcriptional activity even in the absence of RA and stabilizes RARα in the human promyelogenous leukemic cell line HL60. Moreover, we found that overexpression of TRIM32 in HL60 cells suppresses cellular proliferation and induces granulocytic differentiation even in the absence of RA. These findings suggest that TRIM32 functions as one of the coactivators for RARα-mediated transcription in acute promyelogenous leukemia (APL) cells, and thus TRIM32 may become a potentially therapeutic target for APL.

1. Introduction

Acute promyelogenous leukemia (APL) is a highly malignant subtype of leukemia because of its potentially life-threatening hemorrhagic events due to disseminated intravascular coagulation [1]. One of the most common chromosomal translocations in APL is t(15;17)(q22;q21) resulting in expression of the leukemic-specific chimeric fusion protein PML-RARα [2]. However, the appearance of the differentiating agent all-trans retinoic acid (ATRA), an isoform of retinoic acid, has dramatically improved prognosis of APL [3]. Actually, ATRA likely targets *PML-RARα* gene fusion protein for degradation. Since ATRA therapy showed a high complete remission rate in patients with primary APL and relapsed patients, differentiation therapy with ATRA has now become an established method of treatment in APL [4]. However, the detailed molecular mechanism of ATRA action in APL has not been fully understood.

ATRA plays crucial roles in cell proliferation [5], differentiation [6], tumorigenesis, and in regulation of apoptosis [7]. The biological effects of ATRA are mediated by two types of nuclear receptors: retinoic acid receptors (RARs) and retinoid X receptors (RXRs). Retinoic acid binds to a transcription complex composed of RARs and RXRs, and the heterodimeric pair binds to a specific DNA sequence called a retinoic acid-response element (RARE) [8]. It has been reported that RARs and their fusion proteins such as PML-RARα protein in APL are degraded by the ubiquitin–proteasome pathway [9], which possibly inhibits the complete differentiation of leukemic cells in response to ATRA. On the other hand, there are several reports that ubiquitin-mediated degradation of short-lived regulatory proteins including cell cycle regulatory proteins is essential for ATRA-induced proliferation or differentiation [10,11].

Ubiquitination is a pivotal posttranslational modification system used by eukaryotic cells [12]. It has been reported that gene activities of some nuclear receptors, such as RARs [9,13,14], estrogen receptor [15] and androgen receptor [16,17], are modulated strictly by the ubiquitin–proteasome system, and ubiquitination of those transcriptional factors plays a role not only in the degradation signal but also in the activation signal at a certain transcriptional stage. Tripartite motif (TRIM) proteins are characterized by the presence of a RING finger, one or two zinc-binding motifs called B-boxes, and an associated coiled-coil region (RBCC) [18–20]. It has been reported that TRIM32 has an E3 ligase activity for actin [21], Piasy [22], dysbindin [23], Abi-2 [24], and Argonaute-1 [25]. Point mutation of human TRIM32 has been reported in two genetic disorders: limb-girdle muscular dystrophy type 2H (LGMD2H) [26,27] and Bardet-Biedl syndrome (BBS) [28]. We previously showed that TRIM32 is one of the regulators of RARα by using a comprehensive luciferase reporter assay for *RARE* in neuroblastoma cells [29]. Furthermore, it is important to clarify TRIM32-mediated RARα activity not only in neural differentiation but also in hematopoiesis and leukemogenesis.

* Corresponding author. Address: Department of Biochemistry, Hokkaido University Graduate School of Medicine, Kita 15, Nishi 7, Kita-ku, Sapporo, Hokkaido 060-8638, Japan. Fax: +81 11 706 5169.
E-mail address: hatas@med.hokudai.ac.jp (S. Hatakeyama).

0006-291X/$ - see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2011.12.012

In this study, with the aim of elucidating the molecular function of TRIM32 in promyelogenous leukemic cell differentiation, we analyzed the function of TRIM32 in RARα regulation in the human promyelogenous leukemic cell line HL60. We showed that TRIM32 suppresses cellular proliferation of HL60 cells via transcriptional activation of RARα. We found that TRIM32 acts as a coactivator of RARα not only in neural cell lines but also in human APL cell lines and that overexpression of TRIM32 promotes APL cell differentiation without ATRA, suggesting that TRIM32 is one of the key regulators for APL cell differentiation.

2. Materials and methods

2.1. Cell culture and treatment

HL60, K562, CMK, HEL, KG-1, NKM-1, Namalwa and Jurkat cells (ATCC) were cultured under an atmosphere of 5% CO_2 at 37 °C in RPMI 1640 medium (Sigma) supplemented with 10% fetal bovine serum (Invitrogen). HeLa cells (ATCC) were cultured by the same method in DMEM (Sigma) supplemented with 10% fetal bovine serum (Invitrogen). All-trans retinoic acid (Sigma) was dissolved in dimethyl sulfoxide.

2.2. Cloning of cDNA and plasmid construction

Human *TRIM32* and *RARα* cDNA was isolated and subcloned as previously described [29]. Retinoic acid reporter-luciferase (RAR-Luc) was kindly provided by Dr. Kondo (Hokkaido University).

2.3. Transfection and immunoblot analysis

HL60 cells were electroporated with linearized pCAG-puro-FLAG-TRIM32 plasmid at 275 V and 950 μF once by using Gene Pulser X cell (Bio-Rad Laboratories). HEK293T cells were transfected by the calcium phosphate method. These cell lines were lysed in a solution containing 50 mmol/L Tris–HCl (pH 7.4), 150 mmol/L NaCl, 1% Nonidet P-40, leupeptin (10 μg/mL), 1 mmol/L phenylmethylsulfonyl fluoride, 400 μmol/L Na_3VO_4, 400 μmol/L EDTA, 10 mmol/L NaF, and 10 mmol/L sodium pyrophosphate. The cell lysates were centrifuged at 16,000*g* for 15 min at 4 °C and then boiled in SDS sample buffer. Immunoblot analysis was performed with the following primary antibodies: anti-FLAG (1 μg/mL; M2 or M5, Sigma), anti-HA (1 μg/mL; HA11, Covance), anti-TRIM32 (mouse polyclonal, Abnova), anti-Hsp90 (1 μg/mL; 68, Transduction Laboratories), anti-β-actin (0.2 μg/ml; AC15, Sigma), anti-c-Myc (1 μg/ml; 9E10, Covance) and anti-RARα (Rabbit polyclonal, Cell signaling,). Immune complexes were detected with horseradish peroxidase-conjugated antibodies to mouse or rabbit IgG (1:10,000 dilutions, Promega) and an enhanced chemiluminescence system (GE Healthcare Bioscience Corp).

2.4. Dual-luciferase assay

Cells were seeded in 24-well plates at 1×10^5 cells per well (HEK293T and HeLa cells) and incubated at 37 °C with 5% CO_2 for 48 h. Retinoic acid reporter-luciferase (RAR-Luc) plasmid was transfected with TRIM32 and/or RARα expression vectors into HeLa cells using Fugene HD reagent (Roche). Transfected cells were incubated in DMEM (Sigma) supplemented with 10% fetal bovine serum (Invitrogen) for 24 h and then incubated with ATRA (1 μM) for 24 h, harvested, and assayed for luciferase activity with a Dual-Luciferase Reporter Assay System (Promega). The luminescence was quantified with a luminometer (Promega).

2.5. Cell differentiation assessment and immunofluorescence staining

Smears of mock and TRIM32-transfected HL60 cells were stained with May-Grünwald solution (Merck) for 2 min, rinsed with distilled water, and stained with Giemsa solution (Sigma) for 10 min. Slides were rinsed with distilled water again and air dried. Cell morphology was observed by light microscopy (LABOPHOT, Nikon) under immersion. HL60 cells expressing FLAG-tagged TRIM32 were fixed for 10 min at room temperature with 2% formaldehyde in PBS and then incubated for 1 h at room temperature with a primary antibody to FLAG (Rabbit polyclonal, Sigma) or human CD11b (mouse monoclonal, eBioscience) in phosphate-buffered solution (PBS) containing 0.1% bovine serum albumin. They were then incubated with Alexa488-labeled goat polyclonal antibody to rabbit immunoglobulin or Alexa546-labeled goat polyclonal antibody to mouse immunoglobulin (Molecular Probes) at a dilution of 1:1000 and stained with Hoechst33258. The cells were covered with a drop of VECTASHIELD (VECTOR) and then photographed with a CCD camera (DP71, Olympus) attached to an Olympus BX51 microscope.

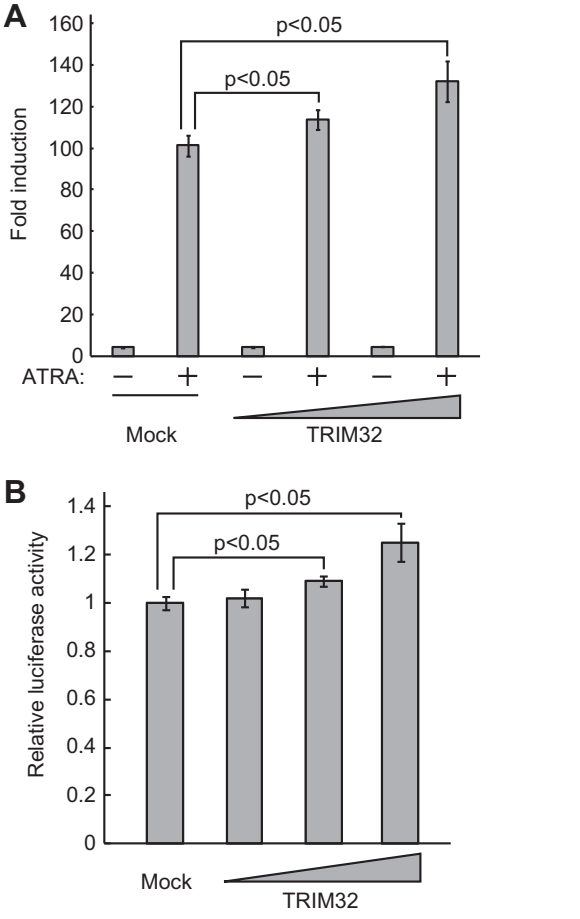

Fig. 1. TRIM32 enhances RARα-mediated transcriptional activity without ATRA. (A) TRIM32 enhances RARα-mediated transcriptional activity in a dose-dependent manner. Expression vectors encoding RARα and TRIM32 or an empty vector with an RAR luciferase reporter vector (RAR-Luc) were transfected into HeLa cells, and then the cells were incubated in a culture medium for 24 h. The cells were incubated with or without ATRA (1 μM) for 24 h and then collected and assayed for luciferase activity. Data are means ± SD of values from three independent experiments. *P* values for the indicated comparisons were determined by Student's *t* test. (B) TRIM32 facilitates RARα-mediated transcriptional activity in a dose-dependent manner without ATRA. RAR-Luc and expression vectors encoding RARα and different amounts of TRIM32 were transfected into HeLa cells. Transfected cells were incubated for 24 h and then incubated without ATRA (1 μM) for 24 h. The cells were then harvested and assayed for luciferase activity.

2.6. Statistical analysis

Student's *t*-test was used to determine the statistical significance of experimental data.

3. Results

3.1. TRIM32 facilitates transcription activity of RARα without ATRA

To determine whether TRIM32 enhances RARα-mediated transcription also in human epithelial carcinoma cell lines, we performed a luciferase reporter assay using an RAR promoter-driven luciferase construct (RAR-Luc) in HeLa cells (Fig. 1A). A TRIM32 expression vector and RAR-Luc were transfected, and luciferase activity was measured with or without ATRA. The luciferase reporter assay showed that TRIM32 facilitates ATRA-dependent RARα-mediated transcriptional activity in a dose-dependent manner in HeLa cells as was found in our previous study using neuroblastoma cells [29] (Fig. 1A). Furthermore, to determine whether TRIM32 in RARα-mediated transcription is dependent on ATRA, we performed a luciferase reporter assay using RAR-Luc without ATRA in HeLa cells. The luciferase reporter assay showed that TRIM32 significantly activates RARα-mediated transcription even without ATRA (Fig. 1B), suggesting that TRIM32 induces differentiation of promyelocytic leukemia cells without ATRA.

3.2. TRIM32 stabilizes the expression level of RARα

It has been reported that *TRIM32* mRNA is expressed in skeletal muscle and in the heart and brain [26]. To clarify the expression profiles of TRIM32 in human leukemic cell lines, we compared the protein levels of TRIM32 by immunoblot analysis in different types of human leukemic cell lines including promyelocytic leukemia cell line HL60, erythroleukemia cell lines K562 and HEL, megakaryocytic leukemia cell line CMK, acute myelogenous leukemia cell lines KG-1 and NKM-1, Burkitt lymphoma cell line Namalwa and T-cell leukemia cell line Jurkat (Fig. 2A). Immunoblot analysis showed that TRIM32 is expressed in various leukemic cell lines regardless of the origins, whereas RARα is highly expressed in promyelocytic leukemia (HL60), erythroleukemia (K562 and HEL) and lymphoblastic leukemia cell lines (Namalwa and Jurkat).

We previously showed that overexpressed TRIM32 physically interacts with RARα in vivo and stabilizes the expression level of RARα in Neuro2a cells and mouse embryonic carcinoma cell line P19. Immunoblot analysis was performed to verify that TRIM32 stabilizes the expression level of RARα not only in neural cell lines but also in HL60 cells without ATRA. An expression vector encoding FLAG-tagged TRIM32 was transiently expressed in HL60 cell lines by electroporation and then immunoblot analysis was performed to evaluate the expression level of endogenous RARα. Immunoblot analysis showed that endogenous RARα was more

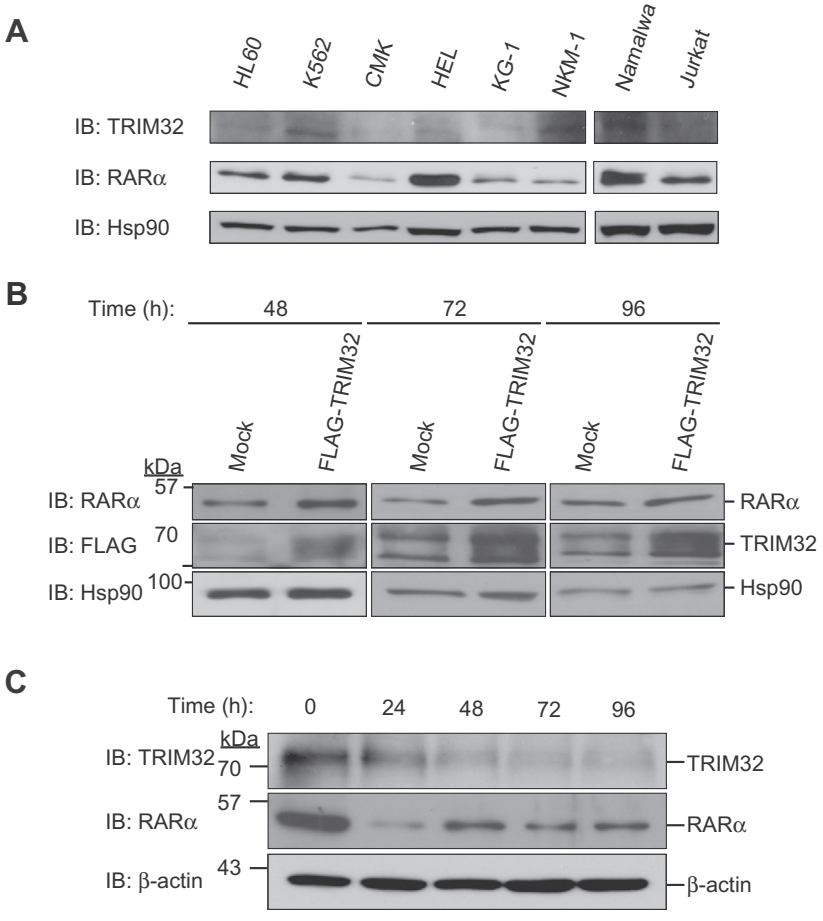

Fig. 2. TRIM32 stabilizes expression levels of RARα. (A) Expression levels of TRIM32 in various human leukemia cell lines. Cell lysates from human leukemia cell lines HL60, K562, CMK, HEL, KG-1, NKM-1, Namalwa, and Jurkat were subjected to immunoblot (IB) analysis with anti-TRIM32, anti-RARα and anti-Hsp90 antibodies. (B) TRIM32 stabilizes expression levels of endogenous RARα. Immunoblot analysis showed expression levels of endogenous RARα in HL60 cells in which FLAG-tagged TRIM32 had been transfected and in mock cells without ATRA. Anti-Hsp90 antibody was used as an internal control. (C) Immunoblot analysis of endogenous TRIM32 and RARα after induction of ATRA in HL60 cells. HL60 cells were seeded at 1×10^6 cells in 60-mm dishes and cultured with ATRA (1 μM). Immunoblot analysis with anti-TRIM32 antibody and anti-RARα antibody was performed at the indicated times after seeding. Anti-β-actin antibody was used as an internal control.

highly expressed in HL60 cells in which FLAG-tagged TRIM32 was expressed (Fig. 2B). Next, to examine whether induction of differentiation with ATRA in HL60 cells affects the expression level of endogenous TRIM32, immunoblot analysis was performed to analyze the expression levels of endogenous TRIM32 after ATRA treatment for the indicated times (Fig. 2C). As previously reported, ATRA caused a decrease in the level of RARα in HL60 cells because of degradation of RARα protein by the ubiquitin–proteasome system [9,13]. Treatment with ATRA in HL60 cells also caused a decrease in endogenous TRIM32 expression. However, TRIM32 was observed until 24 h after ATRA induction. This finding suggests that TRIM32 maintains the activity of RARα-mediated transcription even without ATRA for differentiation of HL60.

3.3. TRIM32 promotes differentiation of HL60 cells without retinoic acid

Since luciferase reporter assays and immunoblot analysis showed that ectopic expression of TRIM32 induces RARα-mediated transcriptional activity and stabilizes RARα even without ATRA, we hypothesized that TRIM32 affects granulocytic differentiation of HL60 cells via RARα-mediated transcription without ATRA. We

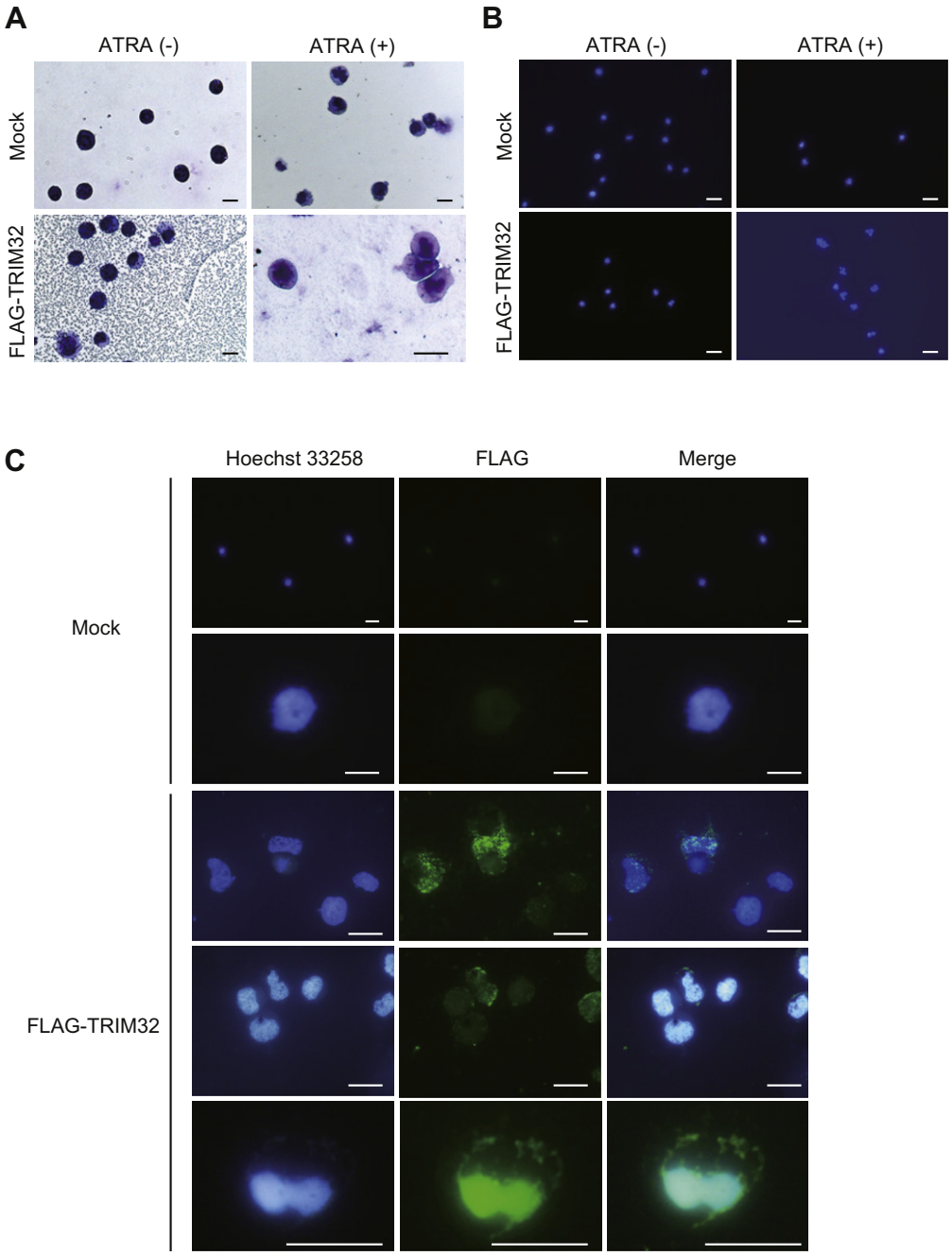

Fig. 3. TRIM32 promotes differentiation of HL60 cells. (A) Morphological changes of HL60 cell lines in which FLAG-tagged TRIM32 had been transfected with or without ATRA. HL60 cells with FLAG-tagged TRIM32 or mock cells were treated with or without ATRA (1 μM) and then stained by May-Grünwald–Giemsa solution. Scale bar, 10 μm. (B) Nuclear morphology of HL60 cell lines in which FLAG-tagged TRIM32 had been transfected. Representative micrographs show Hoechst33258-stained HL60 cells treated with or without ATRA. Scale bar, 20 μm. (C) TRIM32 transfection induces morphological changes of granulocytic differentiation in HL60 cells. HL60 cells were transfected with expression vectors encoding FLAG-tagged TRIM32 or mock by electroporation and then stained with antibodies to FLAG (green) with Hoechst33258 (blue). Scale bar, 10 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

analyzed the effects of TRIM32 on morphological changes of the nucleus in the presence or absence of ATRA (Fig. 3A and B). To test cell morphological changes of HL60 cell lines, an expression vector encoding FLAG-tagged TRIM32 was transiently transfected into HL60 cells by electroporation, and the cells were cultured for 72 h with or without ATRA (1 μM) and then stained by the May-Grünwald–Giemsa method. With ATRA treatment, morphological features of granulocytic differentiation such as condensed lobular nuclei and reduced nuclei in size were observed in both mock cells and TRIM32-overexpressing HL60 cells (Fig. 3A). Interestingly, similar morphological features of granulocytic differentiation such as polynucleated cells with granulated cytoplasms and a decreased cytoplasm/nucleus ratio were observed in a part of the population of ATRA-untreated HL60 cells in which Flag-tagged TRIM32 was transiently expressed. A microscope image of nuclei stained by Hoechst 33258 showed similar results (Fig. 3B).

To further confirm that cells in which FLAG-tagged TRIM32 was overexpressed have a tendency to differentiate to granulocytes

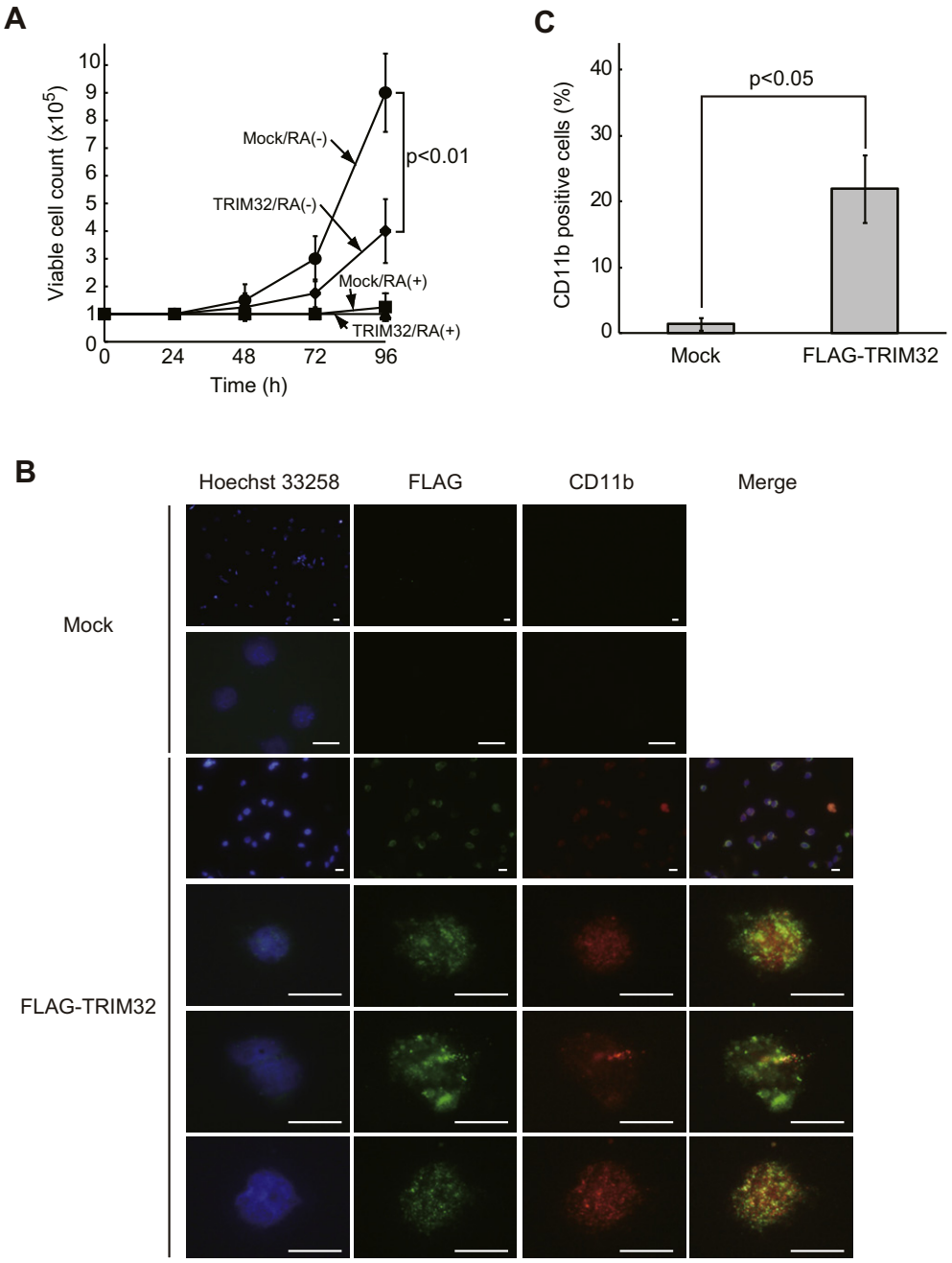

Fig. 4. TRIM32 suppresses proliferation and induces surface expression of CD11b without ATRA in HL60 cells. (A) Overexpression of TRIM32 suppresses HL60 cell proliferation without ATRA. HL60 cells in which FLAG-tagged TRIM32 and mock were transfected by electroporation and seeded at 1×10^5 cells in 60-mm dishes. The cells were cultured with or without ATRA (1 μM) and then counted at the indicated times. Data are means ± SD of values from three independent experiments. *P* values for the indicated comparisons were determined by Student's *t* test. (B) Expression of CD11b by overexpression of FLAG-tagged TRIM32. HL60 cells were electroporated with an expression vector encoding FLAG-tagged TRIM32 or mock without ATRA and then stained with antibodies to FLAG (green) and CD11b (red) with Hoechst33258 (blue) at 72 h after electroporation. Scale bar, 10 μm. (C) Quantification of CD11b-positive HL60 cells. The cells stained in (B) were counted. Data are means ± SD of values from three independent experiments. *P* values for the indicated comparisons were determined by Student's *t* test. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

even in the absence of ATRA, we performed immunofluorescence staining with anti-FLAG antibody. HL60 cells were transfected with an expression vector encoding FLAG-tagged TRIM32 or mock by electroporation and then stained with an antibody to FLAG with Hoechst33258 at 72 h after electroporation. Immunofluorescence analysis showed that granulocytic differentiation with morphological changes was induced in HL60 cells in which Flag-tagged TRIM32 was transiently overexpressed without ATRA (Fig. 3C).

3.4. TRIM32 suppresses HL60 cell proliferation and induces surface expression of CD11b without ATRA

It has been reported that treatment of promyelocytic leukemia cells such as HL60 and NB4 cells with ATRA or As_2O_3 causes differentiation, apoptosis and inhibition of cell proliferation [3,30]. We hypothesized that granulocytic differentiation induced by TRIM32 in HL60 cells is linked to the phenomenon of overexpression of TRIM32 negatively regulating HL60 cell proliferation. To examine whether overexpression of TRIM32 affects cell proliferation, HL60 cells were transfected with a TRIM32 or mock expression vector by electroporation and cultured in the presence or absence of ATRA and then cell numbers were counted at the indicated times. To examine viability of HL60 cells after electroporation, trypan blue staining was performed to count living cells. In the presence of ATRA, HL60 cells in which FLAG-tagged TRIM32 or mock was expressed showed clearly repressed proliferation. Without ATRA, mock cells showed normal rapid proliferation, whereas FLAG-tagged TRIM32 expression delayed cell proliferation (Fig. 4A). These findings suggest that TRIM32 delays cell proliferation and induces granulocytic differentiation via RARα-mediated transcriptional activity even in the absence of ATRA.

Next, we performed immunofluorescence staining to analyze cell surface expression of CD11b antigen as a granulocytic differentiation marker. HL60 cells were transfected with an expression vector encoding FLAG-tagged TRIM32 or mock by electroporation and cultured without ATRA. The cells were stained with antibodies to FLAG and CD11b with Hoechst33258 at 72 h after electroporation to examine whether CD11b expression is higher in TRIM32-transfected cells than in mock cells without ATRA. HL60 cells in which FLAG-tagged TRIM32 (green) was expressed showed high expression level of CD11b (red) in the absence of ATRA (Fig. 4B). We counted the number of cells in which CD11b was expressed. Statistical analysis showed that HL60 cells in which FLAG-tagged TRIM32 was expressed included a significantly higher percentage of CD11b-positive cells than did mock cells (Fig. 4C). These findings suggest that ectopic expression of TRIM32 induces CD11b expression as a granulocyte differentiation marker.

4. Discussion

Emerging evidence has indicated that E3 ubiquitin ligases do not always participate in the regulation of transcriptional activity in the function of proteolytic activity. Moreover, it has been shown that the activity of RARs is regulated by cofactors that either promote or repress RAR-mediated gene transcription and that RARα interacts with several proteins that belong to the family of E3 ubiquitin ligases. In addition, the HECT domain and ankyrin repeat containing E3 ubiquitin–protein ligase (HACE1) has been shown to repress the transcriptional activity of RARα [31], and another E3 ligase, Rnf41, has also been reported to regulate differentiation of hematopoietic progenitors by modulating the steady state of RARα [32]. In this present study, we demonstrated that TRIM32 stabilizes endogenous RARα and induces granulocytic differentiation in HL60 cells regardless of the presence or absence of ATRA.

There are a few reports that regulation of proteasome activation potentiates the differentiating effect of leukemia cells [33]. It has been shown that proteasome inhibitors have the ability to enhance ATRA-induced cell differentiation of HL60 cell lines, suggesting that stabilization of RARα promotes cellular differentiation of leukemic cells. In our study, in the presence of ATRA, we could not show a significant difference in proliferation of HL60 cells in which TRIM32 is overexpressed and mock cells. However, although the ATRA-induced ubiquitin–proteasome system was activated, expression level of TRIM32 was maintained until 24 h after ATRA induction. These findings suggest that the remaining TRIM32 promotes granulocytic differentiation of HL60 cells via an unknown RARα-independent pathway. A proteasome inhibitor would cause retention of TRIM32, and thereby accumulated TRIM32 may activate RARα transcriptional activity and suppress cellular proliferation followed by induction of granulocytic differentiation. It is important to clarify whether inhibition of the ubiquitin–proteasome pathway promotes differentiation of promyelogenous leukemic cells as well as multiple myeloma and to determine whether a proteasome inhibitor in combination with ATRA is more effective for therapy of APL.

Retinoids have important roles in cancer therapy for their potential effects on cell differentiation and apoptosis. ATRA is now used for initial treatment of APL [34]. However, intractable or relapsed APL would need to be treated by several combination therapies including ATRA. In this study, we showed that TRIM32 is a positive regulator of transcriptional activity of RARα, suggesting that TRIM32 regulates the function of RARα or cofactors involved in differentiation of HL60 cells. It is important to analyze TRIM family proteins that regulate transcriptional activity of nuclear receptors related to cancers or metabolic syndromes. Identification of substrates of TRIM32 and drug discovery for RAR-associated ubiquitin ligases would be helpful for patients suffering from malignant diseases.

Acknowledgments

We would like to thank Dr. Takeshi Kondo for the plasmids.

This work was supported in part by KAKENHI (18076001 and 21390087) from the Ministry of Education, Culture, Sports, Science and Technology in Japan, The Sumitomo Foundation and The Suhara Foundation (to S. Hatakeyama).

References

[1] G.B. Zhou, S.J. Chen, Z. Chen, Acute promyelocytic leukemia: a model of molecular target based therapy, Hematology 10 (Suppl. 1) (2005) 270–280.
[2] H. de The, C. Lavau, A. Marchio, C. Chomienne, L. Degos, A. Dejean, The PML-RAR alpha fusion mRNA generated by the t(15;17) translocation in acute promyelocytic leukemia encodes a functionally altered RAR, Cell 66 (1991) 675–684.
[3] Y. Carpentier, P. Mayer, H. Bobichon, B. Desoize, Cofactors in in vitro induction of apoptosis in HL60 cells by all-trans retinoic acid (ATRA), Biochem. Pharmacol. 55 (1998) 177–184.
[4] M.E. Huang, Y.C. Ye, S.R. Chen, J.R. Chai, J.X. Lu, L. Zhoa, L.J. Gu, Z.Y. Wang, Use of all-trans retinoic acid in the treatment of acute promyelocytic leukemia, Blood 72 (1988) 567–572.
[5] M. Dokmanovic, B.D. Chang, J. Fang, I.B. Roninson, Retinoid-induced growth arrest of breast carcinoma cells involves co-activation of multiple growth-inhibitory genes, Cancer Biol. Ther. 1 (2002) 24–27.
[6] C. Rochette-Egly, P. Chambon, F9 embryocarcinoma cells: a cell autonomous model to study the functional selectivity of RARs and RXRs in retinoid signaling, Histol. Histopathol. 16 (2001) 909–922.
[7] L. Altucci, A. Rossin, W. Raffelsberger, A. Reitmair, C. Chomienne, H. Gronemeyer, Retinoic acid-induced apoptosis in leukemia cells is mediated by paracrine action of tumor-selective death ligand TRAIL, Nat. Med. 7 (2001) 680–686.
[8] A.R. de Lera, W. Bourguet, L. Altucci, H. Gronemeyer, Design of selective nuclear receptor modulators: RAR and RXR as a case study, Nat. Rev. Drug Discov. 6 (2007) 811–820.
[9] J. Zhu, M. Gianni, E. Kopf, N. Honore, M. Chelbi-Alix, M. Koken, F. Quignon, C. Rochette-Egly, H. de The, Retinoic acid induces proteasome-dependent degradation of retinoic acid receptor alpha (RARalpha) and oncogenic RARalpha fusion proteins, Proc. Natl. Acad. Sci. USA 96 (1999) 14807–14812.

[10] M.J. Spinella, S.J. Freemantle, D. Sekula, J.H. Chang, A.J. Christie, E. Dmitrovsky, Retinoic acid promotes ubiquitination and proteolysis of cyclin D1 during induced tumor cell differentiation, J. Biol. Chem. 274 (1999) 22013–22018.
[11] B.E. Clurman, R.J. Sheaff, K. Thress, M. Groudine, J.M. Roberts, Turnover of cyclin E by the ubiquitin–proteasome pathway is regulated by cdk2 binding and cyclin phosphorylation, Genes Dev. 10 (1996) 1979–1990.
[12] A. Hershko, A. Ciechanover, The ubiquitin system, Annu. Rev. Biochem. 67 (1998) 425–479.
[13] E. Kopf, J.L. Plassat, V. Vivat, H. de The, P. Chambon, C. Rochette-Egly, Dimerization with retinoid X receptors and phosphorylation modulate the retinoic acid-induced degradation of retinoic acid receptors alpha and gamma through the ubiquitin–proteasome pathway, J. Biol. Chem. 275 (2000) 33280–33288.
[14] M. Gianni, A. Bauer, E. Garattini, P. Chambon, C. Rochette-Egly, Phosphorylation by p38MAPK and recruitment of SUG-1 are required for RA-induced RAR gamma degradation and transactivation, EMBO J. 21 (2002) 3760–3769.
[15] A. Nakajima, S. Maruyama, M. Bohgaki, N. Miyajima, T. Tsukiyama, N. Sakuragi, S. Hatakeyama, Ligand-dependent transcription of estrogen receptor alpha is mediated by the ubiquitin ligase EFP, Biochem. Biophys. Res. Commun. 357 (2007) 245–251.
[16] N. Miyajima, S. Maruyama, M. Bohgaki, S. Kano, M. Shigemura, N. Shinohara, K. Nonomura, S. Hatakeyama, TRIM68 regulates ligand-dependent transcription of androgen receptor in prostate cancer cells, Cancer Res. 68 (2008) 3486–3494.
[17] M. Kikuchi, F. Okumura, T. Tsukiyama, M. Watanabe, N. Miyajima, J. Tanaka, M. Imamura, S. Hatakeyama, TRIM24 mediates ligand-dependent activation of androgen receptor and is repressed by a bromodomain-containing protein, BRD7, in prostate cancer cells, Biochim. Biophys. Acta 1793 (2009) 1828–1836.
[18] A. Reymond, G. Meroni, A. Fantozzi, G. Merla, S. Cairo, L. Luzi, D. Riganelli, E. Zanaria, S. Messali, S. Cainarca, A. Guffanti, S. Minucci, P.G. Pelicci, A. Ballabio, The tripartite motif family identifies cell compartments, EMBO J. 20 (2001) 2140–2151.
[19] G. Meroni, G. Diez-Roux, TRIM/RBCC, a novel class of 'single protein RING finger' E3 ubiquitin ligases, Bioessays 27 (2005) 1147–1157.
[20] S. Nisole, J.P. Stoye, A. Saib, TRIM family proteins: retroviral restriction and antiviral defence, Nat. Rev. Microbiol. 3 (2005) 799–808.
[21] E. Kudryashova, D. Kudryashov, I. Kramerova, M.J. Spencer, Trim32 is a ubiquitin ligase mutated in limb girdle muscular dystrophy type 2H that binds to skeletal muscle myosin and ubiquitinates actin, J. Mol. Biol. 354 (2005) 413–424.
[22] Y. Liu, J.P. Lagowski, S. Gao, J.H. Raymond, C.R. White, M.F. Kulesz-Martin, Regulation of the psoriatic chemokine CCL20 by E3 ligases Trim32 and Piasy in keratinocytes, J. Invest. Dermatol. 130 (2010) 1384–1390.
[23] M. Locke, C.L. Tinsley, M.A. Benson, D.J. Blake, TRIM32 is an E3 ubiquitin ligase for dysbindin, Hum. Mol. Genet. 18 (2009) 2344–2358.
[24] S. Kano, N. Miyajima, S. Fukuda, S. Hatakeyama, Tripartite motif protein 32 facilitates cell growth and migration via degradation of Abl-interactor 2, Cancer Res. 68 (2008) 5572–5580.
[25] J.C. Schwamborn, E. Berezikov, J.A. Knoblich, The TRIM-NHL protein TRIM32 activates microRNAs and prevents self-renewal in mouse neural progenitors, Cell 136 (2009) 913–925.
[26] P. Frosk, T. Weiler, E. Nylen, T. Sudha, C.R. Greenberg, K. Morgan, T.M. Fujiwara, K. Wrogemann, Limb-girdle muscular dystrophy type 2H associated with mutation in TRIM32, a putative E3-ubiquitin-ligase gene, Am. J. Hum. Genet. 70 (2002) 663–672.
[27] K. Borg, R. Stucka, M. Locke, E. Melin, G. Ahlberg, U. Klutzny, M. Hagen, A. Huebner, H. Lochmuller, K. Wrogemann, L.E. Thornell, D.J. Blake, B. Schoser, Intragenic deletion of TRIM32 in compound heterozygotes with sarcotubular myopathy/LGMD2H, Hum. Mutat. 30 (2009) E831–E844.
[28] A.P. Chiang, J.S. Beck, H.J. Yen, M.K. Tayeh, T.E. Scheetz, R.E. Swiderski, D.Y. Nishimura, T.A. Braun, K.Y. Kim, J. Huang, K. Elbedour, R. Carmi, D.C. Slusarski, T.L. Casavant, E.M. Stone, V.C. Sheffield, Homozygosity mapping with SNP arrays identifies TRIM32, an E3 ubiquitin ligase, as a Bardet-Biedl syndrome gene (BBS11), Proc. Natl. Acad. Sci. USA 103 (2006) 6287–6292.
[29] T. Sato, F. Okumura, S. Kano, T. Kondo, T. Ariga, S. Hatakeyama, TRIM32 promotes neural differentiation through retinoic acid receptor-mediated transcription, J. Cell Sci. 124 (2011) 3492–3502.
[30] G.Q. Chen, X.G. Shi, W. Tang, S.M. Xiong, J. Zhu, X. Cai, Z.G. Han, J.H. Ni, G.Y. Shi, P.M. Jia, M.M. Liu, K.L. He, C. Niu, J. Ma, P. Zhang, T.D. Zhang, P. Paul, T. Naoe, K. Kitamura, W. Miller, S. Waxman, Z.Y. Wang, H. de The, S.J. Chen, Z. Chen, Use of arsenic trioxide (As_2O_3) in the treatment of acute promyelocytic leukemia (APL): I. As_2O_3 exerts dose-dependent dual effects on APL cells, Blood 89 (1997) 3345–3353.
[31] J. Zhao, Z. Zhang, Z. Vucetic, K.J. Soprano, D.R. Soprano, HACE1: a novel repressor of RAR transcriptional activity, J. Cell. Biochem. 107 (2009) 482–493.
[32] X. Jing, J. Infante, R.G. Nachtman, R. Jurecic, E3 ligase FLRF (Rnf41) regulates differentiation of hematopoietic progenitors by governing steady-state levels of cytokine and retinoic acid receptors, Exp. Hematol. 36 (2008) 1110–1120.
[33] Y. Fang, X. Zhou, M. Lin, M. Ying, P. Luo, D. Zhu, J. Lou, B. Yang, Q. He, Inhibition of all-trans-retinoic acid-induced proteasome activation potentiates the differentiating effect of retinoid in acute myeloid leukemia cells, Mol. Carcinog. 50 (2011) 24–35.
[34] G. Jiang, A. Albihn, T. Tang, Z. Tian, M. Henriksson, Role of Myc in differentiation and apoptosis in HL60 cells after exposure to arsenic trioxide or all-trans retinoic acid, Leuk. Res. 32 (2008) 297–307.