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Trib1 and Trib2 inhibit granulocytic differentiation by suppressing Akt pathway

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Abstract

Background :
Overexpression of Tribbles homolog 1 (Trib1) and Tribbles homolog 2 (Trib2) in hematopoietic stem/progenitor cells evokes acute myeloid leukemia (AML) in murine transplantation models. Degradation of CCAAT-enhancer-binding-protein α (C/EBPα) plays a crucial role in Trib1- or Trib2-induced AML. However, because C/EBPα knockout mice do not develop AML, it is likely that Trib1 and Trib2 influence other signaling pathways besides C/EBPα. Elevated Akt phosphorylation is considered to contribute to the development of AML. In contrast, two groups recently reported that reduced Akt activity is involved in the pathogenesis of leukemia. We performed this study to reveal the role of Akt signaling in Trib family-induced AML.
Methods :
G-CSF-induced granulocytic differentiation of 32D cells was assessed morphologically and phenotypically. G-CSF-induced signaling was assessed by Westernblotting.
Results :
Overexpression of Trib1 or Trib2 inhibited G-CSF-induced granulocytic differentiation of 32D cells, which was accompanied by reduced Akt phosphorylation. Also, an Akt inhibitor API-2 blocked G-CSF-induced granulocytic differentiation independently of C/EBPα degradation. Furthermore, retroviral C/EBPα restoration did not completely abolish the differentiation block caused by Trib1 and Trib2.
Conclusion :
Trib1 and Trib2 block granulocytic differentiation, at least partially, by suppressing Akt phosphorylation.
Key words : Trib1, Trib2, C/EBPα, Akt, AML, differentiation

Introduction

Acute myeloid leukemia (AML) is a genetically and phenotypically heterogeneous disease, which is commonly characterized by the proliferation, survival and differentiation block of hematopoietic cells.[1] Typical genetic events of AML are chromosomal translocations involving transcription factors necessary for normal myeloid differentiation. Point mutations also play an important role in the pathogenesis of AML. Activating point mutations in receptor tyrosine kinases (e.g. FLT3 and c-KIT) and/or intracellular signaling molecules such as Ras constitutively transmit a proliferative and/or survival signal in hematopoietic stem/progenitors. In contrast, loss of function point mutations of hematopoietic transcription factors such

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as GATA-1, RUNX1, and C/EBPα block differentiation. The combination of these abnormalities leads to the excessive proliferation and survival of undifferentiated AML cells.[2]

Tribbles was originally identified as a regulator of String/CDC25 in *Drosophila*.[1–3] Tribbles also promotes the degradation of slbo, a *Drosophila* homolog of the C/EBP family.[6] Subsequently, three mammalian homologues, Trib1, Trib2 and Tribbles homolog 3 (Trib3), have been identified.[7] They share a conserved amino acid motif, which is commonly observed in the catalytic domains of serine/threonine kinases. It remains unresolved whether Trib proteins act as nonfunctional pseudokinases, decoy kinases, or true kinases.[8] As for the roles of Trib proteins in hematologic malignancies, the expression of Trib1 was found to be elevated in samples from patients with AML and myelodysplastic syndromes (MDS) through its gene amplification.[9–11] Also, Trib2 was overexpressed in a subset of patients with AML[12] and T-ALL.[13] In addition, a mutation of Trib1 was recently identified in human acute megakaryocytic leukemia.[14] In accord with these findings, overexpression of Trib1 and Trib2 in hematopoietic stem/progenitors cells by retrovirus transduction evokes AML in murine transplantation models.[15,16] In this model, Trib1 and Trib2 were found to cooperate with homeobox gene A9 (HoxA9) and accelerate the onset of AML.[15,17] Also, Trib1 was reported to enhance extracellular signaling-regulated kinase (ERK) phosphorylation through the direct interaction with MEKK1 and thereby induce C/EBPα degradation.[18] Retroviral transduction of Trib2 into mouse bone marrow (BM) stem/progenitor cells inhibited their granulocytic differentiation and enhanced self-renewal activity. Also, Trib2 was reported to associate with and degrade C/EBPα in a proteasome-dependent manner using COP1 as an E3 ubiquitin ligase.[16,19] According to these previous data, degradation of C/EBPα appears to play an important role in the pathogenesis of murine AML evoked by Trib1 and Trib2. However, the fact that mice bearing knockout or mutated C/EBPα alleles do not develop AML[20–22] suggests that Trib1 and Trib2 may influence other signaling pathways besides C/EBPα.

Akt is a pivotal molecule that transmits cell survival signals from growth factors, cytokines, and other cellular stimuli.[23] Deregulated Akt activation is frequently observed in a wide variety of cancers.[24] In particular, elevated Akt phosphorylation is observed in about 50% of AML cases and considered to contribute to the development of AML.[25] However, two interesting papers were recently published indicating that reduced Akt activity was involved in the pathogenesis of AML and myeloproliferative neoplasms. In one paper, nuclear localization of Foxo3a caused by decreased Akt phosphorylation was shown to be important for the maintenance of leukemia-initiating cells in a murine model.[26] In another paper, it was shown that phosphorylation of Akt was reduced in MLL-AF9-introduced murine leukemic cells.[27] These new data prompted us to investigate the role of Akt in the pathogenesis of Trib1- and Trib2-induced AML.

Here, we show that Trib1 and Trib2 inhibit G-CSF-induced differentiation in a murine myeloid cell line 32D, which was accompanied by C/EBPα degradation and decreased phosphorylation of Akt. Also, we found that C/EBPα restoration did not completely abolish this differentiation block and that an Akt inhibitor API-2 blocked G-CSF-induced granulocytic differentiation in 32D cells independently of C/EBPα degradation. These data indicate that Trib1 and Trib2 would block granulocytic differentiation, at least partially, by suppressing Akt phosphorylation.

Materials And Methods

Cell culture

A murine myeloid cell line 32D-cl3 (32D) was purchased from RIKEN BRC (Tsukuba, Japan) and was maintained according to the manufacturer's instructions. WEHI 3B was also purchased from RIKEN BRC and maintained as instructed. PLAT-E, a packaging cell line, was a kind gift from Dr. T. Kitamura (The University of Tokyo) and was maintained in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% FCS.

Plasmid constructs and Retroviral gene transduction

The entire cDNAs of Trib1[28] and Trib2 were kind gifts from Dr. M. Yamamoto (Osaka University). They were subcloned into a retrovirus vector pMXs-SBPc-IRES-puro.[29] MSCV-C/EBPα-IRES-GFP retrovirus vector was a kind gift from Dr. H. Hirai (Kyoto University).

Retroviral infection of 32D cells was performed as previously described,[30] except that PLAT-E packaging cells and a 6-well plate coated with RetroNectin (Takara, Otsu, Japan) were used instead of Polybrene. After 48-hour culture with the supernatant of PLAT-E containing the retrovirus, 32D cells transduced with the retrovirus vector were selected in 1.5 μg/ml puromycin containing medium.

Proliferation assay (ATP assay)

Cell proliferation was assayed using the CellTiter-Glo Luminescent Cell Viability Assay (Promega) according to the manufacturer's instructions.

Differentiation assay for 32D cells

32D cells maintained in 10% WEHI 3B cell-conditioned medium were washed twice and replated in 10% WEHI 3B cell-conditioned medium for IL-3 condition or rG-CSF (100 ng/ml ; KYOWA KIRIN)- containing medium. Morphological differentiation was assessed by staining the cytospin preparations with May-Grunwald-Giemsa (Muto Pure Chemicals ; Wako Pure Chemical Industries) after 6-day culture. Phenotypic granulocytic differentiation was analyzed by a FACSCalibur (Becton Dickinson) after 4-day culture.

Flow cytometry, cell sorting and antibodies

Cell suspensions were incubated with immunoglobulin Kenketsu Venilon-I (Teijin Pharma) to block Fc receptor. Then, analytical flow cytometry was performed on a FACSCalibur (Becton Dickinson) and analyzed by FlowJo software (Treestar). FITC- and PE-conjugated anti-CD11b monoclonal antibodies (mAb) were purchased from BD Pharmingen. Dead cells were excluded by staining with 7AAD (BD Pharmingen). 32D cells transduced with retrovirus vectors containing the GFP gene were sorted by a FACSVantage SE DiVa cell sorter (Becton Dickinson) 48 hours after gene transduction.

Western blotting and Abs

Cells were lysed in RIPA buffer as described.[14] Protein concentration was measured by the Pierce BCA Protein Assay Kit (Takara Bio Inc.). The following antibodies were used : anti-SBP mAb (clone20 ; Merck Millipore), anti-C/EBPα mAb (Sc-61 ; Santa Cruz), anti-Actin mAb (Sc-1616 ; Santa Cruz), anti-phospho-Akt Ser473 mAb (#4058 ; Cell Signaling Technology), anti-Akt mAb (#4691 ; Cell Signaling Technology), anti-phospho-ERK mAb (#9101 ; Cell Signaling Technology), anti-ERK mAb (# 4695 ; Cell Signaling Technology), and anti-GAPDH mAb (#2118 ; Cell Signaling Technology).The signal was detected with ECL Prime Western Blotting Detection Reagent (GE Healthcare) and ImageQuant LAS 4010 (GE Healthcare).

Real-time quantitative reverse-transcribed polymerase chain reaction (RQ-PCR)

RNA was isolated with the RNeasy Mini Kit (QIAGEN). cDNA was synthesized with the High Capacity cDNA Reverse Transcription Kit (Life Technology). RQ-PCR was performed with PCR Master Mix or SYBR GREEN PCR Master Mix (Life Technology) and signal detection was carried out on the Step One real-time PCR system (Life Technology). Primers for Trib1 were purchased from Life Technology. Primers for Trib2 were designed using primer3 software (Life Technology) and customized and purchased from Sigma Genosys.

Results

Effect of Trib1 and Trib2 on cell proliferation and cell death

To investigate the effect of Trib1 and Trib2 on myeloid differentiation, we utilized an IL-3-dependent myeloid cell line, 32D. 32D cells proliferate exponentially in the presence of IL-3 without differentiation and differentiate into mature granulocytes in the presence of G-CSF. First, we retrovirally transduced an empty pMXs vector or pMXs vectors, each containing SBPc-tagged Trib1 and SBPc-tagged Trib2. After selection with puromycin, we obtained puromycin-resistant cells from each trasfectant. To exclude bias among the clones in each transfectant, we did not separate out the clones but utilized puromycin-resistant whole cell populations ; each cell population was designated as 32D/Mock, 32D/Trib1, and 32D/Trib2, respectively. We initially examined the expression levels of Trib1 and Trib2 by Western blotting with anti-SBPc mAb. As shown in Fig. 1A, SBPc-tagged Trib1 and Trib2 were abundantly expressed in 32D/Trib1 and 32D/Trib2 cells. Also, RQ-PCR analysis showed that Trib1 and Trib2 were highly expressed in 32D/Trib1 and 32D/Trib2, respectively, while their expression was hardly detected in 32D/Mock cells (Fig. 1B, C).

First, we examined the effect of Trib1 and Trib2 on the growth of 32D cells in the presence

of IL-3. However, the growth of 32D/Trib1 and 32D/Trib2 cells was almost identical to that of 32D/Mock cells, indicating that Trib1 and Trib2 do not influence cell proliferation in the presence of IL-3 (Fig. 1D). Next, we analyzed the effects of Trib1 and Trib2 on the survival of 32D

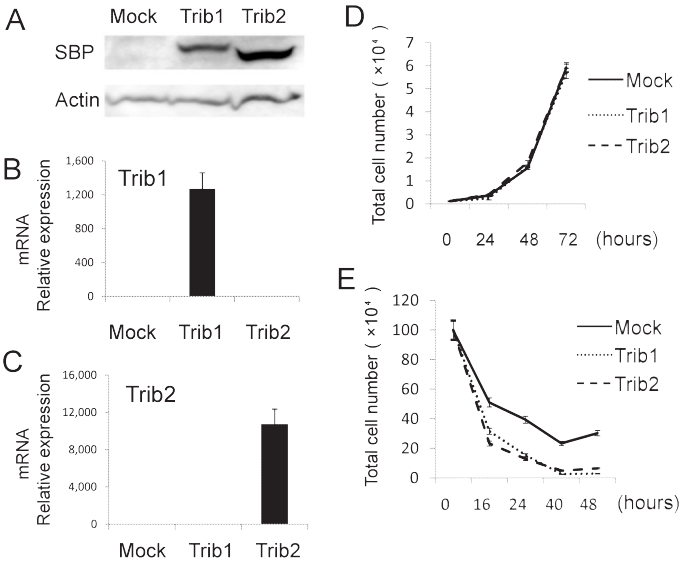

Fig. 1 Effect of Trib1 and Trib2 on cell proliferation and cell death.
32D cells were retrovirally transfected with SBPc-tagged-Trib1, SBPc-tagged-Trib2, or pMXs-IRES-puro control vector. Then, retrovirus-transduced cells were selected by culture with puromycin. (A) The expression of Trib1 and Trib2 protein was analyzed by Western blot analysis with anti-SBP Ab. Anti-β-actin blotting was used for the loading control. (B), (C) The expression levels of Trib1 and Trib2 were measured in the indicated cells by quantitative RT-PCR analysis. (D) The growth of the indicated cells in the presence of IL-3 was assessed by an ATP assay at the indicated time points. (E) After deprivation of IL-3, the indicated cells were cultured without cytokine, and total viable cell numbers were assessed by an ATP assay at the indicated time points. Representative data from three independent experiments are shown as the mean $\pm$ SD of triplicate cultures.

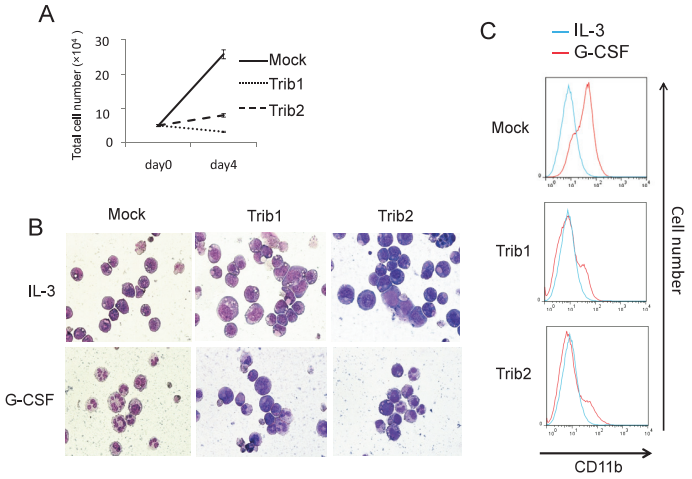

Fig. 2 Effect of Trib1 and Trib2 on G-CSF-induced granulocytic differentiation.
32D/Mock, 32D/Trib1 and 32D/Trib2 cells were cultured with G-CSF. (A) To analyze the proliferation, the cell number of each transformants was determined by ATP assay at the indicated time points. (B) After 6-day culture, morphological analysis was performed by staining cytospin preparations with May-Grunwald-Giemsa. (C) The expression of CD11b was assessed by flow cytometric analysis after 4-day culture with G-CSF. Representative data from three independent experiments are shown.

cells after IL-3 deprivation. As shown in Fig. 1E, total viable cell number of 32D/Trib1 and 32D/Trib2 cells decreased more rapidly than 32D/Mock cells, indicating that both Trib1 and Trib2 promote factor-deprived apoptosis in 32D cells.

Trib1 and Trib2 block granulocytic differentiation

Next, we examined the effects of Trib1 and Trib2 on G-CSF-induced granulocytic differentiation. As shown in Fig. 2A, although 32D/Mock cells gradually proliferated in the presence of G-CSF, 32D/Trib1 and 32D/Trib2 cells hardly proliferated in this condition, indicating that growth and/or survival signals from G-CSF were interrupted by Trib1 and Trib2. Then, we performed morphologic analysis using these cells. Interestingly, cytospin preparations of these cells showed that 32D/Trib1 and 32D/Trib2 cells were bigger than 32D/Mock cells in the culture with IL-3 (Fig. 2B, upper panels), whereas there was no difference in IL-3-dependent growth among these transfectants (Fig. 1D). After 6-day culture with G-CSF, 32D/Mock cells underwent apparent granulocytic differentiation, while 32D/Trib1 and 32D/Trib2 cells remained undifferentiated (Fig. 2B, lower panels). In accord with these morphological findings, G-CSF-induced CD11b expression was suppressed on 32D/Trib1 and 32D/Trib2 cells compared to that on 32D/Mock cells (Fig. 2C). Together, these results indicate that both Trib1 and Trib2 also block G-CSF-induced granulocytic differentiation of 32D cells in our system, as previously reported.[32]

C/EBPα restoration is not sufficient to abolish Trib1- and Trib2-induced differentiation arrest

In the previous paper, it was shown that degradation of C/EBPα was an important mechanism for Trib1 and Trib2 to block granulocytic differentiation,[32] so we examined the effect of Trib1 and Trib2 on the expression level of C/EBPα in our 32D cells. As shown in Fig. 3A, the expression of C/EBPα was apparently reduced in 32D/Trib1 and 32D/Trib2 in both forms (p42 and p30). To further investigate the significance of C/EBPα degradation in Trib1- or Trib2-mediated differentiation arrest, we transfected the bicistronic retroviral expression vector for wild-type C/EBPα and GFP and the control (CTL) GFP expression vector into 32D/Mock, 32D/Trib1, and 32D/Trib2 cells. After transfection, we sorted GFP-positive cells to isolate doubly transfected 32D cells (each transfectant was designated as 32D/Mock/CTL, 32D/Mock/C/EBPα, 32D/Trib1/CTL, 32D/Trib1/C/

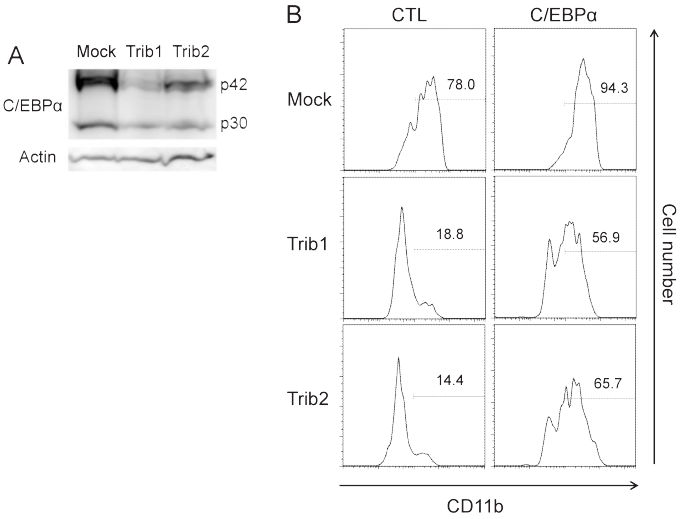

Fig. 3 Roles of C/EBPα in differentiation block caused by Trib1 and Trib2.
(A) The expression of C/EBPα protein was assessed by Western blot analysis in 32D/Mock, 32D/Trib1, and 32D/Trib2. Anti-β-actin blotting was used as the loading control. (B) The expression vector for C/EBPα or an empty control vector containing the expression cassette for GFP was further transfected into 32D/Mock, 32D/Trib1, and 32D/Trib2 cells. The indicated double-transfected cells were sorted from GFP-positive fractions and cultured with G-CSF for 4 days. Then, the expression of CD11b was assessed by flow cytometric analysis. Presented numbers in the histograms indicate percentage of CD11b-positive cells. Representative data from three independent experiments are shown.

EBPα, 32D/Trib2/CTL, and 32D/Trib2/C/EBPα, respectively). As shown in Fig. 3B, when these cells were cultured with G-CSF, the expression of CD11b was enhanced on 32D/Mock/C/EBPα cells as compared with 32D/Mock/CTL cells, indicating that overexpressed C/EBPα enhanced G-CSF-induced granulocytic differentiation. In contrast, the expression of CD11b was induced by G-CSF at a very low level on 32D/Trib1/CTL and 32D/Trib2/CTL cells, the levels of which were almost the same as those observed on their parental cells (32D/Trib1 and 32D/Trib2, Fig. 2C). In contrast, CD11b expression was substantially induced by G-CSF on 32D/Trib1/C/EBPα and 32D/Trib2/C/EBPα cells. However, their expression levels (56.9% on 32D/Trib1/C/EBPα and 65.7% on 32D/Trib2/C/EBPα cells) were significantly lower than those on 32D/Mock/CTL (78.0%) and 32D/Mock/C/EBPα cells (94.3%). Together, these results indicate that overexpressed C/EBPα cannot completely abolish the differentiation block caused by Trib1 or Trib2, and raised the possibility that an additional mechanism besides C/EBPα degradation underlies this differentiation block. This possibility is supported by the previous finding that disruption of C/EBPα by itself does not cause AML in mice.[20–22]

Trib1 and Trib2 suppressed G-CSF-induced Akt phosphorylation

To identify the additional mechanism, we analyzed the effects of Trib1 and Trib2 on G-CSF signaling. For this purpose, we stimulated 32D/Mock, 32D/Trib1 and 32D/Trib2 cells with G-CSF and examined the changes in the phosphorylation of Akt and ERK up to 30 min. As shown in Fig. 4A, G-CSF-induced Akt phosphorylation was suppressed in 32D/Trib1 and 32D/Trib2 cells compared with 32D/Mock cells, while ERK phosphorylation was similar or rather enhanced in these cells. Densitometric analysis showed that the relative levels of G-CSF-induced Akt phosphorylation at 15 min were 17% and 20% in 32D/Trib1 and 32D/Trib2 cells, respectively, compared with that in 32D/Mock cells (Fig. 4B). This result prompted us to speculate that the decreased activation of Akt might be involved in the differentiation block by Trib1 and Trib2.

Pharmacological inhibition of Akt blocked G-CSF-induced granulocytic differentiation of 32D cells

To examine the hypothesis that Trib1 and

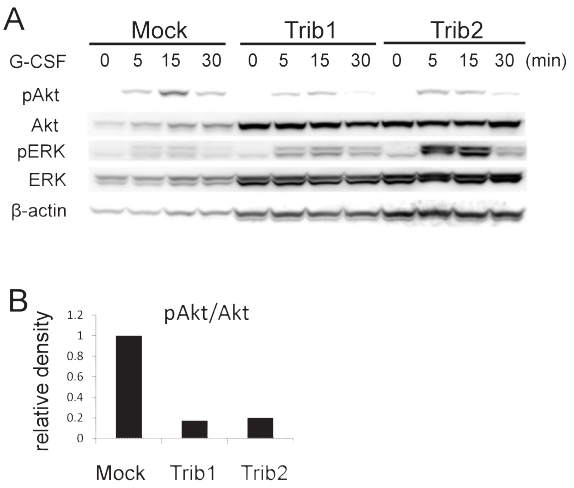

Fig. 4 Effects of Trib1 and Trib2 on G-CSF signaling
(A) 32D/Mock, 32D/Trib1, 32D/Trib2 cells were deprived of IL-3 for 12 hours, and then stimulated with G-CSF for the indicated periods. The kinetics of phosphorylation of Akt and ERK caused by G-CSF ligation was assessed by Western blot analysis. Blotting with anti-Akt Ab and anti-ERK Ab was used as loading controls. (B) Relative density of phosphor-Akt and Akt at 15 min was measured by a densitometer. Representative data from three independent experiments are shown.

Trib2 might inhibit G-CSF-induced granulocytic differentiation through the reduced phosphorylation of Akt, we took advantage of a highly specific Akt inhibitor, Akt/protein kinase B signaling inhibitor-2 (API-2), also known as Triciribine[31]. We cultured 32D cells with API-2 or DMSO (as a control) in the presence of IL-3 or G-CSF. After 6-day culture with G-CSF, morphological analysis of the cytokine preparations showed that API-2 but not DMSO inhibited G-CSF-induced granulocytic differentiation in 32D cells (Fig. 5A). Also, flow cytometric analysis revealed that G-CSF-induced CD11b expression was apparently impaired in API-2-treated 32D cells compared with DMSO-treated cells. This result indicates that Akt activation is required for G-CSF-induced granulocytic differentiation in 32D cells (Fig. 5B). Then, we further examined whether API-2-treated 32D cells were alive and remained undifferentiated with potential to grow. As a result, when these cells were washed and resuspended in culture medium containing IL-3 without API-2, they proliferated again, indicating that API-2 induced neither apoptosis nor terminal differentiation, which abolish the growth potential of cells (data not shown). To examine whether C/

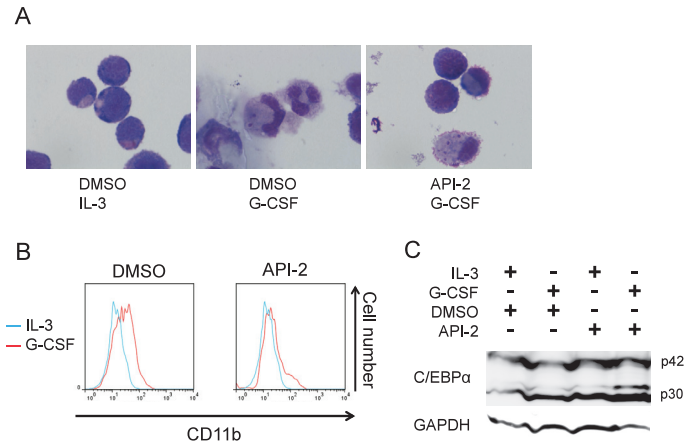

Fig. 5 Effects of an Akt Inhibitor on G-CSF-induced granulocytic differentiation
32D cells were treated with an Akt inhibitor, Akt/protein kinase B signaling inhibitor-2 (API-2), or DMSO in culture with IL-3 or G-CSF. (A) After 6-day culture under the indicated conditions, cultured cells were subjected to cytospin preparations and stained with May-Grunwald-Giemsa for morphological analysis. (B) CD11b expression was assessed by flow cytometric analysis after 4-day culture under the indicated conditions. (C) 32D cells cultured under the indicated conditions for 4 days were assessed for C/EBPα protein expression by Western blot analysis. GAPDH is a protein loading control. Representative data from three independent experiments are shown.

EBPα degradation was involved in API-2-induced differentiation block in 32D cells, we examined the expression level of C/EBPα by Western blot analysis. As shown in Fig. 5C, the expression of C/EBPα was maintained in API-2-treated 32D cells at almost similar levels to that in DMSO-treated 32D cells, indicating that API-2 inhibited G-CSF-induced granulocytic differentiation through another pathway, independent of C/EBPα degradation.

Discussion

In the present study, we initially found that neither Trib1 nor Trib2 influenced IL-3-dependent growth of 32D cells. Also, neither molecule conferred factor-independent growth on 32D cells but enhanced their factor-deprived apoptosis. In addition, in accordance with the previous report,[32] both Trib1 and Trib2 inhibited G-CSF-induced granulocytic differentiation. In this report,[32] Trib1 and Trib2 were shown to degrade C/EBPα and thereby inhibit granulocytic differentiation. Furthermore, it was shown that the restoration of C/EBPα was sufficient to abolish the Trib2-mediated differentiation block,[16] indicating that degradation of C/EBPα was solely responsible for this block. However, in this study,[16] the authors utilized an estrogen-inducible form of C/EBPα, that is, a chimera consisting of C/EBPα and estrogen receptor, C/EBPα-ER. This ER-inducible system is quite artificial in the context of C/EBPα transcriptional activation.[16] Thus, it may exert unexpected effects unrelated to the proper function of C/EBPα and affect cell growth and differentiation, so in the present study, we used the expression vector for wild-type C/EBPα. Although we restored C/EBPα expression in our experiment, neither Trib1- nor Trib2-induced differentiation block was completely abolished. From this result, we speculated that an additional mechanism is involved in this differentiation block, so we investigated the effects of Trib1 and Trib2 on G-CSF signaling and found that G-CSF-induced phosphorylation of Akt was suppressed by Trib1 and Trib2, while that of ERK was hardly affected. Although there are no data about the interaction between Trib family members and Akt in hematopoietic cells, murine Trib2 and Trib3 were reported to interact with Akt and inhibit its activity in adipocytes and hepatocytes, respectively,[33,34] so Trib1 and Trib2 might directly interact with Akt and inhibit its phosphorylation in a similar manner.

Other groups previously showed that both Trib1 and Trib2 possess pro-apoptotic activity in hematopoietic cells.[19,35,36] Also, in the current study, we found that overexpressed Trib1 and Trib2 enhanced factor-deprived apoptosis in

32D cells. Because Akt is well known to play an important role in protecting a variety of cells from apoptosis, enhanced apoptosis caused by Trib1 and Trib2 might be explained by Akt suppression. Meanwhile, it was recently demonstrated that Trib1 gene disruption resulted in a lack of eosinophils and increase of neutrophils.[37] Inversely, Akt activation was reported to inhibit eosinophil differentiation and promote granulocytic maturation.[38] Together with our finding that Trib1 and Trib2 inhibit Akt phosphorylation, these results suggest a possible interaction between Trib1/Trib2 and Akt in the development of normal eosinophils and neutrophils as well as in the pathogenesis of AML.

As for the roles of Akt in the pathogenesis of AML, Akt has been reported to be constitutively activated in a substantial proportion of AML cases and is considered to be a good therapeutic target. In contrast, decreased Akt phosphorylation is observed in a specific type of AML cells, in which enhanced Akt activation promoted their differentiation. Also, nuclear localization of Foxo3a caused by decreased Akt phosphorylation was shown to be important for the maintenance of leukemia-initiating cells in a murine model.[26] On the other hand, for the roles of the PI3K/Akt pathway in normal granulopoiesis, it was reported that the inhibition of PI3K or Akt with LY294002 or 1L-6-Hydroxymethyl-chiro-inositol 2-(R)-2-O-methyl-3-O-octadecylcarbonate (HIMO) blocked G-CSF-induced granulocytic differentiation in human cord blood $CD34^+$ cells.[38] These data are largely consistent with our data that the pharmacological inhibition of Akt by API-2 blocked G-CSF-induced granulocytic differentiation. Importantly, we further found that the expression of C/EBPα was maintained during this inhibition, indicating that API-2 inhibited granulocytic differentiation independently of C/EBPα degradation. However, another group also showed that C/EBPα is phosphorylated and suppressed by GSK-3 and that Akt abrogated this inhibitory phosphorylation and induced granulocytic development.[38] Thus, there is a possibility that Trib1 and Trib2 may inhibit granulocytic differentiation not only by degrading C/EBPα but also by modulating its phosphorylation status through the suppression of Akt activity.

We here showed that Trib1 and Trib2 inhibited G-CSF-induced granulocytic differentiation by degrading C/EBPα and, at least partially, by suppressing Akt phosphorylation. However, further studies are required to fully understand the molecular mechanism of Trib1- and Trib2-induced AML, which would provide useful information to establish a new therapeutic strategy.

Conclusion

Trib1 and Trib2 inhibit G-CSF-induced granulocytic differentiation by degrading C/EBPα and, at least partially, by suppressing Akt phosphorylation.

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