PBK/TOPK mediates promyelocyte proliferation via Nrf2-regulated cell cycle progression and apoptosis

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Abstract. Acute myeloid leukemia (AML) is a disorder involving hematopoietic stem cells, characterized by blockage of hematopoietic cell differentiation and an increase in clonal neoplastic proliferation. AML is associated with poor patient outcome. PBK/TOPK is a protein kinase derived from PDZ-binding kinase (PBK)/T-lymphokine-activated killer (T-LAK) cell-originated protein kinase (TOPK). Previous studies have shown that PBK/TOPK is expressed in hematologic tumors. In the present study, we aimed to investigate the role of PBK/TOPK in promyelocyte proliferation and to clarify the molecular mechanism. PBK/TOPK knockdown (KD) significantly decreased cell proliferation and viability in the NB4 and HL-60 promyelocytes. PBK/TOPK KD resulted in G2/M cell cycle arrest that attributed to a decrease in cdc2 and cyclin B expression. In addition, PBK/TOPK KD caused apoptosis, as evidenced by activation of the mitochondrial apoptotic pathway and an increase in TUNEL-positive cells. PBK/TOPK KD induced mitochondrial dysfunction and ROS generation, and inhibition of mitochondrial dysfunction and ROS production suppressed PBK/TOPK KD-induced cell cycle arrest and apoptosis. Moreover, PBK/TOPK KD decreased Nrf2 expression and ARE-binding activity. Overexpression of Nrf2 inhibited the PBK/TOPK KD-induced decrease in cdc2 and cyclin B expression and cell cycle arrest, and blocked ROS production and apoptosis. Based on literature and our results, it was demonstrated that Nrf2 may be a crucial regulator that mediates PBK/TOPK-exerted promotion of cell proliferation. PBK/TOPK stabilizes Nrf2, strictly regulates the ROS level, promotes cell cycle progression and inhibits apoptosis, contributing to the proliferation of promyelocytes. Our results provide new insights into the molecular mechanism of PBK/TOPK-mediated promyelocyte proliferation and shed light on the pathogenesis of AML.

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Abbreviations: AML, acute myeloid leukemia; APL, acute promyelocytic leukemia; ARE, antioxidant response element; CysA, cyclosporine; KD, knockdown; NAC, N-acetylcysteine; Nrf2, nuclear factor (erythroid-derived 2)-like 2; PBK, PDZ-binding kinase; ROS, reactive oxygen species; TOPK, T-lymphokine-activated killer (T-LAK) cell-originated protein kinase; TUNEL, terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling

Key words: PBK/TOPK, acute myeloid leukemia, proliferation, promyelocytes, Nrf2

Introduction

Acute myeloid leukemia (AML) is a disorder of hematopoietic stem cells, accompanied by obstruction in hematopoietic cell differentiation and increased clonal neoplastic proliferation (1). Acute promyelocytic leukemia (APL) is a subtype of AML, characterized by a block of granulocytic differentiation and accumulation of promyelocytes in the bone marrow and blood (2). APL is believed to be one of the most fatal forms of AML with poor patient outcomes (2). Despite extensive investigations, the pathogenesis of AML is still far from being completely understood (3).

PBK/TOPK is a protein kinase derived from PDZ-binding kinase (PBK)/T-lymphokine-activated killer (T-LAK) cell-originated protein kinase (TOPK) (4,5). Although PBK/TOPK was originally cloned differentially by Gaudet *et al* (4) and Abe *et al* (5), the sequences of both genes were later found to be the same. PBK/TOPK is a 322 amino acid serine-threonine kinase that is reported to be expressed in proliferative cells and tissues and to play an important role in spermatogenesis (6). It has been found that PBK/TOPK is expressed in hematologic tumors such as leukemia, lymphoma and myeloma, and its expression is correlated with the malignant potential of these tumors (7-9). In interphase cells, TOPK is expressesed in the cytosol and nucleus without any significant association with microtubule networks (10). During mitosis, expression of PBK/TOPK was found to be upregulated and phosphorylated (4,10). In addition, PBK/TOPK acts as a substrate of cdc2/cyclin B and it possesses a possible phosphorylation site by cdc2, S/T-PX-K/R, at N-terminus (4,10). Once phosphorylated at Thr-9, PBK/TOPK functions to play an important

role in the formation of spindle midzone and in cytokinesis. Compared with non-transformed cells, PBK is expressed at high levels in tumor cell lines (11). PBK/TOPK was shown to promote tumor cell proliferation through activation of p38 MAPK activity (5,11) and regulation of the DNA damage response (11). Moreover, PBK/TOPK expression was found to be decreased during tetradecanoyl phorbol acetate-induced HL-60 leukemic cell differentiation.

In the present study, we aimed to investigate the role of PBK/TOPK in regulating the proliferation of promyelocytes and the possible mechanism. The results showed that knockdown of PBK/TOPK inhibited the proliferation of promyelocytes, induced G2/M cell cycle arrest and apoptosis. Downregulation of nuclear factor (erythroid-derived 2)-like 2 (Nrf2) was identified as being responsible for PBK/TOPK KD-induced G2/M cell cycle arrest, apoptosis and inhibition of cell proliferation.

Materials and methods

Chemicals and materials. α-tubulin and Nrf2 antibodies were purchased from Santa Cruz Biotechnology Inc. (Santa Cruz, CA, USA). Cleaved caspase-3 and -9, cdc2 and cyclin B antibodies were purchased from Cell Signaling Technology (Danvers, MA, USA). DCFH-DA, propodium iodide (PI) and trypan blue were procured from Sigma. All of the other chemicals used were of the highest grade available commercially.

Cell culture. The human APL NB4 and HL-60 cell lines were maintained in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS), 100 U/ml penicillin and 100 mg/ml streptomycin (Gibco, Grand Island, NY, USA) in a humidified incubator at 37˚C with 5% CO_2.

Transfection of lentivirus and plasmids. The PBK/TOPK-RNAi-lentivirus was constructed by Shanghai GeneChem Co., Ltd. (Shanghai, China). NB4 and HL-60 cells were transduced with the PBK/TOPK-RNAi-lentivirus and purified by puromycin treatment. Transduction efficiency of the cells was examined for expression of PBK/TOPK by real-time PCR. In some experiments, cells were transfected with the plasmid vector or plasmid expressing Nrf2 (Shanghai GeneChem) using Lipofectamine 2000 reagent (Invitrogen) according to the manufacturer's instructions.

Analysis of cell cycle distribution. For cell cycle analysis, cells were centrifuged for 5 min at 1,000 rpm. The pellet was resuspended in cold phosphate-buffered saline (PBS) containing 200 μg/ml RNAse A, and kept on ice. Five minutes before the analysis, NP-40 and PI (Sigma) were added at a final concentration of 0.1% and 50 μg/ml, respectively. DNA content was measured in the FL-2 channel using flow cytometry monitored by CellQuest software (BD Biosciences).

Cell proliferation and viability. Cell proliferation and viability were determined by trypan blue exclusion and 3-(4,5-dimethylthiazoyl-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assays, respectively. For trypan blue determination, the cells were cultured in a 24-well plate. To monitor cell growth at intervals, attached cells were removed from quadruplicate wells using trypsin-ethylenediaminetetraacetic acid, and the viable cells were counted in a hemocytometer by trypan blue exclusion. For MTT evaluation, the cells were seeded into 96-well plates. After the treatment, the cell viability was determined by the MTT assay. In brief, the supernatant was discarded, and the cells were rinsed with PBS. After that, the cells were treated with 0.5 mg/ml MTT (dissolved in water and filtered through a 0.2-mm membrane) at 37˚C. Four hours later, the formazan crystals were dissolved in DMSO, and the absorption values were determined using a Bio-Rad microplate reader.

Measurement of apoptosis. Cell apoptosis was determined by terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) assay according to the manufacturer's instructions (Roche). Positive apoptotic cells were counted and the results are shown as a fold of the control.

Oxygen consumption rate. After the treatment, mitochondrial function was assessed by determination of the oxygen consumption rate. In brief, after the treatment, mitochondria were isolated from cells using a commercial kit (Thermo Fisher Scientific). Oxygen consumption rate was measured with a Clark oxygen electrode and is expressed as a percentage of the oxygen consumption.

ROS determination. After the experiment, cells were harvested and resuspended in serum-free medium. DCFH-DA was added to a final concentration of 10 μM and cells were cultured at 37˚C for 30 min in the dark. Subsequently, the cells were washed three times and analyzed using flow cytometry in the FL-1 channel. The ROS level is expressed as a percentage of the control.

Reporter gene assay. Cells were transfected with pGL6-ARE-luciferase (Beyotime Institute of Biotechnology, Haimen, China) and *Renilla* TK (Promega) plasmids using a transfection reagent (TurboFect; Thermo Fisher Scientific). After the experiments, the cells were harvested in passive lysis buffer (Promega), and the reporter assay was performed using the Dual-luciferase reporter assay system (Promega). Firefly luciferase activity was normalized to *Renilla* luciferase and is shown as a ratio of relative light units.

RNA isolation and real-time polymerase chain reaction. In some experiments, total RNA was isolated using a commercial RNA isolation kit (Tiangen Biotech Co., Ltd., Beijing, China) according to the manufacturer's protocols. Subsequently, the concentration of total RNA was determined and then RNA was reverse-transcribed to cDNA using a cDNA synthesis kit (Takara). The samples were analyzed by real-time polymerase chain reaction (PCR) and 1 μl of cDNA was amplified with SYBR Premix Ex Taq (Takara). The results were analyzed using the Bio-Rad RT-PCR System for quantitative evaluation.

Western blot analysis. Briefly, after the treatment, the cells were lysed with cell lysis buffer (50 mM Tris-HCl, pH 8.0, 150 mM NaCl, 1% Triton X-100, 1 mM EDTA, 10 mM NaF, 1 mM Na_3VO_4, and protease inhibitor cocktail) on ice for 30 min. After centrifugation at 20,000 x g for 20 min at 4˚C, the protein contents were determined by the BCA

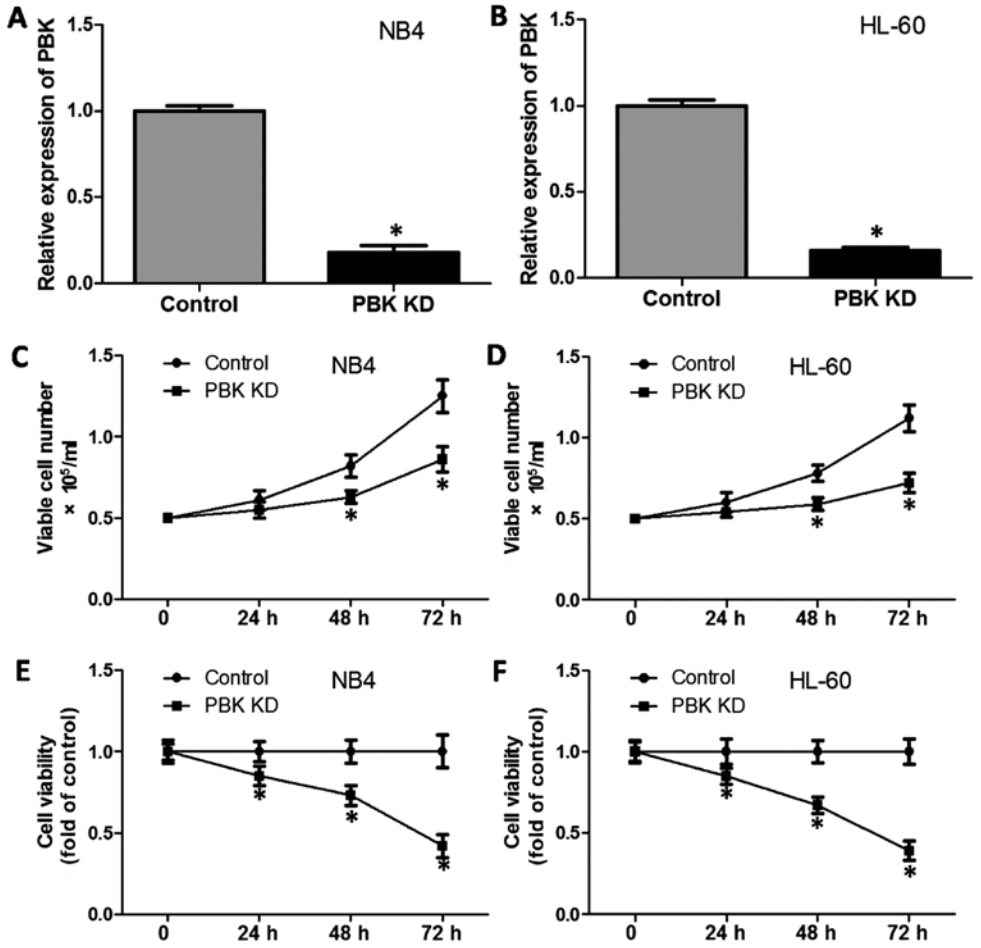

Figure 1. Effect of PBK/TOPK knockdown on cell viability and proliferation of promyelocytes. Promyelocyte NB4 and HL-60 cells were transfected with control vectors or the lentivirus shRNA targeting PBK/TOPK. (A and B) Efficiency of PBK/TOPK knockdown (KD) was evaluated by the measurement of mRNA expression using real-time PCR. (C and D) Cell growth was detected by trypan blue staining. (E and F) Cell viability was determined by MTT assay and expressed as a fold of the control. *P<0.05, compared with the control.

assay kit (Pierce Biotechnology, Inc., Rockford, IL, USA). After boiling for 5 min in a 2X SDS loading buffer, 20 μg of total proteins were subjected to SDS-PAGE, and transferred onto a PVDF membrane. Then, the membrane was blocked and probed with the indicated primary antibodies overnight at 4˚C. After washing for 4 times, the membrane was incubated in the appropriate horseradish peroxidase-conjugated secondary antibody at 37˚C for 30 min. The protein bands were visualized using chemiluminescent reagents according to the manufacturer's instructions and quantified using an image analyzer Quantity One System (Bio-Rad Laboratories Inc., Richmond, CA, USA).

Statistical analysis. Results are expressed as the means ± SD. The statistical analysis was performed by GraphPad Prism software. Statistical analysis was carried out by one-way ANOVA followed by Newman-Keuls multiple-comparison post hoc test. A P-value <0.05 was considered to indicate a statistically significant result.

Results

PBK/TOPK knockdown decreases the cell growth and viability of the promyelocytes. In the present study, NB4 and HL-60 cells were used as *in vitro* models of APL, to investigate the role of PBK/TOPK in the proliferation of promyelocytes. NB4 and HL-60 cells were transduced with the PBK/TOPK-RNAi-lentivirus. Through purification by puromycin treatment, we established cell lines with stable low expression of PBK/TOPK using the NB4 and HL-60 cell lines. Fig. 1A and B shows that the interference effectively decreased PBK/TOPK expression. Then, we evaluated the effect of the knockdown (KD) of PBK/TOPK on cell growth and viability. As shown in Fig. 1C and D, after 48-72 h of incubation, PBK/TOPK KD significantly decreased the proportion of viable cells. Consistently, during a 72-h incubation, PBK/TOPK KD significantly decreased the cell viability in the NB4 and HL-60 cells. After culture for 72 h, PBK/TOPK KD significantly decreased the cell viability to <50% of the respective control (Fig. 1E and F).

PBK/TOPK knockdown induces G2/M cell cycle arrest in the promyelocytes. To test the effect of PBK/TOPK KD on cell cycle progression, cell cycle distribution was analyzed. As reflected in Fig. 2A and B, in both NB4 and HL-60 cells, PBK/TOPK KD decreased the percentage of the cell population in the G0/G1 phase and S phase, and significantly increased the percentage of cell population in G2/M. The results indicated that

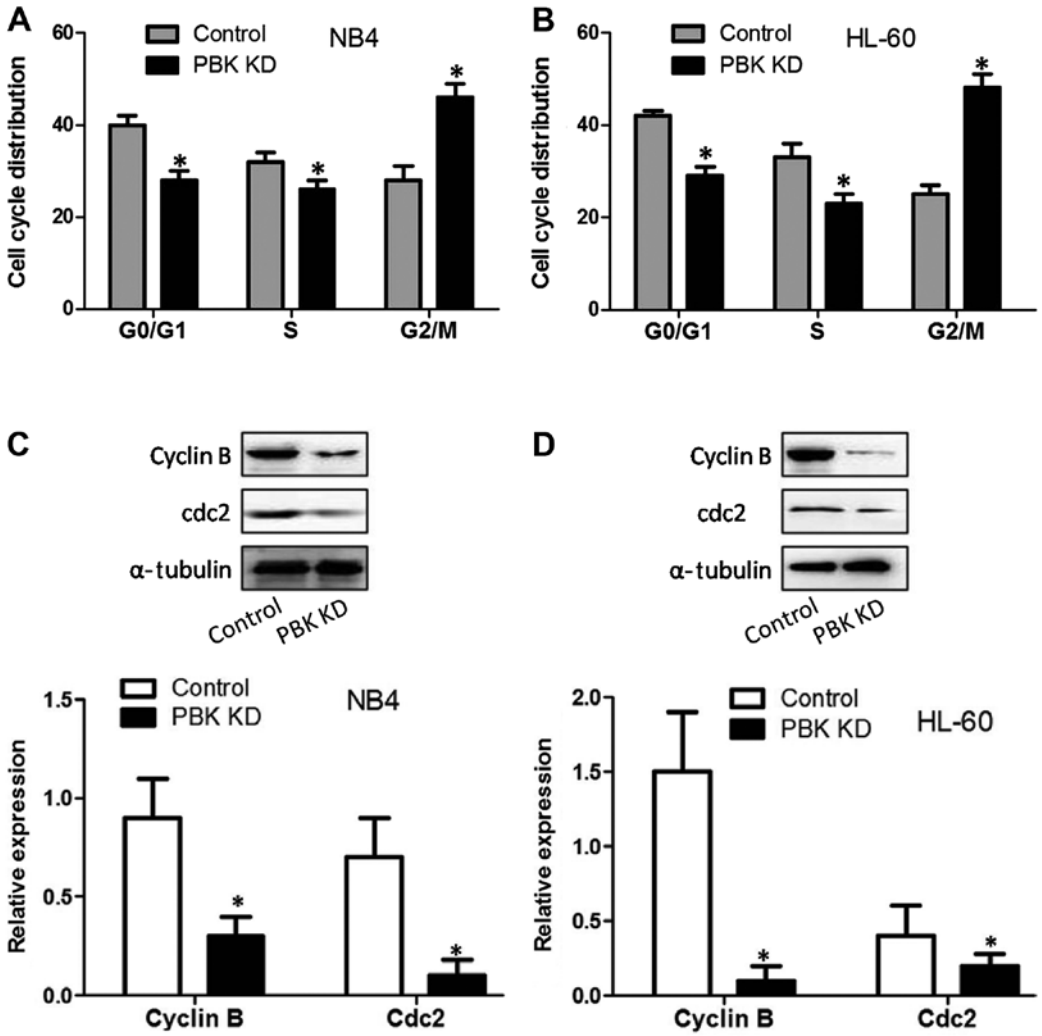

Figure 2. Effect of PBK/TOPK knockdown on cell cycle distribution in the promyelocytes. Promyelocyte NB4 and HL-60 cells were transfected with control vectors or the lentivirus shRNA targeting PBK/TOPK. (A and B) Effect of PBK/TOPK knockdown (KD) on cell cycle distribution was measured by flow cytometric analysis. (C and D) Effect of PBK/TOPK KD on Cdk2 and cyclin B expression was assessed by western blot analysis. $^{*}P<0.05$, compared with the respective control.

PBK/TOPK KD resulted in G2/M cell cycle arrest of the NB4 and HL-60 cells. We next assessed the effect of PBK/TOPK KD on cdc2 and cyclin B expression which are checkpoints of G2/M cell cycle progression. The results showed that in the NB4 and HL-60 cells, PBK/TOPK KD significantly decreased cdc2 and cyclin B expression (Fig. 2C and D).

PBK/TOPK knockdown induces the apoptosis of the promyelocytes. To test the effect of PBK/TOPK KD on apoptosis, TUNEL assay was conducted. As shown in Fig. 3A and B, in both the NB4 and HL-60 cells, PBK/TOPK KD notably increased the proportion of TUNEL-positive cells, indicating the occurrence of apoptosis. Moreover, we assessed the effect of PBK/TOPK KD on molecular cascades in the HL-60 cells. As illustrated in Fig. 3C, Bax mRNA expression was markedly enhanced by PBK/TOPK KD. In addition, PBK/TOPK KD markedly reduced Bcl-2 mRNA expression in the HL-60 cells (Fig. 3D). Furthermore, KD of PBK/TOPK in the HL-60 cells resulted in a significant increase in the cleavage of caspase-3 and -9 (Fig. 3E).

PBK/TOPK knockdown induces mitochondrial dysfunction and ROS generation in promyelocytes. Research has shown that apoptosis is closely associated with mitochondrial dysfunction and reactive oxygen species (ROS) generation. To evaluate the possible role of the mitochondrial pathway in PBK/TOPK KD-enhanced apoptosis, mitochondrial function and the ROS level were determined. As illustrated in Fig. 4A, PBK/TOPK KD in the HL-60 cells resulted in a significant decrease in oxygen consumption ability, as evidenced by decreased oxygen consumption. Moreover, PBK/TOPK KD markedly increased DCFH-DA fluorescence in the HL-60 cells, indicating elevation in the ROS level (Fig. 4B). To test the role of mitochondrial dysfunction and ROS generation in PBK/TOPK KD-induced inhibition of proliferation of the promyelocytes, NB4 and HL-60 cells with PBK/TOPK KD were incubated with cyclosporine (CysA, a mitochondrial protective agent) or N-acetylcysteine (NAC, a potent antioxidant). The results showed that in the presence of CysA and NAC, the inhibitory effect of PBK/TOPK KD on cell growth in the NB4 and HL-60 cells was significantly suppressed

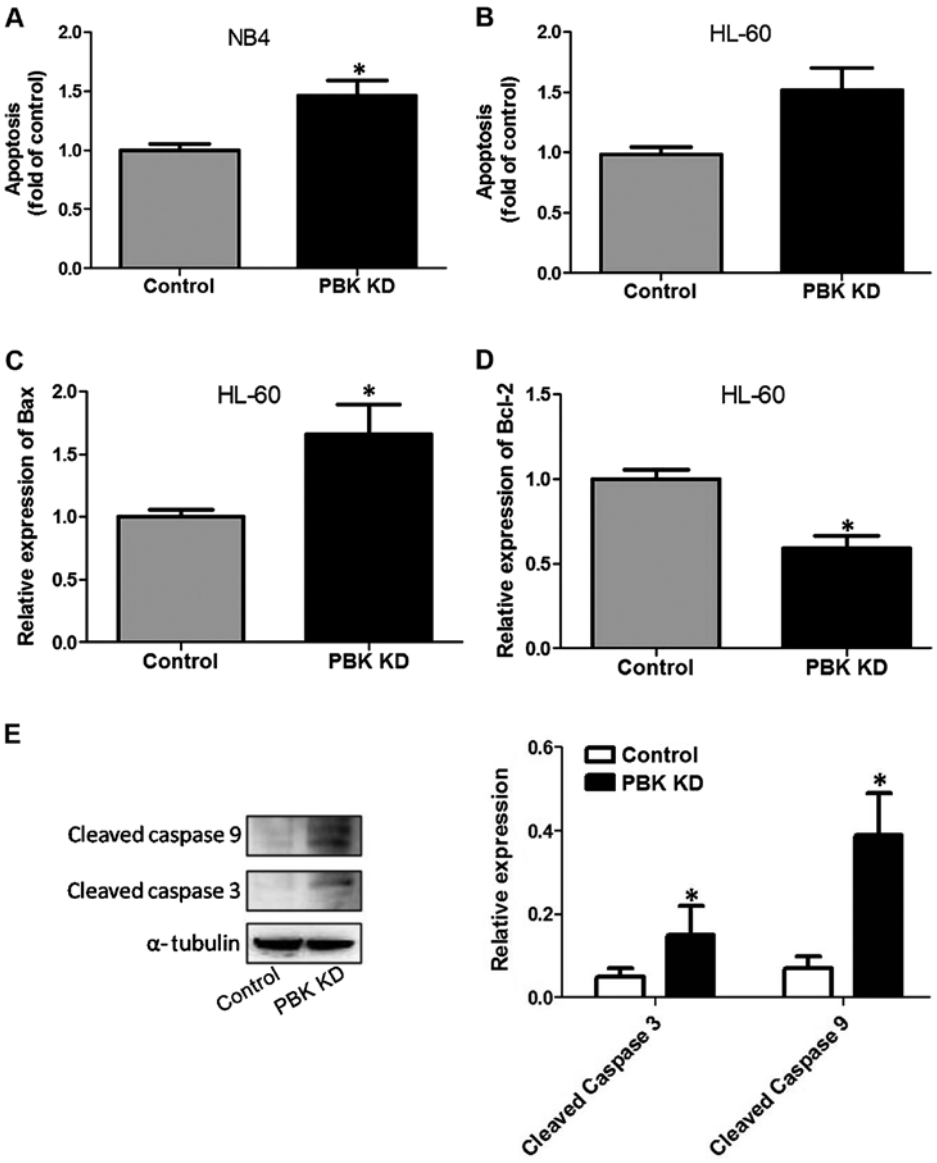

Figure 3. Effect of PBK/TOPK knockdown on apoptosis of the promyelocytes. Promyelocyte NB4 and HL-60 cells were transfected with control vectors or the lentivirus shRNA targeting PBK/TOPK. (A and B) Effect of PBK/TOPK knockdown (KD) on apoptosis was determined by TUNEL assay, and apoptosis was expressed as a fold of the control. (C and D) Effect of PBK/TOPK KD on Bax and Bcl-2 expression in HL-60 cells was determined by real-time PCR. (E) Effect of PBK/TOPK KD on cleavage of caspase-3 and -9 in HL-60 cells was determined by western blot analysis. *P<0.05, compared with the control.

(Fig. 4C and D). PBK/TOPK KD-induced G2/M cell cycle arrest was significantly blocked following treatment of CysA and NAC, as evidenced by a decreased proportion of G2/M phase cells and increased proportion of G0/G1 and S phase cells (Fig. 4E and F). In addition, the increase in TUNEL-positive cell numbers induced by PBK/TOPK KD was notably prohibited by CysA and NAC incubation (Fig. 4G and H).

Role of the reduction in Nrf2 expression and activity in PBK/TOPK KD-induced inhibition of proliferation of the promyelocytes. We next evaluated the effect of PBK/TOPK KD on the expression and activity of Nrf2, an important upstream redox director. The results showed that in both NB4 and HL-60 cells, PBK/TOPK KD notably decreased the protein expression of Nrf2 (Fig. 5A and B). Reporter gene assay was conducted to evaluate the effect of PBK/TOPK KD on Nrf2 transcription activity. As shown in Fig. 5C and D, PBK/TOPK KD significantly decreased ARE-luciferase activity in the NB4 and HL-60 cells, indicating the reduction of Nrf2 transcription activity.

To test the role of the reduction in Nrf2 transcription activity in PBK/TOPK KD-induced inhibition of proliferation of promyelocytes, NB4 and HL-60 cells with PBK/TOPK KD were transfected with a plasmid expressing Nrf2. The results showed that overexpression of Nrf2 significantly suppressed PBK/TOPK KD-induced decreased cell growth, as evidenced by an increase in the viable cell numbers compared with that of the PBK/TOPK KD cells (Fig. 6A). In addition, the effect of the overexpression of Nrf2 on cell cycle checkpoints and distribution was determined. As shown in Fig. 6B, compared with that of the PBK/TOPK KD cells, overexpression of Nrf2 significantly increased cdc2 and cyclin B protein expression. Overexpression of Nrf2 notably decreased the proportion of G2/M phase cells and increased the proportion of G0/G1 and

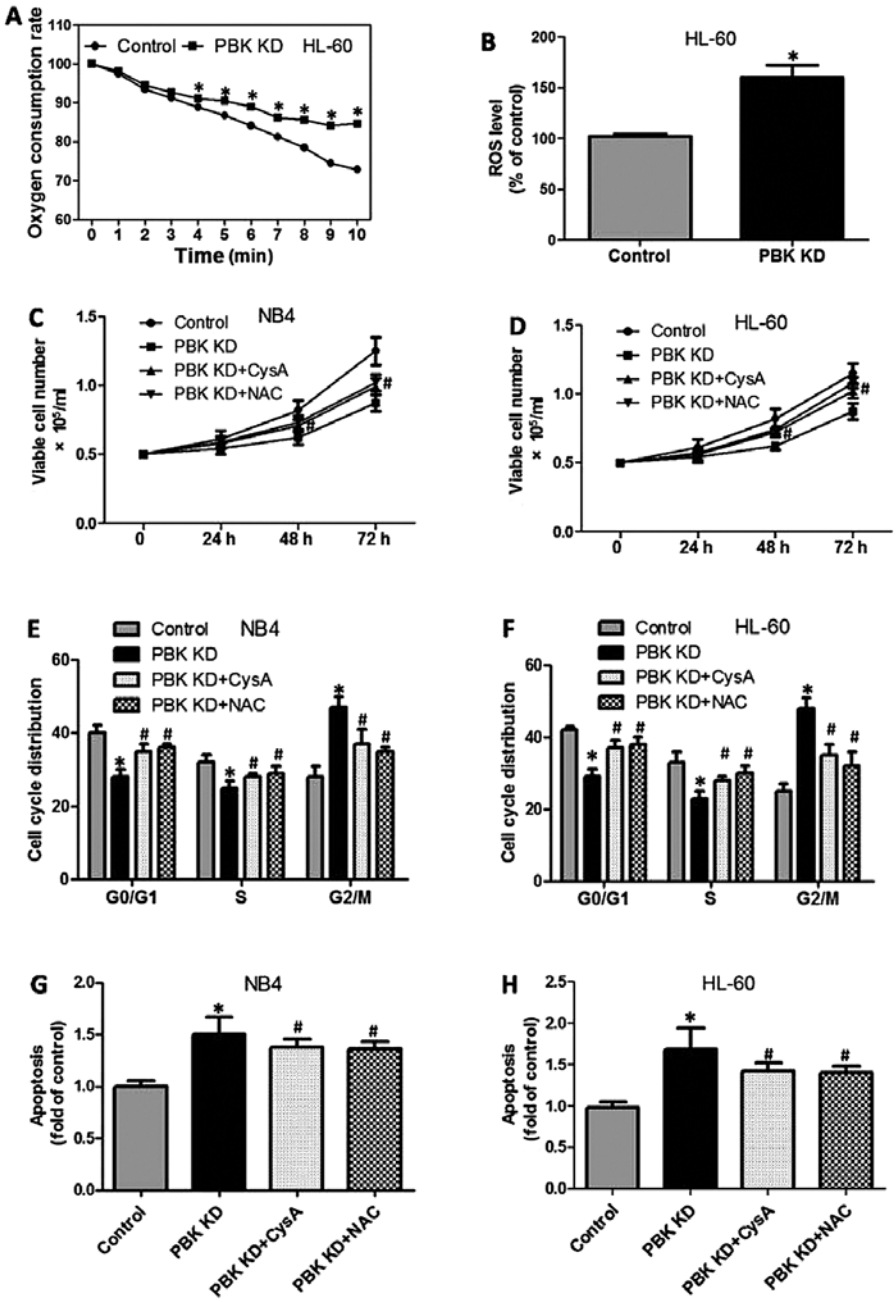

Figure 4. Role of mitochondrial dysfunction and ROS generation in the effect of PBK/TOPK knockdown on the promyelocytes. Promyelocyte NB4 and HL-60 cells were transfected with control vectors or the lentivirus shRNA targeting PBK/TOPK. (A) Effect of PBK/TOPK knockdown (KD) on mitochondrial function in HL-60 cells was evaluated by determination of oxygen consumption (n=3). (B) Effect of PBK/TOPK KD on ROS generation in HL-60 cells was determined using DCFH-DA and is expressed as a percentage of the control (n=3). (C and D) NB4 and HL-60 cells transfected with the lentivirus shRNA of PBK/TOPK were treated with cyclosporine A (CysA, a mitochondrial protective agent) or N-acetylcysteine (NAC). Cell growth was then determined by trypan blue staining (n=5). (E and F) Cell cycle distribution was measured (n=3). (G and H) Apoptosis was determined by TUNEL staining. $^{*}P<0.05$, compared with the control. $^{\#}P<0.05$, compared with PBK/TOPK KD (n=3).

S phase cells (Fig. 6C). Furthermore, the increase in ROS level induced by PBK/TOPK KD was suppressed by overexpression of Nrf2 (Fig. 6D). PBK/TOPK KD-induced apoptosis was inhibited by enhancement of Nrf2 expression (Fig. 6E).

Discussion

Results from our and other laboratories have shown that PBK/TOPK is pivotal for proliferation and malignant transformation of hematologic tumors (7-9). In the present study, we examined the molecular mechanism of PBK/TOPK-mediated proliferation and viability of hematologic cells. We showed that PBK/TOPK KD significantly inhibited cell proliferation and viability in the promyelocytes.

Cell cycle progression is essential for cell proliferation and growth and is controlled by a series of cyclin-cdk complexes. cdc2/cyclin B is a complex controlling G2/M cell cycle transition (12,13). Previous studies have shown that PBK/TOPK is a substrate of cdc2/cyclin B and facilitates mitosis (4,10). Our results showed that PBK/TOPK KD significantly resulted

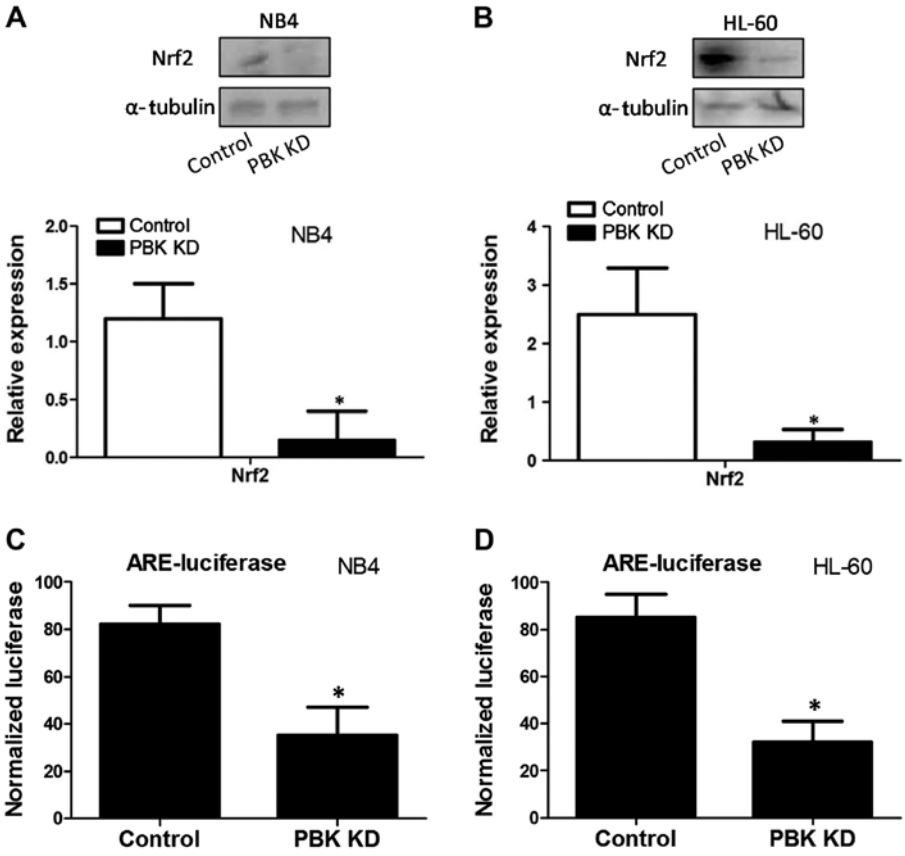

Figure 5. Effect of PBK/TOPK knockdown on Nrf2 in promyelocytes. Promyelocyte NB4 and HL-60 cells were transfected with control vectors or the lentivirus shRNA targeting PBK/TOPK. (A and B) Effect of PBK/TOPK knockdown (KD) on Nrf2 expression was evaluated by western blot analysis. (C and D) Effect of PBK/TOPK KD on Nrf2 transcription activity was determined by measurement of ARE luciferase acitivity. *P<0.05, compared with the control (n=3).

in G2/M cell cycle arrest in promyelocytes, as reflected by a notable increase in the G2/M phase cell proportion. Moreover, the results showed that PBK/TOPK KD contributed to the reduction in cdc2/cyclin B expression, which may be responsible for G2/M cell cycle arrest. These results demonstrated that PBK/TOPK and cdc2/cyclin B complex may form a feedback circle in the regulation of cell cycle progression and thus cell proliferation in promyelocytes (Fig. 7).

Apoptosis, also called programmed cell death, is a common pathway for cell death which is inhibited in tumor development (14,15). In the present study, we also tested the effect of PBK/TOPK KD on apoptosis. The results showed that PBK/TOPK KD significantly increased Bax expression, decreased Bcl-2 expression, promoted cleavage of caspase-3 and -9, and increased apoptotic cell death in the NB4 and HL-60 cells. These results indicated that potential blunting of the apoptotic pathway may be involved in the PBK/TOPK-mediated cell proliferation in promyelocytes.

ROS are an important stimuli of the activation of the apoptotic pathway under both physiological and pathological conditions (16). In cells, ROS are mainly generated by the mitochondria (17,18) and the generation of ROS contributes to mitochondrial damage, release of pro-apoptotic molecules and the activation of caspase cascades (19,20). Mitochondrial dysfunction has been implicated in cellular senescence mainly by promoting oxidative damage-induced cell cycle arrest (21). In addition, mitochondrial ROS generation may influence cell cycle checkpoints resulting in cell cycle arrest (22). In the present study, we evaluated the effect of PBK/TOPK KD on mitochondrial function and ROS generation. The results showed that PBK/TOPK KD significantly increased ROS production and reduced mitochondrial function. Moreover, we used the CysA, a mitochondrial protective agent (23,24), and NAC, a potent antioxidant (25), to test the role of ROS production and mitochondrial dysfunction in PBK/TOPK KD-induced inhibition of proliferation in promyelocytes. The results showed that CysA and NAC significantly inhibited PBK/TOPK KD-induced G2/M cell cycle arrest, apoptosis and blockage of cell proliferation in promyelocytes. These results demonstrated that mitochondrial dysfunction and ROS generation were involved in the PBK/TOPK KD-induced G2/M cell cycle arrest, apoptosis and inhibition of proliferation of promyelocytes (Fig. 7).

Antioxidant response element (ARE)-mediated transcription of cytoprotective genes is essential for cellular protection against excessive ROS generation and related disorders (26,27). With ARE-binding activity, Nrf2 serves as a sensor and director of ROS insult and redox balance (28). In addition, a large amount of evidence supports that Nrf2 is a tumor promoter that promotes oncogenesis under certain circumstances. For example, overexpression of several oncogenes in mice led to increased Nrf2 transcription, increased

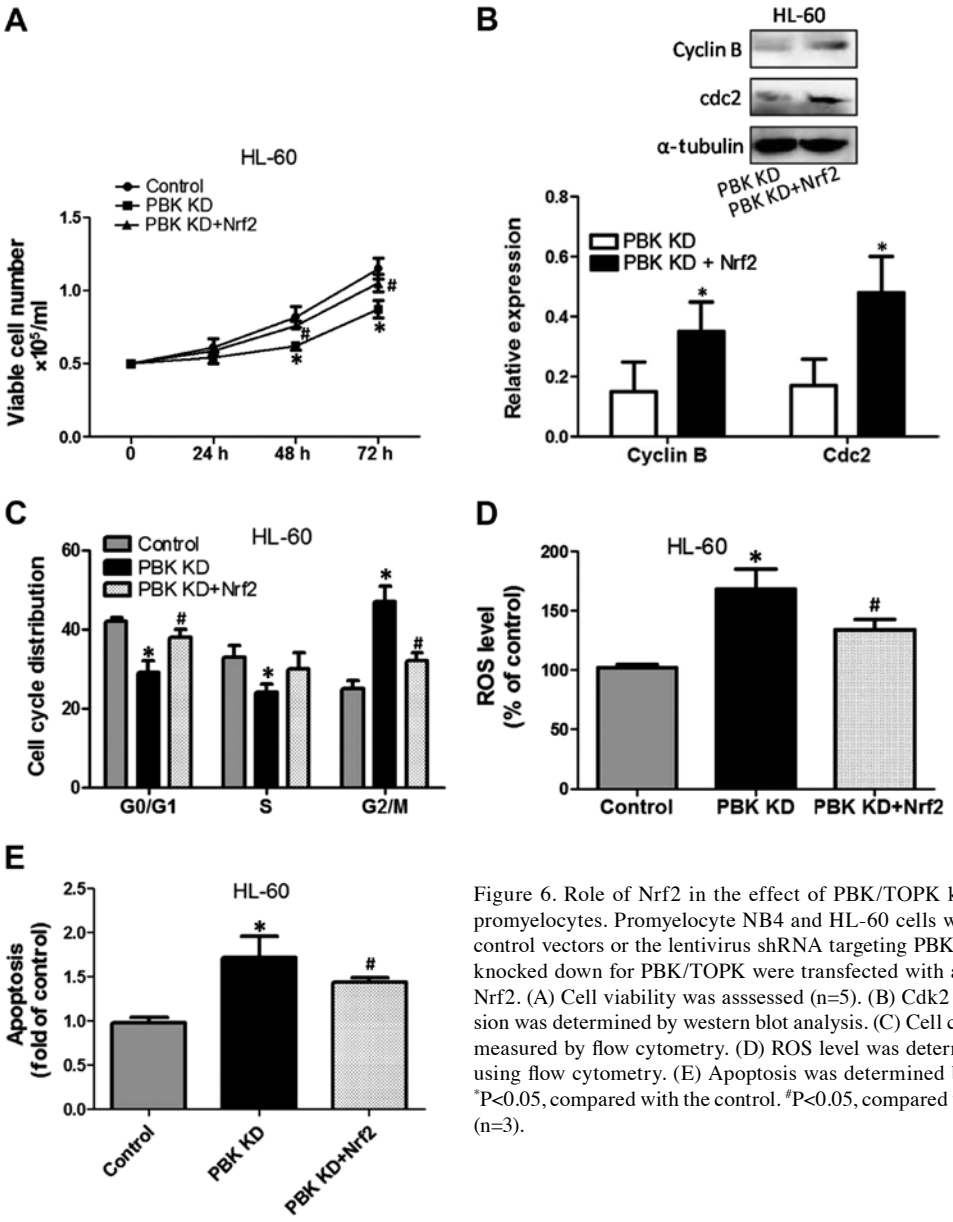

Figure 6. Role of Nrf2 in the effect of PBK/TOPK knockdown (KD) on promyelocytes. Promyelocyte NB4 and HL-60 cells were transfected with control vectors or the lentivirus shRNA targeting PBK/TOPK. HL-60 cells knocked down for PBK/TOPK were transfected with a plasmid expressing Nrf2. (A) Cell viability was asssessed (n=5). (B) Cdk2 and cyclin B expression was determined by western blot analysis. (C) Cell cycle distribution was measured by flow cytometry. (D) ROS level was determined by DCFH-DA using flow cytometry. (E) Apoptosis was determined by TUNEL staining. *P<0.05, compared with the control. #P<0.05, compared with PBK/TOPK KD (n=3).

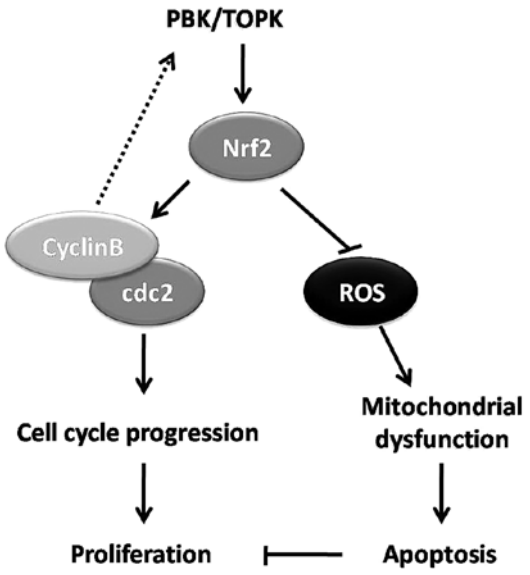

Figure 7. Possible mechanism underlying PBK/TOPK-regulated proliferation of promyelocytes.

basal expression of Nrf2, and decreased ROS, leading to oncogenesis (29). It has also been reported that Nrf2 upregulates transcription of anti-apoptotic genes encoding Bcl-2 and Bcl-xL (30). Moreover, the cell cycle is considered to be a redox cycle which is regulated by ROS and Nrf2 (31). It is noted that Nrf2 activity is upregulated in several types of leukemia where it contributes to leukemogenesis (32). In AML, ROS usually mediate the cytotoxic effect of therapeutic agents (33). Thus, high basal nuclear expression of Nrf2 in AML reduces sensitivity to proteasome inhibitors (33). In the present study, we showed that PBK/TOPK KD caused a decrease in Nrf2 expression and its binding activity with ARE. On the one hand, a decrease in Nrf2 and activity resulted in an increase in ROS generation, which contributed to elevation of the ROS level and activation of the mitochondrial apoptotic pathway. On the other hand, downregulation of Nrf2 decreased cdc2 and cyclin B expression, leading to G2/M cell cycle arrest. However, whether cdc2 and cyclin B were regulated by Nrf2 itself or ROS indirectly and how did PBK/TOPK regulate Nrf2 remain to be elucidated.

Based on previous literature and our results, it was demonstrated that Nrf2 may be a crucial regulator that mediates PBK/TOPK-exerted promotion of cell proliferation. PBK/TOPK stabilizes Nrf2, strictly regulates the ROS level, promotes cell cycle progression and inhibits apoptosis, contributing to proliferation of promyelocytes. Our results provide new insights into the molecular mechanism of PBK/TOPK-mediated promyelocyte proliferation and the pathogenesis of AML.

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