RESEARCH COMMUNICATION

PBK/TOPK Expression During TPA-Induced HL-60 Leukemic Cell Differentiation

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Abstract

Objective: This study concerns expression of PBK/TOPK during differentiation of HL-60 leukemic cells induced by tetradecanoyl phorbol acetate (TPA). Methods: Wright-Giemsa staining was performed to observe morphological changes in the HL-60 cells, and flow cytometry was used to assess the cell cycle and CD11b, CD14, CD13, and CD33 expression. PBK/TOPK levels were determined by Western blot analysis. Results: After treating HL60 cells with 5.1 × 10^{-9} mmol/L of TPA for three days, the number of nitroblue-tetrazolium-positive cells and CD11b, CD13, and CD14 expression increased, whereas the PBK/TOPK levels decreased. Conclusions: TPA can inhibit proliferation and induce differentiation of HL60 cells of the granulocytic or monocytic lineage. PBK/TOPK expression was downregulated during this process, whereas the Pho-PBK/TOPK expression was increased.

Keywords: HL-60 cells - PBK/TOPK - differentiation - TPA

Asian Pacific J Cancer Prev, **13**, 2145-2148

Introduction

Leukemia is a malignant tumor that poses a serious threat to human life, especially young people. Different drugs open up new methods of hematologic cancer treatment, and further research on the mechanisms behind the disease offers the hope for finding a complete cure. Recent studies show that signal transduction pathways are involved in abnormal tumors (Nandi et al., 2004; Liu et al., 2011). PBK/TOPK is a protein kinase from PDZ-binding kinase (PBK)/T-lymphokine-activated killer (T-LAK) cells. PBK was cloned from HeLa cells in 2000 by Gaudet et al. (2000), whereas the T-LAK cell-originated protein kinase (TOPK) was cloned by Abe et al. (2000). The sequences of both genes were later found to be the same. PBK/TOPK contains 322 amino acids and is an important signal that mediates the cytotoxic function of T and T-LAK cells. LAK cells have cytotoxic effects on tumor cells, whereas T-LAK cells express membrane-associated lymphocytotoxin. When T-LAK cells are inactivated, the lymphocyte toxins disappear.

PBK/TOPK has NXXXT, and its aspartic acid replaces the first serine or threonine. It has no catalytic activity. Thus, the phosphorylation of an individual amino acid residue, such as the ninth tyrosine, is needed to provide the catalytic activity (Simons-Evelyn et al., 2001). PBK/TOPK is mainly expressed in human testicular, placental, myocardial, and pancreatic tissues and is overexpressed in various cases of Burkitt's lymphoma and one case of leukemia (Gaudet et al., 2000). In this study, we used acute myeloblastic HL-60 cells to explore the role of PBK/TOPK in the cell differentiation mechanism induced by the phorbol ester, tetradecanoyl phorbol acetate (TPA).

Materials and Methods

Cell culture

HL-60 cells were cultured in RPMI1640 medium, which contains 100 mL/L of inactivated serum, at 37 °C in a 50% CO_2 incubator. The cells were subcultured every two to three days, and cells in the logarithmic growth phase were used for the experiment.

Nitroblue tetrazolium (NBT) reduction positive rate

The control and HL-60 cells were treated with 5.1 nmol/L of TPA for three days, centrifuged, collected, and then resuspended in the medium at a cell density of 1 × 10^6 cells/L. Thereafter, 1 g/L of NBT and 100 μg/L of TPA were added into the suspension, and the cultures were incubated at 37 °C for another 1 h and centrifuged to collect the cell smear. Wright-Giemsa staining was then performed. Approximately 200 cells immersed in oil were observed under a microscope, and the cytoplasms of the NBT-positive cells were found to have blue-purple particles. Finally, the positive-cell rate was calculated.

Determination of the cell cycle

The HL-60 cells (1×10^6) before and after TRA

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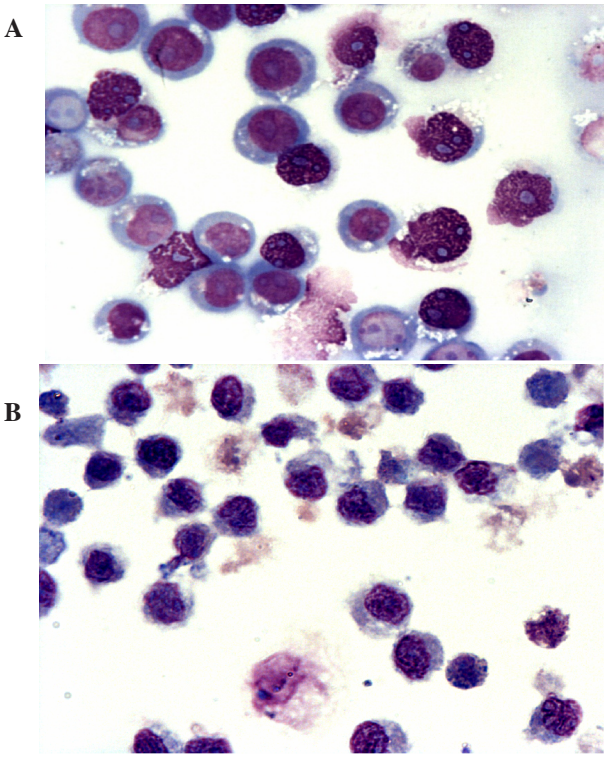

Figure 1. A) HL-60 Cell Untreated by Drug; B) HL-60 Cell Treated by 5.1 nmol/L TPA for Three Days

treatment were collected, suspended, rinsed twice with PBS, and centrifuged to remove the supernatant. Then, 1 mL of PBS and 2 mL of absolute ethyl alcohol were added and fixed for 1 h. Subsequently, the cells were rinsed and immersed in a DNA-Prepstain staining solution. The fluorescent intensity of the cell cycle was detected using a flow cytometer (BD Company, Germany) and statistically analyzed using the DNA Multi Cycle software.

Detection of cell membrane surface antigen

The experimental and control groups were prepared from 100 μL of living cell suspensions (1×10^6 cells). Thereafter, anti-mouse antibodies CD11b, CD13, and CD14 were added into the suspensions and blocked with 10, 20, 20, and 20 μL of CD33-FTTC fluorescent working liquid labeled as mAb and 5 μL of normal rabbit serum. The samples were then mixed thoroughly and placed in the dark for 30 min. The cells were washed twice with DPBS (50 mL/L of FCS and 2 g/L of NaN3) for 5 min, the supernatants were removed, and the cells were resuspended in 500 μL of formaldehyde (20 g/L) before flow cytometry. The CD11b, CD13, CD33, and CD14 FTTC fluorescent labeled monoclonal antibodies were purchased from Immunotech Company (USA).

Western blot analysis

The total protein content was extracted using T-PER (Pierce, USA) and quantified using the BCA assay kit (Pik-day Biotechnology Co., Ltd.). The proteins were transferred into a film and blocked after electrophoresis. The PBK/TOPK primary antibody (1:800, Cell Signal Technology Company, USA) was then added for hybridization, followed by the secondary antibody goat anti-rabbit IgG (Pierce, USA). Subsequently, electrochemiluminescence detection was conducted using

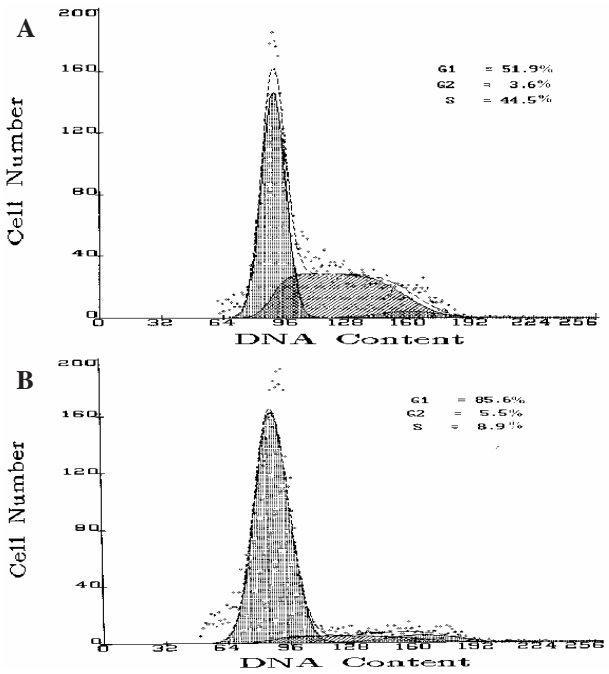

Figure 2. A) Cell Cycle of HL-60 Untreated by Drug; B) Cell Cycle of HL-60 Treated by 5.1 nmol/L TPA for Three Days

the SuperSignal® West Dura Extended Duration Substrate. The film was developed and fixed after it was exposed in the darkroom, and the optical density of the film was scanned using a gel scanner. The experiment was repeated thrice, and the average was calculated.

Statistical analysis

The results are presented as mean ± standard deviation ($\bar{x} \pm s$). One-way ANOVA was used to detect the differences among the experimental groups using the SPSS 16.0 statistical software, and differences of $P < 0.05$ were considered statistically significant.

Results

Cell morphology

Three days after TPA induction, the HL-60 cell volumes and nucleus-to-cytoplasm ratios decreased. The nucleoli either became fuzzy or completely disappeared, whereas the nuclear chromatins appeared dense. The nuclear morphologies were distorted, folded, or segmented, and the cytoplasmic morphologies also tended to change (Figures 1A, 1B).

Positive rate of NBT reduction

Three days after TPA induction, the positive rate of the NBT reduction of the HL-60 cells in the experimental group increased to 39.12% ± 4.87%, which was significantly different from that of the control group at 3.67% ± 1.08% ($P < 0.05$).

Cell cycle

Three days after TPA induction, the percentage of the experimental group cells in the G1 stage increased from 51.9% to 85.6%, those in the control group decreased from 44.5% to 8.9% (Figures 2A, 2B).

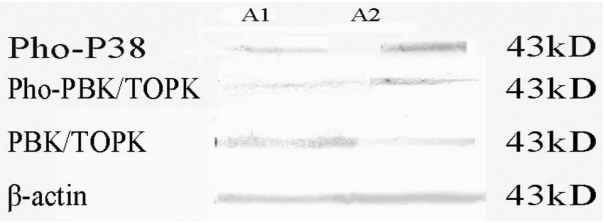

Figure 3. PBK/TOPK, p38 Protein Expression

Table 1. Cell Surface Antigen Expression Changes of HL-60 Cells Induced by TPA for 3 Days ($\bar{x} \pm s$, n=3)

Group	CD11b	CD13	CD14	CD33
Control	10.9±0.27	6.9±0.21	18.9±0.24	98.5±0.70
Treatment	96.4±1.75*	53.7±1.30*	71.1±1.49*	97.0±1.36

Vs control group, *P<0.05

Cell membrane surface antigen

The CD11b, CD13, and CD14 expression rates in the control group cells were 10.9%, 6.9%, and 18.9% respectively. Three days after TPA induction, these rates significantly increased to 96.4%, 53.7%, and 71.1%, respectively. However, CD33 had no statistically significant changes before and after the treatment (Table 1).

Western blot analysis

Western blot analysis showed that the PBK/TOPK expression decreased three days after TPA induction, whereas the phosphorylated Pho-PBK/TOPK and Pho-p38 expressions slightly increased (Figure 3).

Discussion

PBK/TOPK mRNA expression was not detected in normal peripheral blood cells. However, high levels of PBK/TOPK mRNA expression can be found in GA-10 Burkitt's lymphoma lines, human myeloid white blood cells (HL-60), and breast cancer (MDA321), colon cancer (Caco-2), leiomyosarcoma, thymic carcinoma (TC797), renal cell carcinoma (RC298), and Ewing tumor cells (Simons-Evelyn et al., 2001). PBK/TOPK mRNA expressions have also been detected in normal adult testicular, placenta, myocardial, and pancreatic tissues. The highest level of PBK/TOPK expression can be found in the placenta. Meanwhile, low PBK/TOPK expression levels have been detected in skeletal muscle, kidney, liver, and lung tissues (Gaudet et al., 2000). PBK/TOPK can effectively distinguish bile duct epithelial cells from liver cells. Thus, it is expected to be a new tumor-specific marker for bile duct epithelial cells (He et al., 2010).

The PBK/TOPK expression in a cell cycle is mainly regulated by E2F, CREB/ATF, and PBK/TOPK promoter-specific loci binding (Nandi and Rapoport, 2006). The PBK/TOPK-histone H3 signaling pathway can promote mitotic progression and breast cancer cell proliferation (Park et al., 2006).

Cancer is usually caused by the aberrant differentiation of normal cells, excessive proliferation, and inhibition of apoptosis (Köbel et al., 2005; Liu et al., 2011; Nakaya et al., 2012). Studies have found that some cellular signal transduction pathways are closely related with tumorigenesis, where the MAPK signaling pathways play an important role and become an important prognostic factor and drug targets (Drew et al., 2012). MAPK is activated through a series of cellular phosphorylation cascades. Different extracellular signals stimulate different signal transduction pathways, which then activate MAPK through Ras-dependent or Ras-independent mechanisms, forming the MAPKKK-MAPKK-MAPK cascade. ERK1/2 is usually activated by the phosphorylation of growth factors through the Ras-Raf-MEK or TPA-mediated PKC-Raf-MEK pathways (Reddy et al., 2003). JNK/SAPK, p38, and BMK1/ERK5 can also be activated by different kinds of signals inside and outside the cell, such as UV light, oxidative stress, and inflammatory factors (Dong et al., 2001; Raviv et al., 2004). The activated MAPK enters the nucleus, where it regulates the expression of a series of transcription factors, such as ELK1, SAP-1, c-Fos, c-Myc, ATF-2, NF-kappaB, and HSF-1 (Obata et al., 2000; Meinhardt et al., 2002; Ayllon and O'connor, 2007). These transcription factors, in turn, regulate cell proliferation, differentiation, and apoptosis, thus playing important roles in angiogenesis and tumor cell adhesion, invasion, and metastasis.

PBK/TOPK promotes the growth of tumor cells by stimulating effective tumor-cell repair mechanisms and participating in the activation of DNA damage repair mechanisms.

The mechanisms of leukemic cell differentiation are still unclear as it involves the effect of a differentiation agent on the cell membrane, cytoplasm, and nucleus, as well as gene activity, expression, and regulation (Macfarlane and Manzel, 1994; Yamamoto et al., 2000; Beltran et al., 2006).

The proliferation capacity of tumor cells is closely related to their degree of differentiation. Poorly differentiated cells are often more active than well-differentiated ones. Thus, the purposes of tumor therapy can be achieved by reducing cell proliferation and inducing differentiation in adult cells. Nandi et al. (2004) found that PBK/TOPK protein expression was reduced significantly within 24 h after HL-60 cells differentiated into mononuclear macrophages via TPA induction. In the current study, we used TPA to induce HL-60 cell differentiation in vitro and observed that the number of NBT-positive cells increased. Moreover, cell scatter reverted to growth adhesion, and the CD11b, CD13, and CD14 expressions increased. The cells were blocked in the S phase, hence inhibiting cell proliferation and promoting cell differentiation. The PBK/TOPK expression decreased, whereas the Pho-PBK/TOPK and Pho-P38 expressions slightly increased.

The mechanism for the TPA-induced directional differentiation of HL-60 cells into granulocyte or mononuclear cells is unclear. Previous studies have found that HL-60 differentiation is usually related to a change in the expression of receptors on the cell surface, which regulated the cell differentiation (Cho et al., 2005; Huang et al., 2006). In addition, the tumor suppressor gene p27 is also involved in cell proliferation and differentiation by inhibiting cell-cycle protein kinase activity and its

own ectopic expression. PBK/TOPK can induce the phosphorylation of p38 MAP kinase in COS cells, but not that of ERK or JNK. Both PBK/TOPK expression and c-Myc phosphorylation are obviously related to malignant tumors in blood, as PBK/TOPK participates in the regulation of malignant tumor cell proliferation (Cote et al., 2002; Yuryev and Wennogle, 2003).

In conclusion, this study showed that PBK/TOPK protein expression occurs in the germinal center of active B lymphocytes. Moreover, PBK/TOPK activity reduced significantly in the terminal differentiation of HL-60 cells. However, the phosphorylated Pho-PBK/TOPK and Pho-P38 expression slightly increased.

Acknowledgements

The author(s) declare that they have no competing interests.

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