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Dimethylsulfoxide induces upregulation of tumor suppressor protein PTEN through nuclear factor-κB activation in HL-60 cells

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Abstract

Dimethylsulfoxide (DMSO) has been known to differentiate HL60 cells into neutrophil like cells. Here, we provide an evidence for the involvement of tumor suppressor PTEN, an antagonist of phosphatidylinositol 3-kinase (PI3K) in the DMSO-induced differentiation of HL60 cells. DMSO upregulated PTEN with unaffecting the expression of PI3K. The upregulation of PTEN by DMSO lead to the decrease of Akt phosphorylation, a downstream of PI3K. The DMSO-induced upregulation of PTEN might be mediated by NF-κB activation, which was evidenced by the blockage of DMSO-induced PTEN upregulation with an NF-κB inhibitor, pyrrolidine dithiocarbamate (PDTC).

Keywords: Dimethylsulfoxide; PTEN; Leukemic cell differentiation; NF-κB

1. Introduction

Dimethylsulfoxide (DMSO) is a simple amphipathic molecule, with a polar domain and two non-polar groups. Although its biological effects have not been clearly defined, DMSO is still used in a variety of fields, and is commonly used as a cryoprotectant of cultured cells, as well as has a solvent for hydrophobic compounds in biological studies. Recently, DMSO has been used as a convenient cryoprotectant for stem cells in their transplantation using peripheral or umbilical cord blood. It is also widely used as a vehicle for drug therapy for various diseases, including interstitial cystitis, dermatological disorders, gastrointestinal disorders, amyloidosis, pulmonary adenocarcinoma, brain edema, schizophrenia, chronic prostatitis and rheumatologic disorders [1]. In particular, DMSO has been used in the treatment of cancer for several decades, and leukemia cells lose their proliferative properties when treated with it as a cellular differentiation agent [1,2]. Furthermore, DMSO can arrest the cell cycle of several human lymphoid cell lines at the G_1 phase [3,4]. Thereby, it is likely that DMSO could be used as a drug in the differentiation therapy for leukemia. However, to our knowledge, there have only been a few previous reports explaining the DMSO-induced differentiation mechanism of leukemia cells [5,6].

The novel tumor suppressor gene, *PTEN/MMAC1*, on the chromosome 10q23 protein product (PTEN), is a lipid phosphatase that controls a variety of cellular functions, including cell growth, cell survival and the immune reaction [7–9]. Mutations of PTEN have been found in cancers, and are associated with their invasiveness and metastatic properties [8,10].

Abbreviations: PI 3-kinase, phosphoinositol 3-kinase; PIP_3, phosphoinositol 3,4,5-trisphosphate; NF-κB, nuclear factor-kappaB; PTEN/MMAC, phosphatase and tensin homologue/mutated in multiple advanced cancers; PDTC, pyrrolidine dithiocarbamate

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Recently, it has been shown that PTEN expression is associated with neuronal differentiation [11,12], and in particular, the PTEN induced G_1 phase cell cycle arrest in several cell lines [13,14]. HL60 cells, a human promyelocytic leukemic cell line, control the progression of its cell cycle through the activation of the phosphatidylinositol 3-kinase (PI3K)/Akt signaling pathway [15,16]. Therefore, there is a possibility that DMSO, an HL60 differentiation agent, could affect the PI3K/Akt signaling pathway.

Taken together, these findings suggest that PTEN could be a target for DMSO in leukemia cells. The present study was designed to evaluate whether DMSO controls PTEN expression, and investigate the mechanism of DMSO in the control PTEN expression in HL60 cells.

2. Materials and methods

2.1. Materials

The anti-PTEN monoclonal goat antibody was purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA) and the phospho-AKT (p-AKT) polyclonal rabbit antibody from Cell Signaling Technology (Minneapolis, MN, USA). Pyrrolidine dithiocarbamate (PDTC) was obtained from Calbiochem (Germany).

2.2. Cell culture

The human promyelocytic leukemic cell line, HL60 cells, were obtained from the American Type Culture Collection (Rockville, MD, USA), and cultured in RPMI 1640, containing 10% fetal calf serum, 2 mM glutamine, antibiotics (penicillin G 60 mg/l, streptomycin 100 mg/l, and amphotericin B 50 μl/l), in a humid 5% CO_2, 95% air atmosphere.

2.3. Western blot analysis

Human leukemia cells (5×10^5 cells) were seeded in 6-well culture plate, and harvested in phosphate-buffered saline (PBS). After washing with PBS, the cell pellets were lysed, with a lysis buffer, containing 1% Triton X-100, 1 mM phenylmethylsufonyl fluoride (PMSF), 10 μg/ml aprotinin, 1 μg/ml pepstatin, 10 μg/ml leupeptin (10 μg/ml), 10 mM NaF and 1 mM Na_3VO_4. After incubation for 30 min, at 4 °C, the cellular debris was removed by centrifugation at 100,000 × *g* for 30 min, and the supernatants analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The protein concentration was determined with Bio-Rad protein assay regent (Bio-Rad Laboratories, USA). Samples (30 μg) were prepared with four volumes of 0.5 M Tris buffer (pH 6.8), containing 4% SDS, 20% glycerol and 0.05% bromophenol blue, at 95 °C, for 5 min. The SDS-PAGE was performed in a 10% slab gel. Proteins were transferred to nitrocellulose paper, and the membrane washed by shaking in blocking buffer (10 mM Tris–HCl, pH 8.0, 150 mM NaCl and 5% fat-free milk), for 60 min, at room temperature and then with TBST (TBS, 0.01% Tween-20). It was incubated with the primary antibody, at 4 °C, for 4 h. The secondary HRP-conjugated antibodies were goat anti-mouse IgG (PTEN) and goat anti-rabbit IgG (p-AKT) (Santa Cruz, CA, USA). The reactive proteins were detected using enhanced chemiluminescence (ECL, Amersham Life Sciences, Arlington Heights, IL).

2.4. Reverse transcription-polymerase chain reaction (RT-PCR) for PTEN expression

To examine the transcriptional control in the PTEN caused by DMSO, RT-PCR was performed by using an RNA PCR Kit (GeneAmp, Applied Biosystem, USA). The total RNA was isolated from the HL60 cells using TRIzol reagent, following the manufacture's instructions. Five micrograms of total RNA was transcribed into the cDNA in reaction buffer (10 mM Tris–HCl, 50 mM KCl, 1.5 mM $MgCl_2$ and 1 mM of each dNTP) and 2.4 μM oligo-d(T)16-primer, 1 unit RNase inhibitor and 2.5 units M-MLV RNase H-reverse transcriptase, in a final volume of 20 μl, by incubation for 15 min, at 70 °C, and a further 50 min at 42 °C. The reaction was stopped by incubation at 95 °C for 10 min. Aliquots of the synthesized cDNA were added to 45 μl of a PCR mixture, containing 10 mM Tris–HCl, 50 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM of each dNTP, 2 units Taq DNA polymerase and 0.4 μM of each PCR primer: sense primer, human PTEN (5′-CCGGAATTCATGACAGCCATCATCAAAGA-3′), anti-sense primer, human PTEN (5′-CGCGGATCCTCAGACTTTTGTAATTTGTG-3′). The amplification was initiated with 3 min of denaturation at 94 °C, followed by 26 cycles at 94 °C for 1 min, 94 °C for 1 min, 60 °C for 1 min and 72 °C for 1 min. After the last cycle of amplification, the samples were incubated for 5 min at 72 °C. The β-actin PCR was performed with 2.5 μl aliquots of synthesized cDNA, using the primers at a concentration of 0.15 μM: sense primer, human β-actin (5′-CCACGAAACTACCTTCAACTCC-3′), anti-sense primer (5′-TCATACTCCTGCTGCTTGCTGATCC-3′). The PCR products obtained were analyzed on ethidium bromide-stained agarose (2%) gels.

2.5. Preparation of nuclear extract

Cells were immediately washed twice, scraped into 1.5 ml of ice-cold PBS (pH 7.9), and pelleted at 12,000 × *g* for 30 s at 4 °C. The cell pellet was suspended in ice-cold hypotonic lysis buffer (10 mM Hepes, 1.5 mM $MgCl_2$, 0.2 mM KCl, 0.2 mM PMSF and 0.5 mM dithiothreitol (DTT)), vortexed for 10 s, and then centrifuged at 3000 × *g* for 5 min at 4 °C. The packed cells were resuspended in ice-cold hypotonic lysis buffer, in the presence of 50 μl of 10% Nonidet P-40, and then incubated on ice for 25 min. The nuclear fraction was precipitated by centrifugation at 4000 × *g* for 15 min at 4 °C.

The nuclei pellet was resuspended in 50–100 μl of the low salt extraction buffer (20 mM Hepes, pH 7.9, 1.5 mM