BIOCHEMICAL PHARMACOLOGY 76 (2008) 1404–1416

available at www.sciencedirect.com

ScienceDirect

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

SH-5, an AKT inhibitor potentiates apoptosis and inhibits invasion through the suppression of anti-apoptotic, proliferative and metastatic gene products regulated by IκBα kinase activation

Gautam Sethi, Kwang Seok Ahn, Bokyung Sung, Ajaikumar B. Kunnumakkara, Madan M. Chaturvedi, Bharat B. Aggarwal *

Cytokine Research Laboratory, Departments of Experimental Therapeutics, The University of Texas M. D. Anderson Cancer Center, Houston, TX 77030, United States

ARTICLE INFO

Article history:
Received 18 March 2008
Accepted 16 May 2008

Keywords:
TNF
AKT
NF-kappaB
Apoptosis
Signal pathway

ABSTRACT

Because the phosphatidylinositol-3-kinase-AKT pathway is emerging as an important regulator of tumor cell survival, inhibitors of this pathway have enormous potential in cancer treatment. A specific inhibitor of AKT, [D-3-deoxy-2-O-methyl-myo-inositol-1-[(R)-2-methoxy-3-(octadecyloxy)propyl hydrogen phosphate]] (SH-5) has been recently synthesized, but little is known about its effects on cytokine signaling. We found that SH-5 potentiated the apoptosis induced by tumor necrosis factor (TNF), as indicated by intracellular esterase staining, annexin V staining, and caspase-3 activation. This effect of SH-5 correlated with downregulation of various gene products that mediate cell survival, proliferation, metastasis, and invasion, all known to be regulated by NF-κB. SH-5 also blocked NF-κB activation induced by TNF-α, lipopolysaccharide, phorbol ester, and cigarette smoke but not that activated by hydrogen peroxide and RANK ligand, indicating differential requirement of AKT. Inhibition of NF-κB correlated with abrogation of phosphorylation and degradation of IκBα through the inhibition of activation of IκBα kinase (IKK). This led to suppression of the phosphorylation and translocation of p65 and also of NF-κB reporter activity induced by TNFR1, TRADD, TRAF2, NIK, and IKKβ but not that induced by p65 transfection. Thus, our results clearly demonstrate that inhibition of AKT leads to potentiation of apoptosis through modulation of NF-κB signaling.

1. Introduction

AKT, a serine–threonine kinase also known as protein kinase B, is a central signaling molecule in the phosphatidylinositol-3-kinase (PI3K) pathway. [1–3]. This kinase is activated by mitogens and cytokines that function as survival factors. AKT mediates its effects by phosphorylating substrates that decrease the activity of pro-apoptotic proteins or increase the activity of anti-apoptotic proteins [4–7]. Activation of PI3K/AKT signaling results in a disturbance of control of cell proliferation and apoptosis, resulting in competitive growth advantage for tumor cells. Blockade of the PI3K–AKT pathway has been found to sensitize various tumor cell types to apoptotic cell death induced by a variety of chemotherapeutic

* *Corresponding author*. Tel.: +1 713 792 3503/6459; fax: +1 713 794 1613.
E-mail address: aggarwal@mdanderson.org (B.B. Aggarwal).
0006-2952/$ – see front matter
doi:10.1016/j.bcp.2008.05.023

agents [8,9]. Hence, this pathway is an attractive target for the development of novel anticancer strategies. However, the molecular mechanisms for such enhanced induction of tumor cell apoptosis by the combination of a PI3K–AKT inhibitor and anticancer agents have remained largely unknown.

In addition to directly phosphorylating and inactivating pro-apoptotic protein targets, AKT can stimulate signaling pathways that regulate the activity of transcription factor NF-κB (10–16). [10–15]. NF-κB is a family of Rel domain-containing proteins present in the cytoplasm of all cells, where they are kept in an inactive state by a family of anchorin domain-containing proteins, which includes IκBα, IκBβ, IκBγ, IκBε, Bcl-3, p105, and p100. Under resting conditions, NF-κB consists of a heterotrimer of p50, p65, and IκBα in the cytoplasm; only when activated and translocated to the nucleus is the sequence of events leading to activation initiated. Most carcinogens, inflammatory agents, and tumor promoters, including cigarette smoke, phorbol ester, okadaic acid, H_2O_2, and tumor necrosis factor (TNF), have been shown to activate NF-κB. The activation of NF-κB involves the phosphorylation, ubiquitination, and degradation of IκBα and phosphorylation of p65, which in turn leads to the translocation of NF-κB to the nucleus where it binds to specific response elements in the DNA. The phosphorylation of IκBα is catalyzed by IκBα kinase (IKK), which is essential for NF-κB activation by most agents [16–18].

However, the mechanism(s) by which NF-κB-AKT interaction contributes to survival in tumor cells is unknown. In the current study, we used a recently discovered inhibitor of AKT, the phosphatidylinositol ether lipid analogue [D-3-deoxy-2-O-methyl-myo-inositol-1-[(*R*)-2-methoxy-3-(octadecyloxy)propyl hydrogen phosphate]] (SH-5) [19] to investigate the role of NF-κB as a putative mediator of the anti-apoptotic function of AKT in TNF-induced cell signaling. Our results demonstrate that AKT inhibitor potentiates the TNF-induced apoptosis through downregulation of NF-κB-regulated anti-apoptotic gene products and the NF-κB activation pathway.

2. Materials and methods

2.1. Reagents

The phosphatidylinositol ether lipid analogue SH-5 (Fig. 1A) was obtained from Alexis Biochemicals (San Diego, CA, USA). A 50 mM solution of SH-5 was prepared with dimethyl sulfoxide, stored as small aliquots at −20 °C, and then diluted as needed in cell culture medium. Bacteria-derived human recombinant human TNF, purified to homogeneity with a specific activity of 5×10^7 U/mg, was kindly provided by Genentech (South San Francisco, CA, USA). Cigarette smoke condensate (CSC), prepared as previously described [20], was kindly supplied by Dr. C. Gary Gairola (University of Kentucky, Lexington, KY, USA). Penicillin, streptomycin, RPMI 1640 medium, and FBS were obtained from Invitrogen (Carlsbad, CA). Phorbol 12-myristate 13-acetate (PMA), hydrogen peroxide (H_2O_2), lipopolysaccharide (LPS) and anti-β-actin antibody were obtained from Aldrich–Sigma (St. Louis, MO, USA). N-Acetyl-leucyl-leucyl-norleucinal (ALLN) was purchased from EMD Biosciences, Inc. (San Diego, CA, USA). Antibodies against p65, p50, IκBα, cyclin D1, MMP-9, PARP, IAP1, Bcl-2, Bcl-xL, AKT, and TRAF1 were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Anti-COX-2 and anti-XIAP antibodies were obtained from BD Biosciences (San Diego, CA, USA). Phospho-specific anti-IκBα (Ser32/36), and phospho-specific anti-p65 (Ser536) were purchased from Cell Signaling (Beverly, MA, USA). Anti-IKK-α, anti-IKK-β, and phospho-AKT (Ser 473), antibodies were kindly provided by Imgenex (San Diego, CA, USA).

2.2. Cell lines

Cell lines KBM-5 (human myeloid), H1299 (lung adenocarcinoma), and A293 (human embryonic kidney) were obtained from American Type Culture Collection (Manassas, VA, USA). The H1299 cells were cultured in RPMI 1640 medium, the KBM-5 cells were cultured in IMDM medium with 15% FBS, and the A293 cells were cultured in DMEM medium supplemented with 10% FBS. All culture media were also supplemented with 100 U/ml penicillin and 100 μg/ml streptomycin.

2.3. Cytotoxicity assay

Cytotoxicity was assayed by the modified tetrazolium salt 3-(4-5-dimethylthiozol-2-yl)2-5-diphenyl-tetrazolium bromide (MTT) assay [21] with following modification. Briefly, the cells (5000 per well) were incubated in triplicate in a 96-well plate in the presence or absence of indicated test samples in a final volume of 0.1 ml for 24 h at 37 °C. Thereafter, 20 μl MTT solution (5 mg/ml in PBS) was added to each well. After a 2-h incubation at 37 °C, 0.1 ml extraction buffer (20% SDS, 50% dimethylformamide) was added; incubation was continued overnight at 37 °C; and then the optical density (OD) at 570 nm was measured by means of a 96-well multiscanner autoreader (Dynatech MR 5000, Chantilly, VA) [23],

2.4. Live/Dead assay

To measure apoptosis, we used the Live/Dead cell viability assay (Invitrogen, Carlsbad, CA), which determines intracellular esterase activity and plasma membrane integrity [22].

2.5. Clonogenic assay

H1299 cells were seeded in six-well plates at 500 cells/well in RPMI 1640 medium containing 10% serum. After 12 h, cells were treated with medium containing indicated concentrations of SH-5 and TNF (1 nM). The medium with SH-5 and TNF was replaced after every 5 days. After 12 days of incubation, colonies were stained with 0.3% crystal violet solution (dissolved in 1:1 mixture of methanol and H_2O) for 2 min, washed once with Dulbecco's phosphate-buffered saline, air-dried, and manually counted. Each point was a mean of three replicate wells.

2.6. Annexin V assay

Annexin V assay was performed as described previously [23].