

Primary research

PI3-K/Akt pathway contributes to IL-6-dependent growth of 7TD1 cells

JiYan Zhang, Yan Li and BeiFen Shen*

Address: Department of Molecular Immunology, Institute of Basic Medical Sciences, 27 Taiping Road, P. O. Box 130(3), Beijing 100850, P. R. China

Email: JiYan Zhang - jiyanzhang@hotmail.com; Yan Li - liyan@nic.bmi.ac.cn; BeiFen Shen* - shenbf@mx.cei.gov.cn

* Corresponding author

Published: 17 February 2003

Received: 21 November 2002

Cancer Cell International 2003, **3**:1

Accepted: 17 February 2003

This article is available from: <http://www.cancerci.com/content/3/1/1>

© 2003 Zhang et al; licensee BioMed Central Ltd. This is an Open Access article: verbatim copying and redistribution of this article are permitted in all media for any purpose, provided this notice is preserved along with the article's original URL.

Abstract

Background: Recently, growing evidence suggests the involvement of PI 3-K/Akt in IL-6-dependent survival and proliferative responses in several types of cells. However, whether PI 3-K/Akt plays the same role in IL-6-dependent growth of 7TD1 mouse-mouse B cell hybridoma is not known.

Methods: We investigated the activation status of Akt in 7TD1 cells induced by IL-6. With PI 3-K specific inhibitor wortmannin, we also investigated the biological roles of Akt activation in 7TD1 cells.

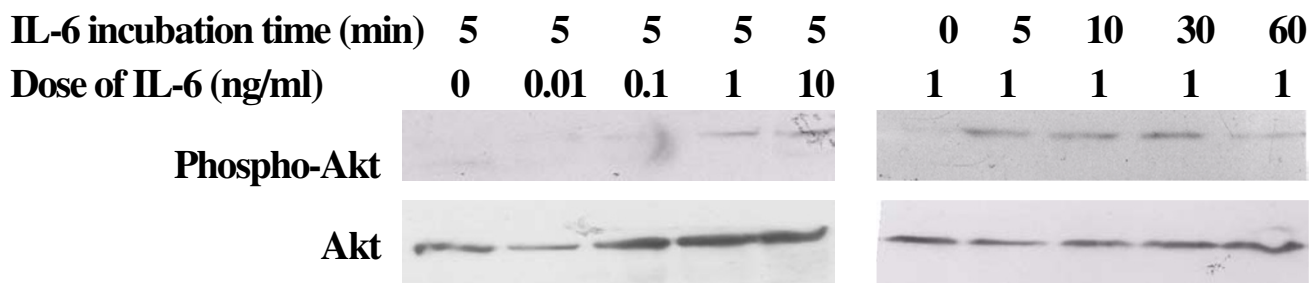
Results: IL-6 stimulated phosphorylation of Akt in a dose- and time-dependent manner in 7TD1 cells. Wortmannin significantly reduced IL-6-induced phosphorylation of Akt and IL-6-dependent growth of 7TD1 cells. Furthermore, wortmannin blocked IL-6-induced up-regulation of XIAP, but not Bcl-2 in 7TD1 cells.

Conclusion: The data suggest that IL-6-induced PI 3-K/Akt activation is essential for the optimal growth of 7TD1 cells through up-regulation of anti-apoptosis proteins such as XIAP.

Background

Interleukin-6 (IL-6) is a pleiotropic cytokine. The binding of IL-6 to its receptor induces the activation of multiple signal transduction pathways such as JAK/STATs (Janus tyrosine kinase/signal transducers and activators of transcription) pathway, Ras/ERK (extracellular signal-regulated kinase) pathway, and PI 3-K (phosphatidylinositol 3-kinase)/Akt pathway via gp130 tyrosine phosphorylation [1]. The roles of JAK/STATs pathway and Ras/ERK pathway in the biological effects of IL-6 have been extensively investigated [1,2]. However, what role PI 3-K/Akt plays in IL-6 signaling is less clear. Akt is a serine (Ser)/threonine (Thr) protein kinase which resides within the

cytosol in a catalytically inactive state in quiescent or serum-starved cells. After stimulation of cells with growth factors and cytokines, Akt is catalytically activated by phosphorylation at Thr308 and Ser473. Activated Akt in turn phosphorylates downstream target molecules and induces the expression of anti-apoptosis proteins, which promote induction of its anti-apoptosis effect [3]. Recently, growing evidence suggests the involvement of PI 3-K/Akt in IL-6-dependent survival and proliferative responses in several types of cells [3–6]. Still, whether PI 3-K/Akt plays the same role in IL-6-dependent growth of 7TD1 mouse-mouse B cell hybridoma is not known. In our previous work, we showed that ERK cascade but not STAT3

**Figure 1**

IL-6 stimulated phosphorylation of Akt in a dose- and time-dependent manner in 7TD1 cells. 7TD1 cells were starved in IL-6-free medium for 6 h. Then the cells were cultured in the presence of different dose of IL-6 for 5 min or in the presence of 1 ng/ml IL-6 for 0, 5, 10, 30, 60 min respectively. After the cells were collected and washed, whole-cell extracts were prepared and subjected to Western-blotting assay. This figure is representative of 3 separate experiments.

contributed to IL-6-dependent growth of 7TD1 cells [7]. Here we report that IL-6 triggers activation of PI3-K/Akt signaling in 7TD1 cells, and that IL-6-induced PI 3-K/Akt activation is essential for the optimal growth of 7TD1 cells.

Methods

Cells and cell culture

7TD1 cells were obtained from the American Type Culture Collection. The cells were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum. β -mercaptoethanol and recombinant human IL-6 (10^7 U/mg) were added to the medium at final concentration of 5×10^{-5} mol/L and 5 ng/ml, respectively. Wortmannin (Sigma) was dissolved in DMSO to 2.5 mM and stored at -20°C . DMSO was added to control cells to keep concentrations of DMSO ($<0.1\%$) equal in all samples. The cells were pre-treated with wortmannin for 30 min at 37°C before IL-6 was added to the medium.

MTT assay

The cells were seeded into 96-well plate and cultured in the presence of different dose of IL-6 for 72 h. Afterwards, 10 μl MTT (Sigma, 5 mg/ml) was added to each well, 4 h later, an equal volume of 10%SDS-10 mM HCl was added to dissolve the blue crystals of formazan. The samples were measured at OD 570 nm by an ELISA reader (Dynatech Laboratories, Inc. U.S.A.).

Western-blotting assay

After protein determination, total cell lysates (5×10^5 cell/sample) were boiled in $2 \times$ reducing SDS loading buffer of equal volume for 10 min. The samples were subjected to one-dimensional SDS-PAGE. After electrophoresis, proteins were transferred to a 0.45 μm pore-size nitrocellu-

lose membrane at 40 V for 2 h. Non-specific binding sites on the nitrocellulose membrane were blocked by incubation in blocking buffer (5% w/v, non-fat dried milk) for 1 h at 37°C . The blots were washed once with Tris-buffered saline (10 mM Tris/HCl pH 7.5, 150 mM NaCl) and incubated with the primary antibody for 1 h at 37°C or overnight at 4°C . After the removal of excess primary antibody with three washes, the blots were incubated with a secondary antibody (goat anti-mouse or goat anti-rabbit antibodies conjugated with horseradish peroxidase). The membrane was developed with enhanced chemiluminescence reagent and exposed to Hyperfilm-ECL[®] (Amersham Life Science Corp.) for detection. Akt and phospho-Akt (Ser473) antibodies were products of New England BioLabs (Beverly, MA, U.S.A.). Anti-(human XIAP) antibody was a product of Medical and Biological Laboratories (Naka, Nagoya, Japan). Anti-Bcl-2 and anti-Caspase-3 antibodies were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, U.S.A.).

Results

IL-6 stimulated phosphorylation of Akt in a dose- and time-dependent manner in 7TD1 cells

The survival and proliferation of 7TD1 hybridoma cells depend on IL-6. IL-6 induced phosphorylation (Ser473) of Akt, a downstream effector of PI 3-K, in 7TD1 cells. This activation by IL-6 occurred as early as 5 min, and was dependent on the dose of IL-6. However, IL-6 had little effect on the protein amount of Akt (Fig. 1).

PI 3-K specific inhibitor wortmannin significantly reduced IL-6-induced phosphorylation of Akt and IL-6-dependent growth of 7TD1 cells

To investigate what role PI 3K/Akt plays in the signal transduction of IL-6 in 7TD1 hybridoma cells, we deter-

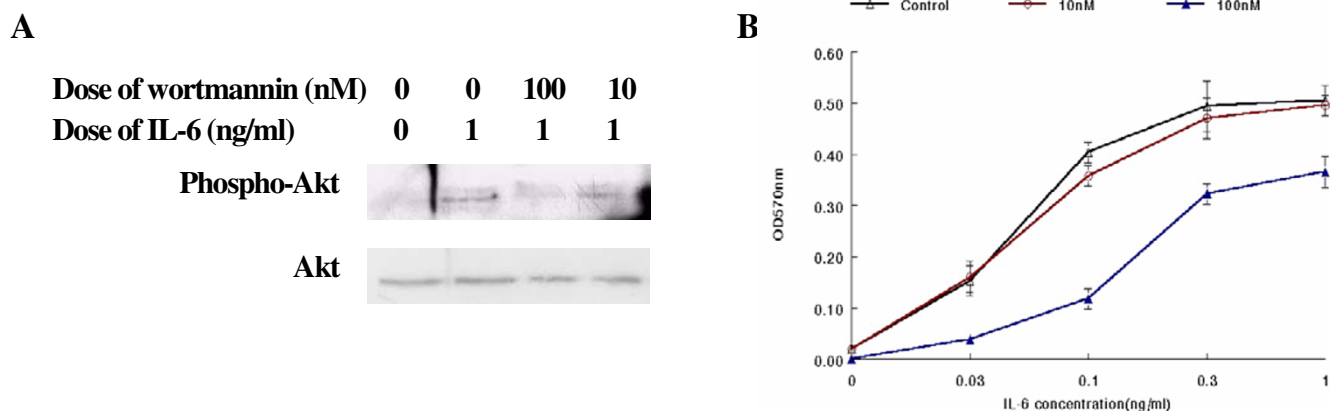


Figure 2

Effects of wortmannin on IL-6-induced phosphorylation of Akt (A) and IL-6-dependent growth of 7TD1 cells (B).

7TD1 cells were starved in IL-6-free medium for 6 h. Then the cells were pretreated with wortmannin or DMSO of equal volume for 30 min at 37°C before IL-6 was added into the medium. (A) The cells were cultured for 5 min in the presence of IL-6. After the cells were collected and washed, whole-cell extracts were prepared and subjected to Western-blotting assay. (B) The cells were seeded into 96-well plate (2×10^3 cells per well) and cultured in the presence of different dose of IL-6 for 72 h. Afterwards, MTT assay was performed to determine the effect of wortmannin on IL-6-dependent growth of 7TD1 cells. This figure is representative of three separate experiments.

mined the effects of wortmannin, a PI 3-K specific inhibitor at 10–100 nmol/L [8], on IL-6-induced phosphorylation of Akt and IL-6-dependent growth of 7TD1 cells. It was found that wortmannin significantly antagonized IL-6-induced phosphorylation of Akt and IL-6-dependent growth of 7TD1 cells and the inhibitory effects of wortmannin were dependent on its concentration. These data confirm that activation of Akt is mediated by a PI 3-K-dependent mechanism and suggest that IL-6-induced PI 3-K/Akt activation is essential for the optimal growth of 7TD1 cells (Fig. 2).

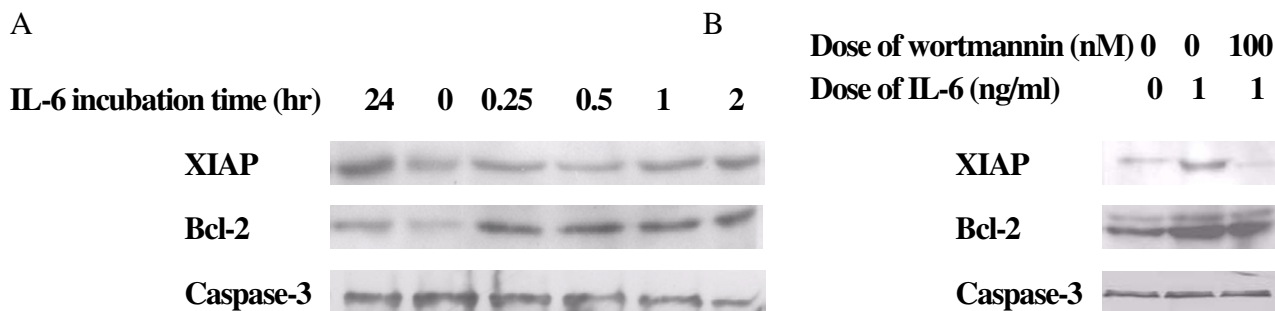
IL-6 induced up-regulation of X chromosome-linked inhibitor of apoptosis protein (XIAP) through PI 3-K/Akt activation

The preceding experiments suggest that IL-6-induced PI 3-K/Akt activation is essential for the optimal growth of 7TD1 cells. Next we try to investigate the underlying mechanism. Recent evidence has indicated that proteins of the inhibitor of apoptosis (IAP) family, whose expression might be under the regulation of NF- κ B, can block apoptotic events by directly binding and inhibiting selected caspases. A potent mammalian IAP is X-linked IAP (XIAP), for which the mechanism of action involves the direct binding and inhibition of caspase-3 and caspase-7, two key effector proteases of apoptosis [10]. What role XIAP plays in IL-6-mediated anti-apoptosis mechanism is of interest. It is reported that the level of Bcl-2, but not Bcl-

X_L and Mcl-1, decreased after IL-6 deprivation [9]. To examine the effects of IL-6-induced PI3-K/Akt activation on these apoptosis-related proteins, we further studied IL-6-induced expression of Bcl-2 as well as XIAP and Caspase-3 in 7TD1 cells by Western-blotting assay with Bcl-2, XIAP, and Caspase-3 antibodies, with or without wortmannin. Untreated 7TD1 cells displayed significant levels of these apoptosis-related gene products (Fig. 3). IL-6 significantly up-regulated the levels of XIAP and Bcl-2 but had little effect on the level of caspase-3. Both constitutive and IL-6-induced expression of XIAP in 7TD1 cells was inhibited by wortmannin. However, wortmannin had little effect on IL-6-induced expression of Bcl-2. Taken together, these data suggest that IL-6 might induce up-regulation of XIAP through PI 3-K/Akt activation.

Discussion

The growth of 7TD1 B cell hybridoma is dependent on the survival factor IL-6. IL-6 inhibits physiological cell death and allows expansion of populations of serum-stimulated cells. How IL-6 can promote the growth of 7TD1 cells remains elusive. In our previous work, we showed that ERK cascade but not STAT3 contributed to IL-6-dependent growth of 7TD1 cells [7]. However, activation of ERK cascade seems not to be sufficient since MEK inhibitor PD098059 pretreatment resulted in partial blockade of IL-6-induced growth of 7TD1 cells although IL-6-induced activity of ERK cascade can be completely blocked by

**Figure 3**

Effects of IL-6 and wortmannin on the expression of apoptosis-related proteins in 7TD1 cells. (A) 7TD1 cells were starved in IL-6-free medium for 6 h. Then the cells were cultured in the presence of 1 ng/ml IL-6 for various periods as indicated. At the end of the incubation period, whole-cell extracts were prepared and subjected to Western-blotting assay. (B) 7TD1 cells were starved in IL-6-free medium for 6 h. Then the cells were pretreated with wortmannin or DMSO of equal volume for 30 min at 37°C before IL-6 was added into the medium. The cells were cultured for 1 h in the presence of IL-6. After the cells were collected and washed, whole-cell extracts were prepared and subjected to Western-blotting assay.

PD098059 of the same concentration. In this work, we show that IL-6-induced PI 3-K/Akt activation is also essential for the optimal growth of 7TD1 cells. Taken together, our data suggest that IL-6 promotes the growth of 7TD1 cells via activation of multiple signal transduction pathways including ERK cascade and PI 3-K/Akt pathway.

Recently, growing evidence suggests the involvement of PI 3-K/Akt in IL-6-dependent survival and proliferative responses in several types of cells [3–6]. Our data are consistent with these findings, further confirming the important roles of PI 3-K/Akt in IL-6 signaling. Furthermore, we found that XIAP, but not Bcl-2, might be a downstream target molecule of Akt since both constitutive and IL-6-induced expression of XIAP, but not Bcl-2, in 7TD1 cells was inhibited by wortmannin. Recent evidence has indicated that the expression of XIAP might be under the regulation of NF-κB. PI 3-K/Akt pathway is thought to be involved in the full activation of NF-κB through phosphorylation of the Rel proteins [11]. Therefore IL-6 might induce the expression of XIAP through PI 3-K→Akt→NF-κB cascade. This is the first report that XIAP is involved in IL-6-mediated anti-apoptosis mechanism.

Acknowledgements

This work was supported by national fund for distinguished young scholars Grant No. 39925019 to Yan Li.

References

- Hirano T, Nakajima K and Hibi M **Signaling mechanisms through gp130: a model of the cytokine system.** *Cytokine & Growth Factor* 1997, **8**:241-252

- Hirano T, Ishihara K and Hibi M **Roles of STAT3 in mediating the cell growth, differentiation and survival signals relayed through the IL-6 family of cytokine receptors.** *Oncogene* 2000, **19**:2548-2556
- Hideshima T, Nakamura N, Chauhan D and Anderson KC **Biologic sequelae of interleukin-6 induced PI3-K/Akt signaling in multiple myeloma.** *Oncogene* 2001, **20**:5991-6000
- Wei LH, Kuo ML, Chen CA, Chou CH, Cheng WF, Chang MC, Su JL and Hsieh CY **The anti-apoptotic role of interleukin-6 in human cervical cancer is mediated by up-regulation of Mcl-1 through a PI 3-K/Akt pathway.** *Oncogene* 2001, **20**:5799-5809
- Kunioku H, Inoue K and Tomida M **Interleukin-6 protects rat PC12 cells from serum deprivation or chemotherapeutic agents through the phosphatidylinositol 3-kinase and STAT3 pathways.** *Neurosci Lett* 2001, **309**:13-16
- Kuo ML, Chuang SE, Lin MT and Yang SY **The involvement of PI 3-K/Akt-dependent up-regulation of Mcl-1 in the prevention of apoptosis of Hep3B cells by interleukin-6.** *Oncogene* 2001, **20**:677-685
- Zhang JY, Li Y and Shen BF **The effects of Ras/MAPK/NF-IL-6 pathway in the IL-6-dependent proliferation of 7TD1 hybridoma cells.** *Shanghai Journal of Immunology* 2000, **20**:273-276
- Thiemermann C and Ruetten H **Interleukin-13 is a more potent inhibitor of the expression of inducible nitric oxide synthase in smooth muscle cells than in macrophages: a comparison with interleukin-4 and interleukin-10.** *Shock* 1997, **8**:409-414
- Rahman Z, Yoshikawa H, Nakajima Y and Tasaka K **Down-regulation of Pim-1 and Bcl-2 is accompanied with apoptosis of interleukin-6-depleted mouse B-cell hybridoma 7TD1 cells.** *Immunol Lett* 2001, **3**:199-208
- Deveraux QL and Reed JC **IAP family proteins – suppressors of apoptosis.** *Genes Dev* 1999, **13**:239-252
- Madrid LV, Wang CY, Guttridge DC, Schottelius AJ, Baldwin AS Jr and Mayo MW **Akt suppresses apoptosis by stimulating the transactivation potential of the RelA/p65 subunit of NF-κB.** *Mol Cell Biol* 2000, **20**:1626-1638