Inhibition of Phosphatidylinositol 3-Kinase by c-Abl in the Genotoxic Stress Response*

(Received for publication, June 19, and in revised form, July 21, 1997)

Zhi-Min Yuan, Taiju Utsugisawa, Yinyin Huang, Takatoshi Ishiko, Shuji Nakada, Sureshkar Harkanda, Ralph Weichselbaum‡, and Donald Kufe

From the Department of Cancer Pharmacology, Dana-Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts 02115 and the ‡Department of Radiation and Cellular Oncology, University of Chicago, Chicago, Illinois 60637

Activation of phosphatidylinositol (PI) 3-kinase by growth factors results in phosphorylation of phosphatidylinositol lipids at the D3 position. Although PI 3-kinase is essential to cell survival, little is known about mechanisms that negatively regulate this activity. Here we show that the c-Abl tyrosine kinase interacts directly with the p85 subunit of PI 3-kinase. Activation of c-Abl by ionizing radiation exposure is associated with c-Abl-dependent phosphorylation of PI 3-kinase. We also show that phosphorylation of p85 by c-Abl inhibits PI 3-kinase activity in vitro and in irradiated cells. These findings indicate that c-Abl negatively regulates PI 3-kinase in the stress response to DNA damage.

The product of the c-abl gene is a nonreceptor tyrosine kinase that shares certain structural features, including SH3, SH2, and catalytic domains, with the Src kinase family. In addition, c-Abl contains nuclear localization signals, a bipartite DNA-binding domain, and F- and G-actin binding domains (reviewed in Ref. 1). Cells exposed to ionizing radiation (IR)1 and certain DNA-damaging agents respond with activation of c-Abl (1–4). Signals downstream of c-Abl activation include induction of the stress-activated kinase protein and p38 mitogen-activated protein kinase (1, 2, 5). The activation of c-Abl by genotoxic stress is also associated with interaction of c-Abl with the p53 tumor suppressor protein in the G1 arrest response (6, 7). Other studies have provided support for involvement of c-Abl in DNA damage-induced apoptosis (8).

One issue concerning the genotoxic stress response is how DNA damage is converted into informational intracellular signals that affect cell behavior. Whereas IR induces DNA double-strand breaks (9), the DNA-dependent protein kinase (DNA-PK) is activated by double-strand breaks and other DNA lesions (10–12). Recent studies have demonstrated that DNA-PK phosphorylates and activates c-Abl (13). In a potential feedback mechanism, c-Abl phosphorylates and inhibits DNA-PK activity (13). Other work has shown that c-Abl interacts with the product of the ataxia telangiectasia mutated (ATM) gene and that ATM may also be responsible for activating c-Abl as a consequence of genotoxic stress (14, 15). The findings that cells defective in DNA-PK or ATM are hypersensitive to the lethal effects of IR (16, 17), whereas c-Abl-deficient cells are resistant to IR-induced killing (8), has suggested that functional interactions between c-Abl and DNA-PK or ATM may contribute to cell fate.

The DNA-PK catalytic subunit and ATM are related to members of the PI 3-kinase family, such as Tor1p, Tor2p, FKBP-rapamycin-associated protein, and Schizosaccharomyces pombe Rad 3, involved in cell cycle control and DNA repair (18). PI 3-kinase is activated by growth factor receptors and has been implicated in transducing survival signals (19). Inositol lipids phosphorylated at the D3 position by PI 3-kinase function in part in the activation of the Akt (protein kinase B) serine/threonine protein kinase (20–23). The PI 3-kinase/Akt pathway inhibits the induction of apoptosis by serum withdrawal (24) and the c-Myc protein (25). Activation of Akt thus promotes survival, whereas inhibition of PI 3-kinase with wortmannin induces apoptosis (24, 26). These findings suggest that signaling mechanisms that down-regulate PI 3-kinase would be pro-apoptotic. However, whereas autophosphorylation of PI 3-kinase on serine inhibits PI 3-kinase activity (27, 28), little is known about other signals that negatively regulate this kinase.

The present studies demonstrate that c-Abl associates with PI 3-kinase. Phosphorylation of the p85 subunit of PI 3-kinase by c-Abl inhibits PI 3-kinase activity. The results also show that c-Abl phosphorylates and inhibits PI 3-kinase in IR-treated cells.

MATERIALS AND METHODS

Cell Culture—Human U-937 myeloid leukemia cells (ATCC, Rockville, MD) were grown as described (29). AblΔ1–30 (30) and AblΔ1 (1) fibroblasts were grown in Dulbecco’s modified Eagle’s medium supplemented with 10% heat-inactivated fetal bovine serum. MCF-7 cells were transfected with pSRαMSVH/Kneo or pSRαMSV-AblK/rTkneo (31) as described in G418 (6). Irradiation was performed at room temperature using a Gammacell 1000 (Atomic Energy of Canada) under aerobic conditions with 137Cs source emitting at a fixed dose rate of 0.76 Gy min−1 as determined by dosimetry.

Immunoprecipitation and Immunoblot Analysis—Immunoprecipitation was performed as described (1). Soluble proteins were incubated with anti-c-Abl (Ab-3; Oncogene Science) or anti-p85 (06–195; Upstate Biotechnology Inc.) and precipitated with protein A-Sepharose for an additional 1 h. The immune complexes were washed with lysis buffer (50 mM HEPES, pH 7.5, 0.5% Nonidet P-40, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1 mM sodium orthovanadate, 1 mM dithiothreitol, 1 mM NaF, 2 mM phenylmethylsulfonyl fluoride, and 10 μg/ml each of pepstatin, leupeptin, and aprotonin), separated by electrophoresis in SDS/polyacrylamide gels and then transferred to nitrocellulose paper. The filters were incubated with anti-p85 (P13020; Transduction Laboratories), anti-c-Abl, or anti-P-Tyr (4G10; Upstate Biotechnology Inc.). The antigen-antibody complexes were visualized by enhanced chemiluminescence (ECL, Amersham Corp.).

Phosphorylation of PI 3-Kinase—Recombinant kinase active c-Abl

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‡ The abbreviations used are: IR, ionizing radiation; PI, phosphatidylinositol; DNA-PK, DNA-dependent protein kinase; ATM, ataxia telangiectasia mutated; Gy, gray; GST, glutathione S-transferase; HI, heat-inactivated.

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Vol. 272, No. 38, Issue of September 19, pp. 23485–23488, 1997

Printed in U.S.A.

THE JOURNAL OF BIOLOGICAL CHEMISTRY

This paper is available on line at http://www.jbc.org

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RESULTS AND DISCUSSION

c-Abl interacts with the PI 3-kinase-related DNA-PK and ATM proteins (13, 15). To determine if c-Abl associates with PI 3-kinase, anti-c-Abl immunoprecipitates were analyzed by immunoblotting with an antibody against the p85 subunit of PI 3-kinase. The results demonstrate constitutive binding of c-Abl and PI 3-kinase (Fig. 1A). The activation of c-Abl by IR treatment (1) had little effect on the interaction (Fig. 1A). The finding that the p85 protein detected in the anti-c-Abl immunoprecipitates exhibits a slightly decreased electrophoretic mobility compared with that in total cell lysates supports binding of c-Abl to only certain post-translationally modified forms or specific isoforms of p85. Analysis of anti-p85 immunoprecipitates with anti-c-Abl in the reciprocal experiment confirmed binding of c-Abl and PI 3-kinase in control and irradiated cells (Fig. 1B). Incubation of cell lysates with GST fusion proteins demonstrated binding of p85 to full-length c-Abl and to Abl SH3 but not to the N-terminal SH3 domain of Grb2 (Fig. 1C). The c-Abl SH3 domain binds to proline-rich sequences with the PXG consensus (33, 36). The presence of potential sequences for c-Abl binding in the p85 subunit suggested that there may be a direct interaction. The finding that GST-p85 binds to recombinant c-Abl purified from baculovirus by cleavage of the GST with thrombin (32). The recombinant c-Abl was incubated with GST or GST-p85. The adsorbates were analyzed by immunoblotting with anti-c-Abl.

To assess the functional significance of the interaction between c-Abl and p85, we asked if PI 3-kinase is a substrate for c-Abl. Incubation of recombinant kinase-active c-Abl with GST-p85 resulted in phosphorylation of p85 (Fig. 2A). c-Abl exhibited no detectable phosphorylation of GST alone (data not shown), and there was no phosphorylation of p85 with heat-inactivated c-Abl (Fig. 2A). To determine whether DNA damage induces c-Abl-dependent phosphorylation of p85, anti-P-Tyr immunoprecipitates were assayed from mouse fibroblasts subjected to immunoprecipitation was used as a positive control for the immunoblot analysis. C, cell lysate was incubated with 5 μg of GST-Grb2 SH3, GST-Abl SH3, or GST-c-Abl (full-length) (32) for 2 h at 4 °C. The adsorbates were analyzed by immunoblotting with anti-p85. Lysate was used directly as a positive control. D, recombinant c-Abl was purified from GST-c-Abl prepared in baculovirus by cleavage of the GST with thrombin (32). The recombinant c-Abl was incubated with GST or GST-p85. The adsorbates were analyzed by immunoblotting with anti-c-Abl.

**FIG. 1.** c-Abl associates with PI 3-kinase. A and B, U-937 cells (1) were exposed to 20 GY of IR and harvested at 1 h. Lysates from control (C) and irradiated (IR) cells were subjected to immunoprecipitation with anti-c-Abl or anti-p85. The immunoprecipitates were separated in 8% SDS-polyacrylamide gels, transferred to nitrocellulose, and analyzed by immunoblotting (IB) with anti-p85 or anti-c-Abl. Lysate not subjected to immunoprecipitation was used as a positive control for the immunoblot analysis. C, cell lysate was incubated with 5 μg of GST-Grb2 SH3, GST-Abl SH3, or GST-c-Abl (full-length) (32) for 2 h at 4 °C. The adsorbates were analyzed by immunoblotting with anti-p85. Lysate was used directly as a positive control. D, recombinant c-Abl was purified from GST-c-Abl prepared in baculovirus by cleavage of the GST with thrombin (32). The recombinant c-Abl was incubated with GST or GST-p85. The adsorbates were analyzed by immunoblotting with anti-c-Abl.

**FIG. 2.** c-Abl phosphorylates PI 3-kinase in vitro and in irradiated cells. A, II and kinase-active recombinant c-Abl were incubated with GST-p85 and [γ-32P]ATP. Phosphorylated proteins were separated in 8% SDS-polyacrylamide gels and analyzed by autoradiography. B, Abl+/− and Abl−/− cells were exposed to 20 GY of IR and harvested at the indicated times. Cell lysates were subjected to immunoprecipitation with anti-P-Tyr. The immunoprecipitates were analyzed by immunoblotting (IB) with anti-p85. Lysates from Abl+/− and Abl−/− cells not subjected to immunoprecipitation were used as controls for the immunoblot analysis. C, MCF-7/p/SR and MCF-7/c-Abl(K-R) cells were exposed to 20 GY of IR and harvested at 1 h. Lysates were subjected to immunoprecipitation with anti-p85, and the precipitates were analyzed by immunoblotting with anti-P-Tyr.

Lipid Kinase Assays—PI 3-kinase activity was measured as described (35) in lipid kinase buffer (10 mM Tris, pH 7.5, 100 mM NaCl, 1 mM EDTA, 100 μM sodium vanadate, 1 mM dithiothreitol) containing 2 μg/ml phosphatidylinositol (Avanti Polar Lipids, Alabaster, AL) and [γ-32P]ATP. The reaction was stopped by the addition of 6 N HCl and chloroform/methanol (1:1). The organic layer was separated and spotted on a Silica Gel-60 plate (Sigma). Phosphorylation was assessed by thin layer chromatography and autoradiography.
Down-regulation of PI 3-Kinase by c-Abl

The demonstration that cells deficient in c-Abl are resistant to IR-induced apoptosis has supported a pro-apoptotic function for c-Abl in the cell death response to DNA damage (8). By contrast, Bcr-Abl expression inhibits the apoptotic response to DNA-damaging agents (41). The Akt/protein kinase B kinase is a downstream effector of PI 3-kinase that participates in suppression of apoptosis (20, 21, 24, 25, 42, 43). Taken together with the functional interaction found between c-Abl and PI 3-kinase in the present studies, these findings suggest that c-Abl could contribute to the regulation of cell fate through down-regulation of PI 3-kinase. The present results also provide the first evidence for a tyrosine kinase that negatively regulates PI 3-kinase.

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J. Biol. Chem. 1997, 272:23485-23488.
doi: 10.1074/jbc.272.38.23485

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