Available online at www.sciencedirect.com
ScienceDirect
Biochemical and Biophysical Research Communications 352 (2007) 431–436
BBRC
www.elsevier.com/locate/ybbrc

Tyrosine 311 is phosphorylated by c-Abl and promotes the apoptotic effect of PKCδ in glioma cells

Wei Lu [a,1], Susan Finnis [a,1], Cunli Xiang [a], Hae Kyung Lee [a], Yael Markowitz [b], Hana Okhrimenko [b], Chaya Brodie [a,b,*]

[a] *William and Karen Davidson Laboratory of Cell Signaling and Tumorigenesis, Hermelin Brain Tumor Center, Department of Neurosurgery, Henry Ford Hospital, Detroit, MI, USA*

[b] *The Mina and Everard Goodman Faculty of Life Sciences Faculty of Life-Sciences, Bar-Ilan University, Ramat-Gan 52900, Israel*

Received 7 November 2006
Available online 16 November 2006

Abstract

In this study we characterized the phosphorylation of tyrosine 311 and its role in the apoptotic function of PKCδ in glioma cells. We found that c-Abl phosphorylated PKCδ on tyrosine 311 in response to H_2O_2 and that this phosphorylation contributed to the apoptotic effect of H_2O_2. In contrast, Src, Lyn, and Yes were not involved in the phosphorylation of tyrosine 311 by H_2O_2. A phosphomimetic PKCδ mutant, in which tyrosine 311 was mutated to glutamic acid (PKCδY311E), induced a large degree of cell apoptosis. Overexpression of the PKCδY311E mutant induced the phosphorylation of p38 and inhibition of p38 abolished the apoptotic effect of the PKCδ mutant. These results suggest an important role of tyrosine 311 in the apoptotic function of PKCδ and implicate c-Abl as the kinase that phosphorylates this tyrosine.

Keywords: PKCδ; Tyrosine phosphorylation; Apoptosis; c-Abl; Src; H_2O_2; Glioma

Protein kinase Cδ is a ubiquitously expressed novel PKC isoform which regulates various cellular functions. PKCδ plays a major role in the regulation of cell apoptosis in a cellular and stimulus-dependent manner [1]. Thus, PKC regulates cell apoptosis of various cell types in response to etoposide [2,3], cisplatin [4], UV radiation [5], and oxidative stress [6]. In contrast to its pro-apoptotic effect, PKCδ has been also reported to act as an anti-apoptotic kinase; in FGF-treated granulosa cells [7], in TRAIL-treated and Sindbis virus-infected glioma cells [8,9], and in NO-treated macrophages [10].

One of the important factors that impacts the activity and functions of PKCδ is its phosphorylation on tyrosine residues. PKCδ has been reported to undergo tyrosine phosphorylation in response to a large variety of stimuli including PMA [11,12], PDGF [12,13], substance P [14], and activation of the IgE receptor [15]. Tyrosine phosphorylation of PKCδ is one of the earliest events that occur in response to various apoptotic stimuli and it has been shown to play a major role in the apoptotic function of PKCδ [1]. Indeed, tyrosines 311, 332, and 532 are phosphorylated in response to H_2O_2 [16,17], and tyrosines 311 and 332 are phosphorylated in response to ceramide [18]. In addition, phosphorylation of PKCδ on tyrosines 187 and 64 mediate the apoptotic effect of etoposide [3]. Although tyrosine phosphorylation is currently recognized as a critical event in the activation of PKCδ, the identification of tyrosine kinases that phosphorylate PKCδ and the role of specific tyrosine residues in the activation, localization, and function of this isoform are just beginning to be understood.

In this study, we characterized the phosphorylation of tyrosine 311 in glioma cells and examined its role in glioma cell apoptosis. We found that tyrosine 311 is phosphory-

* Corresponding author. Fax: +1 313 916 8755.
E-mail address: nscha@neuro.hfh.edu (C. Brodie).
[1] These authors contributed equally to this work.

0006-291X/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2006.11.028

lated by H_2O_2 in glioma cells and that c-Abl is involved in its phosphorylation. In addition we found that a PKCδY311F mutant inhibited glioma cell apoptosis induced by H_2O_2, whereas a PKCδY311E mutant induced cell apoptosis. Finally, we found that the PKCδY311E mutant induced phosphorylation of p38 and that inhibition of this phosphorylation abolished the apoptotic effect of the PKCδ mutant.

Materials and methods

Materials. Polyclonal anti-PKCδ (C-20 and C-17) anti-Src, anti-Lyn, anti-Yes, and anti-c-Abl antibodies were obtained from Santa-Cruz (Santa-Cruz, CA). Anti-phospho-PKCδ Tyr311 antibody was obtained from Biosource, Invitrogen (Carlsbad, California). Anti-PARP, p38, phospho-p38, JNK, phosphor-JNK, Erk1/2, phospho Erk1/2, XIAP, AKT, and phosphor-AKT antibodies were obtained from Cell Signaling (Beverly, MA). PP1, PP2, and SB203580 were obtained from EMD Biosciences (San Diego, CA) and STI571 was kindly provided by Novartis Switzerland.

siRNA transfection. Small interfering siRNA duplexes for Src, Yes, Lyn, and c-Abl were obtained from Dharmacon (Lafayette, CO). A scrambled sequence was used as a negative control. Transfection of siRNAs was performed using OligoFectamine (Invitrogen, Carlsbad, California). Experiments were performed 72 h after transfection.

Site-directed mutagenesis of PKCδ. PKCδ cloned into the pEGFP plasmid served as a template vector for the site-directed mutagenesis using the QuikChange Site-Directed Mutagenesis Kit (Stratagene, La Jolla, CA). Conversion of tyrosine residues at sites 311 into phenylalanine (Y311F) or to glutamic acid (Y311E) was performed using the following primers. Y311F: forward 5′-ca gag tct gtc gga ata TTC cag gga ttt gag aag aag-3′; reverse 5′-ctt ctt ctc aaa tcc ctg GAA tat tcc gac aga ctc tg-3′. Y311E: forward 5′-ca aca gag tct gtc gga ata GAA cag gga ttt gag aag aag cc-3′; reverse 5′-gg ctt ctt ctc aaa tcc ctg TTC tat tcc gac aga ctc tgt tg-3′. The mutations were confirmed by DNA sequencing. PKCδ and the PKCδ mutants were fused into the N-terminal enhanced GFP vector pEGFP-N1 (Clontech Laboratories, Palo Alto, CA) as previously described [3].

Cell apoptosis assays. Cell apoptosis was measured using propidium iodide staining and analysis by flow cytometry as previously described [3,8] and by ELISA using anti-histone antibodies.

For anti-histone ELISA (Cell Death Detection ELISA kit; Roche Applied Sciences, Indianapolis, IN), cellular extracts containing histone-associated DNA fragments were incubated in 96-well plates coated with anti-histone antibodies. Plates were then incubated with anti-DNA antibodies conjugated to peroxidase for 2 h. Substrate solution was added and absorbance was measured at 405 nm.

Immunoblot analysis. Lysates were resolved by SDS–PAGE and were transferred to nitrocellulose membranes. The membranes were blocked with 5% dried milk and subsequently stained with the primary antibody. Specific reactive bands were detected using a goat anti-rabbit IgG conjugated to horseradish peroxidase (Bio-Rad, Hercules, CA) and the immunoreactive bands were visualized by the ECL Western blotting detection kit (Amersham, Arlington Heights, IL).

Statistical analysis. The results are presented as values means ± SE. Data were analyzed using ANOVA and a paired Student's *t* test to determine the level of significance between the different groups.

Results

The phosphorylation of PKCδ on tyrosine 311 in response to H_2O_2 is not mediated by Src, Yes, or Lyn

Oxidative stress induces tyrosine phosphorylation of PKCδ in various cellular systems [16,17]. Here, we examined the phosphorylation of PKCδ on tyrosine 311 in response to H_2O_2 in glioma cells. We found that H_2O_2 (1 mM) induced phosphorylation of PKCδ in the U87 cells already after 5 min and it persisted up to 30 min thereafter (Fig. 1A). Similar results were obtained in the A172 glioma cells (data not shown).

To identify the tyrosine kinase that phosphorylates PKCδ in response to H_2O_2 we first employed the Src family inhibitor PP1 (10 μM) and found that it significantly blocked the phosphorylation of tyrosine 311 by H_2O_2 (Fig. 1B). Similar results were obtained with PP2 (data not shown). To further examine the role of Src in the phosphorylation of tyrosine 311 we silenced Src expression

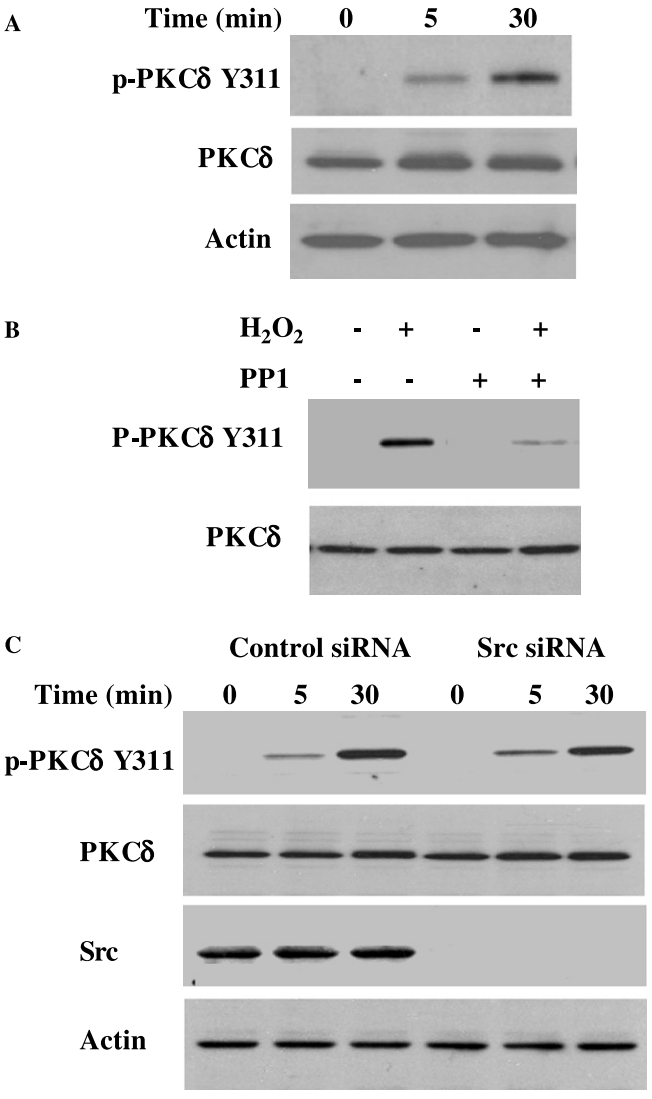

Fig. 1. Role of Src in the phosphorylation of tyrosine 311 in response to H_2O_2. U87 cells were treated with H_2O_2 (1 mM) and the phosphorylation of PKCδ on tyrosine 311 was determined using a tyrosine 311 phospho-specific antibody (A). The cells were pre-treated with PP1 (10 μM) for 30 min and the phosphorylation of PKCδ on tyrosine 311 was determined in cells treated with H_2O_2 (1 mM) for 30 min (B). Cells were transfected with control scramble or Src siRNA duplexes. Following three days, the expression of Src and the phosphorylation of tyrosine 311 in response to H_2O_2 (1 mM, 30 min) were determined (C). The results are from one representative out of four independent experiments.