Proteolytic Activation of Protein Kinase C-ε by Caspase-mediated Processing and Transduction of Apoptotic Signals*

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Several novel protein kinase C (PKC) isozymes have been identified as substrates for caspase-3. We have previously shown that novel PKCε is cleaved during apoptosis in MCF-7 cells that lack any functional caspase-3. In the present study, we show that in vitro-translated PKCε is processed by human recombinant caspase-3, -7, and -9. Tumor necrosis factor-α (TNF) triggered processing of PKCε to a 43-kDa carboxyl-terminal fragment, and cell-permeable caspase inhibitors prevented TNF-induced processing of PKCε in MCF-7 cells. PKCε was cleaved primarily at the SSPD|G site to generate two fragments with an approximate molecular mass of 43 kDa. It was also cleaved at the DDVD|G site to generate two fragments with molecular masses of 32 and 35 kDa. Treatment of MCF-7 cells with TNF resulted in the activation of PKCε and mutation at the SSPD|G (D383A) site inhibited proteolytic activation of PKCε. Overexpression of wild-type but not dominant-negative PKCε in MCF-7 cells delayed TNF-induced apoptosis, and mutation at the D383A site prevented antiapoptotic activity of PKCε. These results suggest that cleavage of PKCε by caspase-7 at the SSPD|G site results in the activation of PKCε. Furthermore, activation of PKCε was associated with its antiapoptotic function.

Protein kinase C (PKC)† is a family of phospholipid-dependent serine/threonine kinases that play a central role in the growth factor signal transduction pathway and regulate a wide variety of cellular functions, including cell proliferation, differentiation, and cell death (1). PKC represents a family of 11 isozymes that have been categorized into three groups: group A or conventional PKCs (α, βI, βII, and γ), group B or novel PKCs (δ, ε, η, and ζ), and group C or atypical PKCs (ζ and λ) (2–4). In addition, PKCδ resembles novel PKCs structurally but resembles atypical PKCs functionally (5). Whereas conventional PKCs require Ca2⁺ and diacylglycerol/phorbol esters for their activities, novel PKCs and atypical PKCs are Ca2⁺-independent. Atypical PKCs are also insensitive to diacylglycerol and phorbol esters.

In our native conformation, the regulatory domain of PKC interacts with the catalytic domain through pseudosubstrate sequences and prevents access of substrates to the catalytic site (3). The binding of cofactors, such as phosphatidylserine, Ca2⁺, and diacylglycerol/phorbol esters, to the regulatory domain induces a conformational change in the enzyme, thereby exposing the substrate-binding site, and catalysis takes place. The proteolytic cleavage of PKC at the hinge region can also separate the regulatory domain from the catalytic domain. The catalytic fragment (PKM) thus generated does not require any activators or cofactors for activation. In the case of conventional PKCs, proteolytic activation can be achieved by Ca2⁺-activated proteases, calpains.

It has been shown that novel PKC isozymes, including PKCδ, -θ, and -μ, are substrates for caspases (6–8), a family of cysteine proteases that specifically cleave proteins after Asp residues and play an essential role in the induction of apoptosis (9, 10). All caspases exist as inactive proenzymes, which are proteolytically processed to the active heterodimeric form. To date, 14 caspases have been identified. Whereas caspase-8, -9, and -10 participate in the initiation phase of apoptosis, caspase-3, -6, and -7 are involved in the execution phase of apoptosis (10). Activation of these executioner caspases results in the cleavage of critical cellular proteins, including poly(ADP-ribose) polymerase (PARP), DNA-dependent protein kinase, and lamin B.

Cleavage of novel PKC isozymes by caspases also generates catalytically active carboxyl-terminal fragments. Proteolytic activation of these novel PKC isozymes has been directly associated with cell death (6–8). No detectable cleavage of PKCα, -β, -ε, and -ζ was noted in these studies, and it was claimed that novel PKCδ and -θ were selectively involved in apoptosis (7). We and others have recently shown that both novel PKCs and atypical PKCζ are also cleaved by apoptotic stimuli (11–13). Atypical PKCζ serves as a substrate for several caspases, including caspase-3, -6, -7, and -8 (13). Caspase-mediated processing of atypical PKCζ generates carboxyl-terminal fragments that are catalytically active. Although the functional significance of atypical PKCζ processing by caspases is not clear, it has been postulated that PKCζ activation may promote cell survival (13).

Novel PKCε plays a very important role in cell survival and cell death (12, 14–19). Although novel PKCδ, -θ, and -μ are

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The abbreviations used are: PKC, protein kinase C; z-DEVD-fmk, benzoxycarbonyl-Asp-Glu-Val-Asp-7-amino-4-fluoromethylketone; z-IETD-fmk, benzoxycarbonyl-Ileu-Glu-Thr-Asp-fluoromethylketone; z-LEHD-fmk, benzoxycarbonyl-Leu-Glu-His-Asp-fluoromethylketone; DN, dominant-negative; MBP, myelin basic protein; PARP, poly(ADP-ribose) polymerase; PI, propidium iodide; TNF, tumor necrosis factor-α; CHAPS, 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonic acid.

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substrates for caspase-3, TNF induced cleavage of PKCe in MCF-7 breast cancer cells that lack functional caspase-3 (19).

Little is known about the regulation of PKCe activity by caspases and the functional significance of caspase-mediated processing of PKCe on cell death. In the present study, we report for the first time that PKCe is cleaved by caspase-7, proteolytic cleavage of PKCe by TNF results in its activation, and inhibition of PKCe activity abrogates its antiapoptotic activity.

EXPERIMENTAL PROCEDURES

Materials—TNF was purchased from R&D Systems (Minneapolis, MN), and cell-permeable caspase inhibitors were obtained from Kamiya Biomedical Co. (Seattle, WA). Calpeptin was from Calbiochem-Novabiochem. Annexin V conjugated to Alexa Fluor 488 and PI were purchased from Molecular Probes (Eugene, OR). Human recombinant caspase-7 and -8 and monoclonal antibody to PARP were from Pharmingen. Human recombinant caspase-2, -6, and -9 and caspase substrates were from BioVision (Palo Alto, CA). MBP was purchased from Sigma. Horseradish peroxidase-conjugated goat anti-mouse and donkey anti-rabbit antibodies were obtained from Jackson ImmunoResearch Laboratory Inc. (West Grove, PA). Polyvinylidene difluoride membrane was from Millipore, and enhanced chemiluminescence detection kit was from Amersham Biosciences.

Cell Culture and Transfection—MCF-7 cells were maintained in RPMI 1640 medium supplemented with 10% heat-inactivated fetal bovine serum and 2 mM glutamine and kept in a humidified incubator at 37 °C with 95% air and 5% CO₂. Cells were transfected using FuGENE 6 transfection reagent (Roche Molecular Biochemicals) according to the manufacturer’s protocol and selected using Geneticin (Invitrogen).

Site-directed Mutagenesis, in Vitro Translation, and Caspase Cleavage Assay—Full-length PKCe was cloned from human muscle cDNA library by PCR and subcloned into pcDNA3. Caspase cleavage sites were mutared using the QuickChange site-directed mutagenesis kit (Stratagene) and the manufacturer’s protocol. Dominant-negative PKCe was generated by mutation of Lys⁵³⁷ to Arg (K457R). Sequences were confirmed by DNA sequencing. [³⁵S]Met-labeled wild-type and mutant PKCe were synthesized by in vitro coupled transcription and translation with the T7 Quick TNT kit (Promega). Labeled proteins were incubated with human recombinant caspases in 50 mM Hepes, pH 7.5, 0.1% CHAPS, 5 mM dithiothreitol, 10% glycerol, and 0.1 mM EDTA at 37 °C for 1 h. Proteins were separated by SDS-PAGE, and autoradiography was performed with the dried gel.

Immunoblot Analysis—Equivalent amounts of total cellular extracts were analyzed by immunoblotting with appropriate antibodies as described.
were electrophoresed by SDS-PAGE and transferred electrophoretically to polyvinylidene difluoride membrane. Immunoblot analyses were performed as described previously (11).

**Immunokinase Assay**—Cells were treated with or without TNF as indicated in the text. PKCε was immunoprecipitated using a polyclonal antibody to PKCε, and immunokinase assay was performed as described previously (20) using MBP as the substrate in the absence of any cofactors. The reaction mixture was separated by SDS-PAGE, and autoradiography was performed with the dried gel.

**Annexin V/Propidium Iodide Binding Assay**—Cells were treated with or without TNF for 12 h. At the end of the incubation, both detached cells and attached cells were collected and washed with phosphate-buffered saline. Cells were then stained with annexin V-Alexa 488 conjugate and propidium iodide according to the manufacturer’s protocol and analyzed using a flow cytometer (Coulter Epics).

**RESULTS**

**Caspase Inhibitors Prevent Proteolytic Processing of PKCε Induced by TNF**—We have previously shown that both DNA-damaging agents and TNF could induce cleavage of PKCε (11, 19). To examine whether proteolytic activation of PKCε is mediated by caspases, we examined the effects of several cell-permeable caspase inhibitors on TNF-induced processing of PKCε. Fig. 1 shows that treatment of MCF-7 cells with TNF resulted in a decrease in full-length PKCε with a concomitant increase in a fragment with an approximate molecular mass of 43 kDa. Because binding of TNF to its receptors induces activation of apical caspase-8, we examined the effect of z-IETD-fmk, a cell-permeable inhibitor of caspase-8, on TNF-induced processing of PKCε. As shown in Fig. 1, caspase-8 inhibitor blocked TNF-induced cleavage of PKCε, z-DEVD-fmk, an inhibitor of caspase-3 and -7, was as effective as z-IELT-fmk in preventing TNF-induced processing of PKCε, whereas z-LEHD-fmk, an inhibitor of caspase-9, exhibited a partial inhibition. In contrast, calpeptin, a cell-permeable inhibitor of calpains, had little effect on the proteolytic cleavage of PKCε, even at 50 μM concentrations (data not shown). These results suggest that TNF-induced activation of caspases results in the processing of PKCε.

**In Vitro-translated PKCε Is Cleaved by Recombinant Caspases**—To determine whether PKCε is in fact a substrate for caspases, we examined the effect of human recombinant caspases on the cleavage of 35S-labeled in vitro-translated PKCε. Fig. 2 shows that PKCε was cleaved by caspase-3, -7, and -9. Human recombinant caspase-8 had only a slight effect, but caspase-2 and -6 were unable to process PKCε (data not shown).

**Comparison of TNF-induced processing of wild-type and mutant PKCε**. MCF-7 cells transfected with wild-type PKCε, or dominant-negative PKCε were treated with 1 nM TNF for the indicated periods of time. PKCε was immunoprecipitated, and an immunokinase assay was performed using MBP as the substrate in the absence of any cofactors. We have used twice as much protein during immunoprecipitation as A (500 μg) as compared with B (250 μg). Proteins were separated by SDS-PAGE, and autoradiography was performed with the dried gel. Equal amounts of total cellular extracts were subjected to Western blot analysis with PKCε antibody.

**Caspase cleavage sites of PKCε.**

| Regulatory domain | Catalytic domain |
|-------------------|-----------------|
| H2N---------------| 383 437 451     |
|                   | D1 K D1         |
|                   | -COOH           |
| 35-kDa            | 43-kDa          |
Caspase-mediated Processing of PKCe

Fig. 10. Effect of wild-type, D383A mutant, and dominant-negative PKCe on TNF-induced cell death. MCF-7 cells transfected with the indicated constructs were treated with or without 1 ng/ml TNF for 12 or 18 h. Cellular morphology was examined under a microscope.

shows). Cleavage of PKCe by human recombinant caspases generated fragments with apparent molecular masses of 52, 43, and 35 kDa. Caspase inhibitors z-DEVD-fmk and z-LEHD-fmk were able to inhibit processing of PKCe by caspase-3/-7 and caspase-9, respectively (Fig. 2). The cleavage pattern of PKCe generated by caspase-3, -7, and -9 was similar, suggesting that there may be overlapping specificity in an in vitro cleavage assay. However, the 52-kDa band was not detectable in the caspase-7-treated lane, presumably because it was further cleaved to 43-kDa and 9-kDa fragments. Because caspase-7 was most effective in processing PKCe, we examined the time course of PKCe cleavage by caspase-7. As shown in Fig. 3, significant processing of PKCe took place by 30 min, and little full-length PKCe remained after incubation of PKCe with caspase-7 for 2 h.

PKCe Is Cleaved at SSPD\(G\) and DDVD\(G\) Sites—To determine the potential caspase cleavage sites in PKCe, we mutated several Asp residues to Ala in PKCe by site-directed mutagenesis. As shown in Fig. 4, the intensity of the band near 43 kDa produced by the treatment of in vitro-translated PKCe with caspase-7 was much greater compared with that of the 52- and 35-kDa fragments. Mutation at the DDVD\(G\) (D451A) site abolished cleavage of PKCe to 52- and 35-kDa fragments, whereas mutation at the SSPD\(G\) (D383A) site prevented generation of fragments near 43 kDa. Cleavage of PKCe at the SSPD\(G\) site presumably produced two fragments of identical molecular mass around 43 kDa. During the TsT assay, sometimes we could detect some faint bands, including one near the 43-kDa region, even without any caspase treatment. This protein results from mRNA-dependent, ribosome-independent addition of \(^{35}\text{S}\)methionine on proteins (21). The important point is that the intensity of the 43-kDa band was similar in control and caspase-treated D383A mutant PKCe. Thus, it was not generated by the cleavage of PKCe by caspases.

To determine the cleavage pattern of PKCe generated by TNF in intact cells, we overexpressed PKCe in MCF-7 cells and treated them with TNF for varying periods of time. We have used an antibody that recognizes the carboxyl-terminal domain of PKCe. Fig. 5 shows that a 6-h exposure to TNF resulted in the generation of a fragment near 43 kDa and that the intensity of the 43-kDa band increased with time. Another band appeared above the 43-kDa fragment after treatment with TNF. The intensity of the band was maximum at 12 h and then rapidly decreased. This band likely represents a phosphorylated form of the 43-kDa fragment because mutation of Asp to Ala of nearby LSFD\(|N\) (D370A) and LGLD\(|E\) (D406A) sites did not prevent caspase-mediated processing of PKCe (data not shown). TNF also induced cleavage of PKCe to a 35-kDa fragment, and the intensity of the 35-kDa band increased when cells were treated with TNF for \(>12\) h. In addition, the intensity of the 35-kDa fragment was much less compared with that of the 43-kDa fragment. A faint band with an approximate molecular mass of 50 kDa was also apparent after treatment of the cells with TNF for 24 h. This could represent the carboxyl-terminal fragment of PKCe processed by caspase-9 (Fig. 2). Thus, PKCe was cleaved primarily at the SSPD\(G\) site in intact cells in response to TNF.

To determine whether mutation of PKCe at the DDVD\(G\) (D451A) or the SSPD\(G\) (D383A) site blocks corresponding cleavage of PKCe in intact cells, we introduced PKCe harboring a mutation at the D451A or D383A site in MCF-7 cells. As shown in Fig. 6, the intensity of the 43-kDa band increased dramatically after treatment of PKCe-overexpressing MCF-7 cells with TNF. TNF also induced cleavage of PKCe to 43-kDa fragments in cells expressing the D451A mutant, but mutation at the D383A site prevented TNF-induced processing of PKCe to 43-kDa fragments. These results corroborate that PKCe is processed primarily at the SSPD\(G\) site in intact cells. The same blot was probed with tubulin to control for loading differences.

TNF Induces Proteolytic Activation of PKCe—To determine whether TNF-induced processing of PKCe results in the activation of PKCe, we performed immunokinase assay using MBP as the substrate. As depicted in Fig. 7, proteolytic separation of the autoinhibitory regulatory domain from the catalytic domain is expected to result in the activation of PKCe in the absence of any cofactors. Fig. 8A shows that TNF caused a slight increase in MBP phosphorylation in vector-transfected MCF-7 cells. In contrast, MBP phosphorylation was almost undetectable in cells expressing DN-PKCe, even when cells were treated with TNF. TNF had a dramatic effect on MBP phosphorylation in PKCe-overexpressing cells (Fig. 8B). We have used twice as much protein during immunoprecipitation of PKCe from vector-transfected and DN-PKCe-expressing cells (Fig. 8A) compared with PKCe-overexpressing cells (Fig. 8B) to allow detection of PKCe activation in vector-transfected cells and to permit comparison between vector-transfected cells and DN-PKCe-expressing cells.
Because the SSPD|G site is localized at the hinge region of PKCα (Fig. 7), we examined whether processing at the SSPD|G site was necessary for TNF-induced activation of PKCα. We compared the ability of TNF to activate PKCα in cells expressing wild-type or mutant PKCα using an immunokinase assay. Fig. 9 shows that mutation at the D451A site that retains the ability of TNF to generate the 43-kDa catalytic fragment of PKCα was not sufficient to prevent activation of PKCα by TNF. In contrast, mutation at the D383A site that prevents generation of the 43-kDa fragment abolished TNF-induced activation of PKCα. Thus, processing of PKCα by TNF at the D383A site results in its activation.

Activation of PKCα Is Necessary for Its Antiapoptotic Action—We have previously shown that PKC activators protect cells against TNF-induced cytotoxicity (22). Fig. 10 shows that when cells transfected with a vector containing neomycin re-
stance gene but without PKCe construct (Fig. 10, Neo) were treated with TNF, cells rounded up and detached from the tissue culture dish. Overexpression of PKCe prevented the morphological changes indicative of apoptosis when cells were treated with TNF for 12 h, although cells started to round up after exposure to TNF for 18 h. Introduction of either D383A mutant or DN-PKCe failed to prevent cell death by TNF, suggesting that activation of PKCe was necessary for the antiapoptotic activity of PKCe.

To quantify cell death by apoptosis, we performed an annexin V binding assay (Fig. 11). When cells undergo apoptosis, phosphatidylserine is flipped from the inner to the outer leaflet of plasma membrane. During an early stage of apoptosis, annexin V binds to phosphatidylserine on the cell surface. Cells were co-stained with the cell-impermeable dye PI to distinguish apoptotic cells from necrotic cells. However, during late-stage apoptosis, annexin V can enter through the membrane, and therefore late-stage apoptosis cannot be distinguished from necrosis. Fig. 11 shows a representative dot blot analysis of annexin V conjugate-PI-stained cells. Cells stained with annexin V conjugate alone (bottom right quadrant) represent apoptotic cells, whereas cells co-stained with annexin V conjugate and PI (top right quadrant) represent late apoptotic and necrotic cells. Viable cells are shown at the bottom left quadrant (negative for both annexin V and PI). We have shown the mean ± S.E. of total percentage of cell death from two to three independent experiments at the top of each panel. A small percentage of cells undergo apoptosis even in the absence of any TNF treatment. TNF increased cell death to ~15% in vector-transfected MCF-7 cells (Neo), but the effect of TNF was attenuated in cells overexpressing PKCe such that ~5% cells underwent apoptosis. In contrast, introduction of mutant PKCe (D383A) caused an increase in apoptotic cell death to 25%. At present, it is not clear why blockage of PKCe cleavage not only inhibited the antiapoptotic effect of PKCe but also potentiated cell death by TNF. Overexpression of dominant-negative PKCe by itself increased cell death to ~8%, and TNF further enhanced cell death to 16%. We have consistently found that when DN-PKCe-expressing cells are cultured for several passages, their ability to potentiate TNF-induced cell death decreases, presumably because cells that are resistant to apoptosis survive selectively over cells that are prone to apoptosis.

Because MCF-7 cells lack functional caspase-3, they do not undergo DNA fragmentation (23). Therefore, we also assessed cleavage of PARP or DNA-dependent protein kinase to monitor cells undergoing apoptosis. Fig. 12 shows that treatment of MCF-7 cells with TNF resulted in the cleavage of PARP, as evidenced by the appearance of two bands with approximate molecular masses of 50 and 38 kDa. Thus, caspase-9 may cleave PKCe to generate a 43-kDa caspase-dependent fragment (11). Whereas caspase-3 and caspase-7 share similar substrate specificities: they both recognize AVP sequence and cleave PARP (24–26). However, PKCe is not processed by apoptotic stimuli in MCF-7 cells, suggesting that there may be some specificity in intact cells (19). Because both caspase-3 and -7 generated similar cleavage products, it is likely that caspase-7 is responsible for the cleavage of PKCe in MCF-7 cells. Human recombinant caspase-9 also generated cleavage products similar to those of caspase-3 and -7, in addition to two faint bands with approximate molecular masses of 50 and 35 kDa. Thus, caspase-9 may cleave PKCe at an additional site, and there may be overlapping specificity in an in vitro cleavage assay. It has been shown that PKCi is processed at the same site by several caspases, including caspase-8, -3, -6, -7 (13).

PKCe contains a DDVD \(\mid\) C site that is recognized by group II caspasers, including caspase-8, -3, -7, and -2 (27). Both caspase-3 and -7, but not caspase-2, cleaved PKCe at the DDVD \(\mid\) C site, although it was predominantly processed at the SSSPD\(\mid\) G site both in vitro and in intact cells. Because a cell-permeable inhibitor of caspase-3/7 was more effective than the inhibitor of caspase-9 in preventing generation of the 43-kDa fragment, it is likely that caspase-7 also cleaves PKCe at the SSSPD\(\mid\) G site. This site was recognized irrespective of the cell type or apoptotic stimuli. For example, TNF induced processing of PKCe to 43-kDa fragments in several breast cancer cells (19). In addition, treatment of HeLa cells with cisplatin, a DNA-damaging agent, also cleaved PKCe to generate a 43-kDa carboxy-terminal fragment (11). Whereas caspase-3 recognized conventional caspase cleavage sites in PKCi and \(-\mu\), it cleaved PKCe at an unconventional CQND\(\mid\) G site, even though PKCe possessed DDND\(\mid\) S and DHED\(\mid\) S sites that could be recognized by caspase-3-like proteases (6–8). PKCi also was cleaved primarily at an unconventional EETD\(\mid\) G site by several caspasers, whereas caspase-3 and -7 processed PKCi at a con-

![FIG. 12. Comparison of the effect of wild-type and D383A mutant PKCe expression on TNF-induced cleavage of PARP, DNA-dependent protein kinase, and PKC\(\mu\). MCF-7 cells transfected with either empty vector or wild-type or D383A mutant PKCe were treated with or without 1.0 nM TNF for 12 h. Western blot analyses were performed with total cell lysates using an antibody against PARP, DNA-dependent protein kinase catalytic subunit, PKC\(\mu\), or tubulin. The arrows indicate the processed form.](image-url)
It is conceivable that the hinge region of PKCs is more accessible to caspases. However, mutation or cleavage of the SSPD site at the hinge region may expose other caspase cleavage sites. This may explain why cleavage of PKCe by caspase-7 was more pronounced in the D383A mutant compared with wild-type PKCe (Fig. 4).

The primary cleavage site for all of the PKC isozymes was at the hinge region between the regulatory domain and the catalytic domain. Because the pseudosubstrate sequence at the regulatory domain interacts with the catalytic domain, caspase-mediated cleavage of PKCs separates the catalytic fragment from the autoinhibitory regulatory domain, resulting in activation of PKCs. Thus, in response to apoptotic stimuli, PKCs may be activated in the absence of any cofactors. Cleavage of PKCe by caspases at the hinge region (Ser-Ser-Pro-Asp383-Gly) would generate a carboxy-terminal fragment containing the catalytic domain. In contrast, cleavage of PKCe at the Asp-Asp-Val-Asp451-Cys site would generate a carboxy-terminal fragment lacking the ATP binding site. We have shown that treatment of MCF-7 cells with TNF results in PKCε activation and that mutation at the D383A site prevents TNF-induced activation of PKCe. Thus, in response to apoptotic stimuli, PKCs may be activated in the absence of any cofactors. Cleavage of PKCe by caspases at the hinge region (Ser-Ser-Pro-Asp383-Gly) would generate a carboxy-terminal fragment containing the catalytic domain. In contrast, cleavage of PKCe at the Asp-Asp-Val-Asp451-Cys site would generate a carboxy-terminal fragment lacking the ATP binding site. We have shown that treatment of MCF-7 cells with TNF results in PKCε activation and that mutation at the D383A site prevents TNF-induced activation of PKCe. In contrast, mutation at the D451A site did not affect TNF-induced activation of PKCe. Therefore, caspase-mediated processing of PKCe at the Ser-Ser-Pro-Asp383-Gly site results in its activation.

PKC activators have been shown to block TNF-induced cell death. In the present study, we have shown that overexpression of PKCe in MCF-7 cells inhibited TNF-induced apoptosis, suggesting that PKCe acts as an antiapoptotic protein. This is consistent with our previous report that overexpression of PKCe in rat embryo fibroblasts delayed apoptosis induced by the DNA-damaging agent cisplatin (17). We have also examined the functional significance of PKCe activation on TNF-induced cell death. In contrast to wild-type PKCe, introduction of DN-PKCe in MCF-7 cells by itself caused some cell death and failed to inhibit TNF-induced apoptosis. Blockage of the caspase cleavage site (D383A) of PKCe prevented antiapoptotic activity of PKCe, suggesting that activation of PKCe was necessary for the antiapoptotic function of PKCe. Thus, our results provide a direct link between activation of PKCe and its antiapoptotic function.

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