Ceramide Inhibits Protein Kinase B/Akt by Promoting Dephosphorylation of Serine 473*

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The second messenger ceramide (N-alkylphosphoglycerol) has been implicated in a host of cellular processes including growth arrest and apoptosis. Ceramide has been reported to have effects on both protein kinases and phosphatases and may constitute an important component of stress response in various tissues. We have examined in detail the relationship between ceramide signaling and the activation of an important signaling pathway, phosphatidylinositol (PI) 3-kinase and its downstream target, protein kinase B (PKB). PKB activation was observed following stimulation of cells with the cytokine granulocyte-macrophage colony-stimulating factor. Addition of cell-permeable ceramide analogs, C₂- or C₆-ceramide, caused a partial loss (50–60%) of PKB activation. This reduction was not a result of decreased PI(3,4,5)P₃ or PI(3,4)P₂ generation by PI 3-kinase. Two residues of PKB (threonine 308 and serine 473) require phosphorylation for maximal PKB activation. Serine 473 phosphorylation was consistently reduced by treatment with ceramide, whereas threonine 308 phosphorylation remained unaffected. In further experiments, ceramide appeared to accelerate serine 473 dephosphorylation, suggesting the activation of a phosphatase. Consistent with this, the reduction in serine 473 phosphorylation was inhibited by the phosphatase inhibitors okadaic acid and calyculin A. Surprisingly, threonine 308 phosphorylation was abolished in cells treated with these inhibitors, revealing a novel mechanism of regulation of threonine 308 phosphorylation. These results demonstrate that PI 3-kinase-dependent kinase 2-catalyzed phosphorylation of serine 473 is the principal target of a ceramide-activated phosphatase.

Generation of the membrane sphingolipid ceramide may constitute an important signaling event activated by cellular stresses. These include activation of tumor necrosis factor receptor, ultraviolet radiation, chemotherapeutic drugs, and hyperosmolarity (1, 2). Mice deficient for acidic sphingomyelinase display resistance to radiation-induced apoptosis (3). Patients with Niemann-Pick syndrome also lack acidic sphingomyelination, and cell lines derived from these patients display resistance to apoptotic stimuli including ultraviolet radiation (3). Furthermore, activation of anti-apoptotic and oncogenic pathways has been reported to decrease sphingomyelinase activity (4). Thus, ceramide accumulation may function as both a primary and secondary initiator of apoptosis by interfacing with the apoptosis machinery directly and by potentiating the apoptotic response.

Relatively little is known about the way in which ceramide acts upon signaling pathways. Ceramide can activate both protein kinase (5) and protein phosphatase 2A-like activities (6–9). Ceramide has also been demonstrated to inactivate certain members of the protein kinase C family (10, 11) while activating others (12, 13). Moreover, one of the mechanisms of growth arrest caused by ceramide-activated protein phosphatase may be through retinoblastoma protein dephosphorylation (14). Recent work suggests that ceramide may induce apoptosis by activating pro-death pathways, possibly through activation of the stress-activated protein kinase c-Jun N-terminal kinase, or JNK (15), and by promoting dephosphorylation of the pro-survival protein Bcl-2 (16) and the pro-apoptotic protein Bad (17, 18). The dephosphorylation of Bcl-2 is proposed to be mediated by ceramide-activated protein phosphatase activity, whereas dephosphorylation of Bad by ceramide may be through activation of the kinase suppressor of Ras (KSR) and subsequent activation of MEK1 and MAPK, leading to decreased PKB activation (17).

Ceramide may also augment the execution of apoptosis by the inactivation of pro-survival pathways. Recently, several reports have supported this notion by demonstrating negative regulation of ceramide on the PI3K signaling pathway. Zundel and Giaccia (18) reported that ceramide specifically inhibits PI3K, which in turn results in the loss of PKB activation, a regulatory target of PI3K activity. Three other reports also demonstrated the negative effects of ceramide on PKB activation (17, 19, 20), but they suggested that the mechanism of inhibition does not involve PI3K inhibition. Because none of these reports actually measured the de novo synthesis of 3′-phosphorylated phosphatidylinositol, the relationship between ceramide and PI3K remains unresolved.

Downstream targets of the PI3K-PKB pathway proposed to be involved in survival include GSK-3 (21), caspase-9 (22), the Forkhead family of transcription factors (23–25), and the Bcl-2 family member Bad (26, 27), although the last may not be

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¶ The abbreviations used are: Bad, Bcl-X-associated death inducer; CAPP, ceramide-activated protein phosphatase; GM-CSF, granulocyte-macrophage colony-stimulating factor; HPLC, high performance liquid chromatography; KSR, kinase suppressor of Ras; MAPK, mitogen-activated protein kinase; MEK, mitogen-activated protein kinase/extracellular signal-regulated kinase kinase; PAGE, polyacrylamide gel electrophoresis; PDK, PI3K-dependent kinase; PI, phosphatidylinositol; PI3K, phosphatidylinositol 3-kinase; PKA, protein kinase A; PKB, protein kinase B; PKC, protein kinase C.
Physiologically relevant (42, 43, 48). Activation of PI3K-dependent pathways other than PKB may include serum- and glucocorticoid-regulated kinase (or SGK) (28), PKC isofoms, Bruton’s tyrosine kinase, and members of the Rh/Rac family of small G proteins, involved in cytoskeletal organization and membrane trafficking (for review, see Ref. 29). The pleckstrin homology domain has been found in an increasing number of signaling proteins and likely functions as a recruitment module for binding of proteins to the lipid products of PI3K (30). Thus, PI3K may be key to many cellular processes, only one of which is the protection of cells from apoptosis.

PI3K is activated by several mechanisms. In resting cells, class I PI3K, which is composed of an SH2 domain-containing p85 subunit and a 110-kDa catalytic subunit, is cytosolic but is thought to redistribute rapidly to the plasma membrane after receptor-activated tyrosine phosphorylation. Once at the membrane, PI3K has access to its substrate, PI(4,5)P2, which it phosphorylates to generate PI(3,4,5)P3. The PI(3,4,5)P3 product is a substrate for SHIP and other 5’-phosphatases (31) and the 3’-phosphatase PTEN (32), which promote the formation of PI(3,4)P2 and PI(4,5)P2, respectively. The loss of PTEN results in elevated PI(3,4,5)P3 and PI(3,4)P2, leading to higher PKB activity, which may contribute to tumorigenesis (33).

PI3K is also activated by interaction with the small G protein Ras (34). The contribution of Ras toward PI3K activation has become the focus of much attention, as the oncogenic potential for activated forms of Ras, and oncogenes that lead to its activation, appear to be mediated to some extent through PI3K activation. Thus, if ceramide generation leads directly to inhibition of PI3K, the effects may be widespread and significant. Here we provide biochemical evidence that ceramide regulates PKB activity not through PI3K modulation, but rather by accelerating the dephosphorylation of PKB selectively at Ser-473, a residue critical for maximal activation. This reduction in phosphorylation could be restored by pretreatment of cells with okadaic acid or calyculin A, two potent phosphatase inhibitors. This study suggests that PI3K-dependent kinase (PDK2)-targeted phosphorylation of PKB may be regulated by ceramide-activated phosphatases.

**EXPERIMENTAL PROCEDURES**

**Materials—**Recombinant human GM-CSF was from R&D Systems (Minneapolis). C2, C2-dihydroxy, and C6-ceramides and LY-294002 were from Calbiochem. Antibodies used were rabbit anti-phospho-Thr-288 PKB (9271), rabbit anti-phospho-Ser-473 PKB (9270), and mouse monoclonal anti-phospho-MAPK (9106), all from New England Biolabs. Sheep anti-PKB (06-558) and Crosstide were from Upstate Biotechnology Inc. Okadaic acid and calyculin A were from Sigma Chemical Co. U0126 was from Promega (Madison, WI).

**Cell Lines and Tissue Culture—** TF-1 cells (American Type Culture Collection) were maintained at 37 °C and 5% CO2 in a humidified incubator, in RPMI 1640 medium supplemented with 10% fetal calf serum and 1% conditioned medium containing recombinant human GM-CSF. Cells were starved of cytokine by overnight incubation in medium without GM-CSF. Alternatively, cells were washed three times and incubated in GM-CSF-free medium for at least 4 h prior to use in the experiment.

**Cell Treatments and Lysis Conditions—** Cells were incubated at 37 °C in HEPES-buffered RPMI immediately prior to assay. Cells were generally treated with ceramides or vehicle for 20 min prior to the addition of 50 ng/ml GM-CSF for various times, which was found previously to induce maximal tyrosine phosphorylation. In experiments involving okadaic acid or calyculin A, compounds were added at least 20 min prior to the addition of ceramide. To stop reactions, cells were pelleted and solubilized in ice-cold lysis buffer containing 20 mM TrisHCl, pH 7.4, 137 mM NaCl, 0.5% Nonidet P-40, 10 mM NaF, 0.2 mM Na3VO4, 1 mM Na2MoO4, 1 μg/ml microcystin-LR, 0.25 mM phenylmethylsulfonyl fluoride, 1 μM pepstatin, 0.5 μg/ml leupeptin, and 10 μg/ml soybean trypsin inhibitor. Nuclei were pelleted by centrifugation at 13,000 rpm for 1 min, and supernatants were transferred to new tubes.

**Immunoblotting—** To visualize phosphorylated and total PKB or MAPK, 50 μg of total protein was fractionated on 9% SDS-polyacrylamide gels and transferred to nitrocellulose. Blots were blocked in 5% skim milk in Tris-buffered saline and then incubated with a 1:1,000 dilution of the appropriate antibody in Tris-buffered saline containing 1% bovine serum albumin. Primary antibody was detected using the appropriate secondary antibody coupled to hors eradish peroxidase, followed by enhanced chemiluminescence as instructed by the manufacturer (Amersham Pharmacia Biotech).

**PKB Assay—** To measure PKB activity, immunocomplex kinase assays were performed as described previously (42). Briefly, PKB was immunoprecipitated from nuclear-free cell extracts using 2 μg of sheep anti-PKB coupled to protein-G Sepharose beads. Beads were resuspended in kinase buffer containing 300 μM ATP, 10 μCi of [γ-32P]ATP, 75 mM MgCl2, and 60 μM Crosstide and incubated at 30 °C for 15 min. 32P-Labeled Crosstide was quantitated by liquid scintillation counting.

**Measurement of PI-3,4,5-P3 and PI-3,4-P2 Levels—** Cells were starved of cytokine overnight, washed, and resuspended at 1 × 107 cells/ml in phosphate-free RPMI containing 20 mM HEPES, pH 7.4, and 1 mM/32P (carrier free, ICN) for 90 min at 37 °C. Cytokines or/and ceramide or other inhibitors were added as indicated, reactions were stopped, and phosphatidylinositol levels were determined by HPLC analysis of glycerophosphoinositides, derived by deacylation of the lipids as described (36). ATP, [H]inositol (1,4,5)Pi (ICN), and [H]inositol (1,3,4,5)Pi (ICN) were used to calibrate the column and eluted at 75, 84, and 106 min, respectively. PI(4,5)P2, which did not change significantly with stimulation, was used as a reference standard to monitor loading onto the HPLC column.

**RESULTS**

**Ceramide Inhibits PKB Activation by GM-CSF—** To investigate the effects of ceramide on PKB activation by cytokines, we began by measuring PKB activity in vitro following exposure of cells to GM-CSF, ceramide, or the PI3K inhibitor LY-294002. In human erythroleukemic TF-1 cells, stimulation with GM-CSF resulted in a large increase in PKB activity after a 1- or 3-min treatment (Fig. 1A). At both times, treatment of cells with C6-ceramide reduced the activation of PKB. Identical re-
sults were obtained when another short chain analog of ceramide, C6-ceramide, was used (data not shown). Inhibition of PI3K by treatment of cells with LY-294002 blocked almost entirely the activation of PKB. In other experiments, treatment of cells with dihydroceramide, an inactive ceramide analog, had no effect (data not shown), consistent with the findings of others (19, 20). C6-ceramide was not reducing the signaling capacity of the GM-CSF receptor nonspecifically because phosphorylation of MAPK was unaffected by ceramide treatment (Fig. 1B). A potent MEK inhibitor, U0126 (35), completely abolished the phosphorylation of MAPK in this experiment, whereas LY-294002 had no effect, as we have shown previously (36). Thus, C6-ceramide specifically reduces the maximal activity of PKB achieved by stimulation of cells with GM-CSF without affecting the parallel MEK-MAPK pathway.

Ceramide Does Not Reduce de Novo Generation of PI(3,4)P2 or PI(3,4,5)P3—We next examined the effects of ceramide on PI3K activity in whole cells. TF-1 cells were metabolically labeled with 32P for 2 h. Cells were either left unstimulated (closed circles) or stimulated with GM-CSF in the presence of ceramide (open circles), LY-294002 (closed triangles), or vehicle alone (closed squares) for 3 min, and lipids were extracted as described under “Experimental Procedures.” Desylated lipids were fractionated by anion exchange chromatography and detected by liquid scintillation counting. Results are representative of two independent experiments.

The results of Fig. 2 suggest that the amounts of both of these lipids above unstimulated cells. PI(3,4,5)P3. Stimulation with GM-CSF caused an increase in C6-ceramide specifically reduces the maximal activity of PKB 294002 had no effect, as we have shown previously (36). Thus, phosphorylation of MAPK in this experiment, whereas LY-potent MEK inhibitor, U0126 (35), completely abolished the phosphorylation of MAPK for the indicated times. Cell lysates were normalized based on protein concentrations, fractionated by SDS-PAGE, and immunoblotted using anti-phospho-Thr-308. Results are representative of four independent experiments. C, cells were stimulated with GM-CSF for 30 s, washed twice with RPMI 1640, and treated for 5 min with vehicle or C6-ceramide. Cell lysates were fractionated by SDS-PAGE and immunoblotted with phospho-Ser-473. Total PKB was immunoprecipitated and fractionated by SDS-PAGE for the indicated times. 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activity of PKB in each of these conditions. Okadaic acid was ineffective in restoring the decrease in activity caused by ceramide. This was consistent with previous reports (19, 20) and at first suggested that ceramide was not affecting PKB through dephosphorylation. However, we noticed that cells treated only with okadaic acid and GM-CSF also had reduced PKB activity. This suggested that okadaic acid may interfere with the normal activation of PKB, which may be independent of the inhibitory actions of ceramide. Immunoblotting for Ser-473 phosphorylation demonstrated that okadaic acid fully restored the decreased phosphorylation of this residue caused by ceramide back to stimulated levels (Fig. 4B). Because okadaic acid was restoring Ser-473 phosphorylation but could not restore kinase activity, we monitored the phosphorylation state of the other regulatory site, Thr-308. Surprisingly, okadaic acid, either in the presence or absence of ceramide, blocked Thr-308 phosphorylation (Fig. 4B). We then performed additional experiments to examine the effects of okadaic acid on PKB phosphorylation. As shown in Fig. 5, okadaic acid did not affect the kinetics or extent of Ser-473 phosphorylation induced by GM-CSF. However, at all times examined Thr-308 phosphorylation was reduced significantly.

To confirm the involvement of a serine phosphatase in these processes, additional experiments were performed with a functionally distinct phosphatase inhibitor, calyculin A. Fig. 6A shows that cells pretreated with ceramide and calyculin A have restored Ser-473 phosphorylation. In another experiment, calyculin A was compared with okadaic acid. Both inhibitors restored Ser-473 phosphorylation equally during ceramide treatment (Fig. 6B). Also, both are shown here to inhibit Thr-308 phosphorylation, demonstrating that this effect is not unique to okadaic acid. As a control to show that these phosphatase inhibitors were not disrupting the generation of PI(3,4,5)P3 following GM-CSF stimulation, lipid analysis was also performed. GM-CSF-stimulated generation of PI(3,4,5)P3 remained unaffected (unstimulated: 190 cpm ± 34, GM-CSF: 738 ± 121, GM-CSF + calyculin A: 698 ± 90, GM-CSF + okadaic acid: 711 ± 88). Together, these results indicate that (i) the effects of ceramide on Ser-473 phosphorylation were through the activation of a phosphatase, and (ii) okadaic acid and calyculin A inhibit an activity necessary for Thr-308 phosphorylation.

DISCUSSION

Recently, three groups have studied the effects of ceramide on PI3K and PKB activation (18–20). Zundel and Giaccia (18) provided evidence that PI3K is a direct target of ceramide, with its inhibition leading to decreased PKB activation. In contrast, Zhou and co-workers (19) and Summers and co-workers (20) provided evidence that ceramide does not affect PI3K activation, but rather inhibits PKB directly. In these three reports, PI3K activity was determined by immunoprecipitating PI3K (either directly or associated with tyrosine-phosphorylated pro-
PKB Regulation by Ceramide

...teins) followed by in vitro kinase assays using PI as a substrate. Because this method does not examine the de novo accumulation of the PI3K reaction products, we felt this issue required further examination, given the importance of PI3K in many different signaling pathways. Therefore, we measured the de novo 3’-phosphoinositide production in the presence of ceramide following stimulation with GM-CSF. In contrast to LY-294002, which completely abolished Pi(3,4,5)P3 and Pi(3,4)P2 generation, ceramide had no effect on the generation of these lipid species. Thus, we conclude that ceramide does not regulate the activation of PI3K, nor does it inhibit PI3K activity directly.

Under the same conditions, we measured PKB activity and found it to be lowered by 50–60% by ceramide. This suggested to us that ceramide was altering the activation of PKB independently of any effect on PI3K. Ceramide could be acting on upstream kinases that regulate PKB, or it may affect the translocation of PKB to the plasma membrane. Closer examination of the regulatory sites of PKB demonstrated that only Ser-473 phosphorylation was largely attenuated by ceramide treatment. Ser-473 is believed to be phosphorylated by a kinase termed “PDK2,” although its identity has not been demonstrated conclusively (38, 39). The phosphorylation of Thr-308 is better understood. It is catalyzed by PDK1, a constitutively active kinase that is responsible for activation loop phosphorylation of PKB, as well as other protein kinases, including the PKCs, PKA, and p70S6K (40). For Thr-308 to become phosphorylated, both PDK1 and PKB must bind with Pi(3,4,5)P3 to induce a conformational change providing access for PDK1. The observation that Thr-308 phosphorylation was not affected by ceramide confirms that PKB retained access to Pi(3,4,5)P3, most likely through translocation to the plasma membrane, consistent with a lack of effect of ceramide on PI3K. Thus, our results here demonstrate that the reduction in PKB activity is caused by reduced overall Ser-473 phosphorylation.

To understand the mechanism by which ceramide was reducing Ser-473 phosphorylation, we asked if this was due to a ceramide-activated phosphatase. In experiments in which ceramide was added to cells that had been stimulated with GM-CSF, Ser-473 phosphorylation was reduced, demonstrating that ceramide could accelerate the dephosphorylation of this residue, possibly by activation of a phosphatase. This result also reinforces the conclusion that ceramide was not acting on components upstream of PKB activation, such as PI3K. Activation of a phosphatase has recently been shown to dephosphorylate Ser-473 of PKB selectively, and not Thr-308, which may be protected (54, 55). This is completely consistent with our findings that ceramide, which can activate a protein phosphatase 2A-like activity (called CAPP), can only cause the acceleration of Ser-473 dephosphorylation, but not Thr-308. This activity is sensitive to the phosphatase inhibitor okadaic acid (7), and okadaic acid has been used to implicate CAPP in the dephosphorylation of Bcl-2, retinoblastoma protein, and PKC isoforms (10, 14, 16). In our experiments, okadaic acid fully restored the reduced phosphorylation of Ser-473 caused by ceramide (Figs. 4–6), in agreement with the hypothesis that the effects of ceramide on Ser-473 phosphorylation were due to phosphatase activation. Additional support for this conclusion was achieved by using a structurally distinct phosphatase inhibitor, calyculin A, which also restored ceramide-inhibited Ser-473 phosphorylation (Fig. 6). Thus, these results are consistent with CAPP being the mediator of PKB dephosphorylation at Ser-473.

We also examined the effects of okadaic acid and calyculin A on Thr-308 phosphorylation. Interestingly, we found that both compounds greatly reduced Thr-308 phosphorylation (Fig. 6), suggesting that a dephosphorylation event is necessary for PDK1-catalyzed Thr-308 phosphorylation. The reduction in Thr-308 phosphorylation by okadaic acid coincided with reduced activity. Thus, our experiments have revealed an unexpected mechanism of regulation of Thr-308 phosphorylation. This was surprising, given that PDK1 is constitutively active. One possible mechanism is that an inhibitor protein of PDK1 exists which is activated by phosphorylation. Yet another possible mechanism of regulation is by phosphorylation of PDK1 itself, which has recently been reported (41). In addition, our findings that PDK1-catalyzed Thr-308, but not PDK2-catalyzed Ser-473 phosphorylation, is restored by these phosphatase inhibitors would argue that the two enzymes are distinct. However, in light of the recent suggestion that PDK2 may actually be PDK1-bound to a modifying protein (39), the role that phosphatase inhibitors play in this model may be complex. In contrast, others have suggested that PDK2 is the integrin-linked kinase (38), distinct from PDK1, and it will be interesting to see if ceramide similarly reduces ILK-phosphorylated substrates through phosphatase activation.

The reduction of PKB phosphorylation at Ser-473 and the parallel decrease in activity caused by ceramide may be significant contributors to ceramide-mediated apoptosis (17–19). At present, the mechanism by which PKB protects against apoptosis is not firmly established. Zundel and Giaccia (18) and Basu and co-workers (17) have suggested that dephosphorylation of Bad is the relevant downstream target conferring ceramide-induced apoptosis, through inactivation of PKB, although they come to different conclusions on the primary mechanism. Basu and co-workers (17) suggest that the ceramide-activated KSR inhibits PKB through activation of the MEK-MAPK pathway, while Zundel and Giaccia (18) suggest that ceramide directly inhibits PI3K, together blocking PKB activity and Bad phosphorylation. Ultimately, both groups demonstrated that Bad phosphorylation is reduced by ceramide treatment. Whether PKB inactivation is responsible for decreased Bad phosphorylation by ceramide is questionable because we (42) and others (43) have provided examples dissociating endogenous Bad phosphorylation from endogenous PKB activation and PI3K-dependent survival. Interestingly, the KSR has recently been shown under some conditions to antagonize the MEK-MAPK pathway and block Ras-induced transformation (44–47). With this in mind, we have recently demonstrated that phosphorylation of Bad on Ser-112 occurs by a MEK-dependent mechanism, and this phosphorylation regulates binding with Bcl-XL (48). Thus, modulation of the KSR by ceramide may block the phosphorylation of Bad on Ser-112 and promote Bcl-XL heterodimers, a condition thought to inactivate Bcl-XL and promote apoptosis (49). This offers a possible alternative explanation for the effect of ceramide on Bad phosphorylation, independent of a role for PKB, although in our experiments we have not observed an effect of ceramide on MAPK activity.

The apoptotic effects of ceramide are probably mediated through other targets in addition to Bad. For example, some of the effects of ceramide on apoptosis may be mediated by Bcl-2 dephosphorylation as proposed by Ruvolo and co-workers (16). With respect to PKB inactivation, ceramide may disrupt the ability of PKB to prevent apoptosis by phosphorylating transcription factors. In Caenorhabditis elegans, genetic experiments have ordered signaling through the insulin-like receptor Daf-2 to Age-1 (PI3K homolog), Pdk-1, and Akt activation, with Daf-16 phosphorylation, which promotes longevity and prevents developmental arrest at the dauer stage (50–53). Null mutations in the pdk-1 gene cause constitutive arrest in the dauer stage, whereas activating mutations rescue null muta-
tions of daf-2 or age-1. Daf-16 is the C. elegans homolog of the mammalian Forkhead transcription factor. In mammalian cells, PKB phosphorylation of Forkhead members has been shown by several groups (23–25). Phosphorylation of Forkhead by PKB stimulates its export from the nucleus. This may prevent the transcription of pro-apoptotic proteins, including up-regulation of the Fas ligand. Thus, ceramide may prevent the nuclear export of Forkhead transcription factors by reducing PKB activation.

In conclusion, we have demonstrated the selective targeting of Ser-473 of PKB by a ceramide-activated phosphatase and have conclusively shown that ceramide has no effect on the lipids generated following activation of PI3K. This ceramide-mediated phosphorylation motif is present in p70s6k (Thr-389) and in the novel and conventional PKC kinases. In contrast, other kinases such as the atypical PKCs and PKC-related kinase (PRK1) contain PKD2-pseudosubstrate domains that do not require phosphorylation for activation. Thus, the activity of these kinases may be immune to ceramide treatment. The possible effect of ceramide on other protein kinases that may be regulated by PKB phosphorylation of Forkhead members has been shown by several groups (23–25). Phosphorylation of Forkhead by PKB stimulates its export from the nucleus. This may prevent the transcription of pro-apoptotic proteins, including up-regulation of the Fas ligand. Thus, ceramide may prevent the nuclear export of Forkhead transcription factors by reducing PKB activation.

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