Ceramide Recruits and Activates Protein Kinase C ζ (PKCζ) within Structured Membrane Microdomains*

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We have previously demonstrated that hexanoyl-γ-erythro-sphingosine (C₆-ceramide), an anti-mitogenic cell-permeable lipid metabolite, limited vascular smooth muscle growth by abrogating trauma-induced Akt activity in a stretch injury model of neointimal hyperplasia. Furthermore, ceramide selectively and directly activated protein kinase C ζ (PKCζ) to suppress Akt-dependent mitogenesis. To further analyze the interaction between ceramide and PKCζ, the ability of ceramide to localize within highly structured lipid microdomains (rafts) and activate PKCζ was investigated. Using rat aorta vascular smooth muscle cells (A7r5), we now demonstrate that C₆-ceramide treatment results in an increased localization and phosphorylation of PKCζ within caveolin-enriched lipid microdomains to inactivate Akt. In addition, ceramide specifically reduced the association of PKCζ with 14–3–3, a scaffold protein localized to less structured regions within membranes. Pharmacological disruption of highly structured lipid microdomains resulted in abrogation of ceramide-activated, PKCζ-dependent Akt inactivation, whereas molecular strategies suggest that ceramide-dependent PKCζ phosphorylation of Akt3 at Ser34 is necessary for ceramide-induced vascular smooth muscle cell growth arrest. Taken together, these data demonstrate that structured membrane microdomains are necessary for ceramide-induced activation of PKCζ and resultant diminished Akt activity, leading to vascular smooth muscle cell growth arrest.

A common response to inflammation or stress is the formation of ceramide, an anti-mitogenic, pro-apoptotic lipid metabolite. Ceramide suppresses vascular smooth muscle (VSM) cell mitogenesis in vitro and in vivo (1, 2). The initial mechanisms by which ceramide leads to suppression of mitogenesis have not yet been defined. Both biophysical (lipid microdomains) and biochemical (ceramide binding targets) mechanisms have been proposed to address this issue. The present study presents an integrated version of these theories.

Published and preliminary data demonstrate that protein kinase C ζ (PKCζ) is a direct and selective target for ceramide (2–6) and that ceramide-activated PKCζ is necessary for inactivation of Akt-dependent mitogenesis in vascular smooth muscle cells (2). These results have been recapitulated in vivo where cell-permeable ceramide analogues function as novel therapeutics to limit VSM cell growth, in part by abrogating trauma-induced Akt activity in a model of neointimal hyperplasia after stretch injury (1).

PKCζ is an atypical isoform of the PKC superfamily and is directly or indirectly regulated by several lipids including ceramides and phosphatidylinositol 3,4,5-trisphosphate but not by diacylglycerol and calcium (7). Generally, PKCs reside in the cytosol and translocate to membranes upon stimulation to interact with activating lipid cofactors. PKCζ may be directly recruited to membranes via interactions with the Cl domain or indirectly recruited to membranes by formation of complexes with PDK1 and scaffolding proteins (7). As lipid micro- and macrodomains have been identified as “signaling hot-spots,” we investigated the roles of ceramide-enriched microdomains in the activation of PKCζ and subsequent inhibition of cellular proliferation via Akt inactivation.

EXPERIMENTAL PROCEDURES

Materials and Cell Culture—Hexanoyl-γ-erythro-sphingosine (C₆-ceramide) was purchased from Avanti Polar Lipids (Alabaster, AL). Hexanoyl-γ-erythro-sphingosine [hexanoyl-6–³H] was obtained from American Radiolabeled Chemicals (St. Louis, MO), and recombinant human PDGF-BB was purchased from R&D Systems, Inc (Minneapolis, MN). Anti-Akt, phosphorylated Akt (Ser473), and phosphorylated PKCζ (Thr410) antibodies were purchased from Cell Signaling Technology, Inc. (Beverly, MA). Anti-PKCζ, 14–3–3 pan, and caveolin-1 antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). The anti-FLAG epitope antibody was purchased from Cayman Chemicals (Ann Arbor, MI). For Western blotting, 4–12% precasted SDS-polyacrylamide gel electrophoresis gradient gels were obtained from Invitrogen, and enhanced chemiluminescence reagent was purchased from Amersham Biosciences. [³H]Thymidine was purchased from MP Biomedicals (Irvine, CA). Methyl-β-cyclodextrin and other chemicals were purchased from Sigma-Aldrich. All cell culture media and reagents were purchased from Invitrogen. Rat embryonic th-
racic aorta smooth muscle cells (A7r5) were obtained from American Type Culture Collection (Manassas, VA) and cultured as described previously (2). Cells were treated as described in the text after 24 h of serum starvation.

Isolation of Buoyant Fractions by Sucrose Gradient Centrifugation—After treatment, low buoyant fractions were isolated by two different methodologies. The first is based upon the detergent-free method originally described by Song et al. (8). Briefly, A7r5 cells were washed twice with cold phosphate-buffered saline followed by the addition of 500 mM sodium carbonate buffer (pH = 11.0) containing 1 mM sodium orthovanadate, 1 mM phenylmethylsulfonyl fluoride, 1 mM β-glycerolphosphate, and a protease inhibitor tablet (Roche Applied Science). Cell lysates were subsequently homogenized using a Dounce glass homogenizer followed by sonicication with three 15-s bursts. The lysates were then mixed with equal volumes of 90% sucrose in MBS buffer (25 mM MES, pH 6.5, 150 mM NaCl) and layered at the bottoms of ultracentrifuge tubes. Samples were then overlaid with 4 ml of 35% and 4 ml of 5% sucrose MBS-carbonate buffer. The samples were centrifuged at 35,000 rpm for 20 h at 4 °C using a Beckman swinging bucket rotor (SW41ti). 1-ml fractions were collected from the top down of each gradient. An additional methodology to obtain buoyant fractions was also employed, based upon Tween 20 solubility, originally described by Ref. 9 with some modifications. Here, after treatment, A7r5 cells were lysed in 2 ml of MBS (25 mM MES, pH 6.5, 150 mM NaCl) and washed twice with cold phosphate-buffered saline followed by the addition of detergent-free lysis buffer (50 mM HEPES, 150 mM NaCl, 5 mM NaF, 1 mM β-glycerolphosphate, protease inhibitor mixture (Roche Applied Science)). Cell lysates were homogenized by Dounce homogenization and then centrifuged at 100,000 × g for 1 h. The supernatant (cytosolic fraction) was collected. The pellet was resuspended in detergent-free lysis buffer and recentrifuged to diminish any cytosolic contamination. The supernatant was removed, and the pellet was resuspended in Nonidet P-40 detergent lysis buffer and sonicated briefly. After incubation on ice for 30 min, the sample was centrifuged at 14,000 rpm for 10 min. The supernatant was collected (membranous fraction) and analyzed by Western blotting.

Western Blot Analysis—Western blots were done as described previously (2) with some modifications. Briefly, after selected treatment, cells were washed once with cold phosphate-buffered saline followed by the addition of cold lysis buffer (1% Nonidet P-40, 20 mM Tris, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 2.5 mM Na3PO4, 1 mM β-glycerolphosphate, 1 mM Na3VO4, protease inhibitor mixture, and 1 mM phenylmethylsulfonyl fluoride in double distilled H2O, pH 7.5) on ice. Cell lysates were lysed for 15 min on ice, and cell lystate was harvested and centrifuged at 14,000 × g for 10 min at 4 °C. Cell lystate or sucrose gradient fractions were loaded in 4–12% precasted SDS-polyacrylamide gel electrophoresis gradient gels, and the resolved proteins were transferred to Hybond C nitrocellulose membranes (GE Healthcare). The membranes were blocked in 5% nonfat milk in Tris-buffered saline containing 0.1% Tween 20 for 1 h and then incubated with the appropriate primary antibody overnight at 4 °C. After incubation, the membranes were washed with Tris-buffered saline containing 0.1% Tween 20, the protein bands were detected by the enhanced chemiluminescence method and quantitated by laser densitometry.

Expression Constructs—Human Akt3 was cloned from human coronary aortic smooth muscle cell cDNA by conventional methodologies. This Akt3 was inserted into pcDNA3.1 (Invitrogen) with a FLAG epitope tag added by PCR to the C terminus. Site-directed mutagenesis was performed to convert serine 34 to glutamic acid (S34E) or alanine (S34A). All constructs were sequence verified by the Molecular Genetics Core Facility of the Section of Research Resources, Penn State College of Medicine. The myristoylated-PKCζ construct was graciously provided by Dr. Alex Toker (10).

Transfections—A7r5 cells were transiently transfected by either an electroporation kit from Amaxa Biosystems (Cologne, Germany) or FuGENE 6 from Roche Applied Science. For FuGENE 6, A7r5 cells were seeded to 50–60% confluency and transfected with a 3:1 ratio of FuGENE 6 to DNA.

Proliferation Assay—DNA synthesis was analyzed essentially as described previously (2). Briefly, transfected cells were plated in a 24-well plate and grown overnight prior to 24 h of serum starvation. Following serum starvation, cells were treated with Cceramide for 1 h followed by treatment ± platelet-derived growth factor (PDGF) for an additional 16 h. Cellular proliferation was assayed with the addition of 0.5 mCi/ml [3H]thymidine for the final 4 h of treatment. Cells were washed once with cold 10% trichloroacetic acid and then incubated with cold trichloroacetic acid for 20 min. Cells were solubilized with 0.4 N NaOH for 30
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**A**

![Graph showing NBD-ceramide incorporation](image)

**B**

![Western blots showing localization of caveolin-1](image)

**C**

![Western blots showing 14-3-3 localization](image)

**FIGURE 1.** Exogenous C₆-ceramide localizes to low buoyant, caveolin-enriched, fractions. A, treatment of A7r5 rat aorta smooth muscle cells with fluorescently labeled NBD-C₆-ceramide results in accumulation within caveolin-enriched fractions. Ceramide accumulated in fractions 4 and 5 of a sucrose gradient, which represent caveolin-1-enriched lipid rafts (caveolae). B, immunoblotting (IB) these sucrose gradient fractions for caveolin-1 demonstrates the localization of caveolin-1, within the raft-containing fractions, fractions 4 and 5, with or without the presence of 10 μM ceramide for 60 min. C, immunoblotting sucrose gradient fractions of A7r5 cells for the scaffolding protein 14-3-3 shows that 14-3-3 does not localize within the raft fractions in the presence or absence of ceramide. Representative blots of n = 3 separate experiments are shown.

**RESULTS**

**Exogenous C₆-ceramide Localizes to Low Buoyant, Caveolin-enriched Fractions**—It has been demonstrated by others that ceramide accumulates in lipid microdomains as a consequence of augmented sphingomyelinase activity or de novo synthesis (11). We now provide evidence showing that microdomains are enriched with exogenously delivered ceramide in A7r5 vascular smooth muscle cells. When A7r5 are treated with fluorescently labeled NBD-C₆-ceramide, the majority of exogenous C₆-ceramide was detected in the low buoyant density fractions (fractions 4 and 5) isolated by a sodium carbonate discontinuous sucrose gradient. C₆-ceramide rapidly accumulated within the low buoyant fractions within 30 min and persisted for at least 4 h after administration (Fig. 1A). Similar results were obtained with a ³H-radio-labeled C₆-ceramide (data not shown). We next assessed the distribution of caveolin-1 (Fig. 1B) and 14-3-3 (Fig. 1C) within a discontinuous sucrose gradient. Caveolin-1 is a common raft-associated protein that is a marker protein for caveolae and also serves as a scaffolding protein (12), whereas 14-3-3 is another important scaffold protein (13). Western blot analysis of individual sucrose fractions confirmed an enrichment of caveolin-1 in fractions 4 and 5 (Fig. 1B), suggesting that we had successfully isolated caveolin-rich/low buoyant microdomains. In contrast, 14-3-3 expression is localized to fractions 9–12 (Fig. 1C). C₆-ceramide (10 μM, 1 h) treatment of A7r5 cells did not appreciably change the distribution or expression of either caveolin-1 or 14-3-3. These data indicate that exogenous C₆-ceramide localizes to the low buoyant fractions that are consistent with caveolin-enriched microdomains.

**Ceramide Dissociates PKCζ from 14-3-3 Scaffold Proteins**—14-3-3 proteins have been demonstrated to regulate signaling proteins, including PKC isotypes (14). Although ceramide did not change the distribution or expression of 14-3-3, it did specifically reduce the association between the scaffolding protein, 14-3-3, and PKCζ, but not PKCα, as determined by co-immunoprecipitation (IP) experiments. In these experiments, either PKCζ or PKCα was immunoprecipitated from A7r5 cells and blotted for 14-3-3. IB, immunoblotted, 8, treatment with 10 μM C₆-ceramide reduces association between 14-3-3 and phosphorylated PKCζ. In these experiments, 14-3-3 was immuno-precipitated and blotted for phospho-PKCζ. This is a representative blot of n = 3 separate experiments, in which ceramide delivered either in a Me₂SO/bovine serum albumin (DMSO/BSA) or in liposomal formulation reduced interactions by 38 ± 2 and 30 ± 3%, respectively.
Ceramide Enhances PKCζ Activation within Caveolin-rich Domains—Confirming earlier published studies (2), we now show that exogenous ceramide, as well as agents that produce endogenous ceramide (interleukin-1β and bacterial sphingomyelinase), increase membrane-bound PKCζ phosphorylation at Thr410 (Fig. 3A). In contrast, PDGF does not increase membrane-associated PKCζ phosphorylation in A7r5 cells. Western blots were reprobed and normalized for total PKCζ protein. It should be noted that ceramide, interleukin-1β, and bacterial sphingomyelinase elevated only membranous PKCζ phosphorylation and did not induce “global” PKCζ translocation from the cytosol to the membrane. It also should be noted that these studies using Thr410 as a surrogate marker for activated PKCζ support our previous work reporting ceramide activation of PKCζ in intact and in cell-free reconstitution assays using either immunoprecipitated or recombinant PKCζ (2).

To evaluate the possibility of intramembranous movement and activation of PKCζ, we utilized discontinuous sucrose gradients and again assessed the phosphorylation state of PKCζ at Thr410. As shown in Fig. 3B, ceramide increased the phosphorylation of PKCζ in the low buoyant fractions 4 and 5. In fact, there was approximately a 7-fold increase (Fig. 3B) in ceramide-induced PKCζ phosphorylation in fractions 4 and 5 when compared with the control levels (2.2 versus 14.7%). Consistent with these findings, bacterial sphingomyelinase co-immunoprecipitation strategy in which we now immunoprecipitated with pan-14-3-3 antibody and reprobed with a co-immunoprecipitation strategy in which we now immunoprecipitated with pan-14-3-3 antibody and reprobed with a anti-Myrs-PKCζ and Myrs-PKCζ Western blots of kinase. These experiments were confirmed when the C6-ceramide-reduced the interaction between 14-3-3 and the activated co-localized PKCζ in the lipid raft-containing fractions. Treatment with ceramide (10 μM, 60 min) increased membrane-bound activated pPKCζ. B, immunoblot of sucrose gradient fractions of A7r5 rat aortic smooth muscle cells treated with 20 μM ceramide for 60 min or vehicle (Veh). The caveolin-1-enriched lipid raft-containing fractions, 4 and 5, are enriched for pPKCζ after treatment with C6-ceramide. Densitometry quantification of pPKCζ in sucrose gradient fractions 4 and 5 in response to ceramide treatment reveals approximately a 7-fold increase in pPKCζ phosphorylation when compared with vehicle-treated controls. C, immunoblot of sucrose gradient fractions of A7r5 rat aortic smooth muscle cells treated with 250 microunits of bacterial sphingomyelinase or vehicle. D, using a second, detergent-inclusive method of sucrose gradient fractionation to isolate lipid microdomains, treatment with exogenous ceramide (10 μM, 60 min) or bacterial sphingomyelinase (250 microunits, 60 min) led to an enhanced amount of pPKCζ found in fractions 5–8. E and F, A7r5 cells were transiently transfected with myristoylated PKCζ, which contains a FLAG epitope tag and is constitutively active. Using both detergent-free (E) and detergent-inclusive (F) sucrose gradient fractionation, a small percentage of myristoylated PKCζ localized in the lipid raft-containing fractions. Treatment with ceramide (10 μM, 60 min) increased the percentage of myristoylated PKCζ in these fractions when compared with vehicle control. Representative blots of n = 3 separate experiments are shown. Myrs-PKCζ, myristoylated-PKCζ.
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mide- and sphingomyelinase-treated cells contained 1.4 and 1.9%, respectively, of the total phospho-PKCζ. Confirming results with the detergent-free procedure, ceramide and sphingomyelinase treatment led to a 7- and 9-fold increase, respectively, in the percentage of total phospho-PKCζ found in the lower buoyant fractions when compared with that of vehicle-treated cells.

To further explore PKCζ activation within ceramide-enriched microdomains, A7r5 cells were transfected with a myristoylated PKCζ construct. Discontinuous sucrose gradient analysis revealed that a small percentage of myristoylated PKCζ localized to the caveolin domain (Fig. 3, E and F). More importantly, exogenous ceramide, which localized within fraction 4 and 5, increased the percentage of myristoylated PKCζ within the caveolin domain. With the detergent-free sucrose gradient fractionation, ceramide treatment led to more than a 50% increase (4.9 versus 7.6%) in the percentage of myristoylated PKCζ detected in fractions 4 and 5 when compared with vehicle-treated cells (Fig. 3E). Similarly, in detergent-inclusive sucrose gradient fractionation, the percentage of myristoylated PKCζ in the raft-containing fractions increased over 2-fold, from 3.3 to 8%, after treatment with ceramide (Fig. 5F). These data further support the hypothesis that the recruitment of PKCζ to the structured ceramide-enriched microdomain may be necessary for PKCζ activation and not just membrane localization.

Lipid Rafts Are Necessary for PKCζ and Akt Activation—Lipid rafts are specialized microdomains that are enriched in specific lipids including cholesterol and sphingolipids. Disruption of the lipid rafts is possible through the addition of methyl-β-cyclodextrin (MβCD), which depletes the cell of cholesterol (15). We have previously demonstrated that ceramide-activated PKCζ inhibits PDGF-stimulated Akt phosphorylation (2).

To test whether discrete ceramide-enriched structured microdomains are necessary for PKCζ-dependent Akt inactivation, we assessed PKCζ Thr410 phosphorylation (Fig. 4A) as well as Akt Ser473 phosphorylation (Fig. 4B) after pretreatment with 1% MβCD. Cα-ceramide modestly stimulated PKCζ Thr410 phosphorylation (Fig. 4A), which correlated with complete inhibition in PDGF-induced phosphorylation of Akt Ser473 (Fig. 4B). Ceramide-induced phosphorylation of PKCζ was significantly reduced in the presence of MβCD (Fig. 4A). Treatment with 1% MβCD also inhibited the PDGF-induced increase in Akt phosphorylation. In the presence of MβCD, ceramide did not further reduce Akt phosphorylation levels (Fig. 4B). Upon cholesterol repletion, ceramide was again able to reduce PDGF-induced Akt activity. It should also be noted that raft disruption also reduced PDGF-induced Akt signaling but not growth factor-induced ERK signaling, which is mediated independent of cholesterol depletion (data not shown). These data support the postulate that ceramide-enriched structured microdomains are necessary for ceramide signaling.

Akt3 Ser344 Is Necessary and Sufficient for Ceramide Inhibition of PDGF-stimulated Akt as well as PDGF-induced Mitogenesis—In contrast to phosphorylations at Thr308 and Ser473, which indicate activation of Akt, post-translational modifications of Akt indicative of inactivation have also been noted. Ceramide-induced activation of PKCζ can lead to inactivation of Akt1 through phosphorylation of Thr344 within the pleckstrin homology domain of Akt1. This phosphorylation in turn inhibits its binding to phosphatidylinositol 3 lipids, a necessary cofactor for Akt activation (16). Here, we proposed that such a mechanism holds true for Akt3, the most abundant homolog found in vascular smooth muscle cells (17), which has an analogous Ser344 in the pleckstrin homology domain. As inac-
Ser34 phosphorylation is also sufficient for ceramide-induced growth arrest. To further delineate this mechanism, we demonstrate that the S34A Akt mutation inhibits C6-ceramide-induced inactivation of PDGF-stimulated Akt. In contrast to wild type overexpression and directly opposite to the S34E mutant construct, A7r5 cells overexpressing the S34A mutant still respond to PDGF, but the ability of ceramide to inactivate Akt, as assessed by Ser473 phosphorylation, is now impaired. Taken together, these data suggest that ceramide-dependent PKCζ phosphorylation of Akt3 at Ser34 is necessary for ceramide-induced vascular smooth muscle cell growth arrest. In addition, these observations support previous data that PDGF antagonizes the growth-arresting actions of ceramide by either inducing ceramide metabolism into pro-mitogenic sphingosine-1-phosphate or activating conventional PKC-dependent signaling cascades (18–20).

**DISCUSSION**

The mechanisms by which ceramide exerts its biological functions, including apoptosis and growth arrest, have been under active investigation. Both biophysical (lipid microdomains) and biochemical (ceramide binding targets) mechanisms have been proposed to address this issue. The present study presents an integrated version of these theories.

We, and others, have previously demonstrated evidence that short chain ceramide analogs as well as physiological ceramide contributes to the activation of PKCζ in vitro and in vivo (biochemical mechanism) (2–4). This in turn leads to inhibition of cell cycle progression through a PKCζ-dependent, phosphatidylinositol 3-kinase-independent inactivation of the pro-survival kinase, Akt, in vascular smooth muscle cells (2). We report now that ceramide results in an increased localization and phosphorylation of PKCζ within lipid microdomains to inactivate Akt, demonstrating both a biophysical and a biochemical mechanism for ceramide-induced growth arrest.

Although the inhibition of Akt by ceramide has been demonstrated by multiple laboratories, including our own, multiple mechanisms have been described. These include mechanisms by which ceramide activates PKCζ (2–6), PP2A (21), and/or...
PTEN (phosphatase and tensin homologue deleted on chromosome ten) (22). We now suggest that a commonality of these multiple mechanisms may be through the localization of ceramide within lipid microdomains. Recently, the Summers laboratory reported that ceramide could inhibit Akt activation in an okadaic acid-(PP2A) sensitive manner and/or via an inhibition of translocation (23). It has been demonstrated that caveolin-1, a resident protein of caveolea microdomains, can directly inhibit PP2A, thus preventing its ability to dephosphorylate/inactivate Akt (24). Furthermore, ceramide has also been demonstrated to recruit PTEN into these microdomains, which can inhibit Akt activation by decreasing phosphoinositide-3-phosphates (22). Here, we have demonstrated that ceramide results in increased phosphorylated PKCζ within lipid microdomains. We have utilized both detergent-free and detergent-inclusive sucrose gradient strategies to isolate both endogenous phosphorylated PKCζ as well as overexpressed myristoylated PKCζ. Moreover, only the detergent-free system utilizes sodium carbonate at pH = 11, whereas the detergent-inclusive system used Tween 20 at pH 6.5. Thus, we confirmed the same phenomenon using two distinct isolation methodologies for two versions of an activated and/or post-translationally modified membrane protein.

We provide evidence that a major affect of ceramide in vascular smooth muscle cells is to localize a bioactive, phosphorylated form of PKCζ within low buoyant caveolin-enriched lipid microdomains. This occurs by the recruitment and disassociation of phospho-PKCζ from 14–3–3, a non-microdomain yet membrane-associated scaffolding protein, to place phospho-PKCζ in close proximity to downstream targets such as Akt to induce growth arrest. We also report that ceramide-activated PKCζ-induced growth arrest is mediated by the phosphorylation of Akt3 at serine 34. This further supports the work previously published by the Hundal laboratory, which demonstrated that ceramide induced PKCζ phosphorylation of Akt1 at Thr34 and subsequent inhibition of activation (16). When the analogous site of Akt3 was mutatized to a glutamic acid to mimic phosphorylation, PDGF-induced proliferation was decreased, and upstream elements in the Akt cascade, including phosphoinositide-dependent kinase (PDK), which interact with and activate PKCζ by phosphorylating Thr310 (10, 28).

Reducing these studies to a common theme suggests that generation and localization of ceramide within discrete microdomain could be the mechanism to achieve optimal interaction with ceramide-binding proteins. Selective ceramide interactions with the putative ceramide-binding domains of signaling targets including PP1, PP2A (29, 30), kinase suppressor of ras (31), c-Raf-1 (32), phospholipase A2 (33), cathepsin D (34), MEKK1 (3, 35), VAV (36), protein kinase (37), calpain (38), and PKC isofoms (6), including PKCζ, have been demonstrated. Thus, the accumulation of ceramide within lipid microdomains may facilitate the ability of ceramide to interact with multiple signaling elements throug a ceramide-binding domain, such as the cytoine-rich loop region known as C1b (PKC, kinase suppressor of Ras (KSR), and c-Raf) (39).

Taken together, understanding the mechanism by which PKCζ is activated within ceramide-enriched microdomains will further define the clinical relevance of ceramide as an anti-proliferative therapeutic for inflammatory diseases. Understanding the biophysics and biochemistry of these lipid-protein interactions could eventually lead to the design of selectice lipid- or peptidomimetic inhibitors of these interactions.

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