Protein Kinase Cδ–Dependent Phosphorylation of Syndecan-4 Regulates Cell Migration

Pinaki Chaudhuri, Scott M. Colles, Paul L. Fox, Linda M. Graham

Abstract—Endothelial cell (EC) migration is a complex process requiring exquisitely coordinated focal adhesion assembly and disassembly. Protein kinase C (PKC) is known to regulate focal adhesion formation. Because lysophosphatidylcholine (lysoPC), a major lipid constituent of oxidized low-density lipoprotein, can activate PKC and inhibit EC migration, we explored the signaling cascade responsible for this inhibition. LysoPC increased PKCδ activity, measured by in vitro kinase activity assay, and increased PKCδ phosphorylation. Decreasing PKCδ activation, using pharmacological inhibitors or antisense oligonucleotides, diminished the antimigratory effect of lysoPC. LysoPC-induced PKCδ activation was followed by increased phosphorylation of the transmembrane proteoglycan, syndecan-4, and decreased binding of PKCα to syndecan-4, with a concomitant decrease in PKCα activity. A reciprocal relationship was noted between the interaction of PKCα and α-actinin with syndecan-4. These changes were temporally related to the observed changes in cell morphology and the inhibition of migration of ECs incubated with lysoPC. The data suggested that generalized activation of PKCδ by lysoPC initiated a cascade of events, including phosphorylation of syndecan-4, displacement and decreased activity of PKCα, binding of α-actinin to syndecan-4, and disruption of the time- and site-specific regulation of focal adhesion complex assembly and disassembly required for normal cell migration. (Circ Res. 2005;97:674-681.)

Key Words: endothelial cell ■ migration ■ lysophosphatidylcholine ■ protein kinase C ■ syndecan-4 ■ α-actinin

Cell migration is a complex process and is thought to require lamellipodial extension, formation of adhesions at the leading edge of the cell, intracellular force generation, and breakdown of adhesions at the rear of the cell.1 Time- and site-specific regulation of these processes is required for normal cell migration. The precise coordination required is evidenced by the need for adhesion contact assembly at the front of the cell and simultaneous disassembly at the back of the cell. Inhibition or uncoordinated activation of one process can disrupt normal movement.

Endothelial cell (EC) migration is essential for angiogenesis and re-endothelialization after arterial injury, but oxidized low-density lipoprotein and lysophosphatidylcholine (lysoPC), a lipid component of oxidized low-density lipoprotein, inhibit EC migration.2,3 LysoPC is abundant in plasma (lysoPC), a lipid component of oxidized low-density lipoprotein and lysophosphatidylcholine.

The mechanisms by which lysoPC inhibits EC migration are not completely understood. LysoPC affects a number of cellular properties, including membrane fluidity, production of reactive oxygen species, intracellular calcium concentration, and other signaling pathways.5-7 LysoPC can activate protein kinase C (PKC) in ECs,8 but the specific isoforms activated have not been reported.

PKC activation is important in the control of cellular migration. PKCα is required for normal EC migration and regulates adhesion formation, lamellipodia extension, and actin organization.10 On wounding of a monolayer of epithelial cells, PKCα is concentrated at the leading edge of lamellipodia and activity increases.31 PKC regulates recruitment of cytoskeletal proteins, including syndecan-4, a transmembrane proteoglycan, to nascent focal adhesion contacts.12 Syndecan-4 interacts with phosphatidylinositol 4,5-biphosphate (PIP3), which stabilizes the oligomeric structure of syndecan-4 and promotes the association of PKCα and syndecan-4.13-15 The catalytic domain of PKCα binds to the variable region of the cytoplasmic domain of syndecan-4, and PKCα is "superactivated.15,16 PKCδ is a novel PKC isoform, which phosphorylates syndecan-4 at Ser183, markedly decreasing its affinity for PIP2 and abolishing its activity. A reciprocal relationship was noted between the interaction of PKCα and α-actinin with syndecan-4. These changes were temporally related to the observed changes in cell morphology and the inhibition of migration of ECs incubated with lysoPC. The data suggested that generalized activation of PKCδ by lysoPC initiated a cascade of events, including phosphorylation of syndecan-4, displacement and decreased activity of PKCα, binding of α-actinin to syndecan-4, and disruption of the time- and site-specific regulation of focal adhesion complex assembly and disassembly required for normal cell migration.
The present study identifies a novel pathway by which lysoPC inhibits EC migration. LysoPC activates PKCδ and initiates a signaling cascade in which syndecan-4 is phosphorylated, decreasing PKCα binding to syndecan-4 with a concomitant decrease in activity. Simultaneously, α-actinin association with syndecan-4 increases. Decreased PKCα binding and increased α-actinin association with syndecan-4 is accompanied by changes in cell morphology, suggesting altered focal contacts or cytoskeletal organization. Prolonged activation of PKCδ disrupts the coordinated assembly/disassembly of focal adhesions and exertion of contractile forces necessary for normal cell migration.

Materials and Methods

EC Culture and Migration Assay
Bovine aortic ECs were isolated from fresh adult bovine aortas. ECs between passages 4 and 10 were grown to confluence in 12-well tissue culture plates in DMEM and Ham F12 nutrient mixture (DMEM/F12; 1:1 vol/vol) containing 10% FCS (HyClone Laboratories). ECs were made quiescent by 24-hour incubation in DMEM containing 0.1% gelatin. EC migration was assessed in a razor scrape assay as described previously. Briefly, a razor blade was pressed through the confluent monolayer into the plastic well to mark a starting line, then swept laterally to remove ECs on one side of that line. At 24 hours, cells were fixed and stained with Wright–Giems stain. An observer blinded to the experimental conditions used NIH Image software to quantitate migration as described previously.

Immunoprecipitation of Intracellular Proteins
ECs were incubated overnight in serum-free DMEM with 0.1% gelatin, treated with PBS, and harvested using 0.05% trypsin-EDTA for 10 minutes. Cell suspensions were washed with 1% Triton X-100 and Complete protease inhibitor (Roche). Insoluble material was removed by centrifugation. Equal amounts of total protein from cells subjected to various treatments were used for immunoprecipitation. The target protein was precipitated overnight at 4°C using an antigen-specific antibody. A razor blade was pressed through the confluent monolayer into the plastic well to mark the starting line, then swept laterally to remove ECs on one side of that line. At 24 hours, cells were fixed and stained with Wright–Giemsa stain. An observer blinded to the experimental conditions used NIH Image software to quantitate migration as described previously.

Immunoblot Analysis of Intracellular Proteins
ECs were cultured under conditions identical to those for migration assay, then harvested and lysed as described above and stored at −20°C until analyzed. Proteins (40 μg per lane) were resolved by 4% to 12% gradient SDS-PAGE, transferred to a polyvinylidene difluoride (PVDF) membrane and detected by antibody specific for the indicated antigen. Antibodies for PKCδ, PKCα, PKCδ, PKCα, PKCδ, PKCα, PKCδ, and PKCδ were from Santa Cruz Biotechnology and used at 1:500 (except PKCα at 1:250) dilution. Other antibodies included phosphospecific anti-PKCδ (1:1000; Cell Signaling Technology Inc.), anti-α-actinin (1:1000; Chemicon), anti-syndecan-4 (N-19; 1:500; Santa Cruz Biotechnology), and phosphospecific anti-syndecan-4 (pS179; 1:500; Biosource International). Signal was developed using a chemiluminescent reagent (Perkin-Elmer) and quantitated by densitometric analysis using NIH Image software. To verify loading equivalency, membranes were reprobed for control proteins, including to actin (1:1000; Chemicon), syndecan-4, and PKCα.

Downregulation of Intracellular PKCδ Using Antisense Oligonucleotides
ECs were transiently transfected with phosphorothioate-mediated oligonucleotides (Integrated DNA Technologies) corresponding to antisense (5’-AGGGTGCCATGATGGA-3’), sense (5’-TGGCACCCT-3’), or scramble (5’-ACGTGATGGGATGCA-3’) oligonucleotides targeted against PKCδ mRNA for 48 hours. ECs were then incubated with lysoPC for 0 to 12 hours and PKCδ phosphorylation assessed as described in D. Representative blots of two (A and E) or three (B through D) separate experiments are shown.

Figure 1. LysoPC activates PKCδ in ECs. A, ECs were incubated with lysoPC (0 to 15 μmol/L) or phosphatidylcholine (PC; 20 μmol/L) for 2 hours. In parallel wells, rotterlin (R; 0.3 μmol/L) was added 1 hour before and with lysoPC. PKCδ was immunoprecipitated (IP) and activity quantitated by a histone H1 phosphorylation assay. B, ECs were incubated with lysoPC for 2 or 12 hours and PKCδ kinase activity quantitated by histone phosphorylation. C, ECs were incubated with 12.5 μmol/L lysoPC for 0 to 12 hours, lysed, and PKCδ immunoblot (IB) analysis performed to determine intracellular level of PKCδ. Stripped blots were reprobed for actin to confirm equal loading. D, ECs were incubated with lysoPC for 12 hours. In parallel wells, 0.3 μmol/L rotterlin was added 1 hour before and with lysoPC. PKCδ was immunoprecipitated using an antibody that recognized nonphosphorylated and phosphorylated forms of PKCδ. Immunoblot analysis was performed using an antibody specific for PKCδ phosphorylated at Thr505. Blots were stripped and reprobed with anti-PKCδ antibody to confirm equal loading. E, ECs were transiently transfected with antisense (ASδ) or scrambled (Scrδ) oligonucleotides targeted against PKCδ mRNA for 48 hours. ECs were then incubated with lysoPC for 0 to 12 hours and PKCδ phosphorylation assessed as described in D. Representative blots of two (A and E) or three (B through D) separate experiments are shown.
sequences for the translation–initiation region of mouse PKCδ mRNA.20 ECs at 80% confluence in 12-well plates were transfected with 2 µg of phosphorothioate-mediated oligonucleotides using Effectene (Qiagen) according to manufacturer directions. The effectiveness of antisense oligonucleotides was verified after 48 hours by immunoblot analysis of intracellular PKC.

**Kinase Activity Assay**

PKCδ kinase activity was determined by immune complex kinase activity assay.21 Briefly, ECs were treated with lysoPC then lysed in buffer (50 mmol/L HEPES, 150 mmol/L NaCl, 1 mmol/L EDTA, 200 µmol/L Na3VO4, 100 mmol/L NaF, 1.5 mmol/L MgCl2, and 10% glycerol, pH 7.4) containing 1% Triton X-100 and Complete protease inhibitor. Lysates were immunoprecipitated with anti-PKCδ antibody. Immunoprecipitates were rinsed and resuspended in 30 L of kinase buffer containing 5 µg histone H1 (an exogenous PKC substrate; Calbiochem) and 30 Ci of [γ32P]ATP (Perkin-Elmer). After 30 minutes, the reaction was terminated and the sample resolved by 4% to 12% gradient SDS-PAGE. Phosphorylated histone was detected by autoradiography and quantitated by densitometry. PKCα kinase activity was performed essentially as described above on samples immunoprecipitated with anti-PKCα antibody. The kinase reaction buffer contained 10 mmol/L diolein and 0.2 mmol/L CaCl2 in addition to components described above.

![Figure 2](image)

**Figure 2.** Activation of PKCδ inhibits EC migration. A, EC migration assay was initiated in the presence or absence of 12.5 µmol/L lysoPC. In parallel wells, 0.3 µmol/L rottlerin was added for 1 hour before and during the migration assay. Migration was quantified at 24 hours. B, EC migration assay was initiated in the presence or absence of ATRA (10 µmol/L). In parallel wells, 0.3 µmol/L rottlerin was added for 1 hour before and during the migration assay. Migration was quantified at 24 hours. C, EC migration assay was initiated in the presence or absence of 12.5 µmol/L lysoPC. In parallel wells, 1 mmol/L hydroxyurea was added for 1 hour before and during the migration assay. Migration was quantified at 24 hours. In all panels, results are expressed as mean±SD (A, n=5; B, n=4; C, n=2; *P<0.0001 compared with control; **P<0.0001 compared with lysoPC or ATRA).

![Figure 3](image)

**Figure 3.** Antisense PKCδ oligonucleotides preserve EC migration in presence of lysoPC. ECs were not transfected (NT) or transiently transfected with antisense (ASδ) or scrambled (Scrδ) oligonucleotides targeted against PKCδ mRNA for 24 hours. A, Migration assay was performed in the presence or absence of lysoPC (12.5 µmol/L). Results are represented as mean±SD (n=4; *P<0.0001 compared with control; **P<0.0001 compared with lysoPC). B, The effect of oligonucleotides on PKCδ level was confirmed in parallel wells at 48 hours by immunoblot analysis using anti-PKCδ antibody. The specificity of the AS oligonucleotides to PKCδ was confirmed by immunoblot analysis of the same sample with anti-PKCα, anti-PKCζ, anti-PKCθ, anti-PKCe, and anti-PKCγ antibodies. Blots were stripped and reprobed with anti-actin antibody to confirm equal loading. Representative blots of two experiments are shown.
**Statistical Analysis**

Data are represented as the mean±SD. Experiments were performed in triplicate with at least three different cell isolates. Data evaluation was performed by *t* test or ANOVA. Differences were considered statistically significant at *P*<0.05.

**Results**

The role of PKC activation in the inhibition of EC migration by lysoPC was assessed using pharmacological inhibitors. Under basal conditions, lysoPC (12.5 μmol/L 1-palmitoyl-2-hydroxy-sn-glycerol-3-phosphocholine; Avanti Polar Lipids) inhibited EC migration to 35% of control. This concentration of lysoPC was not cytotoxic and was below the critical micellar concentration of 40 to 50 μmol/L. General PKC inhibitors chelerythrine chloride and Gö 6983 had a mild inhibitory effect on basal EC migration but blunted the inhibitory effect of lysoPC. PKCα and β inhibitors Gö6976 and pseudosubstrate peptide had an inhibitory effect on basal EC migration and augmented the inhibition of EC migration by lysoPC. A PKCδ inhibitor, rottlerin (0.3 μmol/L, Calbiochem), improved EC migration in the presence of lysoPC. These findings suggested that lysoPC activated PKCδ.

**LysoPC Activated PKCδ**

The ability of lysoPC to activate PKCδ in ECs was studied using an in vitro kinase activity assay. LysoPC increased PKCδ activity in a concentration-dependent fashion, and this was inhibited by rottlerin (Figure 1A). Phosphatidylcholine (Avanti Polar Lipids) had no effect on PKCδ activity. PKCδ activity was 2.1±0.3-fold and 1.4±0.2-fold higher after 2 and 12 hours of lysoPC treatment, respectively, compared with untreated ECs (*P*<0.01 and *P*<0.05; Figure 1B). PKCδ activity in untreated ECs did not change during the 12-hour period (data not shown). The increased activity of PKCδ after lysoPC treatment was not secondary to an increase in the endogenous level of PKCδ in EC (Figure 1C).

To determine whether changes in PKCδ activity were associated with changes in PKCδ phosphorylation, the time course of PKCδ phosphorylation during EC incubation with lysoPC was assessed. LysoPC caused a marked increase in phosphorylated PKCδ by 2 hours (Figure 1D), 5.5±1.4-fold increase over control (*P*<0.02). Phosphorylated PKCδ levels slowly declined, and by 12 hours, were 2.8±1.3-fold higher than baseline (*P*=0.08). Because the level of total PKCδ protein was unchanged, changes represented phosphorylation of endogenous PKCδ. PKCδ phosphorylation in untreated ECs did not change during the 12-hour period (data not shown). PKCδ phosphorylation in response to lysoPC was inhibited by pretreatment of cells with 0.3 μmol/L rottlerin (Figure 1D). Transient transfection of ECs with PKCδ antisense oligonucleotide also decreased the level of phosphorylated PKCδ after lysoPC treatment (Figure 1E).

Figure 4. LysoPC induces syndecan-4 phosphorylation. A, ECs were incubated with lysoPC for the times indicated. Cell lysates were immunoprecipitated (IP) with anti–syndecan-4 antibody. Immunoblot (IB) analysis was performed with an antibody specific for syndecan-4 phosphorylated at Ser179. Blots were stripped and reprobed for syndecan-4 to confirm equal loading. B, In parallel wells, 0.3 μmol/L rottlerin was present 1 hour before and during lysoPC incubation. C, ECs were transiently transfected with antisense (ASδ) or scrambled (Scrδ) oligonucleotides targeted against PKCδ mRNA for 48 hours before the addition of lysoPC. Representative blots of two (C) or three (A and B) separate experiments are shown.
Activation of PKCδ Inhibited EC Migration

The finding that lysoPC activated PKCδ, coupled with inhibitor studies indicating that PKC activation played a role in the antimigratory effect of lysoPC, suggested that activation of PKCδ could inhibit EC migration. LysoPC activated PKCδ and inhibited EC migration to 35% of control (Figures 1 and 2A). All-trans-retinoic acid (ATRA; 10 μmol/L; Calbiochem), which activates PKCδ by Thr505 phosphorylation, inhibited migration to 51% of control (P<0.01; Figure 2B). Rottlerin (0.3 μmol/L) added 1 hour before and during migration preserved EC migration at 56% of control in the presence of lysoPC and 71% of control in presence of ATRA (Figure 2A and 2B). The presence or absence of 1 mmol/L hydroxyurea, a concentration shown to completely block bovine EC proliferation, did not alter the effect of lysoPC (Figure 2C). This suggested that the effect of lysoPC in this assay was purely antimigratory.

Downregulation of PKCδ by Antisense Oligonucleotide Preserved EC Migration in the Presence of LysoPC

The importance of PKCδ activation in the antimigratory activity of lysoPC was explored further by downregulating PKCδ. ECs were transiently transfected with antisense oligonucleotide of PKCδ that reduced intracellular PKCδ protein for 48 hours (data not shown). Migration of ECs transfected with PKCδ antisense was preserved at 59% of control in the presence of lysoPC (Figure 3A). Sense or scrambled antisense oligonucleotides had no effect on EC migration or PKCδ protein level (Figure 3A and 3B). The PKCδ antisense oligonucleotide had no effect on PKCα, PKCθ, PKCe, PKCγ, or PKCη levels (Figure 3B). These observations supported the role of PKCδ in the inhibition of EC migration by lysoPC.

LysoPC Induced Syndecan-4 Phosphorylation

Previously, we observed that lysoPC caused ECs to round, suggesting disruption of focal adhesions. PKCδ can phosphorlylate syndecan-4, a member of the focal adhesion complex. Therefore, we investigated the effect of lysoPC on syndecan-4 phosphorylation. Incubation of ECs with lysoPC had no effect on total syndecan, but by 2 hours, syndecan-4 phosphorylation increased 4.2±0.2-fold over baseline (P<0.01) and remained elevated for ≥8 hours (Figure 4A). Syndecan-4 phosphorylation did not change during 8 hours in untreated ECs (data not shown). Rottlerin and PKCδ antisense oligonucleotide prevented the increase in syndecan-4 phosphorylation in response to lysoPC (Figure 4B and 4C).

LysoPC induced an increase in the association of PKCδ with phosphorylated syndecan-4. In ECs incubated with lysoPC for 2 hours, the association of PKCδ with phosphorylated syndecan-4 was 2.6±0.3-fold greater than baseline (P<0.01; Figure 5A). No enhanced association between total syndecan-4 and PKCδ was observed. Rottlerin and PKCδ antisense oligonucleotides prevented the increase in association of PKCδ and phosphorylated syndecan-4 (Figure 5A and 5B), supporting a role for activated PKCδ in these changes.

LysoPC Increased the Association Between Syndecan-4 and α-Actinin

α-Actinin interacts with syndecan-4 in the variable region, but the effect of syndecan phosphorylation on α-actinin binding has not been reported previously. After EC exposure to lysoPC, α-actinin association with syndecan-4 increased within 2 hours, and the increase persisted for 8 hours, being 1.8±0.2-fold and 1.5±0.2-fold higher than control at 2 and 8 hours, respectively (P<0.01; Figure 6). The association declined to control levels by 12 hours. The increased association showed the same temporal pattern as the PKCδ activation and the syndecan-4 phosphorylation. α-Actinin association with syndecan-4 in untreated ECs did not change during the 12 hours (data not shown). The lysoPC-induced association between syndecan-4 and α-actinin was inhibited by rottlerin and PKCδ antisense oligonucleotide (Figure 6A and 6B), suggesting that activated PKCδ was responsible for the increased association of syndecan-4 and α-actinin, a novel finding.

LysoPC Decreased PKCα Association With Syndecan-4 and Decreased PKCα Activity

PKCα activity is important in maintaining the focal adhesion complex, and interaction with syndecan-4 is reported to...
In ECs incubated with lysoPC for 2 hours, PKCα bound to syndecan-4 decreased to 50±30% of control levels (P<0.05; Figure 6). PKCα interaction with syndecan-4 was determined simultaneously. PKCα interaction decreased, whereas the association of PKCδ and phosphorylated syndecan-4 increased (Figure 7A). Pretreatment with rottlerin or PKCα antisense oligonucleotide prevented the lysoPC-induced decrease in PKCα association and increase in PKCδ association with syndecan-4 (Figure 7A and 7B). The decrease in PKCα association with syndecan-4 was accompanied by a decline in PKCα activity to 40±20% of control after a 2-hour incubation with lysoPC (P<0.03; Figure 7C). In untreated ECs, PKCα activity and association with syndecan-4 did not change during the test period (data not shown). Decreased PKCα activity may lead to loss of focal contacts and decreased migration after EC incubation with lysoPC or other PKCδ activators.
Discussion

The importance of PKCα activation in normal cell migration has long been recognized, but PKCδ activation also controls movement. Normal migration is diminished in smooth muscle cells from PKCδ-deficient mice. Vascular endothelial growth factor (VEGF)–stimulated migration requires early PKCδ activation with phosphorylation of Thr505 within 10 minutes of VEGF exposure. Phosphorylation levels return to baseline by 2 hours, and PKCδ activity decreases below baseline by 8 hours and is maximally inhibited by 16 hours.

LysoPC activates PKCδ in the antimigratory effect of lysoPC. The activation of PKCδ is sustained for >12 hours, as demonstrated by in vitro kinase activity assay and increased phosphorylation of Thr505 in the activation loop, one of the major phosphorylation sites of PKCδ. Inhibition of PKCδ activation, using two distinct approaches, preserves EC migration, supporting the role of PKCδ activation in the antimigratory effect of lysoPC. The specificity of the pharmacological inhibitor rottlerin for PKCδ relative to other PKC isoforms has been related to the concentration used. We show that a low concentration of rottlerin (0.3 μmol/L) blocks PKCδ activation by lysoPC, but basal levels of PKCδ activity persist, and basal migration is unaffected. To confirm the specific role of PKCδ activation in lysoPC-inhibited EC migration in our studies, we also use a molecular approach. Phosphorothioate-mediated antisense oligonucleotide of PKCδ decreases intrinsic levels of PKCδ for up to 48 hours but does not affect other isoforms of PKC. Decreasing the PKCδ level abrogates the inhibitory effect of lysoPC on EC migration, supporting the central role of sustained PKCδ activation in lysoPC-inhibited EC migration. Sustained activation or complete inhibition of PKCδ may inhibit EC migration by disrupting the temporally and spatially regulated formation of new focal contacts at the leading edge of the cell and the disassembly at the trailing edge.

Activation of PKCδ initiates a cascade of events that leads to inhibition of EC migration. The ability of PKCδ to phosphorylate syndecan-4 and the effect of that phosphorylation on PKCα activity have been reported previously. Phosphorylation of syndecan-4 is accompanied by decreased PKCα interaction with syndecan-4 and decreased PKCα activity. These changes are inhibited by rottlerin and PKCδ antisense oligonucleotide. Reduced PKCα binding and activity may decrease stability of focal adhesions and lead to their disruption. This is supported by the finding that expression of a truncated syndecan-4 core protein lacking the PKC binding site decreases spreading, focal adhesion formation, and motility. On the other hand, overexpression of syndecan-4 is accompanied by increased activity of membrane PKCα, increased adhesion formation, and decreased cell motility. Thus, abnormally decreased and increased syndecan-4 interaction with PKCα can inhibit cell migration, emphasizing the exquisite regulation required for normal motility.

PKCα and α-actinin can bind to the variable region of the cytoplasmic portion of syndecan-4. Our studies suggest that lysoPC-induced syndecan-4 phosphorylation increases the association of α-actinin and syndecan-4, a novel finding. Simultaneously, PKCα activity and association with syndecan-4 are decreased. This reciprocal interaction of α-actinin and PKCα with phosphorylated syndecan-4 supports the idea that PKCα and α-actinin compete for binding sites on syndecan-4. Increased α-actinin binding to syndecan-4 may decrease cell motility, similar to the effect of overexpression of α-actinin. Localization of α-actinin to focal adhesion complexes allows the disassembly of these complexes necessary for normal cell migration. The role of α-actinin in disassembly may reflect the associated decrease in PKCα activity that normally maintains focal adhesion complexes. Regulated disassembly is essential for migration, but excessive disruption may contribute to changes in cell morphology observed after EC exposure to lysoPC. In addition, enhanced α-actinin binding to syndecan-4 may strengthen the cytoskeletal-integrin linkages. The effect of increased α-actinin association with syndecan-4 on actin binding remains to be determined.

We propose a model for a sequence of events that culminates in the inhibition of EC migration in lysoPC (Figure 8). LysoPC activates PKCδ, which phosphorylates syndecan-4. Phosphorylated syndecan-4 has increased affinity for α-actinin and decreased affinity for PIP2, with resultant decreased capacity to bind and activate PKCα. The decreased PKCα activity limits normal spreading and lamellipodial extension by decreasing the formation or stability of de novo forward adhesions, thus impeding cell migration. Physiologic PKCδ activation that is subject to feedback downregulation is essential for normal migration, allowing coordinated disassembly of focal adhesion complexes at the trailing end of the cell and assembly at the leading edge. However, sustained...
PKCδ activation by lysoPC inhibits cell movement by disrupting the time- and site-specific nature of these processes. In vivo accumulation of lysoPC in atherosclerotic plaques and prosthetic grafts may inhibit EC movement, delaying restoration of the endothelial lining after an arterial injury such as angioplasty.

Acknowledgments

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References

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