The Direct Interaction of Phospholipase C-γ1 with Phospholipase D2 Is Important for Epidermal Growth Factor Signaling*

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The epidermal growth factor (EGF) receptor has an important role in cellular proliferation, and the enzymatic activity of phospholipase C (PLC)-γ1 is regarded to be critical for EGF-induced mitogenesis. In this study, we report for the first time a phospholipase complex composed of PLC-γ1 and phospholipase D2 (PLD2). PLC-γ1 is co-immunoprecipitated with PLD2 in COS-7 cells. The results of in vitro binding analysis and co-immunoprecipitation analysis in COS-7 cells show that the Src homology (SH) 3 domain of PLC-γ1 binds to the proline-rich motif within the Phox homology (PX) domain of PLD2. The interaction between PLC-γ1 and PLD2 is EGF stimulation-dependent and potentiates EGF-induced inositol 1,4,5-trisphosphate (IP3) formation and Ca2+ increase. Mutating Pro-145 and Pro-148 within the PX domain of PLD2 to leucines disrupts the interaction between PLC-γ1 and PLD2 and fails to potentiate EGF-induced IP3 formation and Ca2+ increase. However, neither PLD2 wild type nor PLD2 mutant affects the EGF-induced tyrosine phosphorylation of PLC-γ1. These findings suggest that, upon EGF stimulation, PLC-γ1 directly interacts with PLD2 and this interaction is important for PLC-γ1 activity.

The epidermal growth factor (EGF)1 signaling pathways have been implicated in cellular proliferation and cytoskeletal reorganization (1). The enzymatic activity of phospholipase C (PLC)-γ1, a downstream signaling component of EGF, is required for the EGF-induced cellular responses (2–4). PLC hydrolyzes phosphatidylinositol 4,5-bisphosphate (PIP2), and one product of this hydrolysis, inositol 1,4,5-trisphosphate (IP3), mobilizes Ca2+ from the intracellular stores, whereas the other product of the hydrolysis, 1,2-diacylglycerol, activates protein kinase C (PKC) (5). Until recently, 11 mammalian PLC isozymes have been identified and classified into four types (6–8). PLC-γ1, unlike the other PLC isozymes, has Src homology (SH) domains: two SH2 and one SH3 domains (9). Tyrosine phosphorylation of PLC-γ1 by the EGF receptor is responsible for inositol phosphate production (10–12). The SH2 domains of PLC-γ1 are important for binding to the EGF receptor and for phosphorylation by the receptor, which is required for activation (13–15). However, little is known about how phosphorylated PLC-γ1 undergoes further processes such as targeting to its PIP2 substrate molecules in the membrane.

Phospholipase D is a membrane-associated enzyme, which hydrolyzes phosphatidylincholine to generate phosphatidic acid (PA) and choline (16). PA has been shown to be an intracellular lipid second messenger involved in many physiological events such as the promotion of mitogenesis, the secretory process, and actin cytoskeletal reorganization (17–20). PLD activity is regulated by protein kinases, small molecular weight G-proteins, and Ca2+ (21), and PKC has been suggested to be a major mediator of PLD stimulation (22). Moreover, PLD requires PIP2 for its enzymatic activity (23). Two types of mammalian PLD isoforms, PLD1 and PLD2, have been reported, which share 55% identity (24). EGF stimulation increases PLD activity (25, 26), but the roles of the domains of PLD in EGF signaling have not been identified.

It has been reported that the EGF receptor is highly enriched in the plasma membrane substructure, caveolae (27, 28), and in our previous study, we found that PLC-γ1 translocates to caveolae upon EGF stimulation (29). PLD isoforms exist both in the caveolae, and the cellular distribution of PLD2, in particular, is largely restricted to caveolae (25, 30). PIP2, the substrate of PLC-γ1 and the activator of PLD, is also highly enriched in caveolae (31). The communication between PLC-γ1 and PLD has been questioned. PKC, a downstream signaling molecule of PLC-γ1, is required for PLD activation (26, 32). Mouse embryo fibroblast lacking PLC-γ1 showed markedly reduced PLD activity (33), and PA, the product of PLD action in the membrane, increased in vitro PLC-γ1 activity (34). These reports suggest that the two phospholipases (PLC-γ1 and PLD2) may affect each other dynamically by changing the membrane phospholipids. Nevertheless, the direct relationship between these phospholipases in cells has not been studied.

In this report, we demonstrate for the first time the direct association between two signaling phospholipases, PLC-γ1 and PLD2. We suggest that their interaction is important for the proper activation of PLC-γ1 after EGF stimulation.

EXPERIMENTAL PROCEDURES

Materials—The enhanced chemiluminescence (ECL) kit was purchased from Amersham Biosciences (Buckinghamshire, United Kingdom); [3H]myristic acid and [3H]inositol 1,4,5-trisphosphate were obtained from PerkinElmer Life Sciences (Boston, MA); Silica Gel 60 thin-layer chromatography plates were obtained from MERK (Darmstadt, Germany); protein A-Sepharose was obtained from RepliGen.
PLD1 and PLD2 were purchased from Kirkegaard and Perry Laboratories, Inc. Peroxidase-conjugated goat anti-rabbit IgG and anti-mouse IgG, IgM, cholic acid was obtained from USB (Cleveland, OH); and horseradish peroxidase Aminex were obtained from Invitrogen (Grand Island, NY); bovine (25, 27)

The major isozyme of PLD in COS-7 cells is PLD2, and PLC-

of the existence of PIP2-utilizing phospholipases in a complex.

be a cofactor of PLD activation (16). We checked the possibility of the interaction of PLC-

and PLD2 in pCDNA3.1, wild type rat PLC-

1 in pFLAG-CMV-2, and the SH3 domain deleted rat PLC-

1 in pFLAG-CMV-2 were prepared as described previously (38–39, 47). Full-length cDNAs of murine PLD2 and its N-terminal deletion mutant (A1–308) were provided generously by Dr. Michael A. Frohman (State University of New York, NY). Anti-FLAG M5 monoclonal antibody and all other chemicals were from Sigma (St. Louis, MO).

Cell Culture and Transfection—COS-7 cells were cultured and transfected using LipofectAMINE (Invitrogen) as described previously (39, 40).

Expression and Purification of PLC-

- Recombinant rat PLC-

1, and human PLD2 were expressed in SF9 cells and purified as described previously (41, 42).

Preparation of GST Fusion Proteins—Glutathione S-transferase (GST) fusion proteins containing the SH domains of PLC-

1 and human PLD2 fragments were prepared as described previously (38, 40, 43). GST fusion SH3 domains of Ab1 and CrkI were kindly provided by Dr. Brian K. Kay (The University of Wisconsin, Madison, WI) (44). GST fusion Phox homology (PX) domains of p40phox and p47phox were kindly provided by Dr. Michael B. Yaffe (Massachusetts Institute of Technology, MA) (45). After harvesting the cells, the GST fusion proteins were purified by standard methods (46) using glutathione-Sepharose 4B (Amersham Biosciences).

Immunoblot Analysis—Proteins were denatured by boiling for 5 min at 95 °C in a Laemmli sample buffer (48), separated by SDS-PAGE, and immunoblot analysis was performed as described previously (39).

In Vitro Binding Analysis—In vitro binding was performed in 300 μl of binding buffer (20 mM Tris/HCl, pH 8.0, 150 mM NaCl, 1 mM MgCl2, 1 mM EGTA, 0.1% Triton X-100) at 4 °C for 3 h.

Immunoprecipitation—Cells were lysed with IP buffer (20 mM Tris/ HCl, pH 8.0, 150 mM NaCl, 1 mM MgCl2, 1 mM EGTA, 1% Triton X-100, 1% cholic acid) by sonication. Cell lysates were centrifuged at 100,000× g at 4 °C for 30 min, and the supernatants were incubated with an antibody immobilized to Protein A-Sepharose beads.

Measurement of PLC Activity in Cells—Cellular IP3 concentration was determined by the [3H]IP3 competition assay using IP3 binding protein (49), which was prepared from bovine adrenal cortex as described previously (50).

Measurement of PLD Activity in Cells—PLD activity was assayed by measuring phosphatidylbutanol formation in the presence of 1-butanol as described previously (51).

Measurement of [Ca2+]i—[Ca2+]i levels were determined using Grynkiewicz et al.’s method with fura-2/AM in Ca2+-free Locke’s solution (154 mM NaCl, 5.6 mM KCl, 1.2 mM MgCl2, 5 mM HEPES, pH 7.3, 10 mM glucose, and 0.2 mM EGTA) (52).

RESULTS

PLC-

1 Is Co-immunoprecipitated with PLD2 in Growing Cells—It has been reported that the EGF receptor, PLD2, and IP3 are enriched in the plasma membrane substructure, caveolae, to which PLC-

1 translocates after EGF stimulation (25, 27–29, 31). PLC-

2, the substrate of PLC-

1, was reported to be a cofactor of PLD activation (16). We checked the possibility of the existence of IP3-utilizing phospholipases in a complex. The major isozyme of PLD in COS-7 cells is PLD2, and PLC-

1 was co-immunoprecipitated with PLD2 from COS-7 cells growing in media containing 10% bovine calf serum (Fig. 1A). However, because the amount of PLD2 in COS-7 cells is very small, the co-immunoprecipitation could be detected after a long time exposure to a film. By transfecting PLD2 into COS-7 cells, the co-immunoprecipitation of PLC-

1 with PLD2 was more easily detected (Fig. 1B). These results indicate that two phospholipases exist in a complex.

PLD2 Increases EGF-induced PLC-

1 Activity, and the Interaction between PLC-

1 and PLD2 Is EGF Stimulation-dependent—It could be deduced from previous reports that EGF stimulation recruits PLC-

1 from the cytosol to the plasma membrane where PLD2 is enriched (25, 29). We observed that increasing the amount of PLD2 in COS-7 cells resulted in increasing EGF-induced PLC-

1 activity, but PLD2 didn’t affect PLC-

1 activity without EGF stimulation or the time course of PLC-

1 activity after EGF stimulation (Fig. 2, A and B). We tested whether the interaction between PLC-

1 and PLD2 is dependent on EGF stimulation. As shown in Fig. 2C, serum starvation abolishes the interaction of PLC-

1 with PLD2, but as soon as cells were stimulated with EGF, PLC-

1 binds to PLD2 and then dissociates from PLD2. The interaction time course correlates with the activity time course of PLC-

1 (Fig. 2B). These results suggest that the interaction of PLC-

1 with PLD2 may contribute to increasing EGF-induced PLC-

1 activity.

PLC-

1 Interacts Directly with PLD2 through the SH3 Domain—To test whether their interaction is direct or not, in vitro binding analysis was performed with purified PLC-

1 and PLD2. Purified PLC-

1, or PLC-

1 for comparison, was incubated with purified PLD2 and immunoprecipitated with an anti-PLD antibody. As shown in Fig. 3B, PLC-

1, but not PLC-

1, was precipitated by PLD2. These results indicate that PLC-

1 binds directly to PLD2. The major differences between PLC-

1 and PLC-

1 lie in the linker region between the X and Y domains. PLC-

1 has two SH2 domains and one SH3 domain, but PLC-

1 has no SH domain (Fig. 3A). To identify which domain of PLC-

1 is responsible for the interaction with PLD2, in vitro binding analysis was performed with purified PLD2 and the GST fusion SH domains of PLC-

1. As shown in Fig. 3C, PLD2 bound to GST-SH223 and GST-SH3
but not to GST-SH2N or GST-SH2C. This result indicates that the SH3 domain of PLC-γ1 interacts with PLD2. For further confirmation, co-immunoprecipitation analysis was performed in cells growing in media containing 10% bovine calf serum. PLD2 was co-transfected into COS-7 cells with PLC-γ1 wild type or PLC-γ1 SH3 domain deletion mutant and immunoprecipitated with an anti-PLD antibody. As shown in Fig. 3D, PLC-γ1 wild type co-immunoprecipitated with PLD2, whereas the PLC-γ1 SH3 domain deletion mutant did not. These results indicate that the SH3 domain of PLC-γ1 is responsible for the interaction with PLD2.

**Proline-rich Motif within the PX Domain of PLD2 Is Important for the Interaction with PLC-γ1**—To identify the region of PLD2 responsible for the interaction with PLC-γ1, we constructed GST fusion PLD2 fragments as shown in Fig. 4A (40). Fig. 4B shows the result of in vitro binding analysis with purified PLC-γ1 and GST fusion PLD2 fragments. PLC-γ1 was found to bind to the F1 fragment (residues 1–314) of PLD2. For further confirmation, co-immunoprecipitation analysis was performed. COS-7 cells were transfected with the empty vector, PLD2 wild type, or the PLD2 deletion mutant (Δ1–308), and immunoprecipitated with an anti-PLD antibody. As shown in Fig. 4C, PLC-γ1 co-immunoprecipitated with PLD2 wild type but not with the PLD2 deletion mutant (Δ1–308). These results indicate that the N-terminal 1–308 residues of PLD2 are important for interaction with PLC-γ1. To determine the binding region more precisely, we prepared four GST fusion constructs, namely, the region 251–308 (Fig. 4A). In vitro binding analysis with purified PLC-γ1 and each GST fusion construct showed that PLC-γ1 binds to PLC-γ1 and PLD2, but not to the other constructs, indicating that the residues from 114 to 166 of PLD2 are responsible for the interaction (Fig. 4D). In the sequence from 114 to 166 of PLD2, there is a proline-rich motif (PSLP: residues 145–148) that can potentially interact with the SH3 domain. For confirmation, two proline residues in the PSLP motif were mutated to leucines, to produce PLD2 or PLC-γ1, respectively. As shown in Fig. 4E, PLC-γ1 bound to PLD2 wild type but not to PLD2 mutant. These results strongly indicate that PLC-γ1 binds to PSLP motif within the PX domain of PLD2.

**The SH3 Domain of PLC-γ1 Specifically Binds to the PX Domain of PLD2**—It is generally known that the selectivity of...
media containing 10% bovine calf serum were lysed with IP buffer, and or GST fusion proteins of PLD2 F1 fragment and C-terminally deleted amino acid-truncated murine PLD2 (PLD2[Δ1-308]). Cells growing in media containing 10% bovine calf serum were lysed with IP buffer, and immunoprecipitation with anti-PLD antibody, and immune complexes were incubated with purified PLC-1. The amounts of GST and GST fusion proteins were visualized on the nitrocellulose membrane by Ponceau staining (lower panel). Data are representative of three independent experiments.

Fig. 4. PLC-γ1 binds to the proline-rich motif within the PX domain of PLD2. A, schematic depiction of PLD2 fragments. B, GST protein alone or the GST fusion proteins of PLD2 fragments were incubated with purified PLC-γ1. The amounts of GST and GST fusion proteins were visualized on nitrocellulose membrane by Ponceau staining (lower panel). Data are representative of three independent experiments. C, COS-7 cells were transiently transfected with the empty vector (Vector), wild type murine PLD2 (PLD2), or N-terminal 308-amino acid-truncated murine PLD2 (PLD2[Δ1-308]). Cells growing in media containing 10% bovine calf serum were lysed with IP buffer, and immunoprecipitation with anti-PLD antibody was performed. Data are representative of two independent experiments. D, GST protein alone or GST fusion proteins of PLD2 F1 fragment and C-terminally deleted constructs of PLD2 F1 fragment were incubated with purified PLC-γ1. The amount of GST and GST fusion proteins were visualized on the nitrocellulose membrane by Ponceau staining (lower panel). Data are representative of three independent experiments. E, COS-7 cells were transfected with the empty vector, wild type human PLD2, or human PLD2 mutant (PLD2/P145L/P148L). Cell lysates underwent immunoprecipitation with anti-PLD antibody, and immune complexes were washed three more times with binding buffer. In vitro binding analysis with purified PLC-γ1 and immune complexes were performed. Data are representative of two independent experiments.

PLD2 activity by PLD2—To identify the role of their interaction in EGF signaling, EGF-induced IP₃ formation and Ca²⁺ increase were measured after transfecting PLD2 wild type or PLD2 mutant (P145L/P148L) into COS-7 cells. EGF-induced IP₃ formation and [Ca²⁺] increase were potentiated in PLD2 wild type-transfected cells but not in PLD2 mutant-transfected cells (Fig. 6, A and B). The basal activities of PLD2 wild type and PLD2 mutant show no difference, but the EGF-induced activity of PLD2 mutant decreased versus PLD2 wild type, indicating that PLC-γ1 activation is crucial for PLD2 activation in these cells (Fig. 6C), and PLC-γ1 bound only to PLD2 wild type and not to mutant after EGF stimulation (Fig. 6D). These results indicate that the interaction between PLC-γ1 and PLD2 may be important for EGF-induced IP₃ formation and [Ca²⁺] increase.

PLD2 Has No Effect on the EGF-induced Tyrosine Phosphorylation of PLC-γ1—There remained a possibility that PLD2 might affect the tyrosine phosphorylation of PLC-γ1 to potentiate IP₃ formation and [Ca²⁺] increase. To test this possibility, we checked the EGF-induced tyrosine phosphorylation of PLC-γ1 after transfecting PLD2 wild type or PLD2 mutant (P145L/P148L) into COS-7 cells. PLD2 transfection was found to have no effect on the EGF-induced tyrosine phosphorylation of PLC-γ1 (Fig. 7). This result indicates that the potentiation of IP₃ formation and Ca²⁺ increase is not due to the change of the tyrosine phosphorylation of PLC-γ1.

DISCUSSION

In this study, we report for the first time upon the phospholipase complex of PLC-γ1 and PLD2. The communication between PLC-γ1 and PLD2 has been questioned because EGF stimulation activates PLC-γ1 and PLD2 both and because in each signaling pathway there are common molecules such as PKC and PIP₂ (22, 23). PIP₂, the substrate of PLC-1, was reported to be a cofactor for PLD activation (16). It was also reported that PLC-γ1 influenced PLD activity through PKC, suggesting that PLC-γ1 might be an upstream regulator of PLD (26, 33, 54). PA, the product of PLD action, increased PLC-γ1 activity in vitro (34). However, these were indirect communications. In the present study, we found that PLC-γ1 binds directly to PLD2, which may provide a new perspective on the communication between PLC-γ1 and PLD. This interaction may increase the efficacy of their communication via other cellular components such as PIP₂ and PKC, and the interaction itself may have an important role. In this report, we propose that PLD2 may function as an adaptor in the redistribution of PLC-γ1 to the membrane region. This is an as yet unidentified function of PLD2, and we suggest that PLD2 can function as an upstream regulator of PLC-γ1 in EGF signaling.

Upon EGF stimulation, PLC-γ1 is recruited from the cytosol to the plasma membrane where EGF receptor is activated (29),
but how PLC-γ1 has access to PIP₂ is not fully understood. In the recruitment of PLC-γ1, two SH2 domains of PLC-γ1 have reported to play critical roles (15). After being phosphorylated at tyrosine residues, PLC-γ1 is redistributed to its substrate-enriched region. We suggest that the SH3 domain of PLC-γ1 may contribute to the redistribution process. Little is known of the roles of the PLC-γ1 SH3 domain in EGF-induced PIP₂ hydrolysis. Our findings suggest that the SH3 domain of PLC-γ1 may increase its substrate accessibility by bringing PLC-γ1 into the vicinity of PIP₂. It was also reported that a PLC-γ1 mutant lacking the SH3 domain showed reduced membrane association and activity (55). The binding partner of the PLC-γ1 SH3 domain in the membrane, however, has not been identified. We found that PLD2 binds to the SH3 domain of PLC-γ1. PLD2 is a membrane protein and requires PIP₂ for its activity, which means that PLD2 should be in the membrane region where PIP₂ resides. Several reports show that PLD2 is mainly localized to the membrane substructure where PIP₂ is enriched (25, 56, 57). We suggest that PLD2 is the binding partner of the PLC-γ1 SH3 domain in its redistribution. Nevertheless, the SH3 domain may not be the only domain responsible for this redistribution, because the deletion of the SH3 domain did not result in the complete inhibition of PLC-γ1 activity (55). Membrane association was also facilitated by the interaction between the C-SH2 or the pleckstrin homology (PH) domain of PLC-γ1 and PIP₃ (58, 59). Prevention of PIP₃ generation by inhibiting phosphatidylinositol 3-kinase with wortmannin or LY294002 resulted in an ~40% decrease in PLC activity (60). Considering our findings and those of previous studies, we suggest that the SH3 domain of PLC-γ1, together with the C-SH2 and the PH domain, play an important role in the redistribution of PLC-γ1 to increase its substrate accessibility.

PLD2 has a region consisting of the PX and the PH domains whose exact roles have not been clarified. Our results demonstrate that PLC-γ1 binds directly to the PX domain of PLD2. This finding suggests that the PX domain of PLD2 serves as an adaptor for other proteins. In the 53-amino acid region responsible for PLC-γ1 binding (Fig. 4D), there is a proline-rich, canonical SH3 domain binding motif. Mutating prolines in this motif to leucines abrogates interaction with PLC-γ1 (Fig. 4D). Although it cannot be said that this mutation has no effect on the conformation of PLD2 PX, it does not change the expression level, subcellular localization, or catalytic properties of PLD2 in vitro (data not shown). These results suggest that the effect of this mutation on the PLD2 structure, if any, is neither dramatic nor significant for the PLD2 function except for the PX domain of PLD2 (P145L) suggests that p47phox binds to its own C-terminal SH3 domain (69). Karathanassis et al. (70) suggested that p47phox-PX would have to undergo a conformational change to interact with its C-SH3 domain. However, this prediction may not fit with PLD2-PX because PLC-γ1 does not interact with the p47phox-PX (Fig. 5B). The PX domain structure of PLD2 may not be exactly the same as that of p47phox. It is also known that PX domains bind to phosphoinositides and
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play critical roles in the intracellular localization of a variety of cell-signaling proteins, including p42/p44 and p40phox (61). Because PLD binds to PI(4,5)P2 requires PI(4,5)P2 for enzymatic activity, and localizes to PI(4,5)P2-enriched membranes (16, 25), the PX domain of PLD2 may exist in proximity to PI(4,5)P2. Considering our findings and previous studies, we suggest that the PX domain of PLD2 contributes to increasing the substrate accessibility of PLC-γ1 by functioning as an adaptor for its SH3 domain.

The mechanism of the interaction between PLC-γ1 and PLD2 is the issue at question. We found that the interaction between PLC-γ1 and PLD2 is dependent on EGF stimulation and is transient (Fig. 2C). In the COS-7 cells, it was estimated that ~0.5% of the total PLC-γ1 translocates to the caveolae after PLD2 transfection. The co-immunoprecipitated PLC-γ1 almost equals this percentage. Without EGF stimulation, PLC-γ1 showed no interaction with PLD2; however, after EGF stimulation, PLC-γ1 bound to PLD2, and this interaction disappeared 1 min after EGF stimulation. We observed that the activity of PLC-γ1 after EGF stimulation was highest at around the time when PLC-γ1 interacts with PLD2 (Fig. 2B). Because the overexpression of PLD2 in COS-7 cells potentiates the EGF-induced IP3 formation and [Ca2+]i increase (Fig. 6, A and B), it is possible that PLD2 augments PLC-γ1 recruitment and its tyrosine phosphorylation. However, as shown in Fig. 7, PLD2 does not affect PLC-γ1 tyrosine phosphorylation levels. PLD2 may contribute to bring PLC-γ1 to the proximity of PI(4,5)P2 and PLC-γ1 may be redistributed to the membrane by binding with PLD2. Our findings show that the interaction between the SH3 domain of PLC-γ1 and the PX domain of PLD2 is important for EGF-induced Ca2+ signaling. These results also suggest that the signaling pathways of PLC-γ1 and PLD2 may cooperate

Cell proliferation plays a fundamental role in the development and maintenance of organisms. EGF is known to influence cell proliferation, and the EGF receptor is the receptor most often found to be up-regulated in a wide variety of human tumors (62). Cell proliferation, however, requires the triggering of numerous downstream signaling pathways. These pathways include those that involve PLC-γ1 and its downstream Ca2+- and PKC-mediated cascades and PLD. In many tumors there is no increase in the number of EGF receptors but, rather, its signaling is up-regulated (63). The enzymatic activity of PLC-γ1 is required for EGF-induced cell cycle progression into the S phase (2). Furthermore, PLC-γ1 signaling is regarded as a convergence point for a number of mitogility- and/or invasion-inducing pathways in cancers (64). Evidence shows that PLC-γ1 and PLD2 are overexpressed in many cancers (65–68), and, in the present study, we found that the direct interaction between PLC-γ1 and PLD2 is important for EGF signaling. The possibility of the involvement of the chicken or mouse homolog of PLC-γ1 in PLD2 in tumor cell mitogenesis or movement is an important question for PLC-γ1 and PLD2 in future studies.
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Cantley, L. G. (1998) J. Biol. Chem. 273, 23750–23757
61. Sato, T. K., Overduin, M., and Emr, S. D. (2001) Science 294, 1881–1885
62. Mendelsohn, J. (2001) Endocr. Relat. Cancer 8, 3–9
63. Wells, A. (1999) Int. J. Biochem. Cell Biol. 31, 637–643
64. Kassis, J., Lauffenburger, D. A., Turner, T., and Wells, A. (2001) Semin. Cancer Biol. 11, 105–117
65. Noh, D. Y., Lee, Y. H., Kim, S. S., Kim, Y. I., Ryu, S. H., Suh, P.-G., and Park, J.-G. (1994) Cancer 73, 36–41
66. Chang, J.-S., Noh, D. Y., Park, I. A., Kim, M. J., Song, H., Ryu, S. H., and Suh, P.-G. (1997) Cancer Res. 57, 5465–5468
67. Zhao, Y., Ehara, H., Akao, Y., Shamoto, M., Nakagawa, Y., Banno, Y., Deguchi, T., Ohishi, N., Yagi, K., and Nozawa, Y. (2000) Biochem. Biophys. Res. Commun. 278, 140–143
68. Min, D. S., Kwon, T. K., Park, W.-S., Chang, J.-S., Park, S.-K., Ahn, B.-H., Ryoo, Z.-Y., Lee, Y. H., Lee, Y. S., Huh, D.-J., Youn, S.-H., Hahn, S. J., Kim, M.-S., and Jo, Y.-H. (2001) Carcinogenesis 22, 1641–1647
69. Hiroaki, H., Ago, T., Ito, T., Sumimoto, H., and Kohda, D. (2001) Nat. Struct. Biol. 8, 529–530
70. Karathanassis, D., Stahelin, R., Bravo, J., Perisic, O., Pacold, C. M., Cho, W., and Williams, R. L. (2002) EMBO J. 21, 5057–5068