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The phospholipase D superfamily as therapeutic targets

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Abstract

The Phospholipase D (PLD) lipid-signaling enzyme superfamily has long been studied for its roles in cell communication and a wide range of cell biological processes. With the advent of loss-of-function genetic mouse models that have revealed that PLD1 and PLD2 ablation is overtly tolerable, small molecule PLD1/2 inhibitors that do not cause unacceptable clinical toxicity, a PLD2 polymorphism that has been linked to altered physiology, and growing delineation of processes subtly altered in mice lacking PLD1/2 activity, the stage is being set for assessment of PLD1/2 inhibition for therapeutic purposes. Based on findings to date, PLD1/2 inhibition may be of more utility in acute rather than chronic settings, although this generalization will depend on the specific risks and benefits in each disease setting.

Keywords

PLD1; PLD2; small molecule inhibitors; cancer; thrombosis; autoimmune disease

Phospholipase D Superfamily Overview

The most commonly studied PLD activity (Figure 1) entails hydrolysis of phosphatidylcholine (PC), the most abundant membrane phospholipid, to yield choline and the second messenger signaling lipid phosphatidic acid (PA). More generally, PLD enzyme superfamily members are transphosphatidylases that conduct headgroup exchange on PA at the terminal phosphodiester bond [1]. In the prototypic reaction, water is used as the nucleophile to exchange an –OH group for the pre-existing choline headgroup [2], but nucleophiles such as primary alcohols (ethanol or 1-butanol) can be used instead of water to generate phosphatidyl-alcohols, and in fact are strongly preferred over water, which has led historically to them being used to commandeer the PLD enzymatic capacity and thus inhibit PA production as will be discussed subsequently.

In mammals, two isoforms found in association with membrane surfaces in the cytoplasm, PLD1 and PLD2, are responsible for the PC-hydrolyzing activity described above [1]. PLD3

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[3] and PLD4 [4] are endoplasmic reticulum (ER) integral transmembrane proteins with a short, N-terminal cytoplasmic tail, and the bulk of the protein, including the hypothetical catalytic domains, in the endoplasmic reticulum (ER) lumen, while PLD6 (MitoPLD) is anchored by an N-terminal transmembrane tail into the outer surface of mitochondria [5]. PLD5, for which there are no publications yet, is most similar to PLD3 and PLD4 but is unlikely to have enzyme activity since the canonical PLD enzymatic catalytic motif is not well conserved in it. Enzymatic activities have not been identified for PLD3 or PLD4 either, and it is possible that they instead have non-enzymatic functions. PLD6 has been reported to both hydrolyze cardiolipin, a mitochondrial-specific lipid, to PA, and to function as an endonuclease (phosphodiesterase) to generate a specialized form of micro-RNAs called piwi-interacting RNA (piRNA) [6].

Interest has become increasingly focused on PLD1 and PLD2 in the context of cancer, cardiovascular, neurodegenerative, and infectious disease, while loss of function of PLD3 has been linked to Alzheimer's disease [7], loss of PLD4 function to autoimmune disease [4, 8, 9], and PLD6 has been found to be required for spermatogenesis [10]. Mice lacking PLD5 have been generated by the International Knockout Mouse Consortium program and subjected to a standardized phenotypic screen involving 25 tests, but no significant abnormalities were observed [11]. A single nucleotide polymorphism (SNP) in the PLD5 gene has been reported to correlate with verbal performance in autism patients [12], but since the SNP is at the center of a large intron, the clinical relevance of this finding is not clear. For different reasons, therapeutic opportunities are not immediately apparent for PLD3, PLD4, PLD5, and PLD6; thus, this review will focus on PLD1 and PLD2.

PLD1 and PLD2, the signaling-activated enzymes

PLD1 and 2 are expressed widely in different tissues and cell types and are activated by a wide variety of G-protein coupled receptors (GPCRs) and receptor tyrosine kinases (RTKs) (Figure 2). Mechanisms regulating PLD1 and PLD2 activity have recently been exhaustively reviewed and can be referred to for more detail on this topic [2].

PA, the second messenger generated by PLD1/2, is pleiotropic in function. PA has a small, negatively-charged headgroup that drives membranes to undergo negative curvature when local concentrations of PA rise sufficiently. This negative curvature is though to lower the activation energy for production of membrane vesicles and for their fusion into target membranes, thus facilitating exocytosis, endocytosis, and membrane vesicle trafficking in general [13]. PA can also act as a lipid anchor, recruiting PA-binding proteins to localized sites of signal transduction [14], examples of which would be the guanine nucleotide exchange factors (GEFs) DOCK2 and SOS, which activate Rac1 and Ras, respectively [15, 16]. In some instances, PA additionally activates the proteins recruited, such as phosphatidylinositol 4-phosphase 5-Kinase (PI4P5K), which phosphorylates PI(4)P to generate PI(4,5)P_2 [17], and mTOR, which regulates many processes including cell hypertrophy, differentiation, and survival [18]. Finally, PA can be dephosphorylated by Lipin to generate diacylglycerol (DAG) [19] or hydrolyzed by phospholipase A (PLA) to generate LysoPA [20, 21], which are both potent signaling lipids as well that can be re-converted to PA by DAG kinases (DGKs) and LysoPA acetyl-transferases (LPAATs),

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respectively (Figure 1). Thus, PA can be generated by PLD1 and/or PLD2 in response to a wide variety of agonists, and once formed, has been reported to affect numerous types of cell biological processes and to be metabolized to other signaling lipids with even more biological roles.

Cell biological roles for PLD have been explored for more than 30 years, the totality of which encompasses almost all signaling-driven processes, such as regulated exocytosis, endocytosis, Golgi-ER trafficking, proliferation, cell migration, autophagy, and apoptosis. Nonetheless, most of these published reports employed tools that represented the only ones available at the time but that are now recognized to be problematic. With the recent development of mice lacking PLD1 and/or PLD2 and potent small molecule inhibitors, a broad-scale reassessment of the field is underway and a considerable narrowing of the scope of function for PLD1 and PLD2 is anticipated.

Tools used to study PLD function and lead therapeutics

As noted above, the finding that primary alcohols were strongly preferred as the nucleophile over water led to their employment to divert PLD from making PA during signaling events, and most reports from 1980 to the mid-2000's used this approach. Awareness of problems with this method grew over the past decade, though, with the observation that the amounts of alcohol required to substantially block PA production by PLD caused substantial cellular toxicity, whereas the lower amounts used in most studies were only modestly affecting PA generation [22, 23]. With the introduction of RNAi (2003) and then small molecule inhibitors (2009), discrepancies between the different inhibitory approaches began to be noted, and a final, definitive report was presented by Kanaho and colleagues in 2013 [24]: By this time, more than 100 articles had been published on the topic of a requisite role for PLD in fMLP peptide signaling-activated superoxide production and degranulation in neutrophils, most if not all based on the use of primary alcohols to block PLD-mediated production of PA [25]. Unexpectedly, however, Sato et al. [24] found that neither pharmacological inhibition of PLD1/2 using the modern small molecule inhibitor FIPI nor genetic deletion of both PLD1 and PLD2 had any effect on superoxide production and degranulation or even PA production; rather, the PA observed with fMLP stimulation was generated by DGK phosphorylation of DAG that in turn had been generated though activation of Phospholipase C (PLC). Thus, ethanol was incontrovertibly shown to be mediating inhibition of superoxide production and degranulation through an unknown mechanism fully separate from effects on PLD activity, and this general caveat is now appreciated to render all prior studies using alcohol-mediated inhibition non-definitive.

Other tools used during this period included catalytically-inactive mutant PLD1 and PLD2 isoforms [26] and RNAi. The catalytically-inactive isoforms are problematic to interpret since they generally do not affect endogenous PLD activation, but can function as dominant-negative alleles if they displace the endogenous isoforms from protein complexes in which they need to function, or can even have undesired, broader negative effects if they sequester other signaling factors. RNAi, which represented an advance at the time of its introduction, nonetheless frequently decreases but does not eliminate expression, has a limited window of

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activity, and can cause cellular stress or off-target effects. The advent of CRISPR/Cas9 genome editing may largely replace it for the creation of cell lines lacking PLD activity [27].

At present, the best tools available for the study of PLD function are the recently developed small molecule inhibitors and mice lacking PLD1 and PLD2. These approaches are complementary since any drug can have unanticipated off-target effects (e.g., one of the PLD1-selective isoforms developed inhibits the P2X7 receptor [28]), whereas the knock-out mice physically lack PLD protein and thus may exhibit effects reflecting loss of a scaffolding function, instability of a protein complex, or lipase-independent functions for the enzyme. Because PLD1 and PLD2 have each been reported to physically interact or be in a complex with about 30 other proteins in different settings ([14]; also see http://www.ncbi.nlm.nih.gov/gene/5337 and /5338), this is a non-trivial issue. Finally, long-term loss of PLD activity may lead to the activation of compensatory mechanisms that would not be observed in the setting of acute pharmacological inhibition, including upregulation of DGKs or LPAATs to facilitate PA production, down-regulation of the enzymes that metabolize PA, or other unanticipated and unknown effects.

The development of a pharmacological approach was sparked by the identification of halopemide, a neuropsychiatric drug, as a robust PLD inhibitor [29, 30]. An analog, denoted FIPI [23], is a potent inhibitor of both PLD1 and PLD2, has a half-life and bioavailability parameters that have permitted it to be used widely in cell culture and animal studies, and thus far has phenocopied outcomes observed with knockout cells and animals and with RNAi [24, 31–34]. While no off-targets effects have yet been reported for FIPI, the potential for ancillary pharmacology actions has been raised based on testing of FIPI in a Ricerca radioligand binding panel of 68 GPCRs, ion channels and transporter in which significant interaction was observed for multiple targets [29]. However, since the assay was conducted at 10 μM, which is more than 100-fold higher than the concentration required for full inhibition using FIPI, and since the interactions have not been validated using functional inhibition assays, it is not presently known whether any of these findings are physiologically relevant or significant. Extensive development of other halopemide analogs has led to isoform-selective compounds that are thought to have low off-target potential effects and to be good candidates for therapeutic development [29]. When possible, use of multiple inhibitors in experimental strategies should be pursued to reduce the possibility that interpretations will be confounded by off-target effects such as that reported for one of the isoform-selective inhibitors [28]. A new class of PLD inhibitors has been developed starting with an approach based on a pyrimidine core structure [35]. While also quite potent, these inhibitors have not been characterized as well. However, they provide another option to confirm phenotypes seen with the halopemide series and might possibly represent lead candidates for clinical use with further development.

Intriguingly, halopemide, which was developed for its dopamine receptor blocking ability and hence use in psychosis, was used clinically at a sufficiently high dose to fully block PLD activity [36], suggesting that PLD inhibition does not cause major unacceptable toxicity even over prolonged periods of time. This observation parallels findings for the PLD1 and 2 single- and double-knockout mice which are viable and overtly normal to inspection [33], indicating that PLD activity is generally dispensable and hence that

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pharmacological inhibition has a reasonable chance of being tolerated in the short-term and potentially even in the long-term, with caveats as discussed subsequently.

General perspective on cell biological roles for PLD gleaned from recent studies with modern tools

As discussed above regarding fMLP-stimulated neutrophil degranulation, some roles long- and well-established for PLD based on the use of older tools such as ethanol-mediated diversion of PLD activity have not been reproduced using PLD small molecule inhibitors and cells genetically ablated for PLD isoforms. In some other settings, non-redundant, partially-redundant, or fully-redundant roles for PLD1 and PLD2 have been reported. As an example of non-redundancy, genetic ablation of either PLD1 or PLD2 decreases macrophage phagocytosis of *Yersinia* bacteria and IgG-coated beads, but the phenotype does not increase in severity when both isoforms are inactivated, suggesting non-redundant functions in successive steps in a sequential pathway [37]. Platelet activation, which will be discussed below in more detail, provides an example of partial redundancy. In this setting, platelets lacking PLD1 have a blunted-activation phenotype while platelets lacking PLD2 appear normal [38]; however, platelets lacking both isoforms have a stronger phenotype than those lacking only PLD1 [39].

Finally, an example of strong redundancy comes from the study of platelet-derived growth factor (PDGF) stimulation of mouse embryo fibroblasts [32]. In brief, PDGF signaling triggers actin cytoskeletal reorganization in the form of peripheral ruffling at the edge of the cell (which models the process of cell motility) and dorsal ruffling at the top of the cell (which models receptor endocytosis and cell invasion, which are important in signaling and in cancer). Genetic ablation of either PLD1 or PLD2 has no effect on these processes – but pharmacological inhibition or genetic ablation of *both* genes fully blocks dorsal ruffling while having no effect on peripheral ruffling. This discordance in the regulation of PDGF-elicited actin cytoskeletal reorganization likely reflects differences in the small GTPases and GEFs involved in the individual ruffling processes, or the ability of an enzyme like DGK to generate PA locally in the absence of PLD activity. Compensatory actions in response to loss of PLD function have also been reported in the context of mTOR regulation [18]. An important conclusion from these studies is that it is not possible to generalize roles for PLD activity in the context of cytoskeletal reorganization or membrane vesicle trafficking; rather, such roles have to be defined in the context of specific cell-types and signaling pathways.

Therapeutic opportunities for PLD inhibition

Given the many reports linking PLD to immune cell function highlighted by the impact on macrophage phagocytosis and migration in mice lacking either PLD isoform [37], the cost-benefit assessment for employing PLD inhibition therapeutically will differ in the acute and chronic settings. Current acute and chronic opportunities (Figure 3) are described following.

Thrombotic disease

The initial PLD loss-of-function phenotype described focused on blunted platelet activation in mice lacking PLD1 [38]. While most aspects of platelet function were unchanged,

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resistance to agonist-stimulated conformational activation of integrin $\alpha_{IIb}\beta_{III}$ was uncovered. Integrin $\alpha_{IIb}\beta_{III}$ activation, which enables the integrin to bind to fibrinogen and thus create a three-dimensional platelet – fibrinogen physical network, is a key step in the formation of vascular thrombi. The integrin activation is dependent on intracellular signaling steps including activation of GEFs and PI(4,5)P_2 synthesis that are known to be regulated in specific instances by PLD, albeit the specific role undertaken by PLD1 in this setting has not yet been determined. Regardless, the decreased integrin $\alpha_{IIb}\beta_{III}$ activation results in blunted thrombus formation, which in turn confers protection in models of pulmonary embolism, aortic thrombosis, and stroke. In a subsequent report, a slightly stronger phenotype was observed in mice lacking both PLD1 and PLD2 [39]. Finally, the PLD small molecule inhibitor FIPI was successfully used to confer protection in the pulmonary embolism and stroke models and was as effective as genetic ablation of both isoforms [33]. Pragmatically, FIPI was much more effective when delivered to the mice prior to the initiation of the thrombotic event and was only mildly effective when delivered simultaneously or shortly afterwards. Taken at face value, this would suggest that PLD inhibition would not be useful in the immediate setting of acute thrombosis such as in stroke, embolic disease, and myocardial infarction, but rather only in more restricted settings such as strokes in evolution or for individuals at high risk for future thrombotic events. However, in the published report, FIPI was delivered intraperitoneally and it is relatively hydrophobic, so it is not known how long it took to achieve adequate serum levels for inhibition. It will be interesting and important to revisit these models using intravenous administration of FIPI or other PLD inhibitors to determine whether rapid effects on thrombosis can be achieved in this setting.

A particular potential advantage for PLD inhibition is that this approach in mouse models did not result in increased bleeding times, suggesting that it might be safer than other anti-thrombotics currently in clinical use. For example, aspirin is used widely for prevention of stroke and myocardial infarctions and has been shown to be effective in secondary prevention, i.e. after an initial stroke and myocardial infarction, despite the increased risk of gastrointestinal hemorrhage [40]. However, the benefit of aspirin in primary prevention, i.e. for individuals at risk for a cardiovascular event, is less clear when weighed against the gastrointestinal hemorrhage risk. A therapeutic lacking hemorrhage risks would provide an important potential advance for the field. However, this is a parameter that would need to be assessed clinically, since mice are less dependent on platelet activation for hemostasis than humans [41].

Another factor that may come into play would limit the use of PLD inhibition to the preventative or acute-injury phase, but not during the post-injury repair phase, since PLD1 deficiency, which blunts immune responses [37], has been reported to hinder immune-driven elements of the repair process after myocardial infarctions [42]. PLD1 may also have cell-intrinsic roles in the cardiac myogenic repair process, since PLD1 has been shown in skeletal muscle to have mTOR-dependent roles in hypertrophy [43], differentiation [44], and second-phase myoblast fusion and regeneration after injury [45].

Nonetheless, the ability of PLD inhibition to block thrombus formation without affecting hemostasis in mice is remarkable in comparison to other proteins targeted to inhibit platelet function, such as GPIb or GPVI, loss of which does result in increased bleeding times.

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Influenza

A whole-genome RNAi screen for host genes required for replication of influenza virus in 2009 identified PLD2 as one of several targetable candidates [46]. A recent study using isoform-selective PLD inhibitors confirmed the RNAi findings and raised the possibility that PLD2 inhibition could be used across a broad spectrum of influenza strains proactively or subsequent to infection to limit the severity of the disease progression [47]. This is an exciting idea given the race to develop and produce an effective vaccine when a new strain emerges and the paucity of generically effective therapeutics, some of which (e.g. amantadine) are already being sidelined by the development of viral resistance. However, compelling as the cellular PLD inhibition studies have thus far been, it will be critical to extend these findings to *in vivo* models for infection, since it is not currently known whether the impacts that PLD inhibition has on immune system function will outweigh the benefits conferred through the suppression of viral replication.

Neurodegenerative disease

Alzheimer's Disease (AD)—PLD1, PLD2, and PLD3 have all been linked to AD [7, 48, 49]. Up to 9% of late-onset AD in European and African populations has been associated with multiple different rare PLD3 polymorphisms and it has been proposed that PLD3 loss-of-function increases pathogenic amyloid peptide secretion [7]. However, since the mechanism of action of PLD3, and even whether it encodes a catalytic activity, is presently unknown, there are no currently obvious therapeutic leads that derive from this intriguing finding. Moreover, it will be important for the linkage of PLD3 variants to late onset AD to be independently validated, since a study of late-onset AD patients from mainland China did not observe the most frequent PLD3 polymorphism (V232M) in this population [50]. PLD1 has similarly been reported to be protective in the context of AD, acting as a negative regulator of β-amyloid formation in cell culture studies [48]. However, since no small molecule PLD1 agonists have been identified to date, the therapeutic opportunities here are not readily apparent either.

The most intriguing studies have focused on PLD2 [49]. PLD2 is activated by amyloid β-peptide (Aβ) in neurons, Aβ loses the ability to suppress long-term potentiation (LTP) in the absence of PLD2, and PLD2 ablation rescues memory deficits and confers synaptic protection in a mouse model of AD. Because PLD2-selective small molecule inhibitors are in development as discussed above, this represents a potential therapeutic avenue. Nonetheless, aside from the extent to which mouse models of AD are predictive for the success of human pharmacological treatment, additional studies are required to address other impacts that PLD2 inhibition may have on brain function. While Oliveira and colleagues [49] did not report differences in LTP or in learning and memory behavioral tasks in normal versus PLD2-deficient mice, another group did observe deficiencies in learning and memory tests and reduced levels of acetylcholine in $PLD2^{-/-}$ mice upon behavioral activation [51]. Whether the differences in the reports reflect the use of distinct measurement approaches or possibly differences in the mouse lines for which PLD2 was ablated using different approaches is not known. As well, even if there are behavioral differences in mice lacking PLD2 from conception, these may be developmental in origin and thus would not manifest

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in adults subsequently treated with a small molecule PLD2 inhibitor, or the changes may be tolerable for patients in comparison to the consequences of further progression of AD.

Multiple Sclerosis—PLD1 ablation has been reported to blunt immune responses [37, 42], which is further supported by the finding that PLD1 ablation markedly reduces symptomatology in experimental allergic encephalomyelitis (EAE), a murine model for multiple sclerosis [52]. Given the lack of overt phenotypes in mice lacking PLD1, PLD1 inhibition may provide a useful approach to augment current multiple sclerosis therapeutics.

Hypertension

A genome-wide analysis study identified a single nucleotide polymorphism (SNP) in PLD2 as negatively correlating with hypertension [53]. The polymorphism, R172C, alters an important amino acid in a region of the protein known as the Phox (PX) domain, which has been shown by many groups to mediate binding to phosphoinositides at the plasma membrane or on endosomes. PLD2 has been reported to facilitate the endocytosis of numerous GPCRs, including AT1R [54], the receptor for angiotensin, and to regulate the synthesis and release of aldosterone, the output of AT1R signaling in adrenal cortex cells [55, 56]. Thus, although not yet specifically demonstrated, the mechanistic components for PLD2 contribution to the regulation of blood pressure have been described. A PLD2-selective inhibitor could be proposed as an anti-hypertensive therapeutic, although whether it would add to the therapeutic value of approaches such as angiotensin-converting-enzyme (ACE) inhibition or be cost-benefit appropriate given the other processes that might be affected by PLD2 inhibition remains to be determined.

Cancer

Roles for PLD in cancer have long been explored. As discussed above, the vast majority of studies more than five years old relied on alcohol-mediated inhibition or other non-specific means of altering PLD activity and thus need to be interpreted with caution. These are discussed in extensive detail in recent reviews [29, 30]. However, studies using modern small molecule inhibitors, animals lacking PLD isoforms, and genetic models are starting to provide a clearer picture of potential utility for PLD inhibitors. Roles for PLD1 have been described in the tumor environment for the ability of vascular endothelial cells to respond to vascular endothelial growth factor (VEGF) released by tumors under hypoxic stress to stimulate tumor vascularization and enable continued growth of the primary cancer [31]. Metastasizing tumor cells also need to interact with platelets to achieve efficient colonization at distant sites [57], and this is hindered in mice lacking PLD1 due to the blunted platelet activation described above; hence there are at least two roles for PLD1 in the tumor environment [31]. Related roles for PLD2 have also been described in hypoxia-induced Hif1-α expression and VEGF secretion by endothelial cells, which similarly results in a reduction of tumor neovascularization and growth when PLD2 is absent, although to a lesser extent than in the absence of PLD1 [58]. Inhibiting both PLD1 and PLD2 may both address redundancy and target non-overlapping roles as well. Inhibition of PLD activity using modern small molecule inhibitors has been shown to affect mTOR-regulated Hif1-α expression and VEGF secretion [59] and survival signaling and invasiveness [60] of glioma tumor cell lines in cell culture studies and to inhibit tumor growth and metastasis in breast,

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lung, and melanoma mouse tumor implant models [31, 61, 62]. Roles for PLD have in fact been proposed in many of the biological processes required for tumor progression based on findings developed using study of cell lines in culture. However, given the complexity of cellular interactions *in vivo* in cancer involving the tumor, local tumor environment, and immune system that can support or oppose tumor growth, as well as potential redundancy and the development of compensatory mechanisms, the actual benefit of PLD inhibitors in cancer will need to be explored using animal models to develop better predictive information regarding their potential therapeutic benefit. Finally, PLD inhibition may be contraindicated in types of cancer where loss of PLD1 activity results in decreased motility that enhances tumor progression. For example, PLD1 facilitates activation of lymphocyte function-associated antigen 1 (LFA-1), an integrin that regulates lymphocyte entry and exit from lymph nodes [63]. A subset of chronic lymphocytic leukemia patients exhibit defective PLD1 activation and blunted LFA-1 activation, causing the tumor cells to arrested in lymph nodes where they encounter increased exposure to proliferation signals in the tissue microenvironment that drive shortened patient survival [64]. In a similar vein, roles *in culture* and *in vivo* for PLD1 in macroautophagy have been reported [34]. The relationship of autophagy to cancer progression is complex and may be either pro- or anti-oncogenic depending on the type and stage of tumor. Taken together, roles for PLD in tumor cells are generally but not always pro-oncogenic, indicating the need to individually define utility for PLD inhibition for specific types of cancer.

Concluding remarks

This is an exciting time with the approaching development of clinically-usable small molecule PLD inhibitors and the growing emergence of genetic and pharmacological animal model studies that are delineating potential utility for PLD inhibition in autoimmune disease, hypertension, infectious disease, cancer, thrombotic disease, and neurodegenerative disease (Figure 3). While acute versus chronic and cost-benefit issues may ultimately decrease enthusiasm in some of these settings, it is likely that PLD inhibitors will find utility in at least several different clinical fields. Current challenges include developing therapeutics with optimal pharmacokinetic parameters, and in the acute setting of thombotic events in evolution, determining if PLD inhibition can be achieved rapidly enough to affect the progression of strokes, myocardial infarctions, and pulmonary embolisms. As well, it will be important to determine if PLD inhibition genuinely offers advantages over existing therapeutics with respect to the risks of bleeding.

PLD suppression of the immune system, while intriguing in the context of many different types of autoimmunity, may create a barrier to using the inhibitors as anti-influenza therapeutics. However, this may be less of an issue for viruses that manifest persistence and for which immunity has already been maximally developed, such as hepatitis C virus. For chronic diseases such as hypertension, cancer, neurodegenerative disease, and long-term prevention of thrombotic disease, it will be important to develop a full characterization of the phenotypes associated with the knockout animals and pharmacological inhibition to assess whether unwanted effects of PLD inhibition outweigh the benefits, and of course this assessment will be different depending on the severity of the disease course.

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In different settings, it may be advantageous to use PLD1-specific or PLD2-specific inhibitors rather than a dual PLD1/2 inhibitor, depending on the extent of redundancy for the individual PLD isoforms in the process that is being inhibited. Finally, thus far there has been a remarkable concordance between phenotypes observed with knockout animals (i.e. long-term absence of PLD function) and the short-term inhibition achieved using small molecules, which suggests that there will be little in the way of compensatory mechanisms that arise with prolonged use of the inhibitors. However, this remains an unexplored topic that will be important to address as therapeutic approaches are developed, in particular in the context of cancer.

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Highlights

- Phospholipase D signaling underlies many cell biological and physiological processes
- PLD ablation and small molecule inhibitors are well-tolerated
- PLD inhibition may be useful in several disease settings including cancer
- Others include autoimmunity, viruses, and thrombotic and neurodegenerative disease

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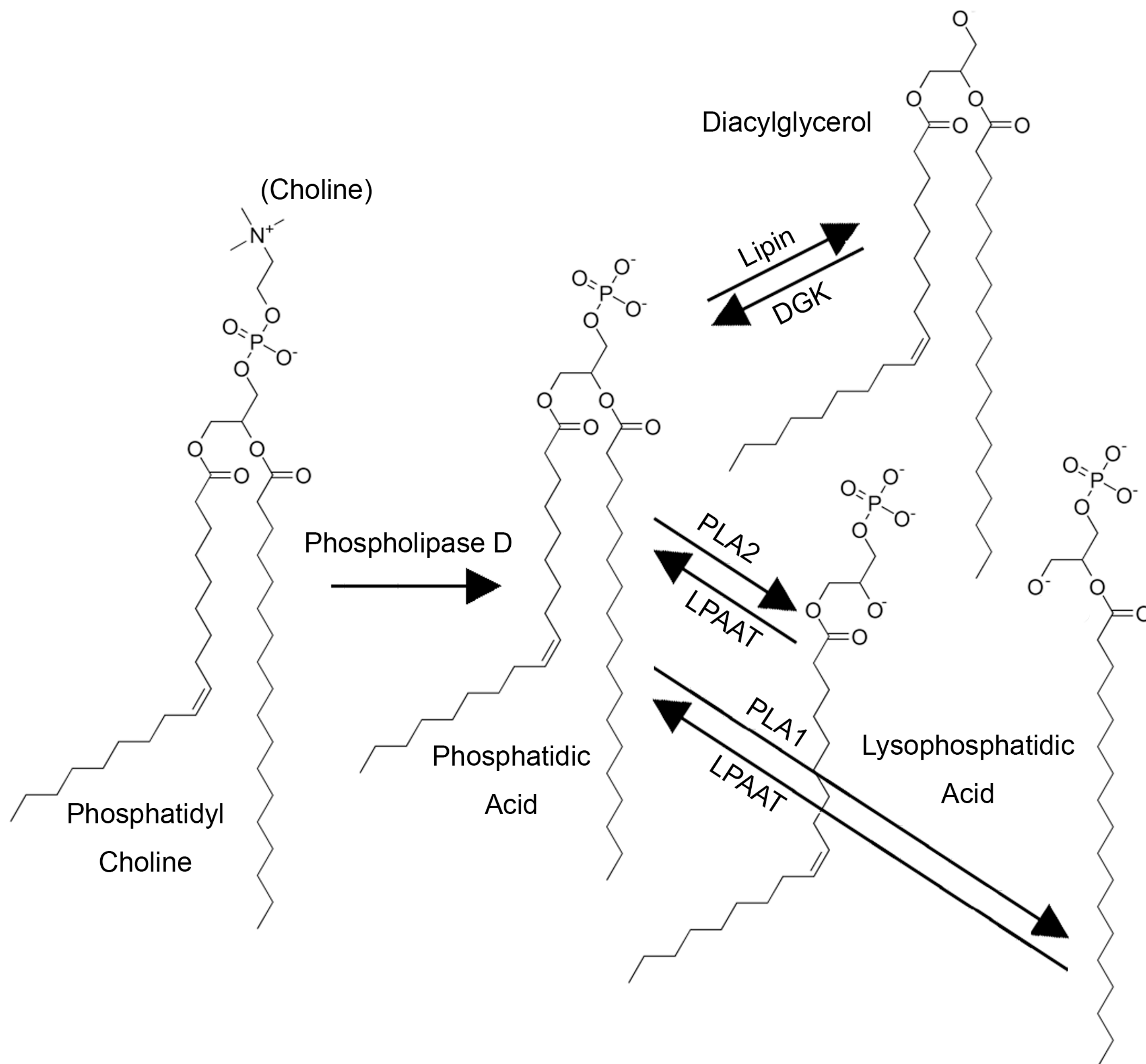

Figure 1.
Schematic depiction of PLD generation of PA and subsequent PA metabolism and regeneration. Phosphatidylcholine is hydrolyzed by PLD to generate phosphatidic acid (PA), which can subsequently be dephosphorylated by Lipin to generate diacylglycerol (DAG) or deacylated by PLA1 or PLA2 to generate LysoPA. DAG and LysoPA are both signaling lipids as well and can serve as substrates to regenerate PA through the actions of diacylglycerol kinases (DGKs) or LysoPA acetyl-transferases (LPAATs), respectively.

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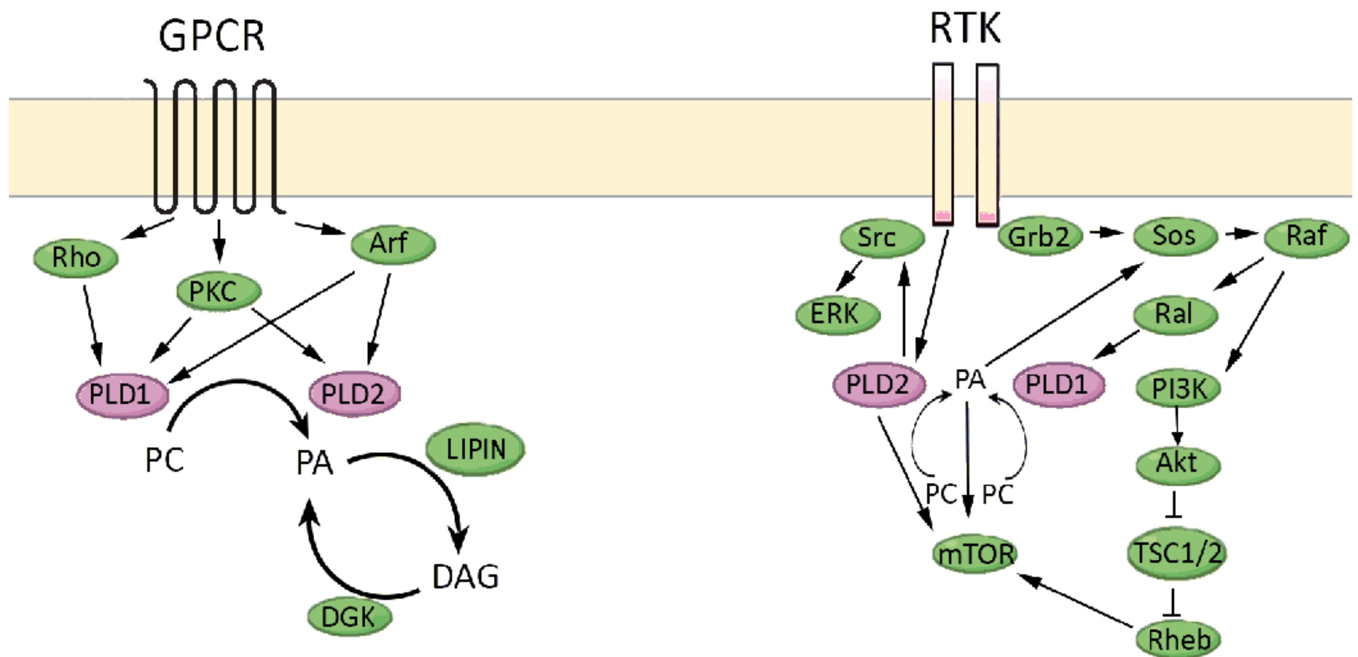

Figure 2.

Simplified schematic for PLD activation by extracellular agonists. A wide variety of hormones, growth factors, and cytokines activate PLD via stimulation of G-protein coupled receptors (GPCR) and receptor tyrosine kinases (RTK). PLD1 is known to be directly activated by protein kinase C (PKC) and the small GTPases RhoA and ARF, whereas PLD2 is probably indirectly activated by some or all of these factors [65]. Binding to PI(4,5)P_2 is also required for PLD activity. PLD2 can also be activated directly by RTKs and PLD1 by Ral. Among many other downstream effector functions, PLD2 can stimulate Src activity, and the PA generated by PLD1 and PLD2 can promote mTOR activity in coordination with many other inputs, such as the small GTPase Rheb.

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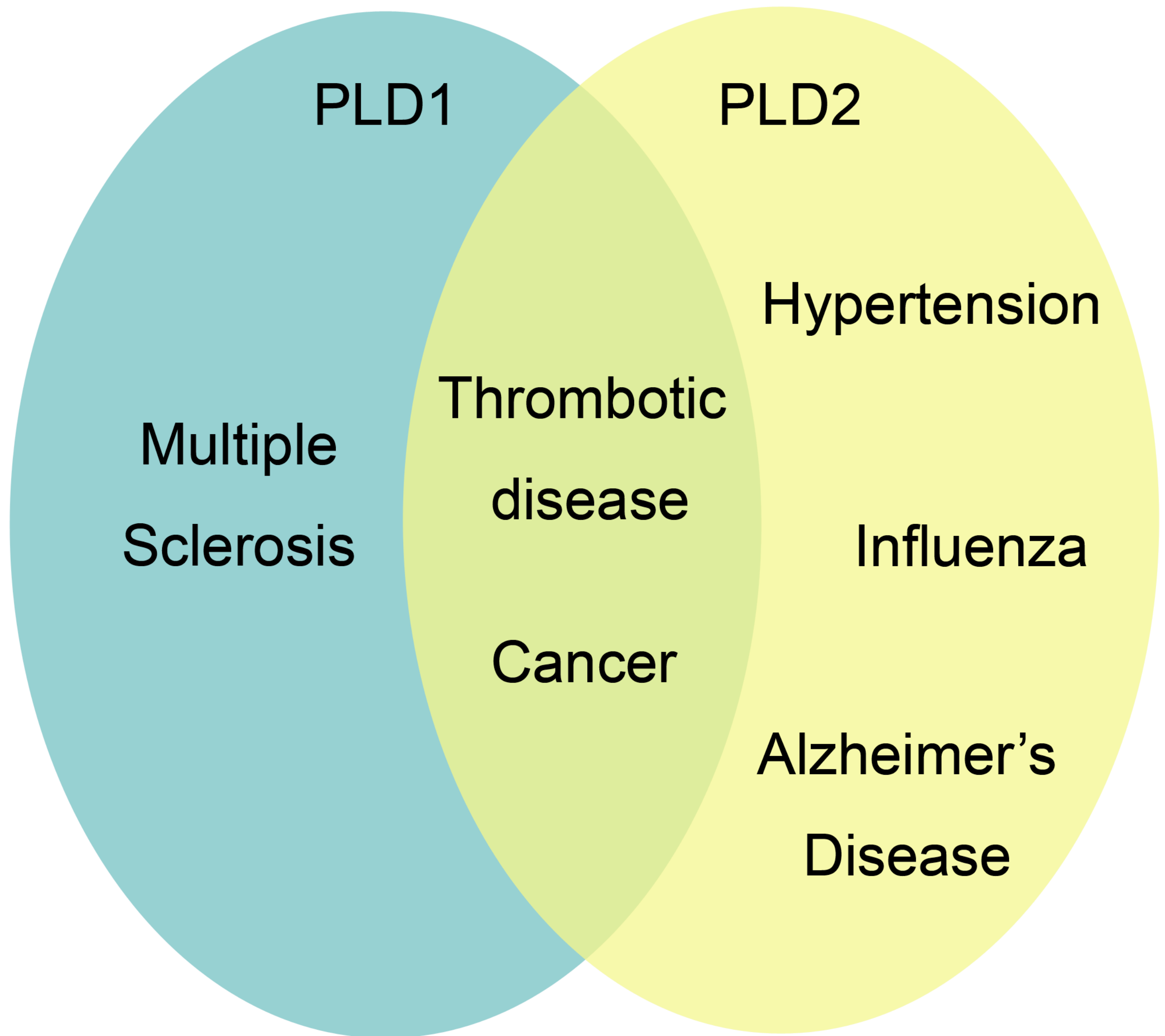

Figure 3.

Therapeutic opportunities for PLD inhibition. The diseases discussed in the text are shown here in relationship to potential benefits that might be achieved from inhibition of PLD1, PLD2, or both isoforms. More generally, PLD1 inhibition may be impactful for many types of autoimmune disease and PLD2 inhibition may affect multiple types of viruses, while PLD inhibition may be potentially useful for some but likely not all types of cancer, and may even be contraindicated in some.