thematic review series

Thematic Review Series: Phospholipases: Central Role in Lipid Signaling and Disease

Phosphoinositide-specific phospholipase C in health and disease

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Abstract Phospholipases are widely occurring and can be found in several different organisms, including bacteria, yeast, plants, animals, and viruses. Phospholipase C (PLC) is a class of phospholipases that cleaves phospholipids on the diacylglycerol (DAG) side of the phosphodiester bond producing DAGs and phosphomonoesters. Among PLCs, phosphoinositide-specific PLC (PI-PLC) constitutes an important step in the inositide signaling pathways. The structures of PI-PLC isozymes show conserved domains as well as regulatory specific domains. This is important, as most PI-PLCs share a common mechanism, but each of them has a peculiar role and can have a specific cell distribution that is linked to a specific function. More importantly, the regulation of PLC isozymes is fundamental in health and disease, as there are several PLC-dependent molecular mechanisms that are associated with the activation or inhibition of important physiopathological processes. Moreover, PI-PLC alternative splicing variants can play important roles in complex signaling networks, not only in cancer but also in other diseases. That is why PI-PLC isozymes are now considered as important molecules that are essential for better understanding the molecular mechanisms underlying both physiology and pathogenesis, and are also potential molecular targets useful for the development of innovative therapeutic strategies.—Cocco, L., M. Y. Follo, L. Manzoli, and P-G. Suh. **Phosphoinositide-specific phospholipase C in health and disease. *J. Lipid Res.* 2015.** 56: **1853–1860.**

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Phospholipases are quite common enzymes that are present in a broad range of organisms, including bacteria, yeast, plants, animals, and viruses. Phospholipase C (PLC) constitutes a class of enzymes that cleave phospholipids on the diacylglycerol (DAG) side of the phosphodiester bond. In plants, a phosphatidylcholine-specific PLC (PC-PLC) has been recently identified: this PLC acts preferentially on phosphatidylcholine, even though it can also act upon other lipids, such as phosphatidylethanolamine, therefore giving rise to a class of nonspecific PLCs (1, 2). PC-PLC isoforms are responsible for phosphatidylcholine hydrolysis, producing phosphocholine and DAG, and they have been isolated but not yet cloned from mammalian sources. However, accruing evidence points to multiple implications of these enzymes in cell signaling through MAPK and oncogene-activated protein kinase pathways, as well as programmed cell death, activation of immune cells, and stem cell differentiation (3). On the other hand, phosphoinositide-specific PLC (PI-PLC) enzymes utilize phosphoinositides as a specific substrate and their metabolism is implicated in a large series of signal transduction pathways.

This review is devoted to highlighting PI-PLC, which plays an important role in cell physiology and particularly in signal transduction pathways in mammals. Thirteen kinds of mammalian PI-PLCs are classified into six isotypes (β, γ, δ, ε, ζ, η), according to their structure. Here, we shall point at the molecular features, function, regulation, and splicing variants of these enzymes and discuss their role in disease.

MOLECULAR FEATURES OF PI-PLC

PI-PLC hydrolyzes phosphatidylinositol-4,5-bisphosphate (PIP2) to produce DAG and inositol-1,4,5-trisphosphate (IP3) (**Fig. 1**) which, in turn, activate protein kinase C

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Abbreviations: DAG, diacylglycerol; GPCR, G protein-coupled receptor; IP3, inositol-1,4,5-trisphosphate; IPMK, inositol polyphosphate multikinase; MDS, myelodysplastic syndrome; PC-PLC, phosphatidylcholine-specific phospholipase C; PDGFR, platelet-derived growth factor receptor; PH, pleckstrin homology; PI3K, phosphoinositide 3-kinase; PIP2, phosphatidylinositol-4,5-bisphosphate; PIP3, phosphatidylinositol-3,4,5-trisphosphate; PI-PLC, phosphoinositide-specific phospholipase C; PKC, protein kinase C; PLC, phospholipase C; RTK, receptor tyrosine kinase; SH, Src homology.

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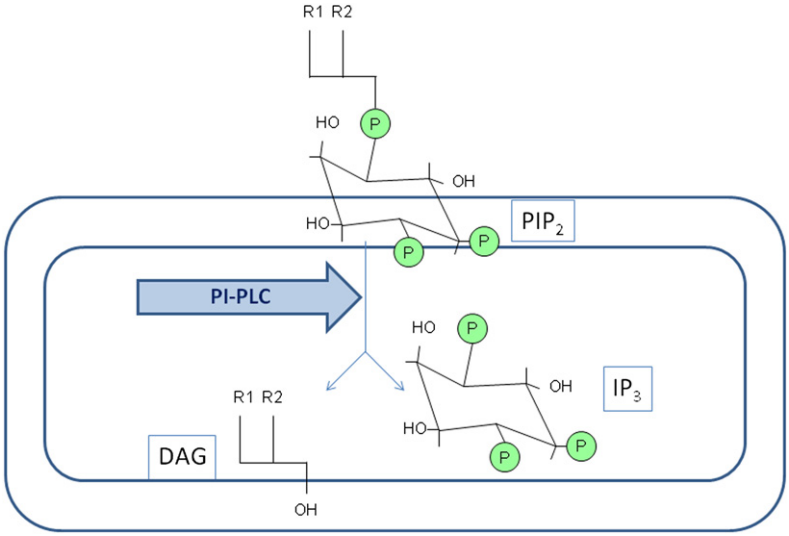

Fig. 1. PI-PLC-mediated enzymatic reaction. PIP2, which is located within the plasma membrane, is cleaved by PI-PLC enzymes, generating the two well-known second messengers, DAG and IP3. DAG remains bound to the plasma membrane, whereas IP3 is located within the cytosol, but both of them can act as second messengers and activate downstream targets.

(PKC) and induce the release of calcium ions from intracellular stores, respectively (4, 5). Since the first report of PI-PLC existence (6), 13 mammal PI-PLC isozymes have been identified and, at a molecular level, they can be divided into six subgroups: PI-PLCβ(1–4), -γ(1 and 2), -δ(1, 3, and 4), -ε, -ζ, and -η(1 and 2). Interestingly, the structure of these PI-PLC isozymes shows highly conserved domains as well as peculiar characteristics (**Fig. 2**). In fact, the X and Y domains are two highly conserved regions, whereas the C2 domain, the EF-hand motif, and the pleckstrin homology (PH) domain are regulatory domains that are mingled in a specific manner in PI-PLC subtypes (7). Therefore, each PI-PLC isozyme shows a unique combination of X-Y and regulatory domains, so that each PI-PLC isozyme can have a different regulation, function, and tissue distribution (8).

The X and Y domains are usually located between the EF-hand motif and the C2 domain, and are composed of α-helices alternated to β-strands, with a structure that is similar to an incomplete triose phosphate isomerase α/β-barrel (9).

Conversely, the PH domain, although being a membrane-phospholipid binding region along with the C2 domain, has other specific functions according to the different isozymes. For instance, in PI-PLCδ1, the PH domain binds PIP2 and contributes to the access of PI-PLCδ1 onto the membrane surface (10). On the other hand, the PH domain specifically binds the heterotrimeric Gβγ subunit in PI-PLCβ2 and PI-PLCβ3 isozymes (11), and mediates interactions with phosphatidylinositol-3,4,5-trisphosphate (PIP3) in PI-PLCγ1, where it is required for inducing a phosphoinositide 3-kinase (PI3K)-dependent translocation and activation (12). As for this latter, it is worthwhile to note that PI-PLCγ1 and PI-PLCγ2 isozymes contain an additional PH domain, which is split by two tandem Src homology (SH)2 and SH3 domains, in order to interact directly with the calcium-related transient receptor potential cation channel 3, thereby providing a direct coupling mechanism between PI-PLCγ and agonist-induced calcium entry (13).

Finally, the C2 and EF-hand motifs are important for the regulation of calcium: the EF-hand motifs, in particular, are helix-turn-helix structural domains that bind calcium ions in order to enhance the PI-PLC enzymatic activity (14, 15).

As described above, the PI-PLC isozymes have peculiar molecular features, with common conserved domains and specific regulatory domains. Interestingly, among the PI-PLC isoenzymes, PI-PLCβ subtypes distinguish themselves also by the presence of an elongated C terminus, consisting of about 450 residues, which contains many of the determinants for the interaction with Gq alpha subunit as well as for other functions, such as membrane binding and nuclear localization (16–18).

FUNCTION AND REGULATION

The activation and regulation of PI-PLC isozymes differ in their subtype. For instance, PI-PLCβ enzymes are usually activated by G protein-coupled receptors (GPCRs) through several mechanisms, while PI-PLCγ subtypes are commonly activated by receptor tyrosine kinase (RTK), via SH2 domain-phospho-tyrosine interaction (8).

Indeed, the regulation of PI-PLCβ isozymes is peculiar. Most of them may have a high guanosine triphosphatase activating protein (GAP) activity, but not PI-PLCβ1, that can also be regulated by a distinct binding region to phosphatidic acid or is specifically activated by MAPK, therefore playing important roles in cell metabolism (19–23). Upon PI-PLCβ1 activation in the nucleus, IP3 generation occurs (**Fig. 3**). IP3 acts as a substrate for inositol polyphosphate multikinase (IPMK), which is located in the nucleus and gives rise to higher inositol phosphates (24).

Moreover, except for PI-PLCβ4, PI-PLCβ isozymes can also be activated by Gβγ dimers (25–28), and the relative sensitivity of PI-PLCβ isozymes to Gβγ subunits differs from that to Gqα subunits, with PI-PLCβ1 being the least sensitive to Gβγ (25, 26).

Although not fully understood, PI-PLCγ1 regulatory mechanisms involve polypeptide growth factor receptors that bind to RTKs, such as the epidermal growth factor receptor (EGFR) and the platelet-derived growth factor

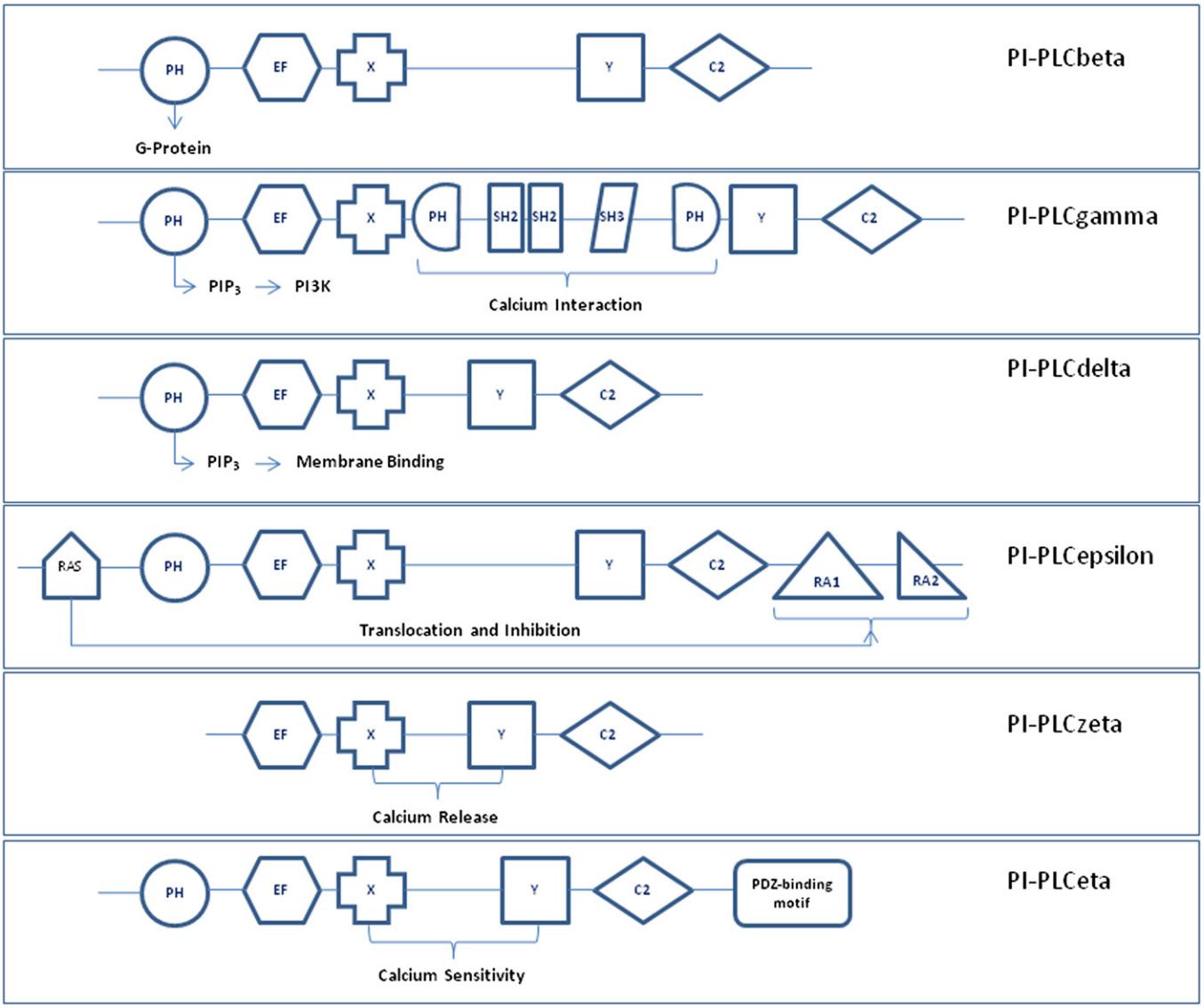

Fig. 2. Molecular structure of PI-PLC isozymes. Each PI-PLC subfamily is characterized by a different pattern and function of PH, EF, X, Y, and C2 domains. In particular, the PH domain of PI-PLCβ enzymes is bound to G proteins, whereas the same PH domain in PI-PLCγ and PI-PLCδ enzymes interacts with PIP3, in order to activate PI3K or favor the membrane binding, respectively. Moreover, the region between the X and Y domains is important for calcium regulation: in PI-PLCζ and PI-PLCη enzymes this region is important for calcium release and sensitivity, while in PI-PLCγ enzymes there are additional specific domains that are important for calcium interaction. As for PI-PLCε enzymes, there are additional RA domains that interact with RAS and modulate both enzyme translocation and inhibition.

receptor (PDGFR). Besides this, the SH2 domains of PI-PLCγ1 can also mediate the binding to auto-phosphorylated tyrosine residues within the intracellular region of the receptor (29). Moreover, it is remarkable that PI-PLCγ1 can also be activated downstream of a series of receptors that lack intrinsic tyrosine kinase activity, including the angiotensin II and bradykinin receptors, cytokine receptors, and the T cell receptor (30–33). This is also the case for PI-PLCγ2, that can be activated downstream of immunoglobulin and adhesion receptors on immune cells, such as B-cells, platelets, and mast cells, by nonreceptor tyrosine kinases interacting with other membrane-localized molecular signaling pathways (34–36).

Interestingly, PI-PLCε isoenzymes can be activated by both GPCR and RTK systems, with distinct activation mechanisms (37). Indeed, several GPCR ligands, such as lipoprotein A, thrombin, and endothelin, can activate PI-PLCε, but PI-PLCε also associates with Rap and translocates to the perinuclear area, where it interacts with activated RTKs (38).

As for PI-PLCδ1 and PI-PLCη1, they are activated via GPCR-mediated calcium mobilization. In particular, the PI-PLCδ1 isozyme is one of the most sensitive enzymes to calcium, suggesting that its activity is directly regulated by calcium (39, 40), whereas PI-PLCη1 specifically acts as a calcium sensor during the formation and maintenance of the neuronal network in the postnatal brain (41). Moreover, both PI-PLCδ1 and PI-PLCη1 subtypes are involved in the positive feedback signal amplification of PI-PLC (39, 42). Indeed, the overall PI-PLC activity may be amplified and

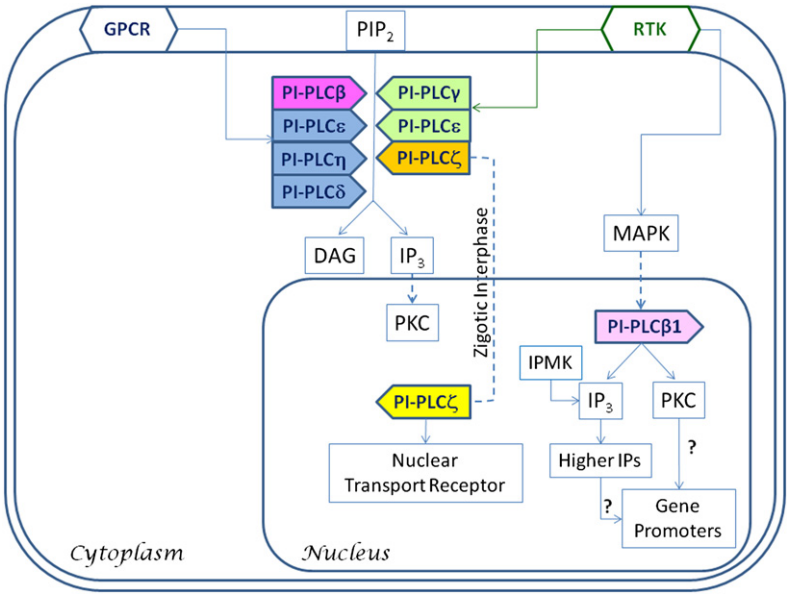

Fig. 3. Function and regulation of PI-PLC isozymes. Most of the PI-PLC isozymes play a role at the plasma membrane. PI-PLCβ enzymes are usually activated by GPCRs through several mechanisms, while PI-PLCγ subtypes are commonly activated by RTK, via SH2 domain-phospho-tyrosine interaction. It is important to note that a specific PI-PLCβ enzyme, that is PI-PLCβ1, can be activated by MAPK and translocate to the nucleus, where it is involved in specific signaling pathways involving IPMK and gene promoter regulation. On the other hand, PI-PLCε isoenzymes can be activated by both GPCR and RTK systems, with distinct activation mechanisms, whereas both PI-PLCδ1 and PI-PLCη1 are activated via a GPCR-mediated calcium mobilization. As for PI-PLCζ, its activation and nuclear translocation mechanisms remain unknown, but it has been described as a sperm-specific protein that, at the nuclear level, has been specifically connected with the molecular activation of oocytes following fertilization in zygotic interphase.

sustained by both intracellular calcium mobilization and extracellular calcium entry, through either a negative or a positive feedback amplification of PI-PLC signaling (43–46).

All in all, it has been suggested that PI-PLCβ and PI-PLCγ isoenzymes (primary PI-PLCs) are primarily activated by extracellular stimuli. On the contrary, secondary PI-PLCs, such as PI-PLCε, are activated by Rho and Ras GTPases, while the activation of other secondary PI-PLCs (mainly PI-PLCδ1 and PI-PLCη1) might be enhanced by intracellular calcium mobilization that amplifies the PI-PLCs activity. As for PI-PLCζ, its activation and nuclear translocation mechanisms remain unknown (Fig. 3).

SPLICING VARIANTS OF PI-PLC

Alternative splicing variants have been reported for several of PI-PLC isozymes, including human and rat PI-PLCβ1, human PI-PLCβ2, rat PI-PLCβ4, rat PI-PLCδ4, and human PI-PLCε (47–52).

Indeed, two splicing variants of the PI-PLCβ1 isozyme have been identified both in rat and mouse, and they differ in their C-terminal sequences (48). As for the human PI-PLCβ1 gene, also in this case there are two alternative splicing variants, with PI-PLCβ1a containing a putative nuclear localization sequence and a nuclear export sequence region and PI-PLCβ1b showing only a putative nuclear localization sequence. Therefore, these variants of PI-PLCβ1 may differ in their cellular localization, suggesting that the transit in and out of the nucleus is finely regulated, and possibly hinting at a different role for these two splicing variants (47).

Also, human PI-PLCβ2 shows two splicing variants: PI-PLCβ2a and PI-PLCβ2b, differing in 15 amino acid residues at the C-terminal region, so that the second transcript variant results in a shorter protein (49, 53).

Interestingly, several alterative splicing variants of the PI-PLCβ4 gene have been reported: two alternative splicing variants were identified from rat and bovine brain (50, 54), while the third splicing variant of rat PI-PLCβ4 has an additional 37 nucleotide exon at the C-terminal region (55). In humans there are also three alternative splicing variants of the PI-PLCβ4 gene, so that variant 1 lacks an internal segment and has a longer and distinct C terminus, variant 2 lacks an alternate in-frame exon in the central coding region, and variant 3 represents the longest transcript (55).

Altogether, all PI-PLCβ genes have at least two alternative splicing variants, which differ mostly in their C-terminal sequences and potentially play different roles in cellular processes.

Also human PI-PLCγ1 gene has two alternative splicing variants that differ in their C-terminal sequences, but in this case the precise function of the two alternative splicing variants is still unknown (56).

Alternative splicing variants of PI-PLCδ isozymes show several different patterns of splicing variants. Indeed, mouse PI-PLCδ1b differs from PI-PLCδ1a by 274 amino acid residues that extend from the catalytic Y domain to the stop sequence, which are replaced with 21 distinct amino acid residues. Moreover, mouse PI-PLCδ1b has a truncated catalytic Y domain, which implies that this variant may have no enzymatic activity. Also, the human PI-PLCδ1 gene has two splicing variants, and the second variant contains an alternate 5′-terminal exon that results in a shorter isoform and a different N terminus, as compared with the wild-type sequence (57).

As for PI-PLCδ4 gene, only the mouse gene shows alternative splicing variants. Two splicing variants have been well-characterized and seem to be functional, whereas the third showed no catalytic activity. In particular, the second variant is slightly different from the wild-type isoform in the 5′-untranslated region but includes an alternate in-frame exon in the coding region, thus resulting in a longer protein that, however, has the same N and C termini as compared with the wild-type isoform. As for the third isoform,

it lacks the linker region between X and Y domains, and instead, contains 32 additional amino acids, so that this isoform shows no catalytic activity (58).

Three splicing variants of the human PI-PLCε1 gene have been reported, with the second variant showing a different N-terminal region, and the third variant using an alternate in-frame splice site in the coding region that results in a shorter protein (52).

As for the PI-PLCζ gene, in this case an alternative splicing variant, named s-PI-PLCζ, has been recently reported (59): structurally, it contains two internal stop codons at the N terminus and lacks one and a half of the EF-hand motifs; functionally, this splicing variant does not affect calcium oscillations.

Finally, three splicing variants of the PI-PLCη1 gene have been reported in both humans and mice (60), whereas five alternative splicing variants of the PI-PLCη2 gene are reported in humans, and in mice there are three of them (61).

PI-PLC IN DISEASE

Given their peculiar roles and their fine regulation in physiology, alterations affecting PI-PLC isozymes have been associated with several diseases that can target different tissues and organs (62–65).

For instance, PI-PLCβ1 plays an important role in brain function and is thus associated with brain disorders (66). In fact, it is highly expressed in the cerebral cortex, hippocampus, amygdala, lateral septum, and olfactory bulb (67, 68), where it regulates both cortical development and synaptic plasticity by specifically modulating hippocampal muscarinic acetylcholine receptor signaling. Moreover, a PI-PLCβ1 gene deletion was observed in orbito-frontal cortex samples from human patients with schizophrenia and bipolar disorders (69–71), and patients with these diseases also showed an abnormal expression pattern of PI-PLCβ1 in specific brain areas (66).

PI-PLCβ isozymes also participate in the differentiation and activation of immune cells that control both the innate and adaptive immune systems (72). In particular, loss of both PI-PLCβ2 and PI-PLCβ3 isozymes is associated with an impaired T-cell migration that is caused by an inability to increase the intracellular calcium. Interestingly, human T-cells from elderly people show a reduced expression of PI-PLCβ2, suggesting that a specific impairment of this enzyme in aged T lymphocytes might contribute to the immune suppression mechanisms in this group of people (72, 73). Moreover, PI-PLCβ2 downregulation plays an important role in M1-M2 macrophage differentiation, whereas PI-PLCβ3 activity is essential for promoting macrophage survival, especially in atherosclerotic plaques, so that PI-PLCβ3 could be a potential specific molecular target for the treatment of atherosclerosis (74).

PI-PLCβ3 deficiency is also linked to the development of myeloproliferative neoplasm in mice. In fact, aged PI-PLCβ3-null mice typically have increased numbers of hematopoietic stem cells and myeloid progenitors in bone marrow and spleen, and their hematopoietic stem cells show an increased proliferation and a reduced apoptosis that have been molecularly associated with Stat5 inhibition (75).

Within the hematological field, not only PI-PLCβ3, but also other PI-PLCβ isozymes have been demonstrated to play a role in the pathophysiology of hematologic diseases (76–79). Indeed, the PI-PLCβ1 gene has been associated with myelodysplastic syndrome (MDS), not only because its lack is linked to MDS progression toward acute myeloid leukemia (80, 81), but also because its expression is regulated by epigenetic mechanisms (82–85). Moreover, PI-PLCβ enzymes have also been implicated in leukemias. In particular, the molecular interaction between PI-PLCβ enzymes and G proteins that induces PI-PLCβ to localize in the cytosol or at the nuclear level has been demonstrated to be determined by the intervention of a binding partner: TRAX (translin-associated protein X), i.e., a nuclease and part of the machinery involved in RNA interference processes (86).

Among the PI-PLC isozymes, PI-PLCγ is important because it can play a specific key role in cell migration and invasion, therefore contributing to carcinogenesis. Indeed, PI-PLCγ is an important enzyme that regulates cell metabolism, so that at first its molecular targeting has been considered as a possible new therapeutic strategy. However, it has been difficult to find specific PI-PLCγ inhibitors that can be effective in cancer treatment. That is why scientists are now trying to identify new specific interacting partners that could become new therapeutic targets for cancer therapy (87). On the other hand, other PI-PLC isozymes have also been demonstrated to play important roles in cancer. This is the case for PI-PLCε, that is specifically linked to tumor suppression (88, 89), mainly in colorectal cancer, where its reduction is associated with a more aggressive disease (90).

PI-PLC isozymes are not only associated with cancer, but their deregulation is also implicated in other diseases and disorders. Another important role for a PI-PLC isozyme has indeed been recently discovered in infertility, where PI-PLCζ, a sperm-specific protein, has been specifically connected with the molecular activation of oocytes following fertilization (91). In fact, the earliest event subsequent to gamete fusion is the onset of a series of intracellular calcium oscillations within the oocyte, which modulate several molecular processes that are known as “oocyte activation”, and together, they represent a fundamental mechanism for the early embryonic development. Importantly, all of these processes are initiated and controlled by calcium release from ooplasmic sources during zygotic interphase in response to PI-PLCζ activity, via the IP3 pathway, thus activating nuclear transport receptors. That is why a correlation between certain types of male infertility and the aberrant expression, localization, structure, and function of PI-PLCζ in human sperm has been determined. The potential therapeutic role of PI-PLCζ could therefore be linked to the identification of male patients that are deficient in PI-PLCζ, and for them an alternative therapeutic approach, based on assisted reproductive technology, could be useful for rescuing the impaired oocyte activation (92).

As for the other PI-PLC isozymes, PI-PLCδ enzymes are a peculiar example of enzymes playing several roles in different tissues and organs. Indeed, PI-PLCδ1 and PI-PLCδ3 share a high sequence homology, so that they can play redundant roles in various tissues. In fact, PI-PLCδ1 is required for the maintenance of homeostasis in skin and metabolic tissues, while PI-PLCδ3 specifically regulates microvilli formation in enterocytes and the radial migration of neurons in the cerebral cortex of the developing brain. Furthermore, it has been shown that the simultaneous loss of PI-PLCδ1 and PI-PLCδ3 in mice causes placental vascular defects, thus leading to embryonic lethality (93).

CONCLUSIONS

PI-PLC isozymes play essential roles in cell metabolism, by regulating calcium and other intracellular signaling pathways that are important for cell proliferation and differentiation. This means that these enzymes have the capability to influence normal and pathological conditions. This is particularly important, because the regulation of PI-PLCs or PI-PLC-dependent signaling pathways can be important for understanding both the normal cellular physiology and the pathogenesis of important diseases, possibly leading to the development of innovative therapeutic strategies or the comprehension of new molecular processes.

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