CHAPTER

2

The role of phospholipases in inflammation, gene expression, and apoptosis

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1 Introduction

Phospholipases are enzymes that possess the capacity to split ester bonds within phospholipids. The resultant effect of the hydrolysis reaction leads to the formation of several lipid products that interfere with cellular signaling (Balboa and Balsinde, 2021). The major categories of phospholipase are phospholipases A (PLA), phospholipases B (PLB), phospholipases C (PLC), and phospholipases D (PLD). PLA and PLB both have a specific potential of hydrolytic cleavage of phospholipid's acyl ester bond at site number 1 or 2 (sn-1 or sn-2). Phospholipases C can act on the cleavage of glycerophosphate bonds, while PLD targets the cleaving of the polar head group. These subfamily members are specific in the display of individual enzymatic activity, physiological state, tissue dispersal, and so on.

Phospholipases A are enzymes that target acyl ester bonds at sn-1 and sn-2 for PLA_1 and PLA_2, respectively (Fig. 1). The PLA_1 subfamily can be grouped based on the site of action into phosphatidyl serine-specific phospholipase A_1 ($PS\text{-}PLA_1$), membrane-bound phosphatidic acid-preferring phospholipase A_1 ($(mPA\text{-}PLA_1)a,b$), hepatic lipase (HL), endothelial lipase (EL), pancreatic lipase-related protein 2 (PLRP2), phosphatidic acid-preferring phospholipase A_1 ($PA\text{-}PLA_1$), human pancreatic lipase (PL), and lipoprotein lipase (LPL). The PLA_2 subfamily can be classified into 15 types of lipases (Baba et al., 2014). The most studied types are secreted phospholipase A_2 ($sPLA_2$), cytosolic phospholipase A_2 ($cPLA_2$), platelet-activating factor-acetylhydrolase (PAF-AH), lysosomal PLA_2, and cytosolic Ca^{2+}-independent phospholipase A_2 ($iPLA_2$). The catalytic activity takes place through membrane homeostasis by recycling

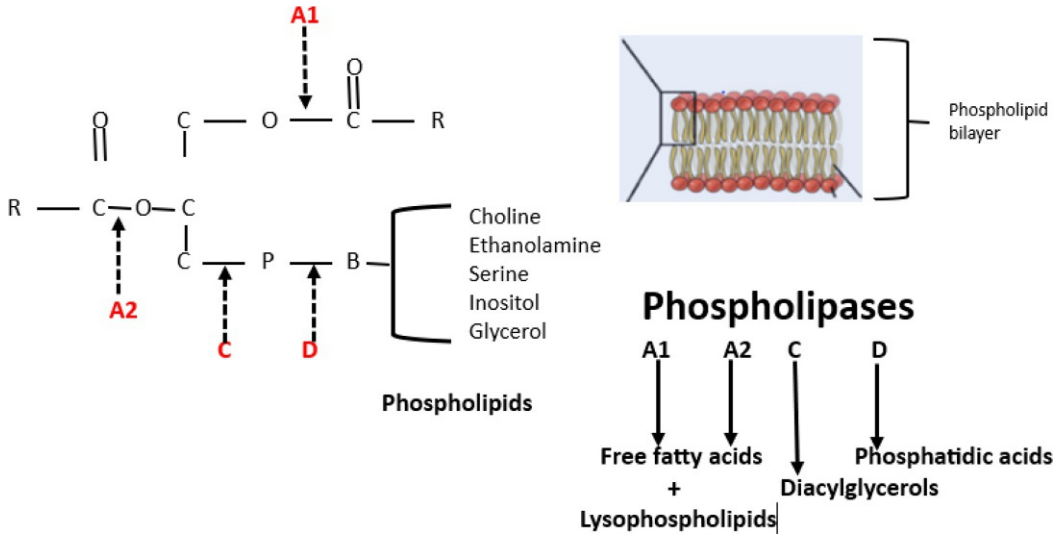

FIG. 1 Schematic of phospholipases and their catalytic sites.

fatty acid moieties by the cleaving of arachidonic acid (AA). AA, which happens to be biologically active, can be a source of starting material in the production of platelet-activating factor (PAF) and eicosanoids. These lipases are Ca^{2+}-dependent and possess a His/Asp dyad at their most active site. This active site (Fig. 1) provides the enzyme (lipase) with the capacity of performing intracellular activity locally and also in distant cells (transporting to other cells via channels).

Phospholipase B is a type of phospholipase that demonstrates hydrolyzing capability on both sn-1 and sn-2. The responsive gene for this action is in the second chromosome and is identified as PLB_1. PLB exhibits the characteristics of both phospholipase A_2 and lysophospholipase. Initially, it is synthesized as a 170 kDa proenzyme and later transformed into an active 97 kDa enzyme through papain treatment. This is present in the sperm, epidermis, and intestine (Maury et al., 2002). Earlier research has shown that the PLB gene is a potential risk gene for rheumatoid arthritis, and further research has indicated the important role of PLB_1 in acrosome exocytosis in sperm by maintaining fertility (Okada et al., 2014).

The PLC family consists of six subfamilies: PLCβ, γ, δ, ϵ, ζ, and η (Gresset et al., 2012). The receptor tyrosine kinase (RTK), the G-protein coupled receptor (GPCR), has been seen to play vital roles in the activation of PLC family members in the sites of hormones and neurotransmitters (Philip et al., 2013). For each of the subfamily identified, there is more than one variant. One of the PLC genes known as $PLCB_1$, when present in neurons, can permit the migration of malignant partial seizures in infancy, schizophrenia, etc. in a state of mutation (De Filippo et al., 2014). In terms of crystallographic data, PLCs possess an X and Y domain for enzyme activity. Differing from the enzyme active site, the subfamilies indicate a Ca^{2+}-dependent phospholipid-binding site, as well as Src sites, which is seen in protein-protein interaction. PLCβ participation has been seen in the separation by regulating cyclin D3 and other cellular activities which include proliferation via the protein kinase C (PKC)α-mediated pathway (Bavelloni et al., 2015).

The PLD family can be grouped into three main types: PLD1, PLD2, and mitoPLD.

MitoPLD was only recently discovered and was found to play a vital role in mitochondrial fission and fusion reactions. PLD1 and PLD2 can be seen to play important cellular roles such as cell division, cell motility, cell survival, apoptosis, and proliferation. PLD possesses a site that can bind to actin, and this helps to improve cell motility at various phases (actin polymerization, spread to substratum, cell adhesion, and contractility) (Rudge and Wakelam, 2009). It also has the potential to hydrolyze phospholipids (phosphatidylcholine) to form choline and phosphatidic acid (PA); this reaction is controlled as cell components include phospholipids. PLD1 is compartmentalized in the secretory granules, endosome, and Golgi complex, while PLD2 is localized to the plasma membrane, and finally mitoPLD is present on the surface of the mitochondria (Oliveira and Di Paolo, 2010). MitoPLD is mainly involved in the formation of PA and the inhibition of transposon mobilization during meiosis in spermatogenesis (Gao and Frohman, 2012).

2 The role of phospholipases in inflammation

Inflammation is a highly regulated physiologic response to injury which is associated with oxidative stress and the bioactive formation of lipids. Those bioactive lipids, such as oxidized phospholipids, platelet-activating factor acetylhydrolase (PAF-AH, also known as lipoprotein-associated phospholipase A_2 (Lp-PLA_2)), and PAF-like substances have been identified in several disease plaques. Lp-PLA_2 was classified as a Ca^{2+}-independent PLA_2, produced by a large range of non- and inflammatory cells. In addition, as Lp-PLA_2 is specific for the breakdown of oxidized fatty acid residues and PAF, PLA_2 is specific for phospholipids containing two long-chain acyl groups (Silva et al., 2011). The enzyme A_2 (PLA_2) is an important factor in some diseases that involve inflammation, such as lung disease (Hurley and McCormick, 2008). Phospholipase A_2 (PLA_2) catalyzes the release of arachidonic acid (AA) from the cell membrane for the generation of lipid mediators of inflammation such as leukotriene and is crucial in diverse inflammatory processes.

Certain $sPLA_2$ regulates an aggregation of biological functions via certain receptors called $sPLA_2$-receptors. Those receptors are mannose-type transmembrane glycoproteins which are related to the C-type animal lectin taxonomic group. Several of the consequential receptor-mediated functions are: (1) group X $sPLA_2$-receptor action for AA left behind by spleen cells; (2) group IB $sPLA_2$-receptor interaction for cell growth and lipid mediator making; (3) group V $sPLA_2$-receptor interaction for antiangiogenic and proangiogenic factors by human neutrophils (Loffredo et al., 2017); (4) group I-receptor action for prostaglandin E2 making; and (5) group IV-receptor action for cytokine liberation (Khan et al., 2016). The $sPLA_2$-receptor interactions are also involved in causing certain diseases including cancer, in addition to physiological functions (Friedemann et al., 2018; Sukocheva et al., 2019).

It has been reported that cells that are deficient in V $sPLA_2$ have impaired eicosanoid synthesis. The remarkable function of these enzymes that likely contribute to inflammatory disease includes the stimulation of chemokines and cytokines, which are critical to the inflammatory and immune responses and generation of bioactive eicosanoids. Lp-PLA_2, a Ca^{2+}-dependent, is a special member of the PLA_2 superfamily that provides materials for the production of different families of compounds which play aggregate roles in inflammation. The inflammatory role of Lp-PLA_2s has been demonstrated in multiple imflammatory disease conditions including cancer, pancreatitis, sepsis, psoriasis, and rheumatoid arthritis (Boilard et al., 2010). PLA_2 contains 15 different groups and several subgroups. These enzymes are distinguished by their capability to hydrolyze ester

bonds at the *sn*-2 position of the glycerol backbone of phospholipid substrates and are allotted to groups settled on molecular weight, sequence, the necessities for Ca^{2+}, disulfide bonding percepts, and other characteristics. The acetyl group hydrolysis at the *sn*-2 location of PAF generates lyso-PAF and acetate, and the capability of Lp-PLA_2 to deactivate PAF has been involved in different types of inflammatory diseases.

The key enzyme that is concerned with inflammation is the Ca^{2+}-dependent IVA phospholipase A_2 group, which is also called cytosolic phospholipase $A_2\alpha$ (c$PLA_2\alpha$). This is as a result of the enzymes's capability to initiate eicosanoids production, such as leukotriene LTB_4, LXs, and hepoxilin HXA_3, serving as a neutrophil chemoattractant, and playing a key role in the inflammatory process (Hurley and McCormick, 2008) by releasing AA preferentially in response to cell activation (Fig. 2). There are several ways by which PLA_2 participates in the inflammatory reaction. When bioactive lipids have been hydrolyzed by Lp-PLA_2, and their biological activity is reduced, the lysophospholipids become the most generated metabolites (Fig. 3). These lipids are implicated in atherosclerotic processes and show a very harmful role of Lp-PLA_2, causing an inflammatory reaction against oxidized lipoproteins. These compounds, which are released by phospholipases A_2 during cell vivification, apoptosis, or injury, are known to distort the diversity of cell types and the function of neutrophils, and can also be generated by phospholipase A_1 and by the action of lecithin-cholesterol acyltransferase (LCAT) or endothelial lipase. There are different types of lysophospholipids, but lysophosphatidylcholine remains the main product of Lp-PLA_2 action; these metabolic action of Lp-PLA2 happens in physiological states (Schmitz and Ruebsaamen, 2010; Silva et al., 2011).

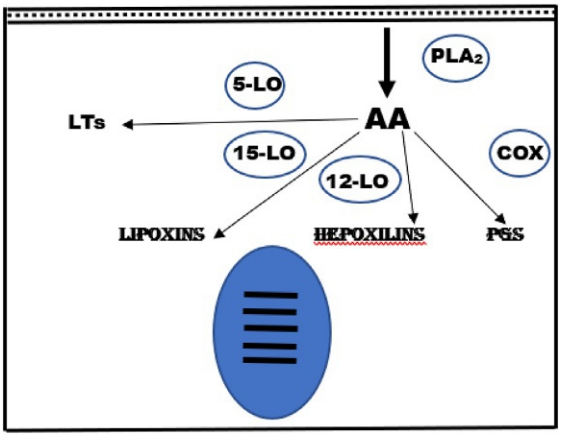

FIG. 2 Eicosanoid synthesis pathways occurring within a mammalian cell.

In inflammation, AA is the most important fatty acid released by PLA_2 from phospholipids. This reaction districts the availability of AA which, in turn, is the rate-limiting precursor for the generation of prostaglandins. AA is also the precursor of leukotrienes that are formed through the lipoxygenase pathway. The bulk of lysophospholipids in some situations amounts to the generation of platelet-activating factor (PAF), which is another influential mediator of inflammation. The essential enzyme for this reaction is platelet-activating factor-acetylhydrolase (PAF-AH) (Khan and Hariprasad, 2020). Due to this property, cytosolic phospholipase $A_2\alpha$ has gained pharmaceutical interest, and is used in the development of antiinflammatory drugs (Balboa and Balsinde, 2021). Due to the complexity of the lipid metabolic pathway, which is often redundant and highly interconnected, as well as the coexistence of several enzymes with phospholipase A_2 activity in the cells with overlapping activation properties, targeting and inhibiting the phospholipase A_2 reaction has proved problematic.

Another group of phospholipases A_2 that is known to take part in some of the functions of macrophages exposed to interleukin 4 (IL-4) is the group V secreted. This group modifies the cells to an antiinflammatory phenotype. This is

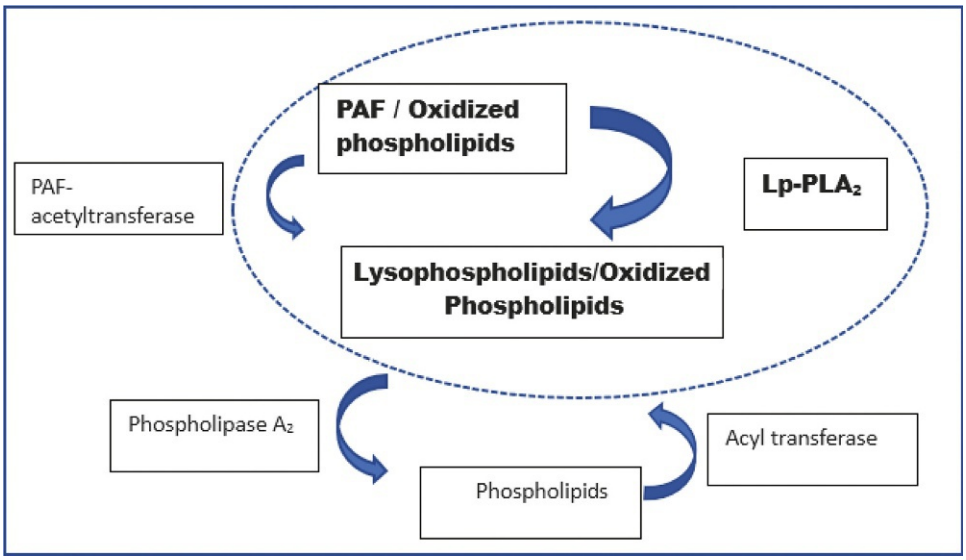

FIG. 3 The role of Lp-PLA_2 in the generation of lysophospholipids.

in accordance with the work of Koganesawa et al. (2021), that the mass spectrometry analysis of phospholipase A_2 substrates and products using macrophages deficient, polarized to either pro-inflammatory (bacterial lipopolysaccharide plus interferon-) phenotypes or antiinflammatory (IL-4-treated). Exclusive changes among the different vivification regimes that function critically for inflammation have been identified that may depend on the group V enzyme. This also suggests the subsistence of novel lipid pathways. The interactions between the secreted phospholipase A_2 and cPLA$_2\alpha$ of the monocytes in terms of lipid droplet biogenesis and eicosanoid response contribute to the initiation of the inflammatory response.

Oxidative stress and inflammation are therefore hallmarks of several diseases and are known to contribute to the initiation, progression, and herniation of lipid-rich vascular lesions (Silva et al., 2011). Lipoprotein-associated phospholipase A_2 is a special associate of the phospholipase A_2 superfamily, which is distinguished by its capacity to specifically hydrolyze truncated and/or oxidized fatty acyl groups, platelet-activating factor, and glycerophospholipids at the *sn*-2 station of the glycerol backbone. Lipoprotein-associated phospholipase A_2 in humans moves around in an active state with high- and low-density lipoproteins as a complex. Reports from clinical studies show that plasma Lp-PLA_2 mass and activity are firmly linked with vascular risk and atherogenic lipids. The hypotheses that restriction of the activity of Lp-PLA_2 could provide a significant therapeutic strategy and that its mass level and/or activity could be utilized as biomarkers of cardiovascular disease are based on these findings (Rosenson and Stafforini, 2012).

Several genetic studies have shown that the diminution of Lp-PLA_2 utility is a risk factor for vascular conditions and inflammation. Animal model studies have also revealed that the overexpression of Lp-PLA_2 demonstrates antiatherogenic and antiinflammatory properties. This is because the production of pro-inflammatory prostaglandins and leukotrienes are been inhibited by the secretory

phospholipase A_2 (PLA_2) inhibitors (Rosenson, 2010). The balance between pro- and anti-inflammatory properties has been regulated by a variety of physiological functions, which are most often redundant mechanisms and diverse stimuli, such as transcriptional activators, receptor ligands, and environmental cues. At the transcriptional level, IFN-γ (the agent of M1 pro-inflammatory phenotypes) decreases the expression of Lp-PLA_2. M-CSF, on the other hand, which is an inducer of an M2-like, antiinflammatory phenotype, increases the monocyte Lp-PLA_2 expression at a greater level when analogized with the M1-inducer GM_CSF (Rosenson and Stafforini, 2012). Blocking the onset of inflammation offers a therapeutic effect in different settings, but improper eclosure of it can cause disastrous short- and/or long-term effects. This is evident in the altered expression of multiple inflammatory gene products, which is associated with the development of cardiovascular disease (CVD). As such, the role of phospholipases should be assessed carefully in individual cases (Libby et al., 2002). Multiple inflammatory cells that are involved in certain disease conditions such as atherogenesis, which secretes Lp-PLA_2, include macrophages, monocytes, reactive mast cells derived from bone marrow, reactive platelets, and neutrophils, of which macrophages/monocytes are the main protagonists in the onset and advancement of such diseases. They have also been declared as novel latent therapeutic reference points for the prevention and treatment of those diseases.

3 The mechanism of action of Lp-PLA_2 on inflammation

The Lp-PLA_2-mediated decrease in the bioactivity of phospholipid substrates, such as PAF and OxPL, is the mechanism accounting for its involvement in inflammation. However, an issue that has eluded clarification despite considerable effort over the years is whether there are generation of products in the in vivo action of Lp-PLA_2 on endogenous substrates (i.e., lysophosphatidylcholine (lyso-PC) plus short and/or oxidized fatty acids) which increase inflammatory reactions in the vascular wall. Specifically, it has not been ascertained if Lp-PLA_2 is detrimental or beneficial, or if the substrates are less, more, or evenly atherogenic than the spin-offs (Rosenson and Stafforini, 2012). Studies have shown that in vitro approaches have been used to distinguish the biological attributes of pertinent products and substrates of the reaction. Both anti- and pro-inflammatory activities have been accredited to glycerophospholipids shielding fragmented and/or oxidized *sn*-2 fatty acyl groups. Similarly, lyso-PC and short and/or oxidized fatty acids have been reported to elicit both detrimental and beneficial effects. For example, low (nanomolar) engrossment of lyso-PC recruit monocytes and induce pro-inflammatory cytokine production in vitro (Olofsson et al., 2008).

Close to 70%–80% of PAF-AH (Lp-PLA_2) circulating is attached to low-density lipoprotein (LDL), and the remainder is connected to high-density lipoprotein (HDL) and several very low-density lipoproteins. The two α-helices aid the enzyme affiliated with these lipoproteins. Low-density lipoprotein has an attraction in the zone between residues 126 and 114, while high-density lipid is linked with the residues 362–369 of the two α-helices. The enzyme possesses a standard lipase α/β-serine hydrolase fold and a catalytic trio comprising Ser273, His351, and Asp296. Ser273 is situated on the N-terminus of an alpha helix and the preserved motif GXSXG standard to other serine esterases and lipases. Ser273 is a nucleophilic constituent reactive for catalysis through other two constituents—His351 and Asp296. The constituents Phe274 and Leu153 act as oxyanion holes and neutralize the negative charge of tetrahedral antepenultimate through their amide nitrogens. The catalytic trio is targeted inside a hydrophobic pocket and situated near its lipid substrate.

Platelet-activating factor (PAF) is a phospholipid-signaling building block that binds its particularized receptor, leading to a succession of proinflammatory signals; therefore, PAF is a conspicuous pro-inflammatory mediator (Khan and Hariprasad, 2020).

Low-density lipoprotein, T-lymphocytes, and macrophages can transmigrate easily to arterial intima as a result of endothelial dysfunction, which is distinguished by increased adhesion molecules expression and by larger spaces between endothelial cells. Through the atherogenic mechanisms pertaining Lp-PLA_2, an individual with obesity, dyslipidemia, insulin resistance, hypertension, and oxidative stress can exist, and thus be highly inclined to atherosclerosis. The LDL particle shows a phenotype that is more atherogenic, being dense and small; these characteristics make it more susceptible to oxidation. In this site, the reduced content of antioxidants favors the high production of free radicals, and consequently oxidative modifications of LDL. Thus, the Lp-PLA_2 will be activated by oxidized phospholipids present in OxLDL.

The enzyme minimizes modifications of OxLDL, hydrolyzing its oxidized phospholipids; this may be interpreted as an antioxidant action. However, during this process, high contents of lysophospholipids and oxidized nonesterified fat acids (OxNEFAS) are produced that promote adhesion molecules expression and attract macrophages to the arterial intima. The OxLDL, lysophospholipids, and OxNEFAS also stimulate cytokines production, for example, TNFα and IL-6, which increase the inflammatory profile in the region of the plate. The activated macrophages, through scavenger receptors and phagocyte OxLDL, gradually turn up in foam cells. The muscle cells are also attracted, and migrate to the intima, where they produce collagen, elastin, and elastases, involving and stabilizing the lipid plaque. Subsequently, the macrophages become apoptotic, as well as the muscle cells, causing the release of lipids in the plaque. In this process, the presence of OxLDL, as well as lysophospholipids and OxNEFAS produced by Lp-PLA_2, continually stimulates the growth of the plaque; these are factors that can be decisive in terms of plaque rupture susceptibility and can culminate in a cardiovascular event.

4 Phospholipases in gene expression

In molecular biology, a gene is generally referred to as the complete nucleic acid sequence that is required for the synthesis of a functional gene product (polypeptide or RNA). Genes are functional units of inheritance as they are made of deoxyribonucleic acid (DNA) or, in the case of some viruses, ribonucleic acid (RNA). Gene expression refers to the events that transfer the information content of the gene into the making of a functional product, usually a protein (Lodish et al., 2003). All the cells regulate gene expression in response to changes in the external environment. The mechanisms of gene expression are described in transcription and translation, which follow the process of initiation, elongation, and termination. A protein that modifies the activity of transcription factors and influences the expression of their target genes is referred to as a modulator. This modulator always acts by binding directly to the transfer factors to affect the expression of a target gene in which phospholipases play a significant role.

During development and differentiation, an individual cell expresses or turns on merely a segment of its genes at any given time, while the remaining genes are repressed or turned off. This process of turning genes on and off is called gene regulation. Genes are regulated using different patterns during development, such as hemoglobin, which is only expressed in developing red blood cells, even though the globin genes are found in all types of cells. Also, gene regulation enables cells to react quickly to

changes in their environments. Regulation of a gene can take place at any point during gene expression, but most commonly happens during transcription (when the information in the DNA of a gene is passed to mRNA). Signals from the environment or other cells activate proteins referred to as transcription factors, which bind to the regulatory regions of a gene, thereby increasing or decreasing the level of transcription.

Phospholipases A_1 (PLA_1), A_2, C, and D act on membrane phospholipids to produce tiny molecules which act as mediators and play vital roles in cellular metamorphosis. As the acyl groups are been cleaved at the sn-1 position by the PLA_1, phospholipases A_2 (PLA_2) are accountable for the acyl groups cleavage at the sn-2 positions of glycerophospholipids producing lysophospholipids (phospholipids containing an acyl chain) that have detergent-like properties. PLA_2s are a large group of the superfamily (more than 50 subtypes) due to their calcium dependence and cellular localization. Out of this group of PLA_2s, the calcium-independent $iPLA_2$, the calcium-dependent cytosolic $cPLA_2$, and the secretory $sPLA_2$, having antithetic molecular structures, cellular determination, and producing lipid mediators with varied functions, are the most studied (Murakami et al., 2020).

5 Biomedical applications of phospholipases in gene expression

Cytosolic phospholipases A_2 ($cPLA_2$s) are intracellular enzymes with a molecular weight of about 85 kDa, containing 749 amino acids. The $cPLA_2$ family has six isoforms: $cPLA_2\alpha$, -β, -γ, -δ, -ε, and -ζ. $cPLA_2$ subgroups possess a highly conserved C_2 domain for binding Ca^{2+} and the C-terminal domain has a catalytic active site which uses the Ser-Asp dyad for catalysis except for $cPLA_2$, which lacks the C-domain. Phosphorylation of $cPLA_2$ results in an increased phospholipid attaching at low calcium levels which assists the translocation of enzymes in the cytoplasm to various intracellular organelles comprising the mitochondrial, nuclear, plasma, and lysosomal membranes.

All the brain cells seem to contain $cPLA_2\alpha$ (comprising astrocytes, microglia, and neurons), which is the most studied subtype of $cPLA_2$. $cPLA_2\alpha$ has been found to stimulate neurons together with excitatory glutamate receptors that could give rise to fast activation of the Ras/Raf/MEK/ERK pathway and, subsequently, phosphorylation of $cPLA_2$. Toll-like receptor galvanization with lipopolysaccharide (LPS) in the microglial cells could lead to ERK1/2-dependent $cPLA_2$ phosphorylation induction and upregulation of the inflammatory pathway involving NFκB. Upregulation of NFκB was found to be associated with inflammatory proteins after treating microglial cells with LPS. Together, these findings show the part played by protein kinases in the $cPLA_2$ phosphorylation causing glial inflammation and neuronal excitation (Sun et al., 2021).

Research has revealed the part that PLA_2 played in the fast emission of polyunsaturated fatty acids (PUFAs) in the brain as a result of diverse types of injuries to the brain. Elevations in oxidative stress, glial cell activation, and neuronal excitation using an animal model have been observed, and these are features that contribute to the galvanization of $cPLA_2$ in cerebral ischemia. Under ischemic conditions, PLA_2-evoked emission of PUFAs is usually worsened by a shortage of oxygen and a decline in the adenosine triphosphate that is needed for the fatty acid transformation to its acyl-CoA. Accordingly, agitation of the deacylation-acylation cycle facilitated by acyltransferases and phospholipases A_2 is considered very significant for changes in cellular PUFAs and phospholipids during cerebral ischemia. An increase in PUFAs in the brain as a result of a stroke can also be seen in plasma, making the investigation of PUFAs in plasma an effective biomarker for

examining the level of brain distortion in patients with stroke.

Alzheimer's disease (AD) is a neurodegenerative disease that is noticeable when amyloid plaques and neurofibrillary tangles are deposited in the brain. The elevating concern on PLA_2 is as a result of a connection to its outputs to synaptic engagement in learning. Recent studies have revealed increased levels of $cPLA_2$ in the brain, brought about by changes in lipid species such as an increase in lysophospholipids in the hippocampus, more aggressive Aβ accumulation, reactive gliosis, and cognitive deficits.

Prostaglandins (PGs) are produced from arachidonic acid (AA) by phospholipase A_2s (PLA_2s) and subsequently by cyclooxygenases. Considering progressive events in reproduction from female ovulation to parturition, PGs are crucial. Cytosolic PLA_2s ($cPLA_2$s) regulated by Ca^{2+}-dependent translocation and phosphorylation usually have a preference for AA in membrane phospholipids, and play a significant role in agonist-induced AA release. The $cPLA_2\alpha$-derived AA is essential for the PG synthesis that is required for on-time implantation.

Cancer is caused by spatially improper and uncontrolled cell proliferation in tissues. Cancer risks are associated with mutations in DNA, perhaps via environmentally influenced and transmissible biomarks on the DNA. New findings have revealed that the organization of biochemical pathways can be delineated in the status of networks comprising of hubs and nodes which are similar to other haphazardly assembled, firm, self-referent organizational structures, and that many oncogenes and tumor suppressor genes react on a number of biochemical pathways. Some classes of lipid mediators are obtained initially through the action of phospholipase A_2 (PLA_2) enzymes on phospholipids, thereby releasing fatty acids and lysophospholipids with biological applications relevant to cancer progression. Each one can be metabolized further—the fatty acids by cyclooxygenase (COX), lipoxygenase (LOX), or cytochrome P450 (CYP) enzymes, or by nonenzymatic oxidation, and the lysophospholipids by lysophospholipases, lysophospholipid acyl-CoA acyltransferases, and lysophospholipid: lysophospholipid transacylases.

A number of the PLA_2 enzymes also demonstrate lysophospholipase and/or transacylase activity, which reveals the relationship of these pathways and the intricacy with which lipid fluidity through them is restrained. More than 100 important and diverse paracrine and autocrine controllers of organ function and normal cell are produced via these pathways which regulate cellular differentiation, growth, apoptosis, and senescence, thus contributing to the homeostatic control of tissue proliferation and vascularization, tissue remodeling, and immune surveillance, particularly in reaction to stress. Together, these cellular utilities are revolutionized by tumor cells which allow them to proliferate locally and metastasize to distant sites. Out of the 22 human PLA_2 enzymes, only 10 have been determined in the human cancer context. Despite the current limitations, several works have led to new hopes that PLA_2 enzymes may be suitable targets for cancer therapy (Murakami et al., 2020).

6 Industrial applications of phospholipases

Phospholipases have many industrial applications including oil refining, dairy, baking, the health food industry, etc. PLC and PLD have chiefly been used for pharmaceutical, medical, and analytical purposes. The microbially produced PLD was used for the synthesis of chemicals by trans-phosphatidylation, which is exchange of the alcohol moiety attached to the phosphatidic acid residue. PLA_2 was reported to be the most suitable for the enzymatic degumming of edible oils and the synthesis of triglycerides enriched in polyunsaturated fatty acids (PUFAs). Genetically modified mammal, plant,

or microbially produced phospholipases are used for these purposes since microbial enzymes are cost-effective and environmentally friendly.

The essential omega fatty acids—γ-linolenic acid, arachidonic acid, and docosahexaenoic acid (DHA)—occur naturally but are usually at low levels when compared with other acids. There is high demand for these acids in industries, and although these materials can be produced by conventional physical and chemical separation methods, they are not always appropriate for nutritional applications. However, this problem has been successfully overcome by exploiting the specificity of enzymes. Phospholipase A_2 can be used to prepare phosphatidylcholine enriched with *n*3PUFA. For example, pig pancreas phospholipase A_2 was used to mediate trans-esterification of soy lecithin containing highly unsaturated fatty acids (at *sn*2 position) to produce lecithin with possible pharmaceutical, nutritional, and industrial applications.

The cholesterol content of a food (usually egg yolk, meat, fish, or dairy products) could be reduced by subjecting the food either to an enzymatic treatment (especially with cholesterol oxidase (EC 1.1 3.6) or a cholesterol oxidase-producing microorganism) or to biochemical treatment (breakdown of cholesterol). These two treatments could also be performed simultaneously. Biochemical, chemical, and/or physical treatment of food substances was improved by treating them with hydrolytic enzymes such as phospholipase A_1, phospholipase A_2, lysophospholipase phospholipase B, or phospholipase to obtain a cholesterol-reduced product (Ramrakhiani and Chand 2011).

Phospholipases (PLs) have been employed for the removal of mucilage and phosphorus components in crude vegetable oil. This is could be achieved by the use of PLA_2 or PLB, but not with PLC or PLD. The phospholipase enzymatic treatment process is used to purify any edible oil containing phospholipids (e.g., vegetable oil such as soybean oil, rape seed oil, and sunflower oil) using two steps: treating the oil with phospholipase (A_1, A_2, or B) to hydrolyze the phospholipids such as lecithin and other accompanying hydrophilic components, then separating an aqueous phase containing the hydrolyzed phospholipids from the oil.

7 Phospholipase and apoptosis

Apoptosis is a form of cell death that does not involve an inflammatory response and occurs in a tightly controlled manner. There are two main pathways through which apoptosis may occur. In the first pathway, apoptosis can be induced by a death receptor such as the tumor necrosis factor receptor; in the second one, apoptosis can be induced by cytotoxic agents that cause a mitochondria-dependent cascade of events (Thornberry and Lazebnik, 1998). In death receptor-mediated apoptosis, a death-inducing signaling complex is formed by the death receptors, adapter proteins, and caspases (cysteinyl aspartate-specific proteases) such as caspase-8 and caspase-10 (Chen and Wang, 2002). In mitochondria-dependent apoptosis, major alterations in mitochondrial membrane function occur. While a proper activation of the apoptotic system is important, so is the recognition and removal of apoptotic cells. Loss of normal phospholipid asymmetry in apoptosis provides an "eat me" signal to macrophages. Meanwhile, phosphatidylserine (PS) exposure is essential for platelets to induce the hemostatic process. Unwanted PS exposure as the result of inadequate removal of apoptotic cells will result in a prothrombotic state, which is often correlated with severe inflammation-induced illness. Secretory phospholipase A2 ($sPLA_2$), an essential secondary messenger in inflammation, generates the building blocks for inflammatory signaling molecules such as eicosanoids and platelet-activating factor (PAF) (Frans, 2022).

In addition, $sPLA_2$ will hydrolyze bacterial and viral membranes, and as such assists in host defense. Unfortunately, $sPLA_2$ will also effectively hydrolyze PS, exposing (apoptotic) cells.

7.1 The role of PLA in apoptosis

Six major types of PLA play specific roles in apoptosis, and these are lysosomal PLA_2, $sPLA_2$, $cPLA_2$, $iPLA_2\beta$, patatin-like phospholipase A_2 ($PNPLA_2$), and phosphatidylserine-specific phospholipase A_1 ($PS\text{-}PLA_1$), all of which have been studied extensively. Other subfamilies and types of PLA mechanisms are still under study, as some have been shown not to initiate cell death.

Apoptosis, which is mostly caused by bacteria, is found in the gastrointestinal (GI) and respiratory tracts. *Escherichia coli*-like bacteria have the capacity to initiate apoptosis on epithelial cells. This is carried out by identifying the phosphatidyl ethanolamine on the plasma membrane and further expression of the outer phosphatidyl serine. The first steps will permit an increase in the accumulation of ceramide in the mitochondria. Ceramides are produced from lipids and stored in the intermembrane space. The ceramides will normally bind to sphingosine and fatty acid, as they are produced in excess during cell apoptosis. The biochemical pathways fostering the accumulation of ceramide lead to the inhibition of ceramide breakdown or activation of acidic sphingomyelinase and activation of de novo synthesis, which is responsible for the hydrolysis of sphingomyelin to ceramide (Schatter et al., 2005). During bacterial-induced apoptosis, the level of ceramide increases due to the inhibition of ceramide acylation catalyzed by PLA_2-like transacylase from phosphatidyl ethanolamine, and phosphatidyl ethanolamine substitutes as a substrate for PLD in the formation of phosphatidic acid (PA). PA serves to appropriate a balance between mitogenic and apoptotic responses (Abul-Milh et al., 2001). Since a higher concentration of PA can be found in the cytosol, there will be an apoptotic response balance shift, and it can also be vice versa. In the final stages involving bacterial-induced margination, apoptosis, nuclear condensation, and membrane/apoptotic blebbing are seen. In this bacterial-induced cell death, the cellular response will undergo more apoptosis than necrosis; during in vitro studies, the apoptosis reaction can end up looking like necrosis. Therefore, proper observation is needed before concluding the specific death pathway initiated.

At the occurrence of an ischemic shock or seizures, there is a temporary release of AA from the brain, which is then present in the nervous system; this is called the "Bazan effect." $cPLA_2$ is a simpler form of intercellular PLA_2 that is found throughout the central nervous system (CNS) (Sun et al., 2004). Prions (or excess glutamate production) are potential triggers inducing $cPLA_2$ activation and neural death. For example, prions can initiate neurodegenerative diseases such as Creutzfeldt-Jakob disease and spongiform encephalitis. On coming into contact with neurons, $cPLA_2$ becomes activated and, in turn, leads to the activation of apoptosis in neural cells. On activation of $cPLA_2$, it is relocated to the cellular neurites through β-III-tubulin and induces the hydrolyzation of AA to prostaglandins (Last et al., 2012). Prostaglandins aid caspase-3 activation and apoptosis. $cPLA_2$ has also been found to play roles in the excitotoxicity initiated by glutamate and oxidative stress induced by the apoptosis of neurons. Oxidative agents elicit $cPLA_2$, $sPLA_2$, and $iPLA_2$ in the CNS, which induces oxidative stress-mediated cell death. $iPLA_2$ is strongly linked with the E and the outer mitochondrial membrane (OMM), which propels the release of cytochrome c (cyt-c) (Lei et al., 2010). *N*-methyl D-aspartate receptor (NMDAR) subgroup activation induces apoptosis in neurons through the further activation of NO_2 synthase and mitochondrial superoxide production (Fig. 4). Other groups including IIA $sPLA_2$ have a major role in the production of mitochondrial superoxide,

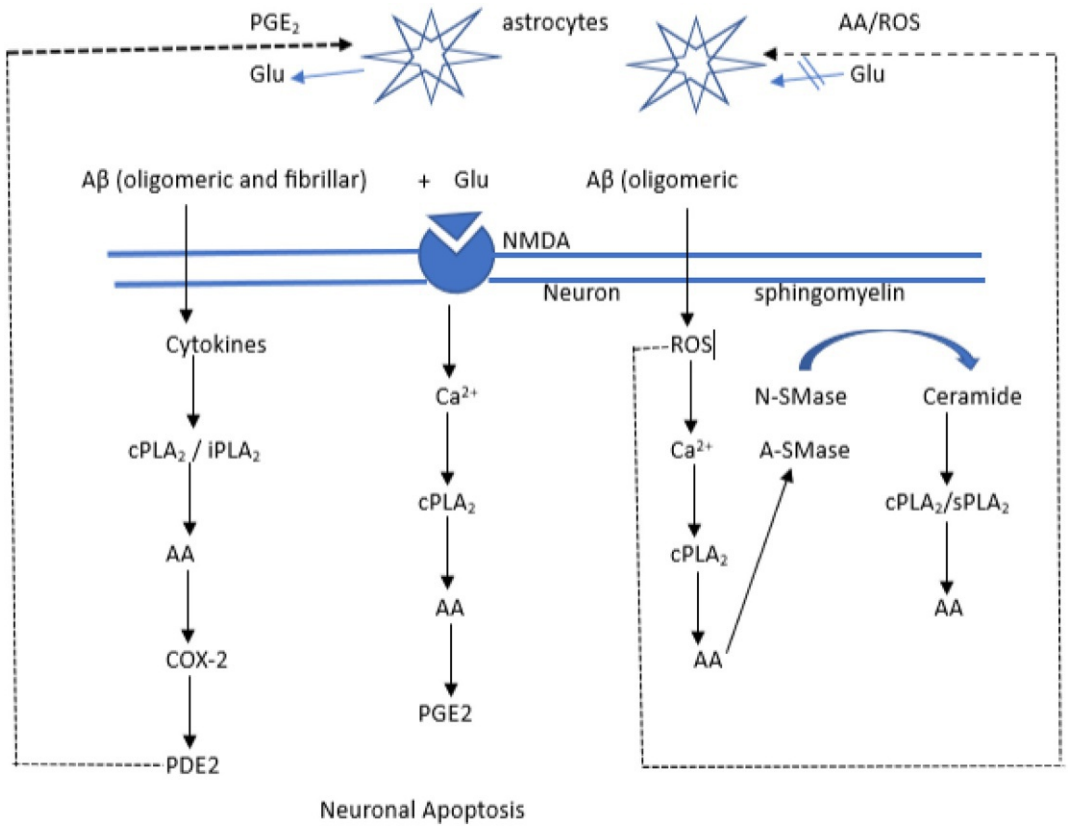

FIG. 4 The role of PLA_2 activation in neuronal apoptosis.

which aids the amplification of the death signal initiated by NMDAR (Chiricozzi et al., 2010).

Tumor necrosis factor alpha (TNFα) and Fas ligand (FasL) are both involved in further triggering apoptosis in cells that possess tumor necrosis factor receptor (TNFR)1/2 or Fas receptor (FasR) on their membrane; this mechanism is realized by the activation of $iPLA_2$. The apoptotic triggers induced by TNFα or FasL must have an activated death domain to initiate the signaling mechanism. Caspase-3 is involved in the important role of the activation and cleavage of $iPLA_2$ at Asp183 (Fig. 5). The trimmed $iPLA_2$ is the cause for the release of AA and the production of lysophosphatidylcholine, a chemo-attractant that permits phagocytic cells to remove apoptotic cells. Caspase-3 can split at a further site, leading to separate variants of $iPLA_2$ (Ravichandran, 2011). Biologically active $iPLA_2$ exists in a homo-tetramer form, and oligomerization is a vital mechanism for regulating $iPLA_2$. $iPLA_2$ can be regulated by oxidants and lipid metabolism, leading to the inactivation of $iPLA_2$ by changing the oligomerization of monomers. $iPLA_2$ also assists in the repair of membrane phospholipids such as cardiolipin in neurons, but the repair mechanism can be altered by reactive oxygen species (ROS)-mediated peroxidation. Research has shown that $iPLA_2$ produces both survival and apoptotic effects in cells of the rat adrenal medulla. Also, when the ER of β-cells is exposed to stress, it leads to the

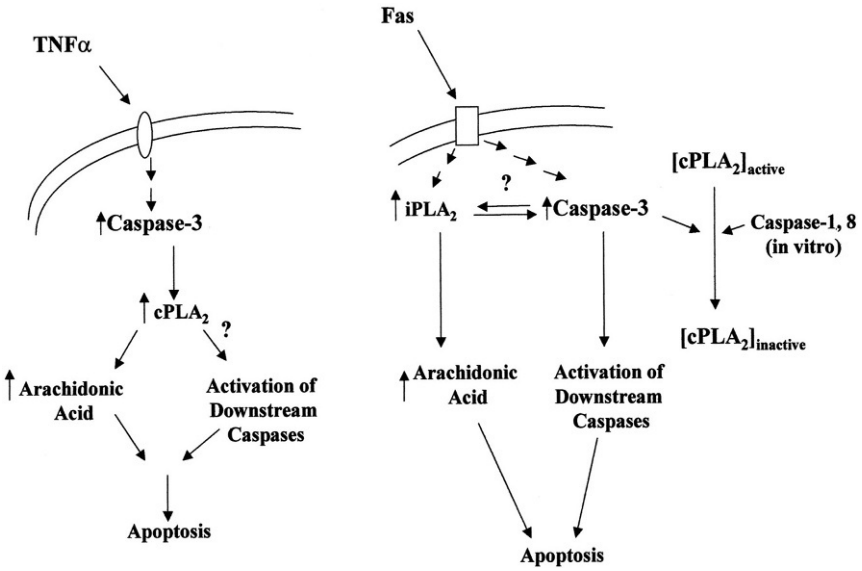

FIG. 5 The roles of PLA_2 in TNFα and Fas-induced apoptosis (Cummings et al., 2000).

reduction of ER Ca^{++} stores and further activation of caspase-12. The activated caspase-12 induces caspase-3 and releases cyt-c and AA from mitochondria. The cells that contain fully developed ER indicate higher vulnerability to ER stress, and $iPLA_2$ was found to be involved in β cell-like cells but did not affect the neuron cells. In β cell-like cells, ceramide expression holds iPLA2 in the "ON" or "ACTIVE" state, instead of deactivating it in the presence of ROS (Lei et al., 2010). $iPLA_2$ assists in the production of ceramide through acidic sphingomyelinase, but on the removal of the C-terminal region from $iPLA_2$VIA, it turns violent toward the cellular membrane.

The determining factor deciding the path that a dying cell will take—either apoptosis or necrosis—relies on lysosomal phospholipase A_2 ($LPLA_2$) activation and lysosomal permeabilization (LP). LP plays a major role in hastening the process of apoptosis induction. This is triggered by sphingosine or by the inhibition of sphingosine kinase 1, which ultimately leads to lysosomal damage. At the stage of lysosomal damage, $sPLA_2$ plays a vital role in eliciting the cell osmosis-sensitive by the production of AA. LP is involved in the release of cathepsin D and the activation of caspases. This can also trigger caspase-independent cell death, where the loss of ability to bind molecules results in anoikis. LP and apoptotic traits are proportionally related (Aits and Jaattela, 2013). The $sPLA_2$ family permits the induction of membrane permeabilization, which in turn allows apoptotic cells to undertake phagocytosis. Normal healthy cells can become resistant to hydrolysis by the actions of $sPLA_2$. This then serves as an early detection that pulls macrophages toward apoptotic cells. On the other hand, PS-PLA_1 activation at the late stages of apoptosis leads to the upregulated expression of phosphatidylserine, which activates the signal "eat me" to phagocytes (Ravichandran, 2011). Extracellular matrix (ECM) interactions in normal cells can lead to several different cell fates, which are formed by integrin. The integrin's focal adhesion kinase (FAK) signal complex has been identified as a factor in regulating anchorage-dependent cell

survival. On the inhibition or downregulation or the absence of ECM, apoptosis occurs when cells enter through anoikis via the p53-dependent pathway, which is normally activated by PKCλ and $cPLA_2$ (Ilić et al., 1998). Senescence occurs in cells over time; $sPLA_2$ is involved in senescence on the activation of the DNA-p53-dependent pathway, which is induced by the accumulation of ROS (Kim et al., 2009).

7.2 The role of PLC in apoptosis

The active PLCs that play several roles in apoptosis are phosphatidylcholine-specific phospholipase (PC-PLC), PLCγ1, PLCγ2, PLCδ1, PLCδ3, and PLCβ3. The ER releases Ca^{++} from the mitochondria, which activates the apoptotic pathways and in turn induces the release of the mitochondrial pro-apoptotic factors. The ER Ca^{++} depletion requires the activation of PLC and also a cytosolic Ca^{++} increase (Marchi et al., 2012). During tissue remodeling, homeostasis, and cytotoxic T-cell killing, Fas/Apo-1 and TNFR1 induce apoptosis. At the point of TNFR1/Apo-1 interlinking, PC-PLC is activated and hydrolyzes phosphatidylcholine into choline and d**iacylglycerol**. The activation of acidic sphingomyelinase requires DAG, which in turn hydrolyzes membrane sphingomyelin. In addition, it will lead to the production of ceramide through de novo synthesis. The accumulation of ceramide leads to the activation of mitogen-activated protein kinase (MAPK) and the C-Jun N-terminal kinase (JNK), which leads to the production of AA by the activation of PLA_2. Other downstream molecular signals such as PLA_2 are involved in the activation of protein phosphatase 2A and the downregulation of c-myc. The downregulation of c-myc leads to cell death by apoptosis. The apoptosis that is induced by TNFα is caused by PC-PLC (studies carried out on acute myelogenous leukemia were quantified (Plo et al., 2000)). This pathway is activated by cytotoxic T-cells to trigger apoptosis as a humoral response. At the end of apoptosis, macrophages will consume the apoptotic materials. At the initial stages of phagocytosis, RTK-like MerTK and phosphatidyl serine identify the apoptotic bodies, and phosphoinositide phospholipase C (PI-PLC) is phosphorylated by tyrosine in the macrophage. The activated PI-PLC further hydrolyzes phosphatidyl inositol 4,5 phosphate into inositol 1,4,5-trisphosphate (IP3) and DAG. The interactions between diacylglycerol and Ca^{+2} leads to the activation of PLCγ2 and further linked with MerTK. The combination of PLCγ2 with MerTK is an important phase of phagocytosis (Todt et al., 2004). When this combination does not occur, apoptotic bodies inflict an internal injury around the surrounding cells and this can induce autoimmunity. The FAK signaling complex contains PLCγ1 and its absence results in anoikis (i.e., the cells begin to lose their ECM or adhesion molecules from surrounding or support cells) (Chattopadhyay and Carpenter, 2002). Severe apoptosis and dysfunctional vascularization of the placenta found in the developmental stages of the embryo are induced by the loss of PLCδ1 and PLCδ3 (Nakamura et al., 2005). PLCδ1- and PLCδ3-deficient embryos induce apoptosis in the cardiomyocytes of the growing fetus, and this leads to an increased heart weight/tibial length ratio (Nakamura et al., 2014).

7.3 The role of PLD in apoptosis

The family of PLD is divided into three types: PLD_1, PLD_2, and mitoPLD. PLD has the capacity to split phosphatidylcholine to PA. Phosphatidyl ethanolamine is a substrate of PLD and plays a vital role in altering the balance between the mitogenic and apoptotic response via the PA level. PC becomes hydrolyzed by PLD into secondary messengers known as PA and choline. On the application of shear stress to cells, the following observations were made: (1) PLD_1 activation; (2) stimulated mammalian target of rapamycin (mTOR) signaling; (3) hypertrophy; and (4) apoptosis. When the shear

stress on the plasma membrane is increased it leads to hypertrophy; cells in hypertrophy also indicate increased c-src phosphorylation and PLD_1 activation, which finally leads to apoptosis. Such apoptosis is found in renal and endothelial cells, which interact directly and regularly with shear force released by fluid motion (Huang et al., 2012). Mitochondrial phospholipase D (MitoPLD) was discovered to be located near the mitochondrial surface. It releases phosphatidic acid (PA) during meiosis of spermatocytes. Mice that are deficient in mitoPLD show a meiotic arrest of apoptosis. MitoPLD plays an important role in apoptosis and neurodegenerative diseases. The increased expression of mitoPLD at the juxtaposition of mitochondria during fusion and RNAi (RNA specific to spermatocytes) leads to mitochondria fragmentation. The apoptosis that occurs in the mitochondria of cells shows as fission rather than fusion. Mitochondrial fission has been implicated in the apoptosis upstream of cyt-c. Dynanin-related protein1 is a vital factor in mitochondrial fission and apoptosis. This type of apoptosis takes place usually during developmental stages. MitoPLD also plays a major role in the termination of mitochondrial fusion, through the activation of Lipin1b-like molecules, which boosts mitochondrial fission by converting PA into DAG.

8 Conclusion

This chapter has highlighted the various classes and subclasses of phospholipases with hydrolytic, lysophospholipase, and transacylase activity which are essential for the modulation and control of diverse cellular processes including signal transduction and inflammation, gene expression, apoptosis, and lipid fluidity. This has served to reveal the relationship of these intricate pathways and the complexity with which they are regulated. These extant multifaceted roles and mechanisms make phospholipases an important group of enzymatic biomolecules that warrant exploitation in the biotechnological and biomedical realm. Furthermore, the fact that biotechnology provides an opportunity for microbes/cells to be specifically engineered and directed for the production of these enzymes supports their position as an important group of biomolecules with future potential and multiple applications spanning the biomedical, pharmaceutical, agriculture, and food industries.

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