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Secretory Phospholipase A₂ Enzymes as Pharmacological Targets for Treatment of Disease

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Abstract

Phospholipase A₂ (PLA₂) cleave phospholipids preferentially at the *sn*-2 position, liberating free fatty acids and lysophospholipids. They are classified into six main groups based on size, location, function, substrate specificity and calcium requirement. These classes include secretory PLA₂ (sPLA₂), cytosolic (cPLA₂), Ca²⁺-independent (iPLA₂), platelet activating factor acetylhydrolases (PAF-AH), lysosomal PLA₂ (LyPLA₂) and adipose specific PLA₂ (AdPLA₂). It is hypothesized that PLA₂ can serve as pharmacological targets for the therapeutic treatment of several diseases, including cardiovascular diseases, atherosclerosis, immune disorders and cancer. Special emphasis has been placed on inhibitors of sPLA₂ isoforms as pharmacological moieties, mostly due to the fact that these enzymes are activated during inflammatory events and because their expression is increased in several diseases. This review focuses on understanding how sPLA₂ isoform expression is altered during disease progression and the possible therapeutic interventions to specifically target sPLA₂ isoforms, including new approaches using nano-particulate-based strategies.

Keywords

secretory phospholipase A₂; phospholipase A₂ receptor; nanoparticles; liposomes; cell signaling; interactomes

1. Introduction

Phospholipase A₂ (PLA₂) enzymes are a diverse class of esterases that preferentially cleave glycerophospholipids at the *sn*-2 position. This results in the liberation of a fatty acid and a lysophospholipid [1]. To date, more than 30 isoforms and related enzymes have been identified. These enzymes range in size, location, function, substrate specificity and calcium requirement. They are subdivided into six families based on their structure, catalytic

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mechanism, localization and evolutionary relationships [2]. These families include cytosolic PLA₂ (cPLA₂), calcium-independent PLA₂ (iPLA₂), secretory PLA₂ (sPLA₂), lysosomal PLA₂ (LyPLA₂), platelet activating factor acetylhydrolases (PAF-AH), and the recently discovered adipose specific PLA₂ (AdPLA₂) [3]. These PLA₂ families include different isoforms that are similar in structure and function. Table 1 summarizes differences and similarity among PLA₂ families [4]. These families are collectively identified as groups, using roman numerals (i.e. Group I to Group XVI), with capital letters to distinguish individual sub-families.

The cPLA₂ family (Group IVA-F) contains six isoforms ranging in size from 60-85 kDa. As the name implies, these isoforms are generally localized to the cytosol. They are active in the presence of μ M levels of calcium and, with the exception of cPLA₂ γ (Group IVC), contain an N-terminal C2 domain for binding two Ca²⁺ ions as well as two conserved phosphorylation sites. They have a conserved Ser/Asp catalytic dyad that is similar in structure to that of iPLA₂, and most cPLA₂ have a preference for choline head groups and arachidonic acid (AA) in the *sn*-2 position. As such, these enzymes play an integral role in prostanoid signaling cascades [2].

Currently, six isoforms of iPLA₂ have been identified (Group VIA-F). The catalytic site of iPLA₂ is similar to cPLA₂. Unlike cPLA₂, however, these do not require calcium to function and they are generally larger in size, ranging from 55-146 kDa with the exception of Group VIF PLA₂ (~28kDa). They are localized either to the cytosol, the inner side of the cell membrane, endoplasmic reticulum (ER) or mitochondrial membrane [5]. iPLA₂ are integrally involved in lipid remodeling and the Land's Cycle, as well as mediating cell growth signaling [2, 3].

In contrast to the above two PLA₂ families, platelet activating factor acetylhydrolases (PAF-AH, Group VIIA and B, and VIIIA and B) are smaller in molecular weight (26-43 kDa) and fewer in number of isoforms. There are four members of this family, three that are expressed intracellularly, and one secreted form that has generated interest as a drug target for atherosclerosis [6]. All members of this family have a catalytic serine and serve the primary function of releasing acetate from the *sn*-2 position of PAF-AH, although they can also catalyze the release of oxidized acyl groups from the *sn*-2 position of phosphatidylcholine (PC) and phosphatidylethanolamine (PE) [2, 3].

There is only one member of the lysosomal PLA₂ family (Group XV). It is a mannose type glycoprotein that localizes to the lysosome and has preference for catalysis in an acidic pH environment. In terms of catalytic activity, this Ly-PLA₂ specifically prefers PC and PE head groups. In addition, the enzyme is ubiquitously expressed in different cell types, but highly expressed in alveolar macrophages. As a result, it plays a role in surfactant metabolism, and specifically in catabolic homeostasis of lung surfactants [7].

The recently discovered adipose-specific PLA₂ (AdPLA₂, Group XVI) is found abundantly in white adipose tissue and appears to be responsible for supplying AA for PGE₂ synthesis within this tissue [8]. Additionally, AdPLA₂ may have roles in energy regulation by cleaving fatty acids from stored triglycerides (TG). Depending on experimental conditions,

AdPLA₂ has also shown the ability to hydrolyze the *sn-1* position of glycerophospholipids, thus the correct classification may be as a PLA_{1/2} rather than a traditional PLA₂ [2].

To date, there are 17 different isoforms of sPLA₂ (Group I-III, V, IX-XIV). sPLA₂ isoforms generally have a lower molecular weight than other PLA₂, ranging in size from 14-19 kDa, except for Group III sPLA₂ that has a molecular weight of 55 kDa [1, 9]. Additionally, sPLA₂ isoforms are calcium-dependent, and require mM concentrations of the ion to function optimally. As a result, sPLA₂ isoforms typically function at the extracellular side of the cell [2, 10]. Among the 17 sPLA₂ isoforms, 11 of them are expressed in mammalian cells. Recent studies suggest that some sPLA₂ isoforms can alter cell function by binding to receptors and other proteins [11]. Binding of sPLA₂ isoforms to these proteins creates an interaction that alters cellular function independent of sPLA₂ enzymatic activity.

Maintaining sPLA₂ homeostasis is suggested to be critical for several physiological functions [12]. For instance, overexpression of some sPLA₂ isoforms is associated with pathological conditions such as atherosclerosis, immune disorders and cancer [3]. The extracellular localization of sPLA₂ isoforms makes them feasible targets for treatment of diseases where sPLA₂ expression is elevated. This review focuses specifically on sPLA₂ biological functions, their role in pathogenesis and the potential of sPLA₂ inhibitors as pharmacological treatment for disease. Special emphasis is placed sPLA₂ receptors and other binding proteins that modulate the action of sPLA₂ isoforms independently of direct inhibition of lipase activity.

2. Secretory Phospholipase A₂

Currently, at least 11 mammalian isoforms of sPLA₂ are identified and belong to Group I, II, III, V, IX, X and XII. Of these, Groups I, II, V and X are considered “conventional” sPLA₂. They share a variety of structural elements including a His/Asp catalytic dyad, a highly conserved Ca²⁺ binding domain and six absolutely conserved disulfide bonds. Groups III and XII, on the other hand, are structurally distinct based on the identity of their protein sequence with Groups I, II, V and X sPLA₂. They only share the aforementioned groups in their Ca²⁺ binding loop and catalytic site [13]. Understanding the structure and function of sPLA₂ isoforms is important to a better understanding the pathology of sPLA₂-related diseases in humans. Unfortunately, to date, only Group IIA and X protein structures have been resolved [14, 15].

Regardless of their structure and substrate preference, most sPLA₂ isoforms require high calcium concentration in the order of mM levels to operate [2, 3, 11]. In contrast, the function and expression of sPLA₂ are isoform specific, and this specificity appears to correlate to certain pathologies. Diseases in which select sPLA₂ isoforms are reported to be overexpressed are listed in Table 1.

Most isoforms of sPLA₂ generally function extracellularly primarily on phospholipids. sPLA₂ hydrolyze a wide range of phospholipids substrates depending on the isoform [2]. The specific structure and enzymatic activity of each sPLA₂ isoform have been the focus of other reviews [3, 11], and will not be repeated in detail here. Rather, this review aims to

emphasize important characteristics of sPLA₂ isoforms that make them regulators in pathogenesis and possible pharmacological targets to treat sPLA₂-associated diseases.

In general, sPLA₂ isoforms have strong preference for negatively charged phospholipid head groups, in particular phosphatidylserine (PS), phosphatidylglycerol (PG) and phosphatidylethanolamine (PE) [16]. This preference is useful for their role as defensive proteins, as PE and PG are major components of bacterial membranes [17].

Among sPLA₂ isoforms, Groups V and X are more efficient in cleaving phospholipids than other sPLA₂ [16]. Some sPLA₂ isoforms such as Groups IB, III and X sPLA₂ are secreted as pro-enzymes and require cleavage at the N-terminus to be fully active [18]. Further, different isoforms have preferences for different membrane and extracellular matrix proteins. For example, Groups II, III and V sPLA₂ have a strong binding affinity for heparan sulfate proteoglycans (HSPG), which provide support and proximity to the cell membrane [19], while Groups IB and X can bind to the M-type phospholipase A₂ receptor (PLA2R1) [20]. Binding of sPLA₂ isoforms to HSPG or PLA2Rs can subsequently transport these enzymes into the cell by a caveola-dependent endocytotic pathway [21]. More sPLA₂ interactomes have been recently discovered, but their roles remain elusive [22].

Understanding the dynamics of the sPLA₂ interactomes may be key to understanding how sPLA₂ isoforms can be targeted for treatment of pathologies in which it is overexpressed.

3. sPLA₂ Expression in Pathologies

sPLA₂ isoforms are commonly overexpressed during bouts of inflammation and in inflammation associated diseases like arthritis, atherosclerosis and sepsis [23-25]. However, select sPLA₂ isoforms are also overexpressed in a variety of cancers including breast, colon pancreas and prostate cancer [26, 27]. Interestingly, it appears that some isoforms of sPLA₂ can have an oncogenic role [28-30]. For example, sPLA₂ overexpression is correlated with poor clinical prognosis in prostate cancers [31]. In contrast, overexpression of select sPLA₂ isoforms in gastric cancer appears to have a beneficial clinical correlation [32]. There are a variety of explanations that may explain the dichotomous role of sPLA₂ isoforms in cancer [33], which all support the hypothesis that the role of sPLA₂ in cancer progression is isoform specific.

Most of the recent studies focusing on the oncogenic role of sPLA₂ isoforms have centered on Group IIA sPLA₂. However, this isoform was originally shown to be involved in host defense and inflammation [34]. It was first identified to have anti-bacterial activity against gram-positive bacteria, which have negatively charged phospholipids on their membrane [35]. It was also shown to be highly upregulated by immune cells during inflammation. The abundance of Group IIA sPLA₂ (up to 500-fold higher in sepsis [36]) in inflammatory fluid further supports it having a prominent regulatory role in inflammation and infectious diseases. In addition, it is possible that Group IIA sPLA₂ is involved in atherosclerosis, obesity, arthritis and cancer. One role of Group IIA sPLA₂ in these diseases may be the recruitment of other sPLA₂, such as Group V and X sPLA₂ [3, 11]. Future studies are needed to confirm this hypothesis.

As mentioned above, the role of Group IIA sPLA₂ in carcinogenesis may be dependent on cancer type. Many studies suggest that Group IIA sPLA₂ promotes breast, lung, pancreatic and prostate cancer cell proliferation *in vitro* and *in vivo* [30, 37]; and enhanced sPLA₂ expression is inversely related to survival time of patients that have some of these cancers [38]. However, other studies report that Group IIA sPLA₂ prolongs survival and has anti-tumor roles in gastric cancer [39]. This divergent role of Group IIA sPLA₂ in different cancer types may be due to differences in the presence of sPLA₂ regulators (i.e. interactomes) within the tumor microenvironment (see below). These include extracellular matrix proteins such as glycosaminoglycans that may protect cancer cells from immune cells [40].

Not all sPLA₂ isoforms have the same functions in disease. For example, the role of Group V sPLA₂ in inflammation is controversial despite its similar expression profile and function with Group IIA sPLA₂. Other studies suggest that Group V sPLA₂ is a pro-inflammatory factor in allergic airway inflammation, acute lung injury and atherosclerosis [41, 42]. In contrast, some studies suggest an anti-inflammatory effect for Group V sPLA₂ via clearing deposition of immune complexes through cysteinyl leukotriene receptor phagocytosis [24]. As is the case for most PLA₂ isoforms, the role of Group V sPLA₂ in inflammation is probably cell-dependent.

Inflammation is not the only pathology for which the role of sPLA₂ is controversial. For example, elevation of Group X sPLA₂ expression is associated with cell proliferation and metastasis in a variety of cancers including lung, breast and colon cancer [43]. However, Group X sPLA₂ expression is inversely related to colorectal cancer metastasis [44]. This inconsistent role suggests that Group X sPLA₂ may not be directly involved in cancer metastasis.

4. sPLA₂ Regulation

A better understanding of the regulation of sPLA₂ isoforms can only lead to a greater potential to identify therapeutic avenues for inhibiting these enzymes. sPLA₂ are regulated by different mechanisms, including cell signaling induced by lipids, and even other PLA₂ (i.e. PLA₂ cross-talk). Similar to their differential role in pathologies, the regulatory mechanisms mediating sPLA₂ expression and activity appear to be isoform and cell specific. Additionally, protein-protein interactions (the sPLA₂ interactome) can modify sPLA₂ activity independently of the sPLA₂ active site. Other studies have focused on the genetic regulation of select sPLA₂ isoforms with more recent data suggesting roles for epigenetic regulation. Finally, some sPLA₂ isoforms also can be regulated by receptor-based mechanisms. These and other regulatory pathways are discussed below.

4.1. Cell Signaling

sPLA₂ can be regulated by lipid metabolites and receptor binding. Products of PLA₂ deacylation reactions, such as AA and lysophospholipids (LP), can trigger signaling cascades by serving as precursors for biosynthesis of eicosanoids [45]. Other PLA₂, such as cPLA₂, are also involved in the production of AA. Depending on the cell type, the role of either sPLA₂ or cPLA₂ in AA production will be different. These reactions have been

covered in great detail in recent reviews [2, 12], thus, only a brief discussion of AA metabolism is given below.

Pathologies such as sepsis can enhance the expression of sPLA₂ isoforms [23, 36], which increases AA release to levels three-fold greater than baseline levels [46]. AA can be further metabolized in cells through cyclooxygenase (COX) and 5-lipoxygenase (5-LOX) pathways to produce pro-inflammatory mediators such as prostaglandins (PGs) and leukotrienes (LTs), respectively [47]. These mediators can stimulate the production of inflammatory cytokines including tumor necrosis factor-alpha (TNF- α) and interleukins (IL) [48]. These soluble factors can then enhance sPLA₂ expression itself [29], leading to signal amplification and intensification of the inflammatory event (Figure 1).

LP can be metabolized by lysophospholipase D (LysoPLD) to produce lysophosphatidic acid (LPA), which has been shown to activate G protein-coupled receptors (GPCRs) to trigger activity of their downstream targets [47, 49]. Stimulation of select GPCRs activates nuclear factor-kappa B (NF- κ B) [50], which is a transcriptional factor for Group IIA sPLA₂ [48] (Figure 1). Furthermore, select GPCRs, such as LPA receptors, contribute to migration and differentiation of inflammatory cells. The chemotactic gradients generated by the above processes recruits immune cells and assists macrophages maturation and differentiation.

Unfortunately, efforts to reduce PG production by inhibiting COX function can lead to increased production of LTs through the LOX pathway [11]. Further, liver toxicity may occur when blocking both COX and LOX pathways [51]. Some of these drawbacks may result from the multiple cellular effects of both COX and LOX metabolites [47]. Inhibiting sPLA₂ overexpression, or activity, would theoretically inhibit the production of either of these metabolites and may result in an improved outcome with fewer side effects.

The role of sPLA₂ isoforms in cell signaling is not inherent to its catalytic activity. Specific sPLA₂ isoforms can also bind to different receptors [22] and glycosaminoglycans [52, 53]. For example, Group IB and X sPLA₂ can bind to M-type phospholipase A₂ receptor (PLA2R1). It has been recently reported that PLA2R1 regulate the JAK/STAT and p53 pathways and sPLA₂ isoforms may be involved in this regulatory mechanism (Figure 2A) [54, 55]. Additionally, Group IIA and V sPLA₂ have a high affinity for selected isoforms of glycosaminoglycans such as heparan sulfate [52], chondroitin sulfate [56] and heparin [53] through electrostatic interactions. These glycosaminoglycans are post-translational modifications of extracellular, transmembrane or GPI-anchored proteins such as perlecan, syndecan and glypican, respectively [3, 56]. These extracellular proteins modulate the function of other membrane-bound receptors including receptor tyrosine kinases (RTKs) and Wnt receptors by interacting with ligands or the receptor itself through glycosaminoglycan chains [52]. Moreover, it is suggested that binding of sPLA₂ isoforms to these proteins is calcium-independent [57]. It is possible that the presence of sPLA₂ isoforms may alter interactions between the receptors and their ligands.

In addition to PLA2Rs and HSPG, some sPLA₂ isoforms can bind to integrins, RTKs (eg. FGFR and VEGFR), K⁺ channels, as well as to intracellular proteins such as calmodulin, v-src kinases and mitochondrial sPLA₂ receptors [22]. Binding of sPLA₂ to these proteins may

be important for their localization and in cellular signaling. As a result, studies identifying modulators of the sPLA₂ interactome may be just as important as those seeking to identify modulators of its activity. At the very least, studies of the sPLA₂ interactome are needed to fully understand sPLA₂ physiological and pathological functions.

4.2. Genetic Regulation of sPLA₂

Genetic factors controlling the expression of sPLA₂ are well defined for certain isoforms, but unclear for others. Most studies have focused on genetic regulation of Group IIA sPLA₂ due to its overexpression in cardiovascular and inflammatory diseases, obesity and different cancers [29, 58, 59], and these studies suggest role for cytokines and enhancer binding proteins (Figure 1). For example, cytokines such as TNF- α and IL-1 β activate inhibitory κ B protein (I κ B), which sequesters nuclear factor-kappa B (NF- κ B) in the cytosol. Phosphorylated I κ B releases NF- κ B to enter the nucleus where it enhances gene transcription of many mammalian genes including Group IIA sPLA₂ [48, 60] (Figure 1). Another positive regulator of Group IIA sPLA₂ gene expression is CCAAT-enhancer-binding proteins (C/EBP) [61].

Studies in rat vascular smooth muscle cells also suggest that peroxisome proliferator-activated receptors (PPAR) can regulate Group IIA sPLA₂ expression. PPAR β ligands can decrease Group IIA sPLA₂ activity, as well as decrease mRNA expression during inflammation by inducing B cell lymphoma-6 (BCL-6), which binds to the sPLA₂ promoter as a transcriptional repressor [62]. Another study demonstrates that this mechanism is also utilized by tumor cells to secrete soluble factors that generate CTL-suppressive macrophages by stimulating PPAR γ , which also inhibit sPLA₂ activity [63]. These studies suggest that PPAR may be pharmacological targets for pathologies that over express sPLA₂ isoforms.

In contrast to Group IIA sPLA₂, relatively little is known about the transcription regulators for other sPLA₂ isoforms. Recent studies suggest that epigenetics mediate the expression of sPLA₂ isoforms including Group IIA, V and X [64]. These same studies suggest that epigenetic regulation of select sPLA₂ isoforms may be important in the regulation of certain cancers, including leukemia and prostate cancer [64].

Despite many documented studies, there is no well-defined study describing translational regulation of sPLA₂ isoforms. Future studies are needed to fill in this knowledge gap and complete our understanding of sPLA₂ regulation. MicroRNAs are small non-coding RNA molecules that can interfere with the translational process of converting mRNA to protein. MicroRNAs have been implicated in mediating numerous diseases including cancer [65]. Investigating the role of microRNA in sPLA₂ protein expression may identify additional therapeutic tools to control expression of these enzymes.

4.3. PLA₂ Cross-Talk

Significant cross-talk exists between sPLA₂ and other PLA₂ isoforms, and this cross talk can regulate sPLA₂ expression. cPLA₂ particularly appears to play a regulatory role in enhancing the activity of sPLA₂ [66, 67]. The reverse may also be true. For example, the release of AA by Group V sPLA₂ in mouse neutrophils is employed to produce pro-inflammatory mediators such as leukotriene B₄ (LTB₄) that will bind to LTR receptors and

activate MAP kinases, such as ERK1 and 2 [68]. MAPK will then phosphorylate cPLA₂ and further enhance intracellular AA production.

While a substantial knowledge base on cross-talk between cPLA₂ isoforms and Group V sPLA₂ exists, less information exists on cross-talk between other types of sPLA₂. The studies that do exist suggest that the role of cross-talk in sPLA₂ regulation may be cell-dependent.

4.4. PLA2Rs and Other sPLA₂ Binding Proteins

As mentioned above, the regulation of some sPLA₂ isoforms may also be regulated by receptor binding. There are two types of phospholipase A₂ receptors (PLA2Rs): neuronal type (N-type PLA2R) and muscle type (M-type PLA2R or PLA2R1). Both N- and M- type receptors belong to the C-type lectin superfamily, which has a carbohydrate recognition domain (CRD), an important element for sPLA₂ binding. However, binding and affinity of the receptor to sPLA₂ isoforms varies depending on species and receptor type [69]. PLA2R1 has recently been proposed to act as tumor suppressor in certain cancer including breast and renal cancer [70].

The binding of sPLA₂ isoforms to PLA2Rs inhibits sPLA₂ catalytic activity. To date, N-type PLA2R have been shown to have a low binding affinity for Group IB, IIA and X sPLA₂, but high affinity for sPLA₂ bee venom (Group III sPLA₂). In contrast, M-type PLA2R does not bind to bee venom sPLA₂ (Group III sPLA₂), but does have high affinity for Group IB and X sPLA₂ [71]. Group IIA sPLA₂ does not bind to human M-type PLA2R, but has low affinity for M-type PLA2R in other species. The binding of sPLA₂ to the receptor mediates endocytosis of sPLA₂ [11, 69] and hence leads to sPLA₂ clearance and inhibition.

Overexpression of PLA2R1 at the cellular membrane of podocytes, and autoimmune anti-PLA2R1 antibodies in circulation are associated with idiopathic membranous nephropathy (IMN) [72]. Recent studies have also implicated PLA2R1 in cardiac rupture after myocardial infarction [73]. Compared to wild-type mice, mice lacking PLA2R are susceptible to higher rates of cardiac rupture due to impaired collagen deposition and reduced α -smooth muscle actin-positive fibroblasts in the infarcted region. This effect can be reversed by injection of wild-type myofibroblasts (PLA2R^{+/+}) to the impaired region. This study suggests that the myoblast dysfunction is not dependent on sPLA₂ enzymatic activity per-se, but rather by its binding to PLA2Rs, as well as its interaction with integrin β 1.

Heparan sulfate (HS), a sulfated glycosaminoglycan, binds Group IIA and V sPLA₂ due to electrostatic interaction that exists due to the abundance of basic amino acids in these proteins. The specific heparan sulfate proteoglycan glypican, a glycosphosphatidylinositol (GPI) anchored protein, is known to be involved in sPLA₂ shuttling through caveolar vesicle formation [11, 22]. Other glycosaminoglycans that can bind to and modulate sPLA₂ activity include sulfated chondroitin, heparin and hyaluronan.

sPLA₂ structures for Group IIA (PDB: 3UB8) and X (PDB: 1LE6) are shown in Figure 2B and C to demonstrate how the charged amino acids in sPLA₂ protein sequence influence sPLA₂ activity, protein binding, and binding with negatively charged phospholipids (eg. PE, PS and PG). In general, the 3-D protein folding structures of these enzymes are similar. They both contain a reserved catalytic dyad (His/Asp) (highlighted red in Figure 2B and C) and calcium binding sites (highlighted in magenta in Figures 2B and C). Interestingly, the distribution of charged amino acids is different between these isoforms. Group IIA contains more positively charged residues (lysine (K) and arginine (R)) that are exposed to the surface of this enzyme, while Group X appears to have less positively charged and more negatively charged residues (glutamic (E) and aspartic (D) acids). As a result, Group IIA sPLA₂ has an overall positively charged surface. This maybe one reason why Group IIA has affinity for sulfated glycosaminoglycan [19, 22]. Additionally, the activity of sPLA₂ IIA can be easily controlled by shielding/blocking the interfacial catalytic surface (ICS) with sulfated glycosaminoglycan such as heparin [53, 75]. The presence of sulfated glycosaminoglycan on the cell surface can hinder the interaction between sPLA₂ Group IIA and phospholipids at the cell membrane, and consequently slow down sPLA₂-induced cell death [4]. However, this same concept does not apply to Group X, which does not have as many positively charged amino acids at the ICS [4]. In contrast, Group V sPLA₂ has a similar distribution of charged amino acids as Group IIA (Figure 2D). This may explain the similar activity and binding targets for these sPLA₂.

Heparan sulfate on glypican-1 can also interact with growth factors such as fibroblast growth factor (FGF), and epidermal growth factor (EGF) and sequester them away from the receptors [74, 75], and hence inhibit receptor activation. In contrast, Wagle et al. showed that sPLA₂ enhance the binding of EGF to its targets on the cell membrane by two-fold, and that this effect is diminished by the presence of heparin [76]. These studies suggest that the presence of Group IIA sPLA₂ contributes to the recruitment of EGF and possibly other growth factors to the cell membrane. They also suggest that sPLA₂ can facilitate the EGFR signaling cascade.

Another endogenous inhibitor of sPLA₂ isoforms, simply termed the sPLA₂ inhibitor, or PLI, is found in animals that are fed sPLA₂ from snake venom [22]. The exact nature and types of PLI are still under study, but they are known to exist in the sera of snakes, where they are believed to inhibit endogenous snake venom sPLA₂ isoforms [77] In human biological systems, heparin secreted by mast cells can act similar to the PLI described above, but its concentrations are not sufficient to effectively inhibit sPLA₂ isoforms like the PLI found in snake venom do [53]. Some glycosaminoglycans, such as chondroitin sulfate and hyarolunan [78], also have inhibitory effect on sPLA₂ by interfering with the access of the phospholipid substrate to the active site, and thus also act similar to PLI.

5. Therapies Targeting sPLA₂

Numerous pharmacological avenues exist to inhibit sPLA₂ activity (Table 2) [2]. The first small molecule sPLA₂ inhibitor for Group IIA sPLA₂ was BMS-181162, which only had modest activity with an IC₅₀ in the micromolar range. This drug was found to inhibit 5-LOX

and reduce the level of LTB₄ and PGE₂, but the chemical structure stability, and limitation in skin penetration resulted in failures in Phase II clinical trials to treat psoriasis [79].

Desirable criteria for sPLA₂ inhibitors include IC₅₀ values at nanomolar concentrations to avoid nonspecific targets. Particular emphasis has been placed on Group IIA sPLA₂ inhibitors. One of the first of these was a N-benzyl indole structure-based sPLA₂ inhibitor referred to as LY311727. This compound possessed an IC₅₀ of approximately 50 nM for Group IIA sPLA₂, but also inhibited Group V sPLA₂ (IC₅₀ of 2000 nM) and Group X sPLA₂ (IC₅₀ of 750 nM) [80]. Unfortunately, this potency did not translate to *in vivo* studies, as high doses were needed for comparable efficacy in mice (30 mg/kg).

LY315920 (Varespladib), another N-benzyl indole structure-based sPLA₂ inhibitor, was shown to have improved potency (IC₅₀ = 9 nM) and selectivity (as compared to LY311727) for Group IIA sPLA₂. Varespladib also had a better outcome in animal studies and only required 16 mg/kg for inhibition in mice [81]. Unfortunately, Varespladib failed in Phase II clinical trials for sepsis due to no enhancement in overall survival outcome [82]. Currently, Varespladib is in Phase II clinical trials for atherosclerosis [83].

Selective and non-selective inhibitors of Group IIA sPLA₂, as well as some other isoforms, are shown in Table 2. Efforts are continuing to develop additional structure-based inhibitors of Group IIA sPLA₂ that have more favorable pharmacokinetics. Some of the more recently developed inhibitors are focused on preventing binding of sPLA₂ to its receptor, as opposed to targeting its active site (see below) [84].

Recent studies highlight the development of therapeutic peptides for inhibition of sPLA₂ activity, as opposed to small molecules [85]. While these peptides offer great promise, especially in the realm of specificity, further studies are needed to characterize their therapeutic efficacy. Of concern is the ability to deliver these peptides to the diseased tissue sites. One promising area is the combination of these peptides with nanoparticle-based drug delivery systems.

While several excellent small molecule inhibitors of sPLA₂ have been developed, many of these have failed in clinical trials due to poor pharmacokinetic profiles. Of concern is the ability to deliver the inhibitors to the diseased tissue sites. Liposomes were first reported by Alec Bangham in the 1960's, and have been extensively modified and used as drug carriers due to their ability to encapsulate both hydrophobic and hydrophilic drugs [86].

Recent studies, including those from our own laboratory, have engineered liposomes to target pathologies that overexpress sPLA₂ and enhance the release of drugs at diseased tissue [87-89]. sPLA₂-targeted nanoparticles are composed of phospholipids that are preferentially cleaved by sPLA₂, such as those with negatively charged and altered levels of phospholipids containing other zwitterionic head groups and shorter fatty acyl chains [89-91]. This preference is likely a result of the many positively charged amino acids present in sPLA₂'s protein sequence (Figure 2B-D), especially at the ICS where these residues are believed to increase the interaction with the negatively charged phospholipid head group. These delivery systems have been used to target pathologies that overexpress sPLA₂ at their inflammatory sites such as arthritis and cancer [91, 92].

There have been many proposed modifications of the phospholipid component of liposomes to improve drug release at sPLA₂-targeted sites [87, 89]. One such modification was the incorporation of low (> 10%) amount of zwitterionic phosphatidylethanolamine (PE) into sterically stable liposomes (SSL). These modified liposomes, termed sPLA₂ responsive liposomes (SPRL), showed enhanced drug release in the presence of Group IIA and III sPLA₂, and were more effective than SSL (which are similar to clinically used formulations) in limiting hormone refractory prostate cancer tumor growth in mouse models [89]. One interesting finding with SPRL is that their activity or effectiveness was not altered by LY3711727. This suggests that liposomal efficacy may not completely depend on sPLA₂ activity. Other mechanisms may be involved including those mediating endosomal uptake facilitated by PLA2R and membrane-bound proteoglycan.

6. Perspectives and Conclusions

A current challenge in the field is elucidating the biological effect of specific sPLA₂ isoforms in diseases as well as identifying biological inhibitors. Further complicating these studies is the fact that these biological inhibitors may be cell-, tissue- and disease-dependent. These biological inhibitors include glycosaminoglycans and PLA2R1. Investigations to identify and characterize the sPLA₂ interactome should be a rewarding area of research to identify drug targets for diseases that involve inflammation, the immune system and cancer.

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Abbreviations

AA	arachidonic acid
AdPLA₂	adipose specific phospholipase A ₂
BCL-6	B cell lymphoma-6
C/EBP	CCAAT-enhancer-binding proteins
COX	cyclooxygenase
cPLA₂	cytosolic phospholipase A ₂
ECM	extracellular matrix
FGF	fibroblast growth factor
FGFR	fibroblast growth factor receptor
GPCR	G protein-coupled receptors
HSPG	heparan sulfate proteoglycan
ICS	interfacial catalytic surface
IκB	inhibitory κB protein

IL	interleukin
IMN	idiopathic membranous nephropathy
iPLA₂	calcium-independent phospholipase A ₂
5-LOX	5-lipoxygenase
LP	lysophospholipid
LyPLA₂	lysosomal phospholipase A ₂
LysoPLD	Lysophospholipase D
NF-κB	nuclear factor-kappa B
PAF-AH	platelet activating factor acetylhydrolases
PC	phosphatidylcholine
PE	phosphatidylethanolamine
PLA₂	phospholipase A ₂
PLA₂R	phospholipase A ₂ receptor
PLA₂R1	M-type phospholipase A ₂ receptor
PPAR	peroxisome proliferator-activated receptors
PS	phosphatidylserine
RTKs	Receptor tyrosine kinases
sPLA₂	secretory phospholipase A ₂
TG	triglycerides
TNF-α	tumor necrosis factor-alpha

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Figure 1. Regulation of sPLA₂ expression by cell signaling and gene regulation

Degradation products from the cleavage of phospholipids by sPLA₂ and cPLA₂ (AAs and LPs) can be further metabolized to second signaling messengers (LPA, PGs, LTs) to trigger cell signaling pathways by activating GPCR and cytokine receptors. These events can result in amplification of pro-inflammatory cytokine production and intensification of inflammation. Activation of NF- κ B, C/EBP, and epigenetic events can also enhance sPLA₂ expression, while activation of PPAR and BCL-6 can suppress their expression at mRNA level. PM = plasma membrane.

Figure 2. sPLA₂ and their interactomes

A. Binding of sPLA₂ to PLA2Rs can trigger endocytosis, migration and differentiation. **B** and **C.** The 3-D structures of sPLA₂ IIA (PDB: 3UB8) (**B**) and X (PDB: 1LE6) (**C**) are similar, but their surface charge is distinctly different due to distribution of positively (K and R amino acids: blue stick/surface/highlight) and negatively charged amino acid (E and D amino acids: orange surface or yellow highlight). These sPLA₂ isoforms have conserved catalytic dyad (His/Asp – red) and calcium binding sites (magenta). **D.** The amino acid sequence comparison between Group IIA, X, and V shows similar distribution of charged amino acid between Group IIA and V. It suggests similar interactions between Group IIA and V with their interactomes based on the electrostatic interaction.

Table 1

Phospholipase A2 Classification and Pathologies Associated with Secretory Phospholipase A2

PLA ₂ Family	Group	Gene Name	Source	MW (kDa)	Catalytic Residues	[Ca ²⁺]	Pathologies
cPLA ₂	IVA to F	PLA2G4A to F	Human/murine	60-85	Ser/Asp	μM	N/A
iPLA ₂	VIA to F	PLA2GVIA to F	Human/murine	28-146	Ser/Asp	None	N/A
PAF-AH	VIIA & VIIB	PLA2G7A, PLA2G7B	Human/murine/porcine/bovine	40-45	Ser/His/Asp	None	N/A
	VIIIA & VIIB	PLA2G8A, PLA2G8B	Human	~26	Ser/His/Asp		N/A
Ly-PLA ₂	XV	PLA2G15	Human	~45	Ser/His/Asp	None	N/A
AdPLA ₂	XVI	PLA2G16	Human Adipocyte	~18	His/Cys	None	N/A
sPLA ₂	IA	PLA2G1A	Cobras and Kraits	~14	His/Asp	mM	N/A
	IB	PLA2G1B	Human/porcine pancreas	~14			Pancreatic acinar carcinoma [93], dry eye disease [94]
	IIA	PLA2G2A	Human synovial/Rattlesnakes	~14			Arthritis, atherosclerosis, sepsis, cancer [20, 23-25, 95]
	IIB	PLA2G2B	Gaboon viper	~14			N/A
	IIC	PLA2G2C	Rat/murine testis	~14			N/A
	IID	PLA2G2D	Human/murine/pancreas/spleen	~14			Chronic obstructive pulmonary disease [96], asthma [97]
	IIE	PLA2G2E	Human/murine/brain/heart/uterus	~14			Ulcerative colitis [98], chronic rhinosinusitis [99]
	IIF	PLA2G2F	Human/murine/testis/embryo	~14			Atopic dermatitis [100], colorectal cancer [101]
	III	PLA2G3	Lizard/bee/human/murine	~55			Colorectal cancer [101], atherosclerosis [102]
	V	PLA2G5	Human/murine heart/lung/macrophage	~14			Arthritis, atherosclerosis, sepsis, cancer, chronic hepatitis [20, 23-25]
	IX	PLA2G9	Metazoan	~14			N/A
	X	PLA2G10	Human spleen/thymus/leukocyte	~17			Arthritis, atherosclerosis, sepsis, cancer [20, 23-25]
	XIA	PLA2G11A	Green rice shoots	~13			N/A
	XIB	PLA2G11B	Green rice shoots	~13			N/A
	XIIA	PLA2G12A	Human/murine	~14			Kuhnt-Junius degeneration [103], malignant glioma [104]
	XIIB	PLA2G12B	Human/murine	~14			Acute pancreatitis [105]
	XIII	PLA2G13	Parvovirus	<10			N/A
	XIV	PLA2G14	Symbiotic fungus/bacteria	13-19			N/A

This table has been adapted from [2, 106]

N/A = Not applicable for the purpose of this review. Only sPLA₂ human-related diseases are considered

Table 2

sPLA2 small molecule inhibitors and peptide inhibitors

Inhibitor	Structure ^d	CAS	IC ₅₀ (nM) ^b				References
			Group IIA sPLA ₂	Group V sPLA ₂	Group X sPLA ₂	Group X sPLA ₂	
YM-2673		144337-18-8	Not tested	110	>1,600	[107]	
LY311727		164083-84-5	8,000	36	Not tested	[108]	
LY315920 (Varespladib)		172732-68-2	Not tested	77	15	[108]	
Indole-based inhibitors (Compound 11c)		258262-50-9	>1600	100	15	[109]	
KH064		393569-31-8	Not tested	>5,000	Not tested	[110]	
(+)-3-O-[4-(4,5-dihydro-5-oxo-1,2,4-4H-oxadiazol-3-yl)phenyl]-2-Otetradecyl-1-Otriphenylmethylglycerol		935851-73-3	575	660	1,250	[111]	
2,2'-biphenyl-3,4'-diylbis(4H-chromen-4-one)		20081-60-1	>100,000	23,200	>100,000	[112]	

^a Structures were collected by searching SciFinder® (<https://scifinder.cas.org/>) using the CAS number for each compound.^b IC₅₀ data are based on data derived with purified human sPLA₂ isolated from bacteria system.