Review Article

Diverse Functions of Secretory Phospholipases A₂

Preetha Shridas and Nancy R. Webb

1 Department of Internal Medicine, Division of Endocrinology and Cardiovascular Research Center, University of Kentucky, 900 S. Limestone, 567 Wethington Building, Lexington, KY 40536-0200, USA
2 Molecular and Biomedical Pharmacology, University of Kentucky, Lexington, KY 40536-0200, USA

Correspondence should be addressed to Preetha Shridas; pshri2@uky.edu

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Phospholipase A₂ enzymes (PLA₂s) catalyze the hydrolysis of glycerophospholipids at their sn-2 position releasing free fatty acids and lysophospholipids. Mammalian PLA₂s are classified into several categories of which important groups include secreted PLA₂s (sPLA₂s) and cytosolic PLA₂s (cPLA₂s) that are calcium-dependent for their catalytic activity and calcium-independent cytosolic PLA₂s (iPLA₂s). Platelet-activating factor acetylhydrolases (PAF-AHs), lysosomal PLA₂s, and adipose-specific PLA₂ also belong to the class of PLA₂s. Generally, cPLA₂ enzymes are believed to play a major role in the metabolism of arachidonic acid, the iPLA₂ family to membrane homeostasis and energy metabolism, and the sPLA₂ family to various biological processes. The focus of this review is on recent research developments in the sPLA₂ field. sPLA₂s are secreted enzymes with low molecular weight (with the exception of GII sPLA₂), Ca²⁺-requiring enzymes with a His-Asp catalytic dyad. Ten enzymatically active sPLA₂s and one devoid of enzymatic activity have been identified in mammals. Some of these sPLA₂s are potent in arachidonic acid release from cellular phospholipids for the biosynthesis of eicosanoids, especially during inflammation. Individual sPLA₂ enzymes exhibit unique tissue and cellular localizations and specific enzymatic properties, suggesting their distinct biological roles. Recent studies indicate that sPLA₂s are involved in diverse pathophysiological functions and for most part act nonredundantly.

1. Introduction

Secreted phospholipases A₂ (sPLA₂s) are secreted from a variety of cells and act in autocrine or paracrine manners on cell membranes and other extracellular phospholipids, including lipoprotein particles, surfactant and dietary lipids, microbial membranes, and microvesicles [1]. Even though sPLA₂s are considered to act as extracellularly requiring millimolar concentrations of Ca²⁺, few in vitro reports also indicate possible intracellular activity prior to or during secretion [2]. To date, eleven sPLA₂ enzymes, group IB (GIB), group IIA (GIIA), group IIC (GIIC), group IID (GIID), group IIE (GIIE), group IIF (GIIF), group III (GI), group V (GV), group X (GX), group XI (GXIA), and group XII (GXIB), have been identified in mammals [3–5]. GIH sPLA₂ is an atypical sPLA₂ that contains unique N-terminal and C-terminal domains and a central sPLA₂ domain, the S domain, which has higher homology with bee venom sPLA₂ (a prototypic group III enzyme) than with other known mammalian sPLA₂s [6]. Another unique member is GXIIIB protein which has structural features similar to those of the GXIIIA sPLA₂ subgroup. However, GXIIIB sPLA₂ has a mutation in the active site, which replaces the canonical histidine by a leucine thus making the enzyme catalytically inactive [7].

Recently some unexpected novel roles for individual sPLA₂s have been described, which form the basis of this review. The review does not provide details of several in vitro studies regarding substrate specificities, biochemical properties, and structure and sequence homology of different sPLA₂s as they have been covered by a number of earlier reviews [4, 8–11] but focuses on several recently described physiological functions of selected sPLA₂s.

2. Functions of sPLA₂s in Asthma and Inflammation

Eicosanoids, including leukotrienes and prostaglandins, play complex roles in the pathogenesis of airway inflammation
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Prostaglandin D2 (PGD2) triggers asthmatic responses [12], while prostaglandin I2 (PGI2) and prostaglandin E2 (PGE2) serve as inhibitors of allergic response [15, 16]. Leukotrienes (LTs) generally promote the development of asthma; 5-lipoxygenase products are found to be necessary for ovalbumin-induced airway responsiveness in mice [17]. In contrast, 12/15-lipoxygenase products are generally considered protective [18, 19]. PGD2; thromboxane A2 (TXA2) and cysteinyl leukotrienes (cys-LTs) are reported to function as bronchoconstrictors while PGE2 is considered to act as bronchodilator [20].

The rate-limiting step in eicosanoid biosynthesis is the release of unesterified arachidonic acid (AA) from the sn-2 position of membrane phospholipids by PLA2s [21]. Some sPLA2s find importance in the development of asthma through the generation of arachidonic acid (AA). However, the role of individual sPLA2s in the disease depends on the metabolic fate of AA released. It is interesting to observe that AA released from the same cell type by different classes of PLA2s is metabolized differently. The reason for this difference could be the difference in cellular localization and mode and rate of release. Using specific inhibitors, it has been demonstrated that sPLA2 can play opposing roles in asthma pathophysiology with sPLA2s linked to the production of the bronchoconstrictor Cys-LT whereas cPLA2 promotes the production of the bronchodilator PGE2. In cells that play a key role in asthma, namely, human eosinophils and basophils, PGE2 is produced from a cPLA2-linked pool [20, 22–24].

sPLA2s are found at high levels in bronchoalveolar lavage (BAL) fluid and bronchotraheal smooth muscle cells [25, 26]. Studies have identified an increase in sPLA2 activity in BAL fluid from subjects with asthma [27] and also an increase in sPLA2 activity after allergen challenge [28–30]. Four sPLA2s (GIB, GIIA, GV, and GX) are reported to be expressed in human lungs, the highest being GX sPLA2, followed by GV- and GIIA-sPLA2s in the BAL fluid of patients with asthma [31]. GX sPLA2 displays the greatest potency among mammalian sPLA2s in hydrolyzing the phosphatidylcholine- (PC-) rich extracellular leaflet of mammalian plasma membranes [1, 32] and has the strongest ability to initiate eicosanoid synthesis in mammalian cells [32]. Immunohistochemical studies indicate that GX sPLA2 is expressed in airway epithelial cells and macrophages in BAL fluid [33]. GX sPLA2 has the highest expression in the airways of patients with asthma [31, 34] and is strongly expressed in the airway epithelium relative to the other sPLA2 genes [31]. Human GX sPLA2 is also found in induced sputum samples in patients with exercise-induced asthma and its levels in BAL fluid correlated with asthma severity [31]. The activity of GX sPLA2 appears to correlate with lung functions, neutrophil recruitment, and prostaglandin levels [31]. GX sPLA2 is known to specifically initiate Cys-LT synthesis by eosinophils [35]. It is also believed that GX sPLA2 is released from airway epithelial cells and may act on eosinophils in a paracrine manner to produce lysophosphatidylcholine (LPC), which in turn triggers Ca2+ influx leading to activation of cPLA2α and thereby production of Cys-LT [35]. The expression of GX sPLA2 is upregulated by cytokines implicated in asthma, including TNF/IL-1β, IL-17, and to a lesser extent IL-13 but is suppressed by IL-4 [36]. Deficiency of GX sPLA2 in mice dampens the development of asthma. Ovalbumin-(OVA-) induced Cys-LT and PGD2 production were near fully blocked in GX sPLA2-deficient mice (GX KO mice), indicating this as a possible mechanism [33]. Insertion of the human GX sPLA2 gene in GX KO mice restored the capacity for the development of airway inflammation, which could further be abolished by an active site-directed inhibitor of human GX sPLA2 [37]. These results indicate inhibition of GX sPLA2 as a novel therapeutic target in asthma.

The action of GV sPLA2 in asthma and other respiratory diseases appears to be complex. GV sPLA2 expressed by both myeloid cells and lung-resident nonmyeloid cells and participates in the innate immune response to pulmonary infection [38]. GV sPLA2 is also induced in bronchial epithelial cells in antigen-challenged mice and intratracheal application of an antibody against GV sPLA2 ameliorates airway inflammation, suggesting a proinflammatory action of this enzyme in the airway [39]. GV sPLA2-deficient mice (GV KO) exposed to an extract of house dust mite Dermatophagoides farinae had markedly reduced pulmonary inflammation and goblet cell metaplasia compared with wild-type (WT) mice [40]. GV KO mice had also impaired Th2-type adaptive immune responses to D. farinae compared with WT mice. Processing of antigen-presenting cells is significantly dampened in GV KO mice and the mice also displayed a reduction of Th2 polarization, thus preventing further propagation of inflammation [40]. Thus, GV sPLA2 appears to function in a dual manner in airway-resident cells, one to facilitate airway inflammation possibly via surfactant degradation and secondly in antigen-presenting cells to regulate antigen processing and thereby the Th2 immune response. In another study, Degousee et al. [38] demonstrated that GV sPLA2 is important for leukocyte recruitment to the lung and for efficient pulmonary clearance of bacteria in a mouse model of E. coli pneumonia. GV KO mice cleared bacteria from lung parenchyma and the alveolar space less efficiently than the wild type (WT) mice after pulmonary infection with E. coli. Similarly, in severe systemic candidiasis, reduced clearance of Candida albicans was observed in GV KO mice compared to similarly treated WT mice. However, cytokine production and eicosanoid generation were not impaired by the lack of GV sPLA2 [41]. Injection of lipopolysaccharide (LPS) into the air pouches of mice results in the attraction of leukocytes and deficiency of GV sPLA2 is found to reduce leukocyte recruitment in this model [42]. GV sPLA2 likely plays a role in hydrolyzing pulmonary surfactants. Transgenic mice that overexpress mouse GV sPLA2 (GV-Tg) die of respiratory failure during the neonatal period [43]. In these mice, excessive hydrolysis of the pulmonary surfactant has been observed, which interrupts respiratory function [43]. The lungs of these mice exhibit atelectasis with thickened alveolar walls and narrow air spaces and pronounced infiltration of macrophages, but with modest changes in eicosanoid levels. This severe pulmonary defect in GV-Tg mice is attributable to marked reduction of the
lungsurfactantphospholipids,dipalmitoyl-PC, andphosphatidylglycerol. Asthma and airway inflammation is thus one example where the two closely related GV and GX sPLA_2s exhibit different modes of action in disease pathophysiology. In contrast with GV-Tg mice, mice overexpressing human GX sPLA_2 (GX-Tg) do not have an overt defect in the lung. The reason for the lack of phenotype is attributed to the existence of GX sPLA_2 as an inactive proenzyme in the transgenic mice, which becomes activated upon inflammatory challenge [43].

sPLA_2s are also involved in other inflammatory diseases. A recent report indicates that GX sPLA_2 protein and mRNA expression are increased in the lungs of mice following H1N1 pandemic influenza infection [44]. Both epithelial cells and leukocytes were found to be sources of GX sPLA_2 during infection and GX sPLA_2 expression was detected in epithelial cells 3 days prior to the infiltration of leukocytes. The targeted deletion of GX sPLA_2 led to increased survival in mice challenged with H1N1. Lack of GX sPLA_2 resulted in decreased levels of PGD2, LTB4, cysLT, PGE2, and Lipoxin A4 and increased adaptive immune responses at 3 days following H1N1 infection indicating the role of GX sPLA_2 in the generation of these lipid mediators following infection [44]. Thus it appears that deficiency of GX sPLA_2 in mice was beneficial to the host during influenza infection.

GX sPLA_2 is not the only isoform associated with inflammation. Several sPLA_2 isoforms are expressed by inflammatory cells such as neutrophils, eosinophils, basophils, T cells, monocytes, macrophages, and mast cells [45, 46]. The expression of some of the sPLA_2s is known to be upregulated with inflammation, while the expression of some other sPLA_2s appears to be constitutive [4, 47, 48]. The level of GIIA sPLA_2 in serum correlates with the severity of inflammatory diseases such as rheumatoid arthritis and sepsis. Its expression is markedly induced by proinflammatory stimuli in a wide variety of cells and tissues of various animal species [5]. Expression of GIIA sPLA_2 is induced by inflammatory stimuli such as IL-6, TNFx, IFN-γ, LPS, cAMP-elevating agents, and phorbol esters [4, 48]. GIIA sPLA_2 is thought to play an important role in innate immunity. Transgenic mice expressing human GIIA sPLA_2 are protected against group B streptococcal (GBS) infection. Consistent with this finding, serum isolated from humans with acute invasive GBS infection has increased levels of GIIA sPLA_2 [49]. Similarly, transgenic mice expressing human GIIA sPLA_2 are found to be resistant to experimental Bacillus anthracis infection [50, 51]. GV sPLA_2 is present in the phagosomes of macrophages and regulates phagocytosis [52]. Mammalian sPLA_2s are also known to participate in host defense against viruses. In vitro studies performed in mammalian cell lines indicate that GV and GX sPLA_2s prevent adenoviral infections in mammalian cell lines by preventing the entry of viruses into the cells, an effect that is mimicked by LPC [53].

3. Role of sPLA_2s in Atherosclerosis

Atherosclerosis is a chronic inflammatory disease of the vessel wall characterized by the accumulation of macrophages filled with lipids and fibrotic material. According to the "response-to-retention" hypothesis, the disease is initiated by the accumulation of modified lipoproteins in the vessel wall, such as oxidized LDL. It has been shown by several investigators that several sPLA_2 isoforms are expressed in atherosclerotic lesions [54, 55], some of which are capable of hydrolyzing phospholipids (PLs) present in low density lipoprotein (LDL). There is now accumulating evidence that several sPLA_2 isoforms, namely GIIA, GIIH, GV-, and GX-sPLA_2s, play significant, distinct, or overlapping roles in one or several steps of atherosogenesis [56–63].

In human aortic tissues, GIIA sPLA_2 is more strongly expressed in the arterial intima of atherosclerotic than of nonatherosclerotic tissue. The majority of GIIA sPLA_2 is localized along the extracellular matrix, associated with collagen fibers and other extracellular matrix structures [64]. GIIA sPLA_2 binds to cell-surface proteoglycans with high affinity and this binding to proteoglycans increases their potency to hydrolyze phosphatidylcholine (PC) present in LDL [65]. Circulating levels of GIIA sPLA_2 are an independent risk factor for cardiovascular events in humans [66]. Studying the in vivo function of GIIA sPLA_2 in host defense and inflammatory conditions has been limited by the fact that inbred C57BL/6 mouse strain is naturally deficient for this sPLA_2 [67]. Most of the studies relating to the in vivo effects of the enzyme have been carried out in transgenic mouse models or mice in which the recombinant enzyme has been injected. Transgenic mice that constitutively express GIIA sPLA_2 exhibit spontaneous atherosclerosis in the absence of hyperlipidemia indicating that this enzyme may take part in atherosogenesis and not just serum marker for the disease [68]. These mice also had elevated VLDL/LDL cholesterol levels and lower HDL levels with decreased paraoxonase activity, suggesting that changes in lipoproteins may play a role in GIIA sPLA_2’s atherogenic effect [68]. The decreased HDL is thought to be due to an increase in hepatic selective uptake of HDL-cholesterol ester and plasma clearance of HDL [69, 70]. Low density lipoprotein receptor-deficient (LDLR-/−) mice overexpressing GIIA sPLA_2 in bone marrow-derived cells demonstrated larger atherosclerotic lesion formation compared to WT mice in both aortic sinus and aortic arch when fed a high fat diet [71]. The transgenic mice had increased collagen deposition in lesions independent of lesion size without any effect on systemic cholesterol levels indicating a proatherosclerotic role for GIIA sPLA_2 [56].

GV sPLA_2 is detected in human and mouse atherosclerotic lesions and hyperlipidemic high fat diet upregulates expression of GV sPLA_2 in mice [72]. In vitro studies using recombinant proteins indicate that GV sPLA_2 is much more potent in hydrolyzing lipoprotein PC than GIIA sPLA_2 [73]. Hydrolysis of LDL by GV sPLA_2 decreases the particle size, induces spontaneous aggregation and enhances foam cell formation in cultured mouse peritoneal macrophages indicating a potential proatherosclerotic role for the enzyme [74]. Enhanced uptake of GV sPLA_2-modified LDL was independent of scavenger receptors SR-A and CD36 but dependent on cell-surface proteoglycans [63]. In vitro studies performed in macrophages indicated that syndecan-4 is involved in the uptake of GV sPLA_2-modified LDL particles [75]. Fatty acids released by GV sPLA_2 hydrolysis of LDL stimulate nuclear
factor-kappaB (NF-κB) activation in macrophages resulting in the production of proinflammatory cytokines [63]. The proatherogenic role of GV sPLA₂ was demonstrated later by gain-of-function and loss-of-function studies in LDLR<sup>−/−</sup> mice; whereas retroviral-vector-mediated overexpression of GV sPLA₂ in bone marrow-derived cells increased lesion size, deletion of GV sPLA₂ in bone marrow-cells reduced atherosclerosis [61]. Subsequent studies demonstrated that lack of GV sPLA₂ in bone marrow-derived cells does not alter lesion development in apolipoprotein E-deficient (apoE<sup>−/−</sup>) mice, possibly due to marked differences in lipoprotein particles that circulate in apoE<sup>−/−</sup> mice compared to LDLR<sup>−/−</sup> mice [76].

GIII sPLA₂-modified LDL, like GV sPLA₂- or GX sPLA₂-treated LDL, facilitated the formation of foam cells from macrophages ex vivo. Accumulation of GIIII sPLA₂ was detected in atherosclerotic lesions of humans and apoE<sup>−/−</sup> mice. Furthermore, following an atherogenic diet, aortic atherosclerotic lesions were more severe in GIII sPLA₂ transgenic mice than in control mice on the apoE<sup>−/−</sup> background, in combination with elevated plasma lysophosphatidylcholine and TXB2 levels. These results collectively suggest a potential functional link between GIII sPLA₂ and atherosclerosis [60].

GX sPLA₂ is present in human atherosclerotic lesions. In vitro studies indicate a proatherogenic role for GX sPLA₂ [55, 57]. Consistent with this belief, our lab has observed that upon angiotensin II infusion, deficiency of GX sPLA₂ in a apoE<sup>−/−</sup> background reduces the development of atherosclerosis as indicated by reduction of lesion size in the aortic arch area [77]. However, a later study performed in LDLR<sup>−/−</sup> mice indicated deficiency of GX sPLA₂ in the bone marrow-derived cells to accelerate the development of atherosclerotic lesions in the aortic sinus through an exacerbated Th1 immunoinflammatory response [78], indicating a protective role for GX sPLA₂ in the development of atherosclerosis. The reasons for the discrepancy between the studies are not very clear, maybe due to differences between the mouse strains (apoE<sup>−/−</sup> vs LDLR<sup>−/−</sup>) or the inducer high fat diet versus angiotensin II between the two models.

In a double-blind, randomized, multicenter trial in patients with recent acute coronary syndrome (ACS), varespladib, a nonspecific pan-sPLA₂ inhibitor, did not reduce the risk of recurrent cardiovascular events and significantly increased the risk of myocardial infarction (MI). It was thus concluded that the sPLA₂ inhibition with varespladib may thus be harmful and is not a useful strategy to reduce adverse cardiovascular outcomes after ACS [79].

4. Effect of sPLA₂ on Male Reproductive System

Male reproductive system consists of organs that act together to produce and deliver functional spermatozoa into the female reproductive tract. After the differentiation process of male germ cells, spermatozoa exit the seminiferous tubules of the testis through the efferent ducts towards the epididymis. During their transition from the caput to the cauda epididymidis, spermatozoa undergo significant morphological and biochemical modifications which lead to acquisition of their forward motility and ability to recognize and fertilize oocytes [80]. Ejaculated mammalian sperm must undergo a maturation process called capacitation before they are able to fertilize an egg. Different sPLA₂s are expressed in male reproductive organs and in sperm cells, among which mouse GX sPLA₂ is found to play an important role in sperm motility and fertilization outcome during capacitation. Several studies have suggested a role for members of sPLA₂ family in capacitation, acrosome reaction (AR), and fertilization. GX sPLA₂ is the major enzyme present in the acrosome of spermatozoa and it is released in an active form during capacitation through spontaneous AR in mice [81]. In their study, Escoffier et al. [81] reported that GX KO male mice produced smaller litters than wild-type male siblings when crossed with GX KO females. Further they reported that spermatozoa from GX KO mice exhibited lower rates of spontaneous AR and that this was associated with decreased in vitro fertilization (IVF) efficiency due to a drop in the fertilization potential of the sperm and an increased rate of aborted embryos. Mouse GX sPLA₂ acts as a potent inhibitor of sperm motility of both capacitated and noncapacitated sperms [82]. Though the mechanism for this regulation is unclear, this effect of GX sPLA₂ is dependent on its catalytic function and hence might involve changes in phospholipid metabolism. It is also found that endogenous GX sPLA₂ is spontaneously released during acrosome reaction and modulates motility of capacitated sperms [82]. Mouse GX sPLA₂ has a unique property of improving fertilization outcome during capacitation [83] and hence has a unique usefulness in improving outcomes in in vitro fertilization. This property of mouse GX sPLA₂ is not mimicked by other sPLA₂s including human GX or GV sPLA₂ or progesterone; this is yet another example of how functions and specificities differ for individual sPLA₂s. Deficiency of GIIII sPLA₂ in mice leads to defective sperm maturation and asthenozoospermia [84]. The enzyme is expressed in mouse proximal epididymal epithelium. The deficient mice had normal spermatogenesis but displayed hypomotility and demonstrated impaired fertilization efficiency. The defect was attributed to impaired phospholipids remodeling in the sperm during epididymal transit. The enzyme was also found to contribute to gonadal 12/15-lipoxygenase metabolites, indicating important role played by this enzyme in phospholipid and eicosanoid metabolism in male reproductive organs.

5. Unexpected Proteolytic Properties of sPLA₂

As summarized above, sPLA₂s have been associated with the development of atherosclerosis, which was related to its ability to hydrolyze phospholipids on lipoprotein particles. A novel report by Cavigiolio and Jayaraman [85] indicated that certain sPLA₂s are capable of acting as proteolytic enzymes. They demonstrated the effect by hydrolyzing the apolipoprotein A1 (apoA1) on HDL. Incubation of sPLA₂ (GII, GIIA, and bee venom GIII sPLA₂) with lipid-free apoA1 produced protein fragments in the range of 6–15 suggesting specific
and direct reaction of sPLA₂ with apoA-I. Mass spectrometry analysis of isolated proteolytic fragments indicated at least two major cleavage sites at the C-terminal and the central domain of apoA-I. ApoA-I proteolysis by sPLA₂ was Ca²⁺- independent, implicating a different mechanism from the Ca²⁺-dependent sPLA₂-mediated phospholipid hydrolysis. Inhibition of proteolysis by benzamidine suggests that the proteolytic and lipolytic activities of PL₂₃ proceed through different mechanisms. The proteolytic potential of other sPLA₂s have not yet been reported.

6. GX sPLA₂ as a Regulator of Liver X Receptor Activation

Our group recently reported a novel role of GX sPLA₂ in suppressing macrophage expression of ATP-binding cassette transporter Al (ABCA1) and ATP-binding cassette transporter GI (ABCG1) [86]. ABCA1 and ABCG1 play key roles in macrophage cholesterol homeostasis by exporting excess cellular free cholesterol to extracellular acceptors [87, 88]. The nuclear hormone receptors, liver X receptors α and β (LXRα and β) act as intracellular cholesterol sensors and induce the expression of target genes including ABCA1 and ABCG1 [89, 90]. We made the novel observation that GX sPLA₂ negatively regulates expression of ABC transporters in macrophages by suppressing LXR activation [86]. Peritoneal macrophages isolated from mice deficient in GX sPLA₂ exhibit significantly increased expression of ABCA1/ABCG1 and increased cellular cholesterol efflux with a consequent reduction in cellular free cholesterol content [86]. This effect of GX sPLA₂ is dependent on its catalytic function and is mimicked by the exogenous addition of free arachidonic acid. Further, the effect of GX sPLA₂ is abolished when LXR α- and β-expression is suppressed in macrophages. Polyunsaturated fatty acids (PUFAs) including arachidonic acid (AA) have been shown to act as antagonists for LXR activation by interacting with the ligand binding domain [91, 92]. PUFAs compete with LXR agonists to block LXR activation and hence their effects are reversed by LXR agonists including T0901317. Like PUFAs, GX sPLA₂ also suppresses LXR activation through a mechanism involving the C-terminal portion of LXR that spans the LXR binding domain. However, the effect of GX sPLA₂ on LXR activity is only partially reversed by LXR agonists indicating that the hydrolytic products generated by GX sPLA₂ do not act in a manner that simply involves direct competition for agonist binding. This effect of GX sPLA₂ was found to be specific to LXR and not observed for other nuclear receptors tested including the glucocorticoid receptor [93].

Cholesterol accumulation in macrophages is associated with a proinflammatory phenotype [94–96]. Changes in plasma membrane free cholesterol/lipid raft content modulate macrophage inflammatory responses through a myeloid differentiation primary response 88- (MyD88-) dependent signaling pathway which is independent of an ER stress response [94, 97]. In macrophages, suppressed expression of ABCA1 and ABCG1 results in decreased cholesterol efflux, increased cellular free cholesterol levels, and enhanced MyD88-dependent signaling. We determined that macrophages overexpressing GX sPLA₂ have significantly increased lipid raft content consistent with GX sPLA₂’s suppressive effect on ABCA1/ABCG₁ expression. This increased cholesterol accumulation in macrophages was associated with enhanced macrophage inflammatory responses. Transgenic overexpression or exogenous addition of recombinant GX sPLA₂ to macrophages results in increased induction of TNFα, IL6, and cyclooxygenase-2 expression in J774 macrophage-like cells in response to lipopolysaccharide (LPS) treatment [98]. This effect is abolished in macrophages that lack either toll-like receptor 4 (TLR4) or MyD88 or when cellular free cholesterol was normalized using cyclodextrin. Mice deficient in GX sPLA₂ demonstrate dampened inflammatory responses as evidenced by significantly reduced plasma concentrations of inflammatory cytokines, including TNFα, IL6, and IL-1β following LPS treatment. Taken together these findings indicate that GX sPLA₂ modulates plasma membrane free cholesterol and lipid raft content by suppressing the expression of LXR target genes ABCA1/ABCG1, thereby contributing to inflammatory responses in macrophages. These findings are consistent with the report by Sato et al. [99] demonstrating that pharmacological inhibition of sPLA₂ significantly attenuates the acute lung inflammation and injury induced by LPS in C57BL/6J mice. The authors also concluded that the effects were most likely due to inhibition of GX sPLA₂ and/or GV sPLA₂. Consistent with the above observations, studies in our lab showed that deficiency of GX sPLA₂ in apoE⁻/⁻ mice significantly reduced abdominal aortic aneurism formation induced by angiotensin II (AngII). ApoE⁻/⁻ mice lacking GX sPLA₂ demonstrated significantly blunted induction of inflammatory mediators in the aorta after AngII infusion compared to apoE⁻/⁻ mice [77].

Another interesting phenotype demonstrated GX KO mice related to LXR activation in adrenal glands. C57BL/6 mice deficient in GX sPLA₂ have ~80% higher basal and ACTH-induced plasma corticosterone levels compared to wild-type controls [100]. GX sPLA₂-deficient mice have no defect in the hypothalamic-pituitary axis as evidenced by normal ACTH levels in response to dexamethasone challenge [100]. Consistent with hypercorticosteronemia, primary adrenal cells from GX sPLA₂-deficient mice showed increased corticosterone release both under basal and ACTH-stimulated conditions. Further mechanistic studies indicated that GX sPLA₂ negatively regulates the expression of steroidogenic acute regulatory protein (STAR) in adrenal cells. STAR is a nucleoerly encoded mitochondrial protein that mediates the rate-limiting step of steroidogenesis by delivering cholesterol to the inner mitochondrial membrane [101]. STAR mRNA expression is under positive and negative regulation by a variety of transcription factors including AP-1, SF-1, C/EBP, Sp-1, GATA, DAX-1, SREBP-1a, and LXR [102, 103]. Administration of a synthetic LXR agonist increases plasma corticosterone levels and adrenal STAR mRNA expression in mice [103, 104]. A functional LXR-response element- (LXRE-) like sequence has been identified in the STAR promoter [103]. Consistent with its effect on
LXR activation as described above, GX sPLA₂ suppresses LXR-mediated StAR activation through a mechanism that is dependent on GX sPLA₂’s catalytic activity. Based on luciferase reporter assays, GX sPLA₂’s regulation of StAR expression requires an intact LXRE-like sequence in the StAR promoter, indicating the LXR-dependency for this effect [100].

LXR activation promotes adipogenesis and triglyceride accumulation in adipocytes [105, 106]. Consistent with the role of GX sPLA₂ in suppressing LXR activation, in vitro studies indicated that GX sPLA₂ suppressed triglyceride accumulation in adipocytes by blunting the expression of adipogenic genes including PPARγ, SREBP-1c, SCD1, and FAS. These genes are either direct or indirect targets of LXR. GX sPLA₂-deficient mice gained more weight than WT mice when fed a normal chow diet due to increased adiposity and increased adipocyte sizes [107]. The difference in body weight was not clearly evident until the mice are at least 4-5 months of age. There was no difference in food intake, energy balance, or oxygen consumption between the wild-type and deficient mice. However, there were alterations in fat storage by adipocytes. Effect on body weight has not been shown in all studies; Fujioka et al. have observed no change in body weight between GX sPLA₂-deficient mice and wild-type control mice [108]; this discrepancy could be due to differences in the type of housing of the mice in the two studies. Figure 1 shows the overall effects of GX sPLA₂’s regulation of LXR activity in mice.

7. Biological Functions of Catalytically Inactive GXIIB sPLA₂

GXIIB sPLA₂ is catalytically inactive due to the presence of a leucine residue in place of a canonical histidine that is essential for the enzymatic activity. GXIIB sPLA₂ is abundantly expressed in liver, small intestine, and kidney in both human and mouse species. Interestingly, the expression of this sPLA₂ is dramatically decreased in human tumors from the same tissues. The absence of enzymatic activity suggests that this protein may exert biological functions by acting as a ligand for as yet unidentified receptor(s) [7].

Hepatocyte nuclear factor-4 alpha (HNF-4α) is an important transcription factor governing the expression of genes involved in multiple metabolic pathways. Guan et al. reported that GXIIB sPLA₂ is a target gene for HNF-4α [109]. HNF-4α agonists induce GXIIB sPLA₂ expression in human hepatocarcinoma cells. Interestingly, GXIIB sPLA₂-deficient mice accumulate triglyceride, cholesterol, and fatty acids in the liver and develop severe hepatosteatosis resembling some of the phenotypes of liver-specific HNF-4α-deficient mice. These defects are in part due to compromised hepatic very low density lipoprotein secretion. Overexpression of HNF-4α in liver by adenoviral vector elevates serum triglycerides level in wild-type but not GXIIB deficient mice [109]. It will be interesting to investigate whether GXIIB sPLA₂ demonstrates proteolytic activity as described earlier, independent of their phospholipase activity which might account for the phenotype observed in the deficient mice.
8. Conclusions

With the increasing number of mouse strains with altered expression of individual sPLA₂s, considerable progress has been made in understanding the physiological functions of each of the isoforms. However, knockout/transgenic strains for many of the isoforms and mice with tissue-specific targeting are still largely unavailable. It is now quite clear that the functions of sPLA₂s are nonredundant, tissue/cell type-specific, and unique. It is therefore crucial to understand the factors contributing to the functional differences between these enzymes especially in a therapeutic point of view.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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