Trends in Cell & Molecular Biology
Vol. 7, 2012

Review

Unraveling the secrets of secreted Phospholipase A_2

Makoto Murakami* **and Yoshitaka Taketomi**

Lipid Metabolism Project, Tokyo Metropolitan Institute of Medical Science, 2-1-6 Kamikitazawa, Setagaya-ku, Tokyo 156-8506, Japan

ABSTRACT

Phospholipase A_2 (PLA_2) enzymes catalyze the hydrolysis of the *sn*-2 position of glycero-phospholipids to liberate free fatty acids and lysophospholipids. More than one third of the PLA_2 enzymes belong to the secreted PLA_2 ($sPLA_2$) family, which contains 11 isoforms. Individual $sPLA_2$s exhibit unique tissue and cellular localizations and enzymatic properties, suggesting their distinct pathophysiological roles. Since $sPLA_2$s are secreted, their target membranes should reside in the extracellular spaces. In comparison with the well-established roles of cytosolic PLA_2, the biological roles of $sPLA_2$s have remained unclear until recently. However, recent studies using transgenic and knockout mice for several $sPLA_2$ enzymes have revealed their distinct contributions to various biological events by producing lipid mediators, by promoting membrane remodeling, by modifying extracellular non-cellular lipid components such as surfactants and lipoproteins, or by degrading foreign phospholipids such as bacterial membranes and dietary lipids. In this review, emerging biological roles of $sPLA_2$ enzymes in pathophysiology will be discussed.

KEYWORDS: Phospholipase A_2, knockout mouse, inflammation, atherosclerosis, phospholipid

INTRODUCTION

The *$sPLA_2$* family represents structurally related, disulfide-rich, low molecular weight enzymes with a His-Asp catalytic dyad that hydrolyzes the ester bond at the *sn*-2 position of glycero-phospholipids in the presence of mM order of Ca^{2+}. In mammals, there are 11 $sPLA_2$ isoforms (IB, IIA, IIC, IID, IIE, IIF, III, V, X, XIIA and XIIB), which are subdivided into a classical group (group I/II/V/X) and two atypical groups (group III and group XII) [1, 2]. The classical *group I/II/V/X $sPLA_2$s* are closely related, 14-19-kDa secreted enzymes with a highly conserved Ca^{2+}-binding loop and a catalytic site as well as conserved disulfide bonds. *Group IB $sPLA_2$* has an N-terminal propeptide, a unique five amino acid extension termed the pancreatic loop in the middle part of the molecule, and a group I-specific disulfide between Cys^{11} and Cys^{77} [3]. *Group IIA $sPLA_2$* has a group II-specific disulfide linking Cys^{50} to the C-terminal Cys and a C-terminal extension of seven amino acids in length [4]. These structural properties are conserved in *group IID* and *group IIE $sPLA_2$s* [5, 6]. *Group IIC $sPLA_2$* has an additional disulfide bond between Cys^{87} and Cys^{93} in an extended loop region, yet it is a pseudogene in humans [7]. *Group IIF $sPLA_2$* has a unique 30-amino acid C-terminal extension with an additional Cys residue [8]. *Group V $sPLA_2$* does not possess the group I- and group II-specific disulfides and the group II-specific C-terminal extension [9]. *Group X $sPLA_2$* has both the group I- and II-specific disulfides, the group II-specific C-terminal extension, and the group I-specific propeptide [10]. *Group III $sPLA_2$* is an unusually large protein (55 kDa) consisting of large and unique N- and C-terminal domains and a central $sPLA_2$ domain, which is structurally distinct from the classical $sPLA_2$s [11]. *Group XII $sPLA_2$s*

*Corresponding author
murakami-mk@igakuken.or.jp

(XIIA and XIIB) are 19-kDa enzymes containing a central catalytic domain with a catalytic dyad, yet the locations of Cys residues outside the catalytic domain are far different from other $sPLA_2$s [12, 13]. As individual $sPLA_2$s display distinct cellular/tissue distributions and substrate preferences, they may play non-redundant, isoform-specific roles *in vivo*. In this article, we will make an overview of pathophysiological functions of four particular classical $sPLA_2$s (group IB, IIA, V and X) and an atypical $sPLA_2$ (group III), as revealed by their transgenic overexpression or gene targeting in mice. Phenotypes revealed by various $sPLA_2$ gene-manipulated mice are summarized in Table 1.

Group IB $sPLA_2$

Group IB $sPLA_2$ is often called "pancreatic $sPLA_2$", since it is abundantly expressed in pancreatic acinar cells [3]. After secretion from the pancreas into the duodenum lumen, an N-terminal hepta-peptide of the inactive zymogen is cleaved by trypsin to yield an active enzyme. The main role of this enzyme is digestion of dietary and biliary phospholipids, and perturbation of this process by gene disruption of its gene (*Pla2g1b*$^{-/-}$) results in decreased lipid digestion and absorption in the gut [14]. Thus, on a high-fat/carbohydrate diet, the intestinal production and thereby absorption of lysophosphatidylcholine (LPC), a hydrolytic product of dietary and biliary phosphatidylcholine (PC), are reduced in *Pla2g1b*$^{-/-}$ mice [15, 16]. Postprandial elevation of circulating LPC causes hepatic insulin resistance, and accordingly, *Pla2g1b*$^{-/-}$ mice are protected from diet-induced obesity, hepatic glucose intolerance, and hyper-lipidemia. Interestingly, oral application of a pan-$sPLA_2$ inhibitor protects mice from diet-induced obesity [17], and the *PLA2G1B* gene is mapped to an obesity susceptibility locus in humans [18].

Group IIA $sPLA_2$

Amongst the $sPLA_2$s, *group IIA* $sPLA_2$ is the only isozyme that can be detected in the circulation, particularly under pathologic conditions. Indeed, its levels in sera or inflammatory exudates correlate with the severity of inflammatory (e.g. rheumatoid arthritis and sepsis) or cardiovascular (e.g. myocardial infarction) diseases, and it is promptly released from secretory granules of inflammatory cells or is robustly induced by pro-inflammatory stimuli in various cells [19, 20]. Since *group IIA* $sPLA_2$ prefers anionic phospho-lipids to PC, the intact cellular plasma membrane, whose outer leaflet is PC-rich, is generally insensitive to the hydrolytic action of this enzyme. When overexpressed, *group IIA* $sPLA_2$ can augment arachidonic acid release and attendant prostaglandin production by cells stimulated with agonists, which may induce membrane rearrangement [21, 22]. Because of these features, *group IIA* $sPLA_2$ is often referred to as an "inflammatory $sPLA_2$". Despite these facts, however, the contribution of *group IIA* $sPLA_2$ to inflammation has remained a subject to debate until recently, since a natural frame-shift mutation in its gene (*Pla2g2a*) in C57BL/6 and 129Sv mice prevents the proper assessment of its functions by a classical gene targeting strategy (see below).

Several *in vivo* functions have been assigned to this enzyme using mice with transgenic over-expression of either human or mouse *group IIA* $sPLA_2$ (*Pla2g2a*-Tg). The first phenotype reported for *Pla2g2a*-Tg mice was skin abnormalities manifested by complete hair loss and epidermal hyperplasia, yet without any sign of inflammation [23]. Subsequently, it was shown that skin-specific *Pla2g2a*-Tg mice developed skin tumor after topical application of a carcinogen [24]. However, since *group IIA* $sPLA_2$ is not intrinsically expressed in the skin of C57BL/6 mice, the physiological importance of the skin abnormalities in *Pla2g2a*-Tg mice remains elusive. Presumably, the artificially overexpressed *group IIA* $sPLA_2$ may mimic the function of other $sPLA_2$ isoform(s) intrinsically expressed in the skin (see below).

The most probable physiologic function of *group IIA* $sPLA_2$ is the degradation of bacterial membranes, thereby providing the first line of antimicrobial defense of the host. In fact, *group IIA* $sPLA_2$ can efficiently kill Gram-positive bacteria (and to a lesser extent Gram-negative bacteria) by degrading bacterial membrane phospholipids (mainly composed of phosphatidyl-ethanolamine (PE)) *in vitro* [25, 26]. Accordingly, *Pla2g2a*-Tg mice or wild-type mice treated with recombinant *group IIA* $sPLA_2$ are resistant to local

Table 1. Summary of $sPLA_2$ gene-manipulated mice.

$sPLA_2$ isoform	Models	Homeostasis and diseases	Phenotypic outputs		Underlying mechanisms	References
IB	Knockout	Obesity and diabetes	Decrease	Protection against high fat diet-induced obesity and obesity-related insulin resistance	Gastrointestinal phospholipid digestion	14
	Knockout	Obesity and diabetes	Decrease	Protection from diet-induced obesity, hepatic glucose intolerance, and hyperlipidemia	Intestinal production and thereby absorption of LPC	15, 16
IIA	Transgenic	Skin homeostasis	Increase	Epidermal hyperplasia, hyperkeratosis and permanent alopecia	N.D.	23
	Transgenic	Antimicrobial defense	Decrease	Resistance to local inflammation, systemic shock or death caused by bacterial infection	Killing of Gram-positive bacteria by degrading bacterial membrane phospholipids	27, 28
	Knockout (natural mutation)	Intestinal tumorigenesis	Increase	Increased susceptibility to colorectal cancer	Maintenance of intestinal microflora?	29
	Transgenic	Intestinal tumorigenesis	Decrease	Reduced incidence of intestinal polyposis	Maintenance of intestinal microflora?	30
	Transgenic (skin-specific)	Skin carcinogenesis	Increase	Increased incidence of papillomas	N.D.	24
	Transgenic	Atherosclerosis	Increase	Development of advanced atherosclerotic lesions	Production of atherogenic small-dense LDL	34
	Transgenic bone marrow transfer	Atherosclerosis	Increase	Increased high cholesterol diet- induced atherosclerosis in *Ldlr*$^{-/-}$ mice	Collagen production in the plaque and thus fibrotic cap development (by macrophage $sPLA_2$-IIA)	35
	Knockout	Arthritis	Decrease	Protection from K/BxN autoantibody-driven inflammatory arthritis	Decreased PGE_2 production?	36
	Transgenic	Arthritis	Increase	Exacerbation of K/BxN autoantibody-driven inflammatory arthritis	Increased PGE_2 production?	36
V	Knockout	Peritonitis	Decrease	Decreased zymosan-induced peritonitis, defective phagocytosis of zymosan	Eicosanoid generation in macrophages	39
	Knockout	Peritonitis	Decrease	Defective phagocytosis and phagosome maturation in macrophages and *Candida albicans* killing	Modulation of phagocytosis and phagosome maturation in macrophages	45

Table 1 continued..

	Knockout	Inflammation	Decrease	Reduced recruitment of LPS-induced leukocytes in the air-pouch model	Altered expression of adhesion molecules	40
	Knockout	Antimicrobial defense	Increase	Impaired phagocytotic clearance of fungi	Phagocytotic killing of fungi	47
	Knockout	Antimicrobial defense	Increase	Severe respiratory acidosis and hypothermia in bacteria-induced pneumonia model, reduced recruitment of neutrophils	Clearance of bacteria	48
	Knockout	Asthma	Decrease	Attenuated antigen-induced asthma	N.D.	42
	Knockout	Asthma	Decrease	Attenuated house dust mite-induced pulmonary inflammation	Regulation of maturation and antigen processing in dendritic cells	46
	Transgenic	Acute lung injury	Increase	Neonatal lethality because of lung dysfunction	Aberrant hydrolysis of surfactant PC	44
	Knockout	Acute lung injury	Decrease	Attenuation of LPS-induced airway injury	N.D.	43
	Knockout	Arthritis	Increase	Exacerbation of K/BxN autoantibody-induced arthritis	Clearance of immune complex by regulating cysLT production	36
	Knockout	Myocardial infarction	Decrease	Decreased myocardial infarct size	Production of LTB_4 and TXA_2 in the myocardium	41
	Knockout (bone marrow transfer)	Atherosclerosis	Decrease	Partial protection from atherosclerotic development in *Ldlr-/-* mice	Production of atherogenic small-dense LDL, Modulation of collagen deposition (by macrophage $sPLA_2$-V)	51
X	Knockout	Asthma	Decrease	Attenuated asthmatic response, with decreased levels of Th2 cytokines and pro-asthmatic eicosanoids	Production of cysLTs	56, 57
	Transgenic (Macrophage-specific)	Acute lung injury	Increase	Neonatal death because of severe lung injury	N.D.	59
	Knockout and knockout bone marrow tranfer	Myocardial infarction	Decrease	Protected from myocardial injury and reduced neutrophil infiltration	LTB_4 production and respiratory burst in neutrophils	62
	Knockout	Aneurysm	Decrease	Attenuated angiotensin II-induced atherosclerosis and aneurysm in $ApoE^{-/-}$ mice	Production of atherogenic small-dense LDL and eicosanoid synthesis	65

Table 1 continued..

	Knockout	Aneurysm	Decrease	Attenuated $CaCl_2$-induced aneurysm	Modulation of recruitment and activation of neutrophils	66
	Knockout	Macrophage function	Increase or decrease	Altered cholesterol efflux and LPS-induced cytokine production	Negative regulation of the LXR signaling by PUFAs	67, 68
	Knockout	Obesity	Increase	Increased obesity, increased plasma corticosterone levels and adrenal expression of StAR	Negative regulation of the LXR-dependent lipogenic programme by PUFAs	69, 70
	Knockout	Obesity	Decrease	Reduced adiposity, improved muscle insulin tolerance, and reduced gastrointestinal phospholipid digestion	Gastrointestinal phospholipid digestion	55
	Knockout	Pain	Decrease	Partially reduced neuritogenesis of DRG neurons, attenuated peripheral pain nociception elicited by a chemical stimulus	Neuritogenesis by LPC production?	54
	Transgenic	Pain	Increase	Increased peripheral pain nociception elicited by a chemical stimulus	Neuritogenesis by LPC production?	54
	Knockout	Male reproduction	Decrease	Reduced litter size, impaired acrosome reaction and fertility	Sperm acrosome reaction by LPC production	55, 71
	Knockout	Hair homeostasis	Decrease	Abnormal hair follicles	Regulation of ORS cell growth	72
	Transgenic	Hair homeostasis	Increase	Development of alopecia, along with epidermal hyperplasia and hair follicle distortion	Massive hydrolysis of PUFA-bearing PE in the skin	72
III	Knockout	Male reproduction	Decrease	Male sterility, impaired sperm maturation	Epididymal sperm maturation through phospholipid remodeling in sperm membrane	77
	Transgenic	Atherosclerosis	Increase	Accelerated atherosclerotic lesion development in *ApoE*$^{-/-}$ mice	Production of atherogenic small-dense LDL and TXA_2	75
	Transgenic	Inflammation	Increase	Spontaneous development of systemic inflammation	Production of PGE_2?	76

N.D. not determined.

inflammation, systemic shock or death caused by bacterial infection [27, 28]. It is thus likely that constitutively high levels of *group IIA sPLA*$_2$ in tears, seminal fluids and intestinal Paneth cells as well as its robust induction in various cells during inflammation may reflect its protective role against invading bacteria.

Some mouse strains with a natural disruption of the *Pla2g2a* gene are more susceptible to intestinal tumorigenesis [29]. Transgenic transfer of the *Pla2g2a* gene into C57BL/6 mice, a strain that intrinsically lacks *group IIA sPLA*$_2$ as noted above, reduces the incidence of intestinal polyposis [30]. Furthermore, expression levels of *group IIA sPLA*$_2$ show inverse correlation with gastric cancer in humans [31]. On the basis of these observations, *group IIA sPLA*$_2$ appears to have an anti-tumorigenic potential in the gut micro-environment. Although the molecular mechanism underlying this effect has not yet been firmly proven, the absence of bactericidal *group IIA sPLA*$_2$ in Paneth cells and colorectal epithelial cells may alter the intestinal bacterial flora, thereby facilitating tumor development. On the other hand, there is ample evidence that the expression levels of *group IIA sPLA*$_2$ is positively linked to the malignancy of prostate cancer [32]. Therefore, the impact of *group IIA sPLA*$_2$ on cancer appears to be tissue-specific.

Clinical evidence that the serum levels of *group IIA sPLA*$_2$ show good correlation with cardiovascular diseases [20] and that *group IIA sPLA*$_2$ is expressed at high levels in atherosclerotic plaques [33] suggests the pathological role of this enzyme in the development of atherosclerosis. Hydrolysis of phospholipids in low-density lipoprotein (LDL) and high-density lipoprotein (HDL) by *group IIA sPLA*$_2$ (or more effectively by *group V* and *X sPLA*$_2$*s*; see below) gives rise to small-dense, LPC-rich, pro-atherogenic lipoprotein particles that facilitate macrophage foam cell formation, and more importantly, *Pla2g2a*-Tg mice develop advanced atherosclerotic lesions with alteration in plasma lipoprotein profiles on an atherogenic diet [34]. Moreover, adoptive transfer of *Pla2g2a*-Tg bone marrow cells into LDL receptor (*Ldlr*)-null mice leads to increased atherosclerosis, indicating that the elevated expression of *group IIA sPLA*$_2$ in hematopoietic cells (likely regional macrophages) is sufficient for its pro-atherogenic effect [35]. Considering that atherosclerosis represents a chronic inflammation in the aorta, *group IIA sPLA*$_2$ can be regarded as a pro-inflammatory molecule in this pathological setting.

Although *group IIA sPLA*$_2$ was originally found in fluids from patients with rheumatoid arthritis [19], its role in the pathology of this autoimmune disease has remained unclear. Recently, the mutated *Pla2g2a* allele in the C57BL/6 strain was delivered onto the BALB/c strain to produce *Pla2g2a*$^{-/-}$ BALB/c mice. Analysis of these mice has finally provided compelling evidence that *group IIA sPLA*$_2$ does play an exacerbating role in inflammatory arthritis. Thus, the autoantibody-induced inflammation in joints is markedly attenuated in *Pla2g2a*$^{-/-}$ mice, whereas it is conversely exacerbated in *Pla2g2a*-Tg mice [36]. The molecular mechanism whereby *group IIA sPLA*$_2$ promotes the process of arthritis remains controversial, although several *in vitro* studies using synovial fibroblasts or chondrocytes have proposed the lipid mediator-dependent and -independent pathways [37].

Group V sPLA$_2$

Since *group V sPLA*$_2$ is capable of releasing arachidonic acid and LPC more efficiently than *group IIA sPLA*$_2$ from cultured cells when overexpressed or added exogenously [38], several investigators have paid attention to its roles in inflammation. *Group V sPLA*$_2$ is expressed in macrophages and mast cells, and its gene ablation in mice (*Pla2g5*$^{-/-}$) results in partial reduction of eicosanoid production in these cells following zymosan stimulation [39]. This eicosanoid-biosynthetic function of *group V sPLA*$_2$ may depend on the activation of cytosolic PLA$_2$ before or after membrane hydrolysis by *group V sPLA*$_2$. In line with the decreased eicosanoid synthesis, the early phase of zymosan-induced peritonitis is partially reduced in *Pla2g5*$^{-/-}$ mice [39]. In the air pouch model, *Pla2g5*$^{-/-}$ mice show less recruitment of LPS-induced leukocytes through reduced expression of adhesion molecules [40]. Among various tissues, *group V sPLA*$_2$ shows the highest expression in the myocardium of the heart [9], and in a myocardial ischemia/reperfusion model, *Pla2g5*$^{-/-}$ mice exhibit a marked decrease in

myocardial infarct size, with attenuated production of eicosanoids [41]. Thus, *group V sPLA$_2$* has a pro-inflammatory potential in these situations, at least partly through regulating lipid mediator levels.

Group V sPLA$_2$ is expressed in bronchial epithelial cells and alveolar macrophages, with robust induction during airway inflammation. Importantly, *Pla2g5$^{-/-}$* mice are significantly protected from airway disorders such as antigen-induced asthma and LPS-induced respiratory distress syndrome [42, 43]. Furthermore, in an asthma model, airway hyper-responsiveness in the allergic mice is partially attenuated by intratracheal application of anti-*group V sPLA$_2$* antibody, whereas intratracheal treatment with *group V sPLA$_2$*, but not with its catalytically weak mutant, increases airway resistance and narrowing along with increased leukocyte infiltration [42]. These results suggest the pro-inflammatory action of *group V sPLA$_2$* in the airway. Pulmonary surfactant, a lipid-protein complex that lowers surface tension along the alveolar epithelium and thereby promotes alveolar stability, is one of the best targets of *group V sPLA$_2$*. Accordingly, transgenic overexpression of this enzyme in mice (*Pla2g5*-Tg mice) causes aberrant hydrolysis of surfactant PC, leading to respiratory distress and neonatal death [44].

However, the roles of *group V sPLA$_2$* in inflammation have recently turned out to be more complex. *Group V sPLA$_2$* is uniquely localized in phagosomal membranes of macrophages and dendritic cells during uptake of exogenous particles, and *Pla2g5$^{-/-}$* macrophages show decreased uptake of the particles [45]. In an asthma model, capture and presentation of a mite antigen by dendritic cells are significantly attenuated in *Pla2g5$^{-/-}$* mice [46]. Accordingly, *Pla2g5$^{-/-}$* mice display a reduction of Th2 polarization, thereby resisting subsequent propagation of asthmatic inflammation. Thus, *group V sPLA$_2$* appears to function in two regulatory steps in asthma; (i) in antigen-presenting cells to regulate antigen processing and maturation and thereby the Th2 immune response, and (ii) in airway-resident cells to facilitate subsequent airway inflammation that may involve surfactant degradation.

Because of the defective phagocytosis, *Pla2g5$^{-/-}$* mice are more susceptible to *Candida albicans* infection, since phagocytotic clearance of the fungus is impaired in *Pla2g5$^{-/-}$* macrophages [47]. In addition, *Pla2g5$^{-/-}$* mice are unable to properly clear *Escherichia coli* infected into the pulmonary tract likely due to reduced recruitment of neutrophils, thereby suffering from more severe respiratory acidosis and hypothermia [48]. In this bacteria-induced pneumonia model, levels of prostaglandins and expression of endothelial adhesion molecules are significantly reduced, while levels of several cytokines are elevated, in the lung of *Pla2g5$^{-/-}$* mice. *Group V sPLA$_2$* expressed in both myeloid cells and lung-resident non-myeloid cells participates in the regulation of the innate immune response to pulmonary bacterial infection. These results provide evidence that *group V sPLA$_2$* plays a defensive role against infection of fungus and bacteria.

Contrary to mice lacking *group IIA sPLA$_2$* (see above), those lacking *group V sPLA$_2$* are more sensitive to inflammatory arthritis, likely because macrophage phagocytosis of the immune complex, a process that depends on cysteinyl leukotrienes, is hampered in the arthritic joints [35]. Thus, it seems that sPLA$_2$-V has an anti-inflammatory, rather than pro-inflammatory, role in certain diseases induced by deposition of the immune complexes, such as rheumatoid arthritis. The fact that the two particular enzymes, *group IIA* and *group V sPLA$_2$s*, exert promoting and protective effects, respectively, on inflammatory arthritis may explain why clinical attempts to treat patients with rheumatoid arthritis with a pan-sPLA$_2$ inhibitor, which blocks both enzymes, have been unsuccessful [49]. These observations provide a rationale for testing two new therapeutic approaches for treatment of immune complex-mediated inflammation, namely the use of a *group IIA sPLA$_2$*-specific inhibitor or that of recombinant *group V sPLA$_2$*.

The hydrolytic action of *group V sPLA$_2$* on LDL phospholipids is much more potent than that of *group IIA sPLA$_2$*, promoting foam cell formation of macrophages [50]. *Ldlr*-deficient mice transplanted with *Pla2g5$^{-/-}$* bone marrow cells are partially protected from atherosclerosis development, whereas those mice with adoptive transfer of *group V sPLA$_2$*-overexpressing bone marrow cells develop more severe lesions [51]. This indicates that *group V sPLA$_2$* expressed in hematopoietic

cells (most likely macrophages) are responsible for the exacerbation of atherosclerosis, although the contribution of this enzyme expressed in non-hematopoietic cells to atherosclerosis remains unknown. In relation to this, a recent tagging single nucleotide polymorphism analysis has revealed an association of the human *group V* $sPLA_2$ gene haplotype with LDL levels in patients with type 2 diabetes [52].

Group X $sPLA_2$

As in the case of *group IB* $sPLA_2$, *group X* $sPLA_2$ is synthesized as a zymogen, and removal of an N-terminal propeptide produces an active mature enzyme [10]. This processing occurs prior to its secretion [53], after secretion [54], or during inflammatory response [44]. Among mammalian $sPLA_2$s, *group X* $sPLA_2$ has the highest binding affinity for PC and thus exhibits the most potent activity to release arachidonic acid and LPC from the plasma membrane of intact cells [54]. Because of this property, many investigators in this research field have speculated that *group X* $sPLA_2$ plays a major role in inflammation. Indeed, this enzyme is expressed at substantial levels in airway epithelial cells and neutrophils, suggesting its involvement in some inflammatory processes. It should be noted, however, *group X* $sPLA_2$ is constitutively expressed at much higher levels in digestive and genital organs than in other places [55], implying its fundamental and homeostatic roles at these locations.

Mice lacking *group X* $sPLA_2$ ($Pla2g10^{-/-}$) are refractory to antigen-induced asthma, with markedly reduced infiltration of eosinophils and lymphocytes, attenuated goblet cell hyperplasia and smooth muscle layer thickening, and decreased levels of Th2 cytokines and pro-asthmatic eicosanoids [56]. The attenuated asthmatic responses in $Pla2g10^{-/-}$ mice are fully restored by knock-in of human *group X* $sPLA_2$, and importantly, treatment of the knock-in mice with an inhibitor specific for human *group X* $sPLA_2$ suppresses the airway inflammation [57]. Mechanistically, *group X* $sPLA_2$ secreted from the airway epithelium may act in a paracrine manner on infiltrating eosinophils to augment leukotriene production in a process harboring LPC-dependent activation of cytosolic PLA_2 [58]. Macrophage-specific transgenic overexpression of human *group X* $sPLA_2$ (*Cd68-Pla2g10*-Tg) results in severe lung inflammation [59]. Moreover, *group X* $sPLA_2$ is one of the major $sPLA_2$ isoforms detected in airway cells of patients with asthma [60]. These findings direct attention to *group X* $sPLA_2$ (as well as *group V* $sPLA_2$; see above) as novel therapeutic targets for asthma.

Group X $sPLA_2$ is stored substantially in azurophilic granules of neutrophils [61]. $Pla2g10^{-/-}$ mice are protected from myocardial damage following ischemia-reperfusion that involves neutrophil recruitment and activation [62]. This phenotype is recapitulated by adoptive transfer of $Pla2g10^{-/-}$ bone marrow cells into wild-type mice, implying the contribution of *group X* $sPLA_2$ in myeloid cells to the myocardial injury. *Group X* $sPLA_2$ is released from neutrophils and damages cardiomyocytes at the infarct myocardium, probably through increasing neutrophilic leukotriene production and respiratory burst. Thus, myocardial *group V* $sPLA_2$ (see above) and neutrophilic *group X* $sPLA_2$ exert pro-inflammatory functions in ischemic heart damage with a common outcome. Administration of a pan-$sPLA_2$ inhibitor capable of blocking both enzymes reduced myocardial infarct size [62], suggesting that $sPLA_2$ inhibition may be an effective strategy for treatment of myocardial infarction.

Of the $sPLA_2$ isoforms, *group X* $sPLA_2$ shows the highest activity toward PC in lipoproteins (LDL and HDL) *in vitro*. As such, *group X* $sPLA_2$-hydrolyzed LDL facilitates foam cell formation of macrophages very efficiently, whereas *group X* $sPLA_2$-mediated hydrolysis of HDL results in reduction of the ability of this particle to pull out cholesterol from cholesterol-loaded cells [63, 64]. In $ApoE^{-/-}$ mice, *group X* $sPLA_2$ is induced in the abdominal aorta in response to angiotensin II, and its deficiency significantly reduces angiotensin II-induced atherosclerosis and aneurysm, with a concomitant reduction of various inflammatory markers including matrix metalloproteinases [65]. In another model of aortic aneurysm induced by $CaCl_2$, *group X* $sPLA_2$ is mainly localized in neutrophils migrating into the aortic wall, and $Pla2g10^{-/-}$ neutrophils have reduced gelatinase and elastase activities [66]. Adoptive transfer of $Pla2g10^{-/-}$ neutrophils into wild-type mice

recapitulates this aneurysm phenotype. These results collectively suggest that neutrophilic *group X sPLA₂* in the aorta promotes local inflammation leading to aneurysm.

Overexpression or exogenous addition of *group X $sPLA_2$* significantly reduces ABCA1 and ABCG1 expression in a macrophage-like cell line, whereas the lack of *group X $sPLA_2$* in mouse peritoneal macrophages is associated with enhanced expression of these cholesterol efflux transporters [67]. This effect appears to depend on the suppression of liver X receptor (LXR) by polyunsaturated fatty acids (PUFAs), which are released by the hydrolytic action of *group X $sPLA_2$*. Moreover, the increase of cholesterol content in *Pla2g10*$^{-/-}$ macrophages eventually leads to modest but significant reduction of LPS/TLR4-dependent inflammatory responses [68]. Reportedly, the *group X $sPLA_2$*-PUFA-LXR axis can also occur in adipose tissue, where the absence of *group X $sPLA_2$* facilitates adipogenesis and age-associated obesity [69], as well as in adrenal glands, where its deficiency pushes steroidogenesis [70]. Controversially, however, other groups have demonstrated that the expression levels of *group X $sPLA_2$* in mouse macrophages and adipose tissue are pretty low or nearly undetectable [55, 66]. Therefore, if these systems would be indeed operative *in vivo*, *group X $sPLA_2$* should be supplied from other neighboring cells through paracrine routes.

While one study has demonstrated the increased obesity in *Pla2g10*$^{-/-}$ mice as mentioned above, the other study has shown that another line of *Pla2g10*$^{-/-}$ mice gradually lose their body weight and adiposity over age [55]. Beyond distinct housing conditions between the two studies, the latter study has shown that *group X $sPLA_2$* is abundantly expressed in the luminal epithelium of the gut and that *Pla2g10*$^{-/-}$ mice show reduced gastrointestinal phospholipid digestion [55]. It is therefore likely that, akin to the situation in *Pla2g1b*$^{-/-}$ mice (see above), the reduced digestion and absorption of dietary and biliary fat are eventually linked to reduced fat accumulation in adipose tissue of *Pla2g10*$^{-/-}$ mice. Thus, the two particular $sPLA_2$s *group IB* and *group X*, both of which can be activated through proteolytic removal of the *N*-terminal propeptide, may play a compensatory role in phospholipid digestion in the gastroenteric lumen. Since the former is abundantly secreted into the duodenum from the pancreatic gland, while the latter is constitutively expressed throughout the gastrointestinal mucosa, these two "digestive" $sPLA_2$s may spatiotemporally control the hydrolysis of dietary and biliary phospholipids and thereby absorption of their hydrolytic products, depending on the quantity and quality of dietary and biliary fat input.

Group X $sPLA_2$ is most abundantly expressed in the testis, where it is stored in acrosomes (secretory granules in sperm head) of sperm cells [71]. Male, but not female, *Pla2g10*$^{-/-}$ mice have a reduced litter size, and *Pla2g10*$^{-/-}$ spermatozoa isolated from the epididymis display impaired acrosome reaction and fertility despite their normal number and motility [55, 71]. Likewise, fertility of wild-type sperm is partially suppressed by addition of a pan-$sPLA_2$ inhibitor or an anti-*group X $sPLA_2$* antibody, and this inhibitory effect is restored by supplementation with LPC. Thus, *group X $sPLA_2$* plays a specific role in sperm activation, boosting the acrosome reaction by producing LPC from sperm membranes in a paracrine or autocrine manner.

Group X $sPLA_2$ is localized in ganglia or neuronal fibers in peripheral tissues of both mouse and human, suggesting its neuronal role [54]. In mouse dorsal root ganglia (DRG), *group X $sPLA_2$* is localized in both A-fiber and C-fiber neurons, with preferential distribution in the former [55]. In neuronal cell culture, *group X $sPLA_2$* facilitates neuritogenesis through production of LPC [54]. Intriguingly, neuritogenesis of *Pla2g10*$^{-/-}$ DRG neurons is partially reduced, whereas that of *Pla2g10*-Tg neurons is increased, in *ex vivo* culture [55]. Moreover, peripheral pain nociception elicited by a chemical stimulus is ameliorated in *Pla2g10*$^{-/-}$ mice and conversely augmented in *Pla2g10*-Tg mice. Thus, modification of neuronal network by *group X $sPLA_2$* may affect the duration of pain sensing in peripheral tissues. It is also possible that this neuronal function by *group X $sPLA_2$* would affect the neuroendocrine regulation of adiposity, the autonomic nervous response for ejaculation in the reproductive organs, and the peristaltic reflex controlled by the enteric nervous system, which could give indirect influence on gastrointestinal lipid digestion.

Lastly, a striking skin phenotype of *Pla2g10*-Tg mice has delineated a unique role of *group X sPLA$_2$* in hair homeostasis [72]. Thus, *Pla2g10*-Tg mice develop alopecia (similar to *Pla2g2a*-Tg mice; see above) during the initial hair cycle, accompanied by epidermal hyperplasia. This phenotype is associated with massive hydrolysis of PUFA-bearing PE in the skin. Hair follicles undergo repeated cycles of growth (anagen), regression (catagen) and rest (telogen) during their life span. In mouse skin, endogenous *group X sPLA$_2$* is confined to the outermost epithelial layer (ORS; outer root sheath) of hair follicles only during the anagen period. Although the gross appearance of coat hairs in *Pla2g10*$^{-/-}$ mice looks normal, they have ultrastructural abnormalities including hypoplasic ORS and reduced melanin granules [72]. Moreover, expression levels of hair-associated genes are substantially reduced in *Pla2g10*$^{-/-}$ skin. These results reveal a novel role of *group X sPLA$_2$* in hair homeostasis within a highly restricted and specialized skin compartment, the hair follicle.

Group III sPLA$_2$

Group III sPLA$_2$, an atypical sPLA$_2$ isoform, is similar to bee venom sPLA$_2$ rather than to other mammalian sPLA$_2$s [11]. The enzyme is processed to the mature, sPLA$_2$ domain-only form that retains full enzymatic activity [73]. Over-expression of *group III sPLA$_2$* in cultured cells increases arachidonic acid and prostaglandin releases [74]. *Group III sPLA$_2$* can also hydrolyze lipoprotein phospholipids very efficiently and facilitates foam cell formation from macrophages [75]. Transgenic overexpression of *group III sPLA$_2$* in mice (*Pla2g3*-Tg) results in increased atherosclerosis on the *ApoE*$^{-/-}$ background due to accelerated LDL hydrolysis and increased thromboxane synthesis [75]. These mice also develop systemic inflammation over age due to elevated eicosanoid formation [76]. Thus, *group III sPLA$_2$* has a pro-inflammatory potential in these contexts.

A recent knockout study of *group III sPLA$_2$ (Pla2g3*$^{-/-}$*)* has revealed an unexplored role of this atypical sPLA$_2$ in male reproduction [77]. During epididymal transit of spermatozoa, PC in the sperm membrane undergoes a dramatic shift in its acyl groups from oleic acid, linoleic acid and arachidonic acid to docosapentaenoic acid (DPA) and docosahexaenoic acid (DHA), and this increased proportion of DHA consequently contributes to an increase of sperm membrane fluidity, and thereby motility. *Group III sPLA$_2$* is expressed in the epididymal epithelium and acts on sperm cells passing through the duct in a paracrine manner to facilitate phospholipid remodeling in sperm membranes. In *Pla2g3*$^{-/-}$ mice, this sperm membrane remodeling in the epididymis is severely compromised, and as a result, cauda epididymal spermatozoa from the null mice contain low DPA/DHA contents, have aberrant acrosomes and flagella with an abnormal axoneme configuration, and display hypomotility and reduced ability to fertilize eggs [77]. In addition, the gonads of *Pla2g3*$^{-/-}$ mice contain less 12/15-lipoxygenase metabolites than those of wild-type mice, implying preferential coupling of the gonadal *group III sPLA$_2$* with a specific arm (the 12/15-lipoxygenase pathway) of the PUFA-metabolic pathway. Taken together, the two particular sPLA$_2$s, *group III* and *group X* (see above), which are expressed in different locations within the male reproductive organs, exert non-redundant but interrelated functions in two major steps of male fertility; the former during sperm maturation in the epididymis and the latter during capacitation and the acrosome reaction, likely after ejaculation into the uterus and oviduct.

CONCLUDING REMARKS

Efforts by several groups including ours over the past decade have prompted reassessment of the biological functions of several if not all sPLA$_2$s and their target substrates using gene-manipulated mice and specific inhibitors. Localizations and functions of individual sPLA$_2$s are summarized in Figure 1. It is now obvious that individual sPLA$_2$s play important and diverse roles in various biological processes, often acting through “classical” mechanisms involving lipid mediators, or in other cases through “alternative” mechanisms that depend upon their unique actions on extracellular phospholipids. Currently, the pan-sPLA$_2$ inhibitor varespladib, which inhibits the classical sPLA$_2$s, can markedly reduce the area of atherosclerotic lesions in experimental animals, and even in humans in early-phase clinical studies [78]. These

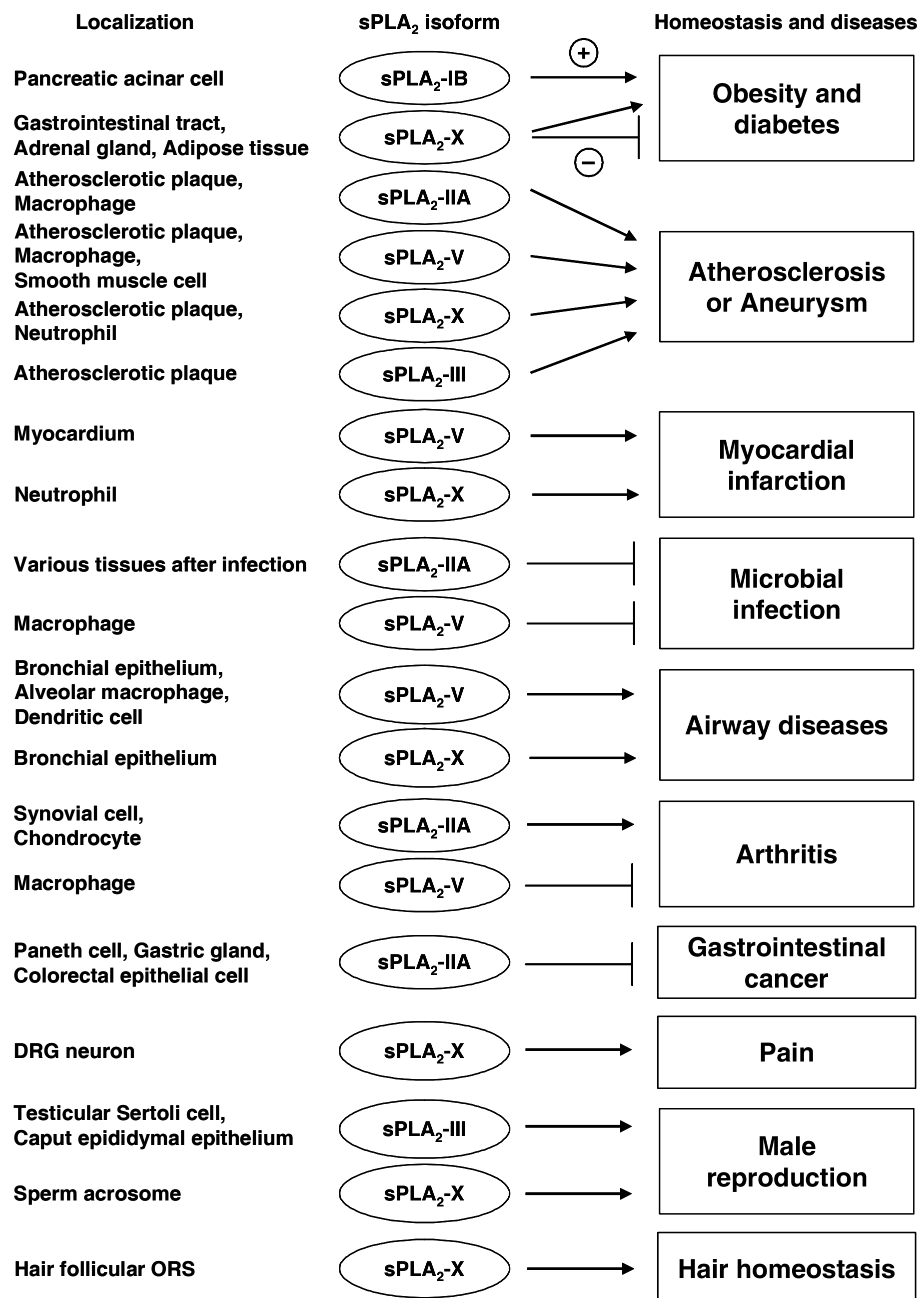

Figure 1. Localizations and functions of $sPLA_2$ isoforms. Biological processes to which individual $sPLA_2$ isoforms contribute, as revealed by gene-manipulated mice, are summarized. Individual $sPLA_2$s take part in various physiological and pathological events in a non-redundant manner.

facts point to the $sPLA_2$ family as a potential therapeutic target for atherosclerosis, and probably other diseases in which one or more $sPLA_2$s are involved, such as asthma, arthritis, and myocardial infarction among others. Hopefully, given all these standpoints, together with continuing efforts to elucidate the functions of other $sPLA_2$ isoforms whose functions have yet to be unresolved, the upcoming full elucidation of the $sPLA_2$ networks underlying each biological event will ensure that $sPLA_2$s come to the central stage of biomedical science in the near future.

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