BIOCHEMICAL PHARMACOLOGY 74 (2007) 949–959

available at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/biochempharm

Phospholipase A_2 as targets for anti-cancer drugs

Brian S. Cummings *

Department of Pharmaceutical and Biomedical Sciences, University of Georgia, Athens, GA 30602, United States

ARTICLE INFO

Article history:
Received 31 January 2007
Accepted 24 April 2007

Keywords:
Phospholipase A_2
Carcinogenesis
Arachidonic acid
Lysophosphatidic acid
Tumor formation
Cell growth

ABSTRACT

Phospholipase A_2 (PLA_2) are esterases that cleave glycerophospholipids to release fatty acids and lysophospholipids. Inhibition of PLA_2 alters cancer cell growth and death *in vitro* and PLA_2 expression is increased in breast, lung, and prostate cancers compared to control tissues. Thus, PLA_2 may be novel targets for chemotherapeutics. However, PLA_2 are a diverse family of enzymes, encompassing 19 members. The selectivity of these individual PLA_2 for phospholipids varies, as does their location within the cell, and tissue expression. Thus, their role in cancer may also vary. This review summarizes the expression of individual PLA_2 in cancers, focuses on the potential mechanisms by which these esterases mediate carcinogenesis, and suggests that select PLA_2 isoforms may be targets for anti-cancer drugs.

1. Introduction

1.1. *Phospholipases A_2 and cancer*

Phospholipase A_2 (PLA_2) are esterases that cleave glycerophospholipids at the *sn*-2 ester bond to release a fatty acid and lysophospholipid ([1,2]; Fig. 1). The activity and expression of several PLA_2 isoforms are increased in several human cancers [3–11], suggesting that these enzymes may be targets for anti-cancer drugs [5]. In order to prove this hypothesis more information is needed about the role of PLA_2 in the mechanisms of carcinogenesis.

A major mechanism by which PLA_2 may mediate carcinogenesis is the release of arachidonic acid, a 20-carbon fatty acid containing 4 double bonds, from glycerophospholipids. Once released, arachidonic acid is metabolized by multiple enzymes into several molecules, most of which induce cancer cell growth and proliferation *in vitro* [12]. In addition, PLA_2 may also mediate carcinogenesis by releasing lysophospholipids (Fig. 1), which can induce cell growth via their metabolism to lysophosphatidic acid (LPA) [13,14]. Thus, multiple mechanisms exist by which PLA_2 can participate in the development of cancer.

PLA_2 inhibitors are attractive anti-cancer targets as they would theoretically decrease the formation of both arachidonic acid and LPA congruently. This would eliminate the shifting of arachidonic acid to alternate pathways, possibly decreasing adverse side effects associated with arachidonic acid metabolism inhibitors [5]. However, PLA_2 are a diverse family of enzymes with at least 19 different individual isoforms [2], some of which have important physiological roles [1,15]. Thus, general inhibitors that target all PLA_2 may

* Tel.: +1 706 542 3792; fax: +1 706 542 5358.
E-mail address: bsc@rx.uga.edu.

Abbreviations: BEL, bromoenol lactone; COX, cyclooxygenase; $cPLA_2$, cytosolic phospholipase A_2; CYPP450, cytochrome P450 monooxygneaes; EET, epoxyeicosatrienoic acid; HETE, hydroxyeicosatetraenoic acid; HPETE, hydroperoxyeicosatetraenoic acid; $iPLA_2$, Ca^{2+}-independent phospholipase A_2; LOX, lipoxygenase; LTC_4, leukotrienes; LysoPLD, lysophospholipid specific phospholipase D; NSAID, nonsteroidal anti-inflammatory drugs; PAF, platelet activating factor; PAF-HA, platelet activating factor-acetylhydrolase; PGE_2, prostaglandins; PGI_2, prostacyclins; PLA_2, phospholipase A_2; PPAR, peroxisomal proliferator activated receptor; $sPLA_2$, secretory phospholipase A_2; TXA_2, thromboxanes

0006-2952/$ – see front matter
doi:10.1016/j.bcp.2007.04.021

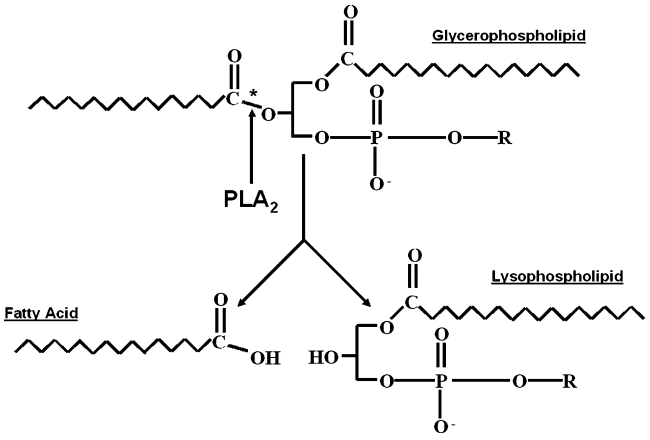

Fig. 1 – PLA_2 cleave glycerophospholipids at the *sn*-2 ester bond to release a fatty acid and a lysophospholipid. The cleavage site for PLA_2 is denoted.

not be practical. Therefore, more studies are needed focusing on the development of inhibition strategies for individual PLA_2 isoforms. Such studies would enhance the development of PLA_2 inhibitors for treatment of cancer.

2. PLA_2 classification, function, and inhibitors

2.1. *Classification of PLA_2*

PLA_2 are broadly defined into three different classes; secretory PLA_2 ($sPLA_2$), cytosolic PLA_2 ($cPLA_2$), and Ca^{2+}-independent PLA_2 ($iPLA_2$) [1,2]. $sPLA_2$ are the oldest class of PLA_2. They are found throughout nature, and were originally characterized in snake and bee venom [1]. They range in size from 13 to 19 kDa, typically require Ca^{2+} for their activity, and utilize histidine to hydrolyze the *sn*-2 ester bond of the glycerol backbone [2]. $cPLA_2$ and $iPLA_2$ are larger in size, typically 66–90 kDa, and utilize serine to facilitate the hydrolytic cleavage of the *sn*-2 fatty acid [2]. While Ca^{2+} facilitates the activity of $cPLA_2$, it is not needed for the hydrolytic cleavage of the fatty acid. Rather, the Ca^{2+} is used to facilitate the translocation of $cPLA_2$ to membranes [2]. In contrast to $sPLA_2$ and $cPLA_2$, $iPLA_2$ do not require Ca^{2+} for either their activity or translocation to membranes.

A newer classification system organizes PLA_2 based on their genetic sequence into 14 distinct groups (designated by a roman numeral), encompassing over 19 individual members (designated by Arabic letters) [2] (Table 1). $sPLA_2$ are represented by Groups I–III, V, and IX–XIV, with Group II having the most members (Groups IIA–F) [2]. $cPLA_2$ are represented by Group IV PLA_2, and include Group IVA, B, and C PLA_2. Group IVC is unique in that it is expressed in the membrane [16]. $iPLA_2$ are represented by Group VI and there are at least 3 known members [15]. Group VIA-1 and A-2 are splice variants of the same gene and are expressed in the cytosol [17]. In contrast, Group VIB is a distinct gene product localized to the endoplasmic, peroxisomal, and mitochondrial membranes [18,19]. A recent report identified three novel $iPLA_2$ called $iPLA_2\varepsilon$, $iPLA_2\zeta$, and $iPLA_2\eta$ [20]. The exact group to which these PLA_2 belong is not yet reported, but may be Group VI. Groups VII and VIII PLA_2 are Ca^{2+}-independent and range in size from 26 to 45 kDa. They are commonly referred to as platelet activating factor-acetylhydrolase (PAF-AH) [21]. PAF-AH utilizes a catalytic serine to hydrolyze the *sn*-2 ester bond, but typically act to deacetylate, and inactivate PAF, as opposed to glycerophospholipids.

Table 1 – Classification of PLA_2

Class	Group	Molecular weight (kDa)	Common name
Histidine active site			
$sPLA_2$	I (A–B)	13–15	N/A
	II (A–F)	13–17	N/A
	III	15–18	N/A
	V	14	N/A
	X	14	N/A
	XI (A–B)	12–13	N/A
	XII	18–19	N/A
	XIII	<10[d]	N/A
	XIV	13–19	N/A
Serine active site			
$cPLA_2$	IVA	85	$cPLA_2\alpha$
	IVB	114	$cPLA_2\beta$
	IVC	61	$cPLA_2\gamma$
$iPLA_2$	VIA-1	84–85	$iPLA_2\beta$ (short)
	VIA-2	88–90	$iPLA_2\beta$ (long)
	VIB	63–88	$iPLA_2\gamma$
PAF-AH	VII (A–B)	40–45	PAF-AH (II)
	VIII (A–B)	26	PAF-AH (IB)

Adapted from Cummings et al. [1] and Balsinde et al. [2]. d: Dalton.

2.2. *Function of PLA_2*

The functions of PLA_2 are as diverse as their classes and include (1) inflammation [22–24], (2) cell death [1,25,26], (3) cell growth [27–31], (4) cell signaling [23,32–34], and (5) maintenance of membrane phospholipids [35–37]. For the most part, specific PLA_2 classes are not assigned to specific functions. This is due, in part, to the significant overlap that exists with regards to the ability of PLA_2 to cleave and release free fatty acids and lysophospholipids (Fig. 1), a process inherent to the function of all PLA_2.

All PLA_2 isoforms are capable of mediating inflammation. However, a majority of studies attribute inflammation to both $sPLA_2$ and $cPLA_2$ [38–40]. $sPLA_2$ tends to be more associated with general inflammatory events while $cPLA_2$ tends to be more associated with chronic inflammation, or that induced by arachidonic acid release [38,39]. These differences may exist due to the fact the $cPLA_2$ prefers phospholipids with arachidonic acid at the *sn*-2 bond [41]. Studies also suggest roles for $iPLA_2$ in acute inflammation [39,40]. Most of these studies focus on PAF-AH [40]. However, at least one study has suggested a role for Group VI $iPLA_2$ ($iPLA_2\beta$) in acute inflammation [39].

PLA_2 can mediate the mechanisms of cell death independently of inflammation [1,24], and all three classes of PLA_2 are known to be involved. Roles for both $cPLA_2$ and $iPLA_2$ are

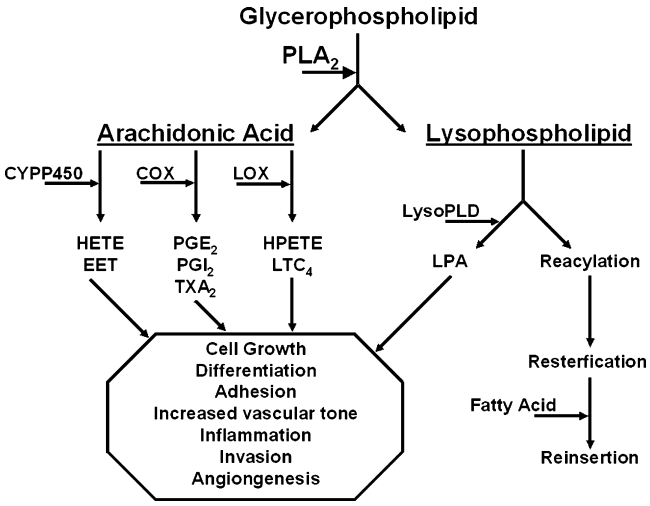

Fig. 2 – Role of PLA_2 in the generation of mitogneic lipid signals. PLA_2 cleave glycerophospholipids to release arachidonic acid and lysophospholipids. LysoPLD can metabolize lysophospholipids to lysophosphatidic acid (LPA), which is reported to be mitogenic in several cells. The lysophospholipid may also act as an acceptor for fatty acids and be inserted into the membrane. Arachidonic acid can be metabolized by cyclooxygenases (COX), lipoxygenases (LOX), or cytochrome-P450 monooxygenases (CYP450). Metabolism of arachidonic acid by COX results in the formation of thromboxanes (TXA_2), prostacyclins (PGI_2), and prostaglandins (PGE_2). Metabolism by LOX results in the formation of hydroperoxyeicosatetraenoic acids (HPETE) and leukotrienes (LTC_4). Metabolism by CYP450 results in the formation of hydroxyeicosatetraenoic acids (HETE) and epoxyeicosatrienoic acids (EET). All of these metabolites are reported to be mitogenic by several mechanisms, which are indicated.

reported in Fas- and TNF-induced cell death in human leukemia cell lines [25,42], and $iPLA_2$ mediates cisplatin-induced cell death in renal proximal tubule cells [43]. Both $sPLA_2$ and $cPLA_2$ mediate oxidant-induced cell death in several cells lines [44].

One of the main mechanisms by which PLA_2 mediate cell death is by the release of arachidonic acid, which is metabolized to several species reported to stimulate caspase activation [1,25] (Fig. 2). However, recent studies demonstrate that arachidonic acid can induce cell death with out metabolism. For example, arachidonic acid can disrupt membrane integrity by acting as a detergent [45]. Further, evidence in isolated mitochondria suggests that Ca^{2+}-induced-PLA_2 activity mediates the release of arachidonic acid, which induces mitochondrial permeability transition [46]. Other studies demonstrate that arachidonic acid can induce mitochondrial swelling and an uncoupling of oxidative phosphorylation by inhibition of NADH-Coenzyme Q oxidoreductase activity [26]. Thus, PLA_2 can mediate cell death via direct alteration of mitochondrial function.

PLA_2 can also function to mediate cell growth. This function is again mediated by cleavage and release of fatty acids and lysophophospholipids. Both the fatty acid and lysophospholid mediate cell growth in a stimulus and cell-dependent manner by activation of both kinases and receptors. It is in this manner that PLA_2 function in cell signaling. The functions of PLA_2 in cell growth and cell signaling are discussed at depth below.

The function of PLA_2 in the maintenance of membrane phospholipids is mainly attributed to $iPLA_2$. In fact, the first defined role of Group IVA PLA_2 ($iPLA_2\beta$) was that of a 'housekeeping' role involving phospholipid remodeling of the cell as part of the Lands cycle [47]. This cycle is a reacylation/deacylation process of incorporating free fatty acids into phospholipids that is integral to the maintenance of a normal cell membrane [48]. In this process, a fatty acid is cleaved by $iPLA_2$ at the *sn*-2 of a phospholipid, creating a lysophospholipid that is re-esterified with another fatty acid by acyl-CoA:lysophosphatide acyltransferase. Balsinde et al. demonstrated in P388D1 macrophages that $iPLA_2$was responsible for providing lysophospholipid acceptors to be re-esterified with arachidonic acid, and reinserted into the cell membrane [47]. The availability of lysophospholipids was the rate-limiting step in the process. This finding was verified using siRNA against $iPLA_2\beta$ [35]. The modulation of $iPLA_2$ activity in correlation with either cell growth or catabolism, strengthens its role as an integral enzyme in maintaining cell membrane homeostasis.

2.3. *Inhibitors of PLA_2*

Several inhibitors of PLA_2 have been developed. These include general inhibitors, those that differentiate between the catalytic serine and histidine of PLA_2 classes, as well as those that can differentiate between individual PLA_2 isoforms within the same class. PLA_2 inhibitors have been extensively reviewed [22,49]. However, a basic understanding of these inhibitors is invaluable when trying to determine the role of PLA_2 isoforms in carcinogenesis (Table 2). General PLA_2 inhibitors include manoalide, and non-specifically decrease the activity of all PLA_2 isoforms. Thy do not display any specificity for different active sites amongst PLA_2 (histidine versus serine) and are typically used at high concentrations (>1 mM) [50]. They have been reported to induce cell death at high concentrations [1].

More advanced PLA_2 inhibitors display specificity for individual PLA_2 classes by targeting the active sites used for the catalytic hydrolysis of the *sn*-2 ester bond. For example, $sPLA_2$ is selectively inhibited by 3-(3-acetamide-1-benzyl-2-ethylindolyl-5-oxy) propane sulfonic acid (LY311727) [13], which targets the catalytic histidine of $sPLA_2$. Because it does not inhibit either $cPLA_2$ or $iPLA_2$ it can be used to test the hypothesis that $sPLA_2$ mediates cancer cell growth.

In contrast to LY311727, methylarachidonyl flourophosphonate (MAFP) and arachidonyl triflouromethyl ketone ($AAOCF_3$) target the catalytic serine active site common to both $cPLA_2$ and $iPLA_2$, but not $sPLA_2$ [1,13]. These inhibitors can be used to distinguish between $sPLA_2$ and $cPLA_2$/$iPLA_2$ activity. The IC_{50} for these compounds against purified $cPLA_2$ or $iPLA_2$ is approximately 0.5 μM [15,51]. However, most studies using these compounds in cell cultures or tissue extracts use 5–10 μM [1,13,19,43,52].

Bromoenol lactone (BEL or (*E*)-6-(1-bromoethyle)tetrahydro-3-(1-naphthalenyl)-2*H*-pyran-2-one)) inhibits $iPLA_2$, but

Table 2 – Inhibitors of PLA_2

Isoform	Inhibitor	IC_{50}[a]	Reference
All	Manoalide	500 μM/1 mM	[22]
$sPLA_2$	3-(3-Acetamide-1-benzyl-2-ethylindolyl-5-oxy) propane sulfonic acid (LY311727)	20–40 nM/1 μM	[13]
$cPLA_2$	Methyl arachidonyl fluorohosphonate (MAFP)	0.5/10 μM	[53]
	Arachidonyl trifluoromethyl ketone ($AACOCF_3$)	0.3/10 μM	[1,13,22]
$iPLA_2$	$AAOCF_3$	0.3/10 μM	[1,13,22]
	MAFP	0.5/10 μM	[1,13,22]
	Bromoenol lactone (BEL)	0.5/5 μM	[37]
$iPLA_2\gamma$	R-Bromonel lactone (R-BEL)	2/2.5 μM	[54,56]
$iPLA_2\beta$	S-Bromoenol lactone (S-BEL)	1/2.5 μM	[54,56]
PAF-AH	Diisopropylfluorophosphate (DFP)[b]	1/1 mM	[55]
	Phenylmethylsulfonylfluoride (PMSF)[b]	1/1 mM	[57,58]
	MAFP	0.25/5 μM	[21]
	Pefabloc (4-[2-aminoethyl]benzenesulfonyl fluoride)[b]	100/200 μM	[58]

[a] x/y: x, Reported IC_{50}; y, dose commonly used in cells and tissues.
[b] General inhibitors of serine-esterase and might inhibit $cPLA_2$ and $iPLA_2$ isoforms.

not $cPLA_2$ or $sPLA_2$, and can be used to distinguish between $cPLA_2$ and $iPLA_2$ [47]. BEL specificity for $iPLA_2$ arises from the fact that it does not modify the serine active site, but rather forms a keto acid hydrolysis product that alkylates cysteines specific to $iPLA_2$ isoforms [53]. Recently R- and S-enantiomers of BEL have been developed that selectively inhibit Group VIB and VIA PLA_2, respectively [52,54]. The mechanisms involved in this specificity are still under study, but demonstrate that pharmacological inhibitors of select individual PLA_2 isoforms are becoming available. However, more are needed, as they would be invaluable for the study of carcinogenesis *in vivo*.

PAF-AH inhibitors include diisopropylfluorophosphate (DFP) and phenylmethylsulfonylfluoride (PMSF) [55] (Table 2). However, these are serine-esterases inhibitors and thus could inhibit $cPLA_2$ and $iPLA_2$. Pefabloc (4-[2-aminoethyl]-benzenesulfonyl fluoride) is also a serine esterase inhibitor that displays increased sensitivity towards PAF-AH, compared to either DFP or PMSF (μM versus mM, respectively) [56]. MAFP also inhibits PAF-AH activity in human coronary artery endothelial cells; however BEL does not [21]. This specificity most likely arises because MAFP targets the active site serine common to PAF-AH, $cPLA_2$, and $iPLA_2$, while BEL does not (see above). Inhibitors that specifically target PAF-AH and not $cPLA_2$ or $iPLA_2$ are still understudy.

The use of anti-sense oligonucleotides, small inhibitor RNA (siRNA), and knock out mice have resulted in valuable inhibition strategies capable of targeting individual PLA_2 isoforms [28,35,57,58]. These techniques remove the drawback of overlapping specificity that exists for many pharmacological inhibitors. However, the effectiveness of some of these techniques vary from model to model, and many, with the exception of mice null for $cPLA_2$ [59], have only been utilized *in vitro*.

3. PLA_2 expression in human cancers

Several studies demonstrate increased expression of PLA_2 in human cancers [3–11]. However, with the exception of Group IIA PLA_2 ($sPLA_2$), the exact roles for many of these PLA_2, and their contribution towards carcinogenesis are not well understood. Further, many studies typically report the expression of a specific class of PLA_2 ($sPLA_2$, $cPLA_2$, or $iPLA_2$), but information regarding the expression of individual group members is lacking, with some exceptions. Table 3 lists PLA_2 isoforms whose expression are altered in human cancers. A discussion of the possible roles of these PLA_2 in multiple human cancers follows.

3.1. *$sPLA_2$ expression in human cancers*

$sPLA_2$ expression and activity is increased in numerous cancers [9] including breast [7–9], pancreatic [60,61], prostate

Table 3 – Cancers in which PLA_2 expression is increased

PLA_2 class	Cancers	Individual isoform	References
$sPLA_2$	Breast, prostate, liver, skin, and pancreatic	Group II, X	[7–11,32–36]
$cPLA_2$	Colorectal, small bowel, and lung	Group IVA	[37,40,41,92]
$iPLA_2$[a]	None to date[b]	?	NA
PAH-AH[c]	Colorectal, lung, thyroid, lung, and brain	?	[3,49–51]

Based on both activity and expression in human tissues. ?: unknown.
[a] Group VI PLA_2 only.
[b] Several studies report expression in cancer cell lines, but no *in vivo* studies with human tissue are reported.
[c] Group VII and VIII PLA_2.

[4,10,11,62], liver [63], and skin [7]. A correlation was reported between $sPLA_2$ expression and colorectal cancer, but further reports failed to provide support for this finding [64], leaving the role of $sPLA_2$ in colorectal cancer unresolved.

Breast cancer was one of the first in which a link between tumor formation and $sPLA_2$ was identified. Yamashita et al. [6–8] measured the activity of microsomal PLA_2 activity in tissue isolated from invasive and benign breast tumors and found significantly higher levels in metastatic tissues, compared to benign breast tumor or normal breast tissue. Microsomal PLA_2 activities were also higher in patients with skin or muscle invasion, vessel involvement, and distant metastasis. This activity was subsequently attributed to Group II PLA_2 ($sPLA_2$) [7,9]. The activity and expression of Group II PLA_2 also correlated to disease reoccurrence and death, leading to the hypothesis that Group II PLA_2 is a prognostic indicator [6,7].

Increased $sPLA_2$ expression is also reported in prostate cancers [4,10,11,62]. This is especially true for Group IIA $sPLA_2$, which is reported to be expressed at levels 22 times higher than paired controls [4]. Group IIA $sPLA_2$ is also increased in seminal fluids in prostate cancer patients [62], suggesting it may serve as a diagnostic tool. In support of this hypothesis Group IIA $sPLA_2$ expression correlated to tumor grade and was highest in the most poorly differentiated, highest-grade, primary human prostate cancers [10]. Alterations in the cellular localization of Group IIA $sPLA_2$ have not been reported to date.

In addition to increased expression, Group IIA $sPLA_2$ DNA is altered in colorectal and intestinal neoplasms [5,65,66]. In these cancers there appears to be a lack of an allele for Group IIA $sPLA_2$ [66], as opposed to a specific mutation. The loss of this allele correlates to deletion of coding sequences on chromosome 4 and altered tumor motility [65,66]. These studies suggest a genetic link between certain tumors types and $sPLA_2$. However, this link appears tissue dependent, as analysis of Group II $sPLA_2$ gene sequences in human colon cancers revealed no mutations, even though mRNA expression was increased over 100-fold [67]. Further, changes in expression of Group IIA $sPLA_2$ mRNA have not been adequately correlated to activity.

3.2. *$cPLA_2$ expression in human cancers*

$cPLA_2$ (Group IVA–C PLA_2) expression is increased in several human cancers including colorectal [68], small bowel [68], and lung [69] cancers. There is some evidence of a role for $cPLA_2$, specifically Group IVA PLA_2, in human prostate cancer [4]. However, $cPLA_2$ expression does not appear to be increased in human prostate tissues [4]. Rather, the role of $cPLA_2$ in prostate cancer may be to facilitate the activation of $sPLA_2$ [4]. Several studies suggest that $cPLA_2$ can mediate cancer cell growth and death in human cancer cell lines [70]. However, further studies are needed to confirm the role of $cPLA_2$ in cancer cell growth and tumor formation *in vivo*.

3.3. *$iPLA_2$ expression in human cancers*

The role of $iPLA_2$ (Group VIA and VIB PLA_2) in human cancer is not as well studied compared to $sPLA_2$ and $cPLA_2$. Reasons for this include the fact that several novel isoforms, such as Group VIB ($iPLA_2\gamma$), have only been identified in the last 7 years [18–20]. Further, antibodies and inhibitors capable of differentiating between different $iPLA_2$ group members (i.e. between Group VIA and VIB PLA_2) have only become available in the last 5 years. While there is a lack of data about the expression of $iPLA_2$ in cancers, several *in vitro* studies demonstrate that both Group VIA and VIB PLA_2 are expressed in human pancreatic [71], kidney [72], and brain [27,52,73] cancer cells. Recent reports also suggest that $iPLA_2$ mediates cell growth in both non-cancerous and cancer cell models [27,28]. However, this work has not been duplicated *in vivo*.

3.4. *PAF-AH expression in human cancers*

With the exception of Group VI PLA_2, less is known about PAF-AH (Group VII and VIII PLA_2) expression in human cancers than other PLA_2 classes. Reports demonstrate increased expression in colorectal cancers [74] and increased activity in thyroid, lung, and brain cancers [56]. PAF-AH deacetylate and inactivate PAF. PAF levels are elevated in breast [76], colorectal, and brain cancers [75]. PAF alters the formation of angiogenic and cytokine networks in these cancers [75], which may promote migration and proliferation. Unlike other PLA_2 classes, the use of PAF-AH inhibitors (DFP, Pefabloc, and MAFP) may be contradictory for the treatment of these cancers, as they would increase the level of PAF. Rather, these data suggest that PAF-AH agonists may be more valuable. This hypothesis is difficult to prove pharmacologically as many PAF-AH inhibitors inhibit $cPLA_2$ and $iPLA_2$. However, studies demonstrated that increased expression of PAF-AH in Kaposi's sarcoma cells implanted in SCID mice, and B16F10 mouse melanoma cells implanted in syngenic C57Bl/6J mice, decreased tumor growth, vascularization, and motility [77].

4. Roles of PLA_2 in the mechanisms of tumor formation and cancer cell growth

The roles of PLA_2 in the mechanisms of carcinogenesis are diverse and somewhat controversial. They include the generation of inflammatory mediators that may to tumor formation [58,62]. In addition, arachidonic acid and lysophospholipids can be metabolized to several molecules that induce cancer cell growth [23,27,28,40,78,79]. Finally, the ability of select PLA_2 to maintain membrane glycerophospholipids may also contribute to cancer cell growth [27,28].

Regardless of the mechanisms involved, PLA_2 roles in carcinogenesis stem from their ability to cleave glycerophospholipids and release fatty acids and lysophospholipids (Fig. 1). These molecules are further metabolized to at least eight different lipid species, all of which alter cell growth in numerous models (Fig. 2). These lipids may also induce cell death [1,2], resulting in controversy as to which lipids are more important in cell growth and which are more important in cell death. The roles of PLA_2 in the mechanisms of inflammation and cell death have been extensively reviewed [1,2,49,80] and will not be covered. In contrast, PLA_2 metabolites as mitogenic signals are stressed.

4.1. *PLA_2-mediated regulation of arachidonic acid*

A majority of mitogenic signals derived from PLA_2 activity arise from the cleavage and release of arachidonic acid from glycerophospholipids. All PLA_2 isoforms are capable of releasing arachidonic acid, provided they have access to phospholipids. After release, arachidonic acid is metabolized by cyclooxygenases I and II (COX-1 and -2, respectively), lipoxygenases (LOX) [1,81], and cytochrome P450 monooxygenases ((CYP450) [23,82]; Fig. 2)). Metabolism of arachidonic acid by COX-1 and -2 results in the formation of cycloperoxides, which can form thromboxanes (TXA_2), prostacyclins (PGI_2), and prostaglandins (PGE_2). These lipids mediate cell death, inflammation, vasoconstriction, and vasodilatation in numerous tissues including platelets [83,84], endothelium [85], and smooth muscle [54]. They also induce proliferation [86]. Metabolism of arachidonic acid by lipoxygenases leads to the formation of hydroperoxyeicosatetraenoic acids (HPETE), which subsequently form leukotrienes (LTC_4), which mediate vascular function during injury and inflammation [2]. Metabolism of arachidonic acid by CYP450 results in the formation of hydroxyeicosatetraenoic acid (HETE) and epoxyeicosatrienoic acid (EET) [23,82]. HETE and EET are reported to mediate vascular tone and ion transport in epithelial cells [82], act as peroxisomal proliferator activated receptor (PPAR) agonists, and as angiogenic and mitogenic signals [87].

4.1.1. *PLA_2-mediated regulation of COX and cancer*

COX metabolize arachidonic acid to cycloperoxides, which can form TXA_2, PGI_2, and PGE_2. COX expression is increased in colon, pancreatic, breast, prostate, lung, skin, urinary bladder, and liver cancers [4,12,86,88,89]. Studies in human cancer cell cultures demonstrate that COX inhibition decreased cell growth and exacerbated chemotherapeutic-induced apoptosis in breast [90], prostate [4], colon, lung, and liver cancer cells [89].

The above studies suggest that inhibition of COX expression or activity would decrease tumor formation *in vivo*. This hypothesis is supported by correlations between the use of nonsteroidal anti-inflammatory drugs (NSAIDs), which inhibit COX activity, and reductions in colorectal, esophageal, breast, lung, and bladder cancers [12,86,88]. The COX-2 inhibitor *Celecoxib*, increased breast cancer cell death *in vitro* [90], and decreased malignancies in human patients [86], suggesting that this isoform may be one chemotherapeutic target for treatment of breast cancer.

COX-mediated generation of PGE_2 may be one mechanism by which this enzyme controls cancer cell growth. PGE_2 levels are increased in several cancers [4,12,86,91] and correlate to tumor formation. PGE_2 actions are initiated upon binding to G-protein coupled receptors, which activate second messengers that induce cell proliferation, migration, apoptosis, or angiogenesis [12,86]. Further, PGE_2 also feedback to increase the expression of COX-2 [92], possibly by mechanisms involving the activation of $cPLA_2$, $sPLA_2$, and PPAR-γ [92]. This positive feedback cycle increases COX-2 activity, leading to increased generation of PGE_2, increased mitogenic signals and increased cancer cell growth.

Regulation of COX activity by PLA_2 is primarily a result of generation of arachidonic acid as a substrate. Studies also suggest that Group IV PLA_2 ($cPLA_2$) and Group II PLA_2 ($sPLA_2$) directly regulate COX expression by mechanisms that are still under study [4,88,93]. However, such regulation does not occur in all cancers, as studies reported no alterations in either COX-1 or -2 expression in mice lacking $cPLA_2$ [58]. Regardless of the mechanisms PLA_2 activity generates the arachidonic acid substrates for COX enzymes. Therefore, studies are needed testing the effect of combinatorial treatments of COX and PLA_2 inhibitors on cancer cell growth and tumor formation.

4.1.2. *PLA_2-mediated regulation of LOX and cancer*

Arachidonic acid is metabolized by LOX to generate HPETE and LTC_4. Multiple LOX isoforms exist including 5-, 12-, and 15-LOX [12,94]. These isoforms are expressed in several cancers including colon, pancreatic, breast, prostate, lung, skin, urinary bladder, and liver cancers [12,4,94,95]. Like LOX expression, HPETE and LTC_4 levels correlate to cancer cell growth, proliferation, and invasion in prostate [96–98], and breast [94] cancer cell lines. The mechanisms involved include the activation of epidermal growth factor receptors [96], inhibition of apoptosis [97], alteration in the activity of protein kinase c [98], stimulation of cell adhesion to extracellular matrices [99], or activation of transforming growth factor-β1-activated protein kinase-1 (TAK1) or mitogen activated protein kinases kinase 6 [99].

Similar to COX-1 and -2, PLA_2 regulate LOX activity by producing arachidonic acid. However, correlations exist between the expression of select PLA_2 and LOX isoforms that go beyond the simple providing of the arachidonic acid substrate [4,12]. Studies demonstrate that increased LOX expression correlates to increased PLA_2 expression [30,100]. For example, $cPLA_2$ (Group IV $cPLA_2$) mediated activation of 12- and 15-LOX results in a signaling cascade involving both MIP-2 and AP-1 activation, resulting in the increased expression of Group IIA $sPLA_2$ [100]. These processes appear to be partially independent of arachidonic acid. Because of these studies, recent reviews suggest that better outcomes in the treatment of cancer may be seen using combinatorial therapies of LOX and PLA_2 inhibitors [4].

4.1.3. *PLA_2-mediated regulation of CYP450 and cancer*

CYP450 metabolizes arachidonic acid to hydroxyeicosatetraenoic acids (HETE) and epoxyeicosatrienoic acids (EET) [23,82]. HETE and EET are reported to mediate vascular tone and ion transport in epithelial cells [82], act on peroxisomal proliferator activated receptors [87], and as angiogenic and mitogenic signals [87]. Several studies demonstrate correlations between the expression of arachidonic acid metabolizing CYP450 and cancer cell growth *in vitro* [101]. These include CYP2W1 [101], CYP2J2 [102], and possibly CYP1A1 and 4A1 (in rats) [103].

Similar to COX and LOX, PLA_2 can regulate CYP450 activity by producing arachidonic acid. However, in contrast to COX and LOX, few studies report links between PLA_2 and CYP450 expression. Selected studies suggests that increases in CYP2C12 expression are regulated by PLA_2-mediated release of arachidonic acid [104]. This regulation may exist due to arachidonic acid-mediated activation of PPARα [105,106]. However, it's not known if these signaling mechanisms are critical in tumor formation or cancer cell growth.

4.2. *PLA_2-mediated regulation of lysophospholipids and cancer*

For every fatty acid generated by PLA_2, a lysophospholipid is also generated (Fig. 1). Lysophospholipids differ in terms of their polar head group, the length of the fatty acid chain on the *sn*-1 position of the glycerol backbone and the saturation of this chain [79]. Once released they can be re-incorporated with fatty acids and re-inserted into the membrane (Fig. 2), or can be further metabolized by a lysophospholipid specific phospholipase D (LysoPLD, also called autoxaxin) to produce lysophosphatidic acid [79]. LPA is degraded by lysophospholipases to monoacylglycerols, free fatty acids, and glycerophosphates [79].

The conversion of lysophospholipids to LPA is most likely how PLA_2-generated lysophospholipids induce cancer cell growth [79]. Both LPA and LysoPLD are increased in ovarian and prostate cancers [79], and increased LPA correlates to cellular proliferation, differentiation, inhibition of cell death, and invasiveness [79,107]. LPA mediates these processes by activating G-protein-coupled receptors leading to increases in several second messengers such as cytosolic calcium and cAMP levels, and activation of Rac and Rho small GTPases, protein kinase C, phosphatidylinositiol 3 kinases, the Ras MAPK, and proteases [79].

PLA_2 contribute to the regulation of LPA by producing lysophospholipids, which are metabolized by LysoPLD. All PLA_2 isoforms can participate in LPA production [14,108]. However, the role of PLA_2 appears to favor the generation of substrates, as opposed to the direct regulation of LysoPLD [108]. To date, it is not know if specific PLA_2 classes have differential roles in LPA production in cancer cells.

4.3. *PLA_2-mediated regulation of glycerophospholipids and cancer*

PLA_2 play major roles in the maintenance and remodeling of glycerophospholipids, especially those that contain arachidonic acid [27,28,35,47]. Both $cPLA_2$ and $iPLA_2$ participate in phospholipid remodeling. Individual isoforms known to be involved include Groups IVA, IVC, VIA, and VIB PLA_2 [27,28]. These PLA_2 do not directly synthesize glycerophospholipids. Rather they are critical in the generation of lysophospholipid acceptors [35,47], which serve as substrates for reacylated fatty acids. In the absence of PLA_2 activity, lysophospholipids are not released, resulting in a decrease in select phospholipids and cell growth [35,47].

The role of PLA_2 in the regulation of phospholipids during cancer cell growth is still being investigated. Studies using both pharmacological and molecular inhibition strategies demonstrate that $iPLA_2$ inhibition decreases arachidonic acid-containing phospholipids and cell growth [27,28]. Decreases in arachidonic-acid containing phospholipids may serve to limit the release of arachidonic acid, leading to a decrease in the generation of mitogenic lipid signals by COX, LOX of CYP450 (Fig. 2). In contrast, decreases in these phospholipids may also reduce the release of lysophospholipids, leading to decreases in the production of LPA (see above).

Recent studies suggests that inhibition of a specific PLA_2, Group VIA $iPLA_2$, or $iPLA_2\beta$ as it's commonly called, decreased both arachidonic acid-containing phospholipids and cell proliferation [27,28]. In contrast, inhibition of Group VIB PLA_2 ($iPLA_2\gamma$) had no effect [28]. These data suggest the hypothesis that $iPLA_2\beta$ may be a novel chemotherapeutic target for inhibition of cancer cell growth.

5. Conclusion

The question can be posed if enzymes that mediate the formation of arachidonic acid and lysophospholipid metabolites are better anti-cancer targets than PLA_2. This is a valid question and these pathways certainly deserve further study. However, some inhibitors of arachidonic acid metabolism, such as COX-2 inhibitors, have severe and unpredictable cardiovascular side effects [86]. This toxicity may be a result of the fact that if arachidonic acid is not metabolized by COX-2 its metabolism may be shifted to other enzymes, such as COX-1, LOX, or CYP450. This may induce inflammation or life-threatening alterations in smooth muscle and vascular tone [15,24,80,82].

PLA_2 represent the primary step in the arachidonic acid signaling cascade and the formation of lsyophospholipids (Fig. 2). Inhibition of PLA_2 would not only decrease the overall release of arachidonic acid, but would also inhibit the activation of epigenetic pathways such as those involving PPARs. Further, inhibition of PLA_2 would decrease the generation of lysophospholipids. These facts, combined with the increased expression of PLA_2 in several cancers, support the hypothesis that PLA_2 isoforms are therapeutic targets for anti-cancer agents. To prove this hypothesis, more studies are needed determining the differential roles of individual PLA_2 isoforms in tumor formation and growth. These studies will aid in the development of specific pharmacological, and molecular, inhibitors for *in vivo* cancer studies. In addition, studies are needed testing the effect of PLA_2 inhibitors in combination with existing chemotherapeutics on cancer cell growth. Finally, studies are needed testing the effect of PLA_2 inhibitors in combination with agents that target COX, LOX, and CYP450, on cancer cell growth.

Acknowledgements

This work was supported by a Georgia Cancer Coalition Distinguished Scholar Grant. I would like to also acknowledge Dr. Shelly Hooks and Dr. Rusty Arnold for their expertise in preparing this manuscript and Brianna Peterson, Bin Sun, and Xiaoling Zhang for their aid in proof reading.

REFERENCES

[1] Cummings BS, McHowat J, Schnellmann RG. Phospholipase A(2)s in cell injury and death. J Pharmacol Exp Ther 2000;294:793–9.
[2] Balsinde J, Winstead MV, Dennis EA. Phospholipase A(2) regulation of arachidonic acid mobilization. FEBS Lett 2002;531:2–6.

[3] Denizot Y, Chianea T, Labrousse F, Truffinet V, Delage M, Mathonnet M. Platelet-activating factor and human thyroid cancer. Eur J Endocrinol 2005;153:31–40.
[4] Dong Q, Patel M, Scott KF, Graham GG, Russell PJ, Sved P. Oncogenic action of phospholipase A2 in prostate cancer. Cancer Lett 2006;240:9–16.
[5] Laye JP, Gill JH. Phospholipase A2 expression in tumours: a target for therapeutic intervention? Drug Discov Today 2003;8:710–6.
[6] Yamashita J, Ogawa M, Sakai K. Prognostic significance of three novel biologic factors in a clinical trial of adjuvant therapy for node-negative breast cancer. Surgery 1995;117:601–8.
[7] Yamashita S, Yamashita J, Ogawa M. Overexpression of group II phospholipase A2 in human breast cancer tissues is closely associated with their malignant potency. Br J Cancer 1994;69:1166–70.
[8] Yamashita S, Yamashita J, Sakamoto K, Inada K, Nakashima Y, Murata K, et al. Increased expression of membrane-associated phospholipase A2 shows malignant potential of human breast cancer cells. Cancer 1993;71:3058–64.
[9] Yamashita S, Ogawa M, Sakamoto K, Abe T, Arakawa H, Yamashita J. Elevation of serum group II phospholipase A2 levels in patients with advanced cancer. Clin Chim Acta 1994;228:91–9.
[10] Graff JR, Konicek BW, Deddens JA, Chedid M, Hurst BM, Colligan B, et al. Expression of group IIa secretory phospholipase A2 increases with prostate tumor grade. Clin Cancer Res 2001;7:3857–61.
[11] Jiang J, Neubauer BL, Graff JR, Chedid M, Thomas JE, Roehm NW, et al. Expression of group IIA secretory phospholipase A2 is elevated in prostatic intraepithelial neoplasia and adenocarcinoma. Am J Pathol 2002;160:667–71.
[12] Cuendet M, Pezzuto JM. The role of cyclooxygenase and lipoxygenase in cancer chemoprevention. Drug Metabol Drug Interact 2000;17:109–57.
[13] Balsinde J, Balboa MA, Insel PA, Dennis EA. Regulation and inhibition of phospholipase A2. Annu Rev Pharmacol Toxicol 1999;39:175–89.
[14] Aoki J. Mechanisms of lysophosphatidic acid production. Semin Cell Dev Biol 2004;15:477–89.
[15] Balsinde J. Roles of various phospholipases A2 in providing lysophospholipid acceptors for fatty acid phospholipid incorporation and remodelling. Biochem J 2002;364:695–702.
[16] Stewart A, Ghosh M, Spencer DM, Leslie CC. Enzymatic properties of human cytosolic phospholipase A2gamma. J Biol Chem 2002;277:29526–3.
[17] Ma Z, Wang X, Nowatzke W, Ramanadham S, Turk J. Human pancreatic islets express mRNA species encoding two distinct catalytically active isoforms of group VI phospholipase A2 (iPLA2) that arise from an exon-skipping mechanism of alternative splicing of the transcript from the iPLA2 gene on chromosome 22q13.1. J Biol Chem 1999;274:9607–16.
[18] Mancuso DJ, Jenkins CM, Gross RW. The genomic organization, complete mRNA sequence, cloning, and expression of a novel human intracellular membrane-associated calcium-independent phospholipase A(2). J Biol Chem 2000;275:9937–45.
[19] Cummings BS, McHowat J, Schnellmann RG. Role of an endoplasmic reticulum Ca(2+)-independent phospholipase A(2) in oxidant-induced renal cell death. Am J Physiol Renal Physiol 2002;283:F492–8.
[20] Jenkins CM, Mancuso DJ, Yan W, Sims HF, Gibson B, Gross RW. Identification, cloning, expression, and purification of three novel human calcium-independent phospholipase A2 family members possessing triacylglycerol lipase and acylglycerol transacylase activities. J Biol Chem 2004;279:48968–75. doi: 10.1074/jbc.M407841200.
[21] Kell PJ, Creer MH, Crown KN, Wirsig K, McHowat J. Inhibition of platelet-activating factor (PAF) acetylhydrolase by methyl arachidonyl fluorophosphonate potentiates PAF synthesis in thrombin-stimulated human coronary artery endothelial cells. J Pharmacol Exp Ther 2003;307:1163–70.
[22] Farooqui AA, Litsky ML, Farooqui T, Horrocks LA. Inhibitors of intracellular phospholipase A2 activity: their neurochemical effects and therapeutical importance for neurological disorders. Brain Res Bull 1999;49:139–53.
[23] Bonventre JV. Phospholipase A2 and signal transduction. J Am Soc Nephrol 1992;3:128–50.
[24] Bonventre JV. Roles of phospholipases A2 in brain cell and tissue injury associated with ischemia and excitotoxicity. J Lipid Mediat Cell Signal 1997;17:71–9.
[25] Atsumi G, Tajima M, Hadano A, Nakatani Y, Murakami M, Kudo I. Fas-induced arachidonic acid release is mediated by Ca^{2+}-independent phospholipase A2 but not cytosolic phospholipase A2, which undergoes proteolytic inactivation. J Biol Chem 1998;273:13870–7.
[26] Farooqui AA, Horrocks LA. Phospholipase A2-generated lipid mediators in the brain: the good, the bad, and the ugly. Neuroscientist 2006;12:245–60. doi: 10.1177/1073858405285923.
[27] Bao S, Bohrer A, Ramanadham S, Jin W, Zhang S, Turk J. Effects of Stable Suppression of Group VIA phospholipase A2 expression on phospholipid content and composition, insulin secretion, and proliferation of INS-1 insulinoma cells. J Biol Chem 2006;281:187–98. doi: 10.1074/jbc.M509105200.
[28] Saavedra G, Zhang W, Peterson B, Cummings BS. Differential roles for cytosolic and microsomal Ca^{2+}-independent phospholipase A2 in cell growth and maintenance of phospholipids. J Pharmacol Exp Ther 2006;318:1211–9. doi: 10.1124/jpet.106.105650.
[29] Longo WE, Grossmann EM, Erickson B, Panesar N, Mazuski JE, Kaminski DL. The effect of phospholipase A2 inhibitors on proliferation and apoptosis of murine intestinal cells. J Surg Res 1999;84:51–6.
[30] Hassan S, Carraway RE. Involvement of arachidonic acid metabolism and EGF receptor in neurotensin-induced prostate cancer PC3 cell growth. Regul Pept 2006;133:105–14.
[31] Teslenko V, Rogers M, Lefkowith JB. Macrophage arachidonate release via both the cytosolic Ca(2+)-dependent and -independent phospholipases is necessary for cell spreading. Biochim Biophys Acta 1997;1344:189–99.
[32] Xu J, Weng YI, Simonyi A, Krugh BW, Liao Z, Weisman GA, et al. Role of PKC and MAPK in cytosolic PLA2 phosphorylation and arachadonic acid release in primary murine astrocytes. J Neurochem 2002;83:259–70.
[33] Balsinde J, Balboa MA, Li WH, Llopis J, Dennis EA. Cellular regulation of cytosolic group IV phospholipase A2 by phosphatidylinositol bisphosphate levels. J Immunol 2000;164:5398–402.
[34] Ramanadham S, Wolf MJ, Li B, Bohrer A, Turk J. Glucose-responsitivity and expression of an ATP-stimulatable, Ca(2+)-independent phospholipase A2 enzyme in clonal insulinoma cell lines. Biochim Biophys Acta 1997;1344:153–64.
[35] Balsinde J, Balboa MA, Dennis EA. Antisense inhibition of group VI Ca^{2+}-independent phospholipase A2 blocks phospholipid fatty acid remodeling in murine P388D1 macrophages. J Biol Chem 1997;272:29317–21.

[36] Balsinde J, Dennis EA. Function and inhibition of intracellular calcium-independent phospholipase A2. J Biol Chem 1997;272:16069–72.
[37] Balsinde J, Dennis EA. Bromoenol lactone inhibits magnesium-dependent phosphatidate phosphohydrolase and blocks triacylglycerol biosynthesis in mouse P388D1 macrophages. J Biol Chem 1996;271:31937–41.
[38] Sun GY, Xu J, Jensen MD, Simonyi A. Phospholipase A2 in the central nervous system: implications for neurodegenerative diseases. J Lipid Res 2004;45:205–13.
[39] Gilroy DW, Newson J, Sawmynaden P, Willoughby DA, Croxtall JD. A novel role for phospholipase A2 isoforms in the checkpoint control of acute inflammation. Faseb J 2004;18:489–98.
[40] Kudo I, Murakami M. Phospholipase A2 enzymes. Prostaglandins Other Lipid Mediat 2002;68–69:3–58.
[41] Clark JD, Lin LL, Kriz RW, Ramesha CS, Sultzman LA, Lin AY, et al. A novel arachidonic acid-selective cytosolic PLA2 contains a Ca(2+)-dependent translocation domain with homology to PKC and GAP. Cell 1991;65:1043–51.
[42] Atsumi G, Murakami M, Kojima K, Hadano A, Tajima M, Kudo I. Distinct roles of two intracellular phospholipase A2s in fatty acid release in the cell death pathway. Proteolytic fragment of type IVA cytosolic phospholipase A2alpha inhibits stimulus-induced arachidonate release, whereas that of type VI Ca^{2+}-independent phospholipase A2 augments spontaneous fatty acid release. J Biol Chem 2000;275:18248–5.
[43] Cummings BS, McHowat J, Schnellmann RG. Role of an endoplasmic reticulum Ca^{2+}-independent phospholipase A2 in cisplatin-induced renal cell apoptosis. J Pharmacol Exp Ther 2004;308:921–8.
[44] Sapirstein A, Spech RA, Witzgall R, Bonventre JV. Cytosolic phospholipase A2 (PLA2), but not secretory PLA2, potentiates hydrogen peroxide cytotoxicity in kidney epithelial cells. J Biol Chem 1996;271:21505–13.
[45] Farooqui AA, Ong WY, Horrocks LA. Biochemical aspects of neurodegeneration in human brain: involvement of neural membrane phospholipids and phospholipases A2. Neurochem Res 2004;29:1961–77.
[46] Kinsey GR, McHowat J, Patrick KS, Schnellmann RG. Role of Ca^{2+}-independent phospholipase A2{gamma} in Ca^{2+}-induced mitochondrial permeability transition. J Pharmacol Exp Ther 2007;321:707–15.
[47] Balsinde J, Bianco ID, Ackermann EJ, Conde-Frieboes K, Dennis EA. Inhibition of calcium-independent phospholipase A2 prevents arachidonic acid incorporation and phospholipid remodeling in P388D1 macrophages. Proc Natl Acad Sci USA 1995;92:8527–31.
[48] Lands WE. Lipid metabolism. Annu Rev Biochem 1965;34:313–46.
[49] Meyer MC, Rastogi P, Beckett CS, McHowat J. Phospholipase A2 inhibitors as potential anti-inflammatory agents. Curr Pharm Des 2005;11:1301–12.
[50] Ackermann EJ, Dennis EA. Mammalian calcium-independent phospholipase A2. Biochim Biophys Acta 1995;1259:125–36.
[51] Lio YC, Reynolds LJ, Balsinde J, Dennis EA. Irreversible inhibition of Ca(2+)-independent phospholipase A2 by methyl arachidonyl fluorophosphonate. Biochim Biophys Acta 1996;1302:55–60.
[52] Kinsey GR, Cummings BS, Beckett CS, Saavedra G, Zhang W, McHowat J, et al. Identification and distribution of endoplasmic reticulum iPLA2. Biochem Biophys Res Commun 2005;327:287–93.
[53] Song H, Bao S, Ramanadham S, Turk J. Effects of biological oxidants on the catalytic activity and structure of group VIA phospholipase A2. Biochemistry 2006;45:6392–406.
[54] Jenkins CM, Han X, Mancuso DJ, Gross RW. Identification of calcium-independent phospholipase A2 (iPLA2) beta, and not $iPlA_2$gamma, as the mediator of arginine vasopressin-induced arachidonic acid release in A-10 smooth muscle cells. Enantioselective mechanism-based discrimination of mammalian iPLA2s. J Biol Chem 2002;277:32807–14.
[55] Jarvi K, Langlais J, Gagnon C, Roberts KD. Platelet-activating factor acetylhydrolase in the male reproductive tract: origin and properties. Int J Androl 1993;16:121–7.
[56] Dentan C, Tselepis AD, Chapman MJ, Ninio E. Pefabloc, 4-[2-aminoethyl]benzenesulfonyl fluoride, is a new, potent nontoxic and irreversible inhibitor of PAF-degrading acetylhydrolase. Biochim Biophys Acta 1996;1299:353–7.
[57] Su X, Mancuso DJ, Bickel PE, Jenkins CM, Gross RW. Small interfering RNA knockdown of calcium-independent phospholipases A2 beta or gamma inhibits the hormone-induced differentiation of 3T3-L1 preadipocytes. J Biol Chem 2004;279:21740–8.
[58] Meyer AM, Dwyer-Nield LD, Hurteau GJ, Keith RL, O'Leary E, You M, et al. Decreased lung tumorigenesis in mice genetically deficient in cytosolic phospholipase A2. Carcinogenesis 2004;25:1517–24.
[59] Ilsley JN, Nakanishi M, Flynn C, Belinsky GS, De Guise S, Adib JN, et al. Cytoplasmic phospholipase A2 deletion enhances colon tumorigenesis. Cancer Res 2005;65:2636–43.
[60] Kiyohara H, Egami H, Kako H, Shibata Y, Murata K, Ohshima S, et al. Immunohistochemical localization of group II phospholipase A2 in human pancreatic carcinomas. Int J Pancreatol 1993;13:49–57.
[61] Oka Y, Ogawa M, Matsuda Y, Murata A, Nishijima J, Miyauchi K, et al. Serum immunoreactive pancreatic phospholipase A2 in patients with various malignant tumors. Enzyme 1990;43:80–8.
[62] Kallajoki M, Alanen KA, Nevalainen M, Nevalainen TJ. Group II phospholipase A2 in human male reproductive organs and genital tumors. Prostate 1998;35:263–72.
[63] Ying Z, Tojo H, Komatsubara T, Nakagawa M, Inada M, Kawata S, et al. Enhanced expression of group II phospholipase A2 in human hepatocellular carcinoma. Biochim Biophys Acta 1994;1226:201–5.
[64] Dimberg J, Samuelsson A, Hugander A, Soderkvist P. Gene expression of cyclooxygenase-2, group II and cytosolic phospholipase A2 in human colorectal cancer. Anticancer Res 1998;18:3283–7.
[65] Cormier RT, Hong KH, Halberg RB, Hawkins TL, Richardson P, Mulherkar R, et al. Secretory phospholipase Pla_2g2a confers resistance to intestinal tumorigenesis. Nat Genet 1997;17:88–91.
[66] MacPhee M, Chepenik KP, Liddell RA, Nelson KK, Siracusa LD, Buchberg AM. The secretory phospholipase A2 gene is a candidate for the Mom1 locus, a major modifier of ApcMin-induced intestinal neoplasia. Cell 1995;81:957–66.
[67] Kennedy BP, Soravia C, Moffat J, Xia L, Hiruki T, Collins S, et al. Overexpression of the nonpancreatic secretory group II PLA2 messenger RNA and protein in colorectal adenomas from familial adenomatous polyposis patients. Cancer Res 1998;58:500–3.
[68] Wendum D, Svrcek M, Rigau V, Boelle PY, Sebbagh N, Parc R, et al. COX-2, inflammatory secreted PLA2, and cytoplasmic PLA2 protein expression in small bowel adenocarcinomas compared with colorectal adenocarcinomas. Mod Pathol 2003;16:130–6.
[69] Kawamoto S, Shoji M, Setoguchi Y, Kato M, Hashizume S, Ichikawa A, et al. Molecular cloning of the 31 kDa cytosolic phospholipase A2, as an antigen recognized by the lung cancer-specific human monoclonal antibody, AE6F4. Cytotechnology 1995;17:103–8.

[70] Pirianov G, Danielsson C, Carlberg C, James SY, Colston KW. Potentiation by vitamin D analogs of TNFalpha and ceramide-induced apoptosis in MCF-7 cells is associated with activation of cytosolic phospholipase A2. Cell Death Differ 1999;6:890–901.
[71] Ma Z, Ramanadham S, Hu Z, Turk J. Cloning and expression of a group IV cytosolic Ca^{2+}-dependent phospholipase A2 from rat pancreatic islets. Comparison of the expressed activity with that of an islet group VI cytosolic Ca^{2+}-independent phospholipase A2. Biochim Biophys Acta 1998;1391:384–400.
[72] Zhang L, Peterson BL, Cummings BS. The effect of inhibition of Ca^{2+}-independent phospholipase A2 on chemotherapeutic-induced death and phospholipid profiles in renal cells. Biochem Pharmacol 2005;70:1697–706.
[73] Peterson B, Knotts T, Cummings BS. Involvement of Ca(2+)-independent phospholipase A(2) isoforms in oxidant-induced neural cell death. Neurotoxicology 2006.
[74] Denizot Y, Truffinet V, Bouvier S, Gainant A, Cubertafond P, Mathonnet M. Elevated plasma phospholipase A2 and platelet-activating factor acetylhydrolase activity in colorectal cancer. Mediators Inflamm 2004;13:53–4.
[75] Denizot Y, De Armas R, Caire F, Pommepuy I, Truffinet V, Labrousse F. Platelet-activating factor and human meningiomas. Neuropathol Appl Neurobiol 2006;32:674–8.
[76] Nigam S, Muller S, Benedetto C. Elevated plasma levels of platelet-activating factor (PAF) in breast cancer patients with hypercalcemia. J Lipid Mediat 1989;1:323–8.
[77] Biancone L, Cantaluppi V, Del Sorbo L, Russo S, Tjoelker LW, Camussi G. Platelet-activating factor inactivation by local expression of platelet-activating factor acetyl-hydrolase modifies tumor vascularization and growth. Clin Cancer Res 2003;9:4214–20.
[78] Seeds MC, Bass DA. Regulation and metabolism of arachidonic acid. Clin Rev Allergy Immunol 1999;17:5–26.
[79] Umezu-Goto M, Tanyi J, Lahad J, Liu S, Yu S, Lapushin R, et al. Lysophosphatidic acid production and action: validated targets in cancer? J Cell Biochem 2004;92:1115–40.
[80] Bonventre JV. Roles of phospholipases A2 in brain cell and tissue injury associated with ischemia and excitotoxicity. J Lipid Mediat Cell Signal 1996;14:15–23.
[81] McHowat J, Creer MH. Catalytic features, regulation and function of myocardial phospholipase A2. Curr Med Chem Cardiovasc Hematol Agents 2004;2:209–18.
[82] Imig JD. Epoxygenase metabolites. Epithelial and vascular actions. Mol Biotechnol 2000;16:233–51.
[83] Barry OP, Pratico D, Lawson JA, FitzGerald GA. Transcellular activation of platelets and endothelial cells by bioactive lipids in platelet microparticles. J Clin Invest 1997;99:2118–27.
[84] Haimovich B, Ji P, Ginalis E, Kramer R, Greco R. Phospholipase A2 enzymes regulate alpha IIb beta3-mediated, but not Fc gammaRII receptor-mediated, pp125FAK phosphorylation in platelets. Thromb Haemost 1999;81:618–24.
[85] Creer MH, McHowat J. Selective hydrolysis of plasmalogens in endothelial cells following thrombin stimulation. Am J Physiol 1998;275:C1498–507.
[86] Wang D, Dubois RN. Prostaglandins and cancer. Gut 2006;55:115–22.
[87] Spector AA, Norris AW. Action of epoxyeicosatrienoic acids (EETs) on cellular function. Am J Physiol Cell Physiol 2007;292:C996–1012.
[88] Yuan CJ, Mandal AK, Zhang Z, Mukherjee AB. Transcriptional regulation of cyclooxygenase-2 gene expression: novel effects of nonsteroidal anti-inflammatory drugs. Cancer Res 2000;60:1084–91.
[89] Liou JY, Aleksic N, Chen SF, Han TJ, Shyue SK, Wu KK. Mitochondrial localization of cyclooxygenase-2 and calcium-independent phospholipase A2 in human cancer cells: implication in apoptosis resistance. Exp Cell Res 2005;306:75–84.
[90] Suh YJ, Chada S, McKenzie T, Liu Y, Swisher SG, Lucci A, et al. Synergistic tumoricidal effect between celecoxib and adenoviral-mediated delivery of mda-7 in human breast cancer cells. Surgery 2005;138:422–30.
[91] Murakami M, Masuda S, Shimbara S, Ishikawa Y, Ishii T, Kudo I. Cellular distribution, post-translational modification, and tumorigenic potential of human group III secreted phospholipase A(2). J Biol Chem 2005;280:24987–98.
[92] Xu L, Han C, Wu T. A novel positive feedback loop between peroxisome proliferator-activated receptor-delta and prostaglandin E2 signaling pathways for human cholangiocarcinoma cell growth. J Biol Chem 2006;281:33982–96.
[93] Wendum D, Comperat E, Boelle PY, Parc R, Masliah J, Trugnan G, et al. Cytoplasmic phospholipase A2 alpha overexpression in stromal cells is correlated with angiogenesis in human colorectal cancer. Mod Pathol 2005;18:212–20.
[94] Jiang WG, Douglas-Jones A, Mansel RE. Levels of expression of lipoxygenases and cyclooxygenase-2 in human breast cancer. Prostaglandins Leukot Essent Fatty Acids 2003;69:275–81.
[95] Kim JH, Hubbard NE, Ziboh V, Erickson KL. Attenuation of breast tumor cell growth by conjugated linoleic acid via inhibition of 5-lipoxygenase activating protein. Biochim Biophys Acta 2005;1736:244–50.
[96] Carraway RE, Hassan S, Cochrane DE. Regulation of neurotensin receptor function by the arachidonic acid-lipoxygenase pathway in prostate cancer PC3 cells. Prostaglandins Leukot Essent Fatty Acids 2006;74:93–107.
[97] Ghosh J. Rapid induction of apoptosis in prostate cancer cells by selenium: reversal by metabolites of arachidonate 5-lipoxygenase. Biochem Biophys Res Commun 2004;315:624–35.
[98] Liu B, Maher RJ, Hannun YA, Porter AT, Honn KV. 12(S)-HETE enhancement of prostate tumor cell invasion: selective role of PKC alpha. J Natl Cancer Inst 1994;86:1145–51.
[99] Nony PA, Kennett SB, Glasgow WC, Olden K, Roberts JD. 15S-Lipoxygenase-2 mediates arachidonic acid-stimulated adhesion of human breast carcinoma cells through the activation of TAK1, MKK6, and p38 MAPK. J Biol Chem 2005;280:31413–9.
[100] Kuwata H, Nonaka T, Murakami M, Kudo I. Search of factors that intermediate cytokine-induced group IIA phospholipase A2 expression through the cytosolic phospholipase A2- and 12/15-lipoxygenase-dependent pathway. J Biol Chem 2005;280:25830–9.
[101] Karlgren M, Ingelman-Sundberg M. Tumour-specific expression of CYP2W1: its potential as a drug target in cancer therapy. Expert Opin Ther Targets 2007;11:61–7.
[102] Jiang JG, Chen CL, Card JW, Yang S, Chen JX, Fu XN, et al. Cytochrome P450 2J2 promotes the neoplastic phenotype of carcinoma cells and is up-regulated in human tumors. Cancer Res 2005;65:4707–15.
[103] Okamoto T, Momose S, Hino O. Suppression of cytochrome P450 1A1 and 4A1 gene expression in renal carcinomas of TSC2 gene mutant (Eker) rats. Int J Oncol 2001;18:147–9.
[104] Tollet P, Hamberg M, Gustafsson JA, Mode A. Growth hormone signaling leading to CYP2C12 gene expression in rat hepatocytes involves phospholipase A2. J Biol Chem 1995;270:12569–77.

[105] Seree E, Villard PH, Pascussi JM, Pineau T, Maurel P, Nguyen QB, et al. Evidence for a new human CYP1A1 regulation pathway involving PPAR-alpha and 2 PPRE sites. Gastroenterology 2004;127:1436–45.
[106] Kim HS, Ishizuka M, Kazusaka A, Fujita S. Alterations of activities of cytosolic phospholipase A2 and arachidonic acid-metabolizing enzymes in di-(2-ethylhexyl)phthalate-induced testicular atrophy. J Vet Med Sci 2004;66:1119–24.
[107] Aoki J, Taira A, Takanezawa Y, Kishi Y, Hama K, Kishimoto T, et al. Serum lysophosphatidic acid is produced through diverse phospholipase pathways. J Biol Chem 2002;277:48737–44.
[108] Eder AM, Sasagawa T, Mao M, Aoki J, Mills GB. Constitutive and lysophosphatidic acid (LPA)-induced LPA production: role of phospholipase D and phospholipase A2. Clin Cancer Res 2000;6:2482–91.