Roles of cPLA$_2$α and Arachidonic Acid in Cancer

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Abstract

Phospholipase A$_2$s (PLA$_2$s) are key enzymes that catalyze the hydrolysis of membrane phospholipids to release bioactive lipids that play an important role in normal cellular homeostasis. Under certain circumstances, disrupted production of key lipid mediators may adversely impact physiological processes, leading to pathological conditions such as inflammation and cancer. In particular, cytosolic PLA$_2$α (cPLA$_2$α) has a high selectivity for liberating arachidonic acid (AA) that is subsequently metabolized by a panel of downstream enzymes for eicosanoid production. Although concentrations of free AA are maintained at low levels in resting cells, alterations in AA production, often resulting from dysregulation of cPLA$_2$α activity, are observed in transformed cells. In this review, we summarize recent evidence that cPLA$_2$α plays a role in the pathogenesis of many human cancers. Much of this evidence has been accumulated from functional studies using cPLA$_2$α-deficient mice, as well as mechanistic studies in cell culture. We also discuss the potential contribution of cPLA$_2$α and AA to apoptosis, and the regulatory mechanisms leading to aberrant expression of cPLA$_2$α.

Keywords

Phospholipase A$_2$; Arachidonic acid; Colon Cancer; Apoptosis; Eicosanoids; Sphingomyelinase

1. Overview

Phospholipase A$_2$s (PLA$_2$) represent an important superfamily of enzymes that catalyze the hydrolysis of membrane phospholipids at the *sn*-2 position to release lipid second messengers that play a fundamental role in inflammation and cancer. There are three major classes of PLA$_2$s: secretory (sPLA$_2$s), Ca^{2+}-independent (iPLA$_2$s), and cytosolic PLA$_2$s (cPLA$_2$s). In addition, there is a closely related group of proteins referred to as the platelet activating factor acetylhydrolases (lipoprotein-associated PLA$_2$s). The characteristics of the PLA$_2$s vary in terms of protein structure, tissue distribution, pH optima, Ca^{2+}-dependency and substrate specificities, thereby conferring their distinct cellular functions. While a comprehensive discussion of PLA$_2$s can be found in several excellent review articles [1-4], we will highlight studies that focus on the role of the PLA$_2$s in the production of arachidonic acid (AA), and how this may relate to cancer pathogenesis.

In recent years, a number of studies have examined the regulatory mechanisms that control the activity of cPLA$_2$α during tumorigenesis. This interest has been stimulated by the high selectivity of cPLA$_2$α on membrane phospholipids containing AA (5.8.11.14: eicosatetraenoic acid). Free AA is rapidly metabolized by the cyclooxygenases (COX), lipoxygenases (LOX) and cytochrome P450s to yield a wide spectrum of eicosanoid metabolites (Figure 1). Because of the potent effects exerted by these bioactive lipids, the enzymatic release of AA from membrane phospholipids must be tightly controlled and its intracellular concentrations maintained at low levels in resting cells [5]. In many types of cancers, however, dysregulation of cPLA$_2$α activation, as well as induction of downstream AA metabolizing enzymes, are often observed. This metabolic imbalance accounts for the high levels of proliferative eicosanoids, such as prostaglandin E$_2$ (PGE$_2$), that are commonly found in tumor cells. These alterations

have provided the rationale for the use of non-steroidal anti-inflammatory drugs (NSAIDs) as chemoprevention agents against certain forms of cancer [6]. Despite their clinical effectiveness, however, NSAID treatment is often associated with adverse side effects, including gastrointestinal bleeding and cardiovascular toxicity [7]. Based on these adverse effects, other components of the AA cascade, including $cPLA_2\alpha$, have been considered as potential targets for therapy and chemoprevention. Although studies correlating increased levels of $cPLA_2\alpha$ and PGE_2 suggest that removing the "source" of proliferative eicosanoids may be effective in blocking tumorigenesis, there are many issues that must be resolved. In recent years, a by-product of AA, lysophosphatidic acid (LPA; 1-acyl-3-phosphoglycerol), and its metabolite, platelet-activating factor (PAF; 1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine), have also stimulated interest as a result of their potent effects on inflammation and mitogenesis [8,9]. The role of these bioactive lipids in tumorigenesis have been reviewed extensively elsewhere, and thus will not be discussed further [8,10].

In the following review, we will summarize studies demonstrating the involvement of the PLA_2s, in particular $cPLA_2\alpha$, in the pathogenesis of cancer, both in animal models and in humans. In addition, we will focus on the contribution of the PLA_2s and AA to apoptosis, and how the intracellular balance of AA is altered in transformed cells.

2. PLA$_2$s and human cancers

Secretory phospholipse A$_2$

In one of the first studies to associate the PLA_2s with cancer, MacPhee *et al*. [11] reported that the gene, *Pla2g2a*, encoding the group IIA PLA_2 (sPLA$_2$IIA), was localized within a chromosomal region associated with the <u>m</u>odifier <u>of M</u>in (*Mom-1*) locus. *Mom-1* locus was first identified by Dietrich et al. [12] using quantitative trait loci (QTL) mapping in an attempt to clarify phenotypic variations found among individuals inheriting adenomatous polyposis coli (Apc) gene mutations [12]. It was further shown that inactivating mutations of *Pla2g2a* enhanced tumorigenesis in the small intestine of Apc^{Min} mice [11], supporting the suggestion that *Pla2g2a* may represent a tumor modifier. These results provided the impetus for several studies to identify a human homologue of *Pla2g2a* with inactivating mutations. For example, Praml et al. [13] mapped the chromosomal position of a *Mom-1* candidate to a region between 1p35 and 1p36.1 that is commonly lost in human cancers. Interestingly, this chromosomal region was also found to harbor loss of heterozygosity (LOH) in 48% of human sporadic colon cancers [13]. Riggins et al. [14] also tested the possibility that the human *Pla2g2a* homologue was in fact the tumor suppressor gene that maps to chromosome 1p. Using a set of polymorphisms, they found that 31% of colorectal cancers (CRC) lost a *Pla2g2a* allele. However, they found no somatic mutations within the remaining allele, and concluded that although *Pla2g2a* was located within a chromosomal region of common loss, it was unlikely to represent the 1p35 tumor suppressor [14]. Spirio et al. [15] then tested the possibility that a mutation in the human homologue might account for a more severe tumor phenotype among individuals with attenuated familial adenomatous polyposis (FAP), a heritable form of colon cancer. Three PLA_2 genes (*Pla2g2a, Pla2g2c, Pla2g5*) were sequenced from subjects presenting clinically with a wide range of polyp sizes. There was no stratification of disease severity among the patient subgroups, however, indicating that mutations of the human homologue of the mouse *Pla2g2a* gene were not likely to be responsible for the phenotypic variations typically observed among FAP patients [15]. Tomlinson et al. [16] reached a similar conclusion regarding *Pla2g2a* in 70 FAP patients from 20 families, although they did report the presence of several polymorphisms and variant forms of the gene. Kennedy et al. [17] examined sPLA$_2$IIA mRNA and protein levels in normal mucosa, as well as in duodenal and colorectal adenomas, from FAP patients. They found a significant increase in the expression of sPLA$_2$IIA in adenomatous tissue relative to the normal intestine, a result that is in direct contrast to the earlier studies in Apc^{Min} mice [17]. Furthermore, there were no mutations found

in the adenomas, suggesting that the effect of sPLA$_2$IIA may be species-dependent [17]. Although overexpression of sPLA$_2$IIA has been found in other human tumors, including prostate, Barrett's adenocarcinoma and signet ring carcinoma [17-21], the role of sPLA$_2$IIA in cancer remains ambiguous.

Cytosolic phospholipase A2

Alterations in the levels and functional activity of cPLA$_2$α have also been associated with cancer pathogenesis. Several of these studies are summarized below in Table 1. Overexpression of cPLA$_2$α has been reported in a variety of human cancers and tumor cell lines such as cholangiocarcinomas (SG231) and non-small lung squamous carcinoma (NSCLC) [22-24]. In Barrett's esophagus, however, cPLA$_2$α mRNA expression was found in only 18% of adenocarcinomas, and an inverse association was reported between cPLA$_2$α expression and depth of tumor infiltration, vascular invasion and perineural invasiveness [20]. Interestingly, cPLA$_2$α expression in NSCLC was associated with the presence of oncogenic *Ras* mutations [23]. Furthermore, constitutively active Ras was sufficient to induce the expression of both cPLA$_2$α as well as COX-2 in normal lung epithelial cells [25], providing a potential mechanism for the high levels of PGE$_2$ often found in this form of lung cancer.

In one of the earliest studies to evaluate cPLA$_2$α status in human CRC, Soydan et al. [26] examined the levels of cPLA$_2$α in surgically excised colon tumors and matched normal tissue. Using Western analysis, they reported increased levels of cPLA$_2$α in a subset of tumors (6 of 17) with a concomitant increase in functional activity [26]. The same group also examined cPLA$_2$α levels in human stomach tumors but found no increase, even when COX-2 levels were highly induced [27]. Osterstrom et al. [28] examined 42 primary colorectal cancers for expression of cPLA$_2$α using DNA dot-blots. Samples were further stratified for the presence of K-ras mutations. Although there was an increase in cPLA$_2$α expression, the levels varied widely and there was no correlation with *K-ras* mutational status [28]. Using semi-quantitative RT-PCR, Dong et al. [29] found an opposite result in five human colorectal cancers, wherein cPLA$_2$α expression was reduced despite increased COX-2 expression in 4/5 tumors. In this study, the mRNA expression data was confirmed by immunohistochemical staining (IHC) of tumor tissue and adjacent normal epithelium [29]. These data revealed intense perinuclear staining of cPLA$_2$α within the normal colonic epithelium, a result that is in agreement with an earlier study in CHO cells showing subcellular localization of activated cPLA$_2$α within the endoplasmic reticular membrane and nuclear envelope [30].

To further explore the potential for coordinated regulation of cPLA$_2$α and COX-2 expression in human colon tumors, our laboratory analyzed a total of 27 human colorectal tumors for cPLA$_2$α and COX-2 expression using IHC and correlated these findings with apoptotic index [31]. The absence of cPLA$_2$α was reported in 84.6% (11/13) of the COX-2-overexpressing tumors, suggesting that COX-2 and cPLA$_2$α expression are not synchronously overexpressed in a significant percentage of human CRC. These findings suggest that up-regulation of COX-2 without a corresponding increase in cPLA$_2$α activity may lead to diminished AA levels, an outcome that would be further exacerbated by its enhanced utilization. This in turn would lead to an imbalance in the cellular pools of AA and the potential loss of several key apoptotic pathways, perhaps conferring a survival advantage that may facilitate tumorigenesis. In a subsequent study, Wendum et al. [32] used IHC to examine cPLA$_2$α and COX-2 expression in a total of 48 colorectal adenocarcinomas and found an opposite result. Overexpression of cPLA$_2$α was found in superficial stromal cells, corresponding to fibroblasts and myofibroblasts. Interestingly, there was correspondence between the number of cPLA$_2$α and COX-2 expressing cells and cPLA$_2$α-positive stromal cells correlated with high microvessel density and VEGF expression [32], leading to speculation that cPLA$_2$α may regulate COX-induced angiogenesis *via* production of AA. More recently, this group tested cPLA$_2$α and

COX-2 expression by IHC in 65 human CRC specimens [33]. Although $cPLA_2\alpha$ expression was not correlated with microsatellite instability, histological pattern or tumor stage, approximately half of the cases showed significant $cPLA_2\alpha$ expression that was associated with COX-2 elevation [33].

3. Genetic deletion of $cPLA_2\alpha$ in mice

Phenotypes of $cPLA_2\alpha$ knockout mice

An important milestone in our understanding of $cPLA_2\alpha$ function was the generation of $cPLA_2\alpha$-deficient mice, accomplished in 1997 [34,35]. These heterozygous and nullizygous mice, created by homologous recombination, have provided a useful *in vivo* experimental model for studying the physiological and pathogenetic roles of the enzyme. The $cPLA_2\alpha$ knockout mice are characterized by a normal lifespan and generally do not display obvious phenotypic defects, with the exception of compromised production of leukotrienes, prostaglandins and PAF [34,35]. Suboptimal production of prostaglandins, most notably prostaglandin $F_2\alpha$ ($PGF_2\alpha$), may underlie the difficulties in embryogenesis and implantation observed in the nullizygous mice [36]. Several additional phenotypic alterations include a failure to negatively regulate insulin-like growth factor-1 (IGF-1) signaling in the skeletal muscle of the heart, and reduced aquoporin-1 expression in proximal tubules [37,38]. Additional studies in $cPLA_2\alpha$-deficient mice show that its absence results in resistance to inflammation, demonstrated by suppression of ischemia-reperfusion injury of the brain [34], collagen-induced arthritis [39], inflammatory bone resorption [40], pulmonary inflammatory responses [41,42], HCl- or endotoxin-induced acute respiratory distress syndrome [42], and experimental autoimmune encephalomyelitis [43]. In contrast, the absence of $cPLA_2\alpha$ has been shown to exacerbate autoimmune diseases such as type-1 diabetes [44]. In one such study, it was shown that non-obese diabetic (NOD) mice deficient in $cPLA_2\alpha$ developed severe insulitis and a higher incidence of diabetes compared to their wild-type littermates [44]. Furthermore, peritoneal macrophages isolated from NOD mice with a $cPLA_2\alpha$ deficiency produced significantly more TNF-α in response to LPS treatment, suggesting that $cPLA_2\alpha$ may be involved in a negative feedback loop regulating TNF-α synthesis [44].

Small intestine

The deletion of $cPLA_2\alpha$ in mice has provided fundamental new insights into its potential role in the pathogenesis of cancer. In the first study to directly test the role of $cPLA_2\alpha$ in cancer, Takaku *et al.* [45] introduced the $cPLA_2\alpha$ deletion into $Apc^{\Delta716}$ mice, a model of human FAP. At 10 weeks of age, the size of small intestinal polyps was reduced by approximately 11-fold, although there were no significant differences in the total numbers of polyps [45]. This result provided the first genetic evidence that $cPLA_2\alpha$ plays a key role in the expansion of intestinal polyps without affecting the initiation of tumors [45]. In contrast to these striking findings in the small intestine, however, colon polyp growth was unaffected by the absence of $cPLA_2\alpha$ [45]. Hong et al. [46] performed a similar study using Apc^{Min} mice. Consistent with the results of the earlier study with $Apc^{\Delta716}$ mice, the deletion of $cPLA_2\alpha$ was found to repress the growth of polyps in the small intestine. In contrast to the earlier study, the number of tumors was also reduced by 83% in the compound mutants [46]. Interestingly, the observed suppression of intestinal tumors in $cPLA_2\alpha$-null mice is consistent with earlier findings in COX-2 knockout mice crossed with Apc^{Min} mice [47], suggesting that $cPLA_2\alpha$ is the predominant source of AA for COX-2 within the small intestinal epithelium [46]. It should also be noted that the Apc^{Min} study [46] showed a trend towards larger numbers and/or size of polyps within the colon, suggesting an organ-specific role of $cPLA_2\alpha$ in modulating intestinal tumorigenesis.

Colon

The reported trend in colon tumorigenesis observed in Apc^{Min} mice [45] was investigated in greater detail using a murine model of colon carcinogenesis. In this study [48], cPLA$_2$α wild-type, heterozygous and nullizygous mice were administered azoxymethane (AOM), an organospecific methylating carcinogen that targets the distal colon [49-51]. AOM treatment of cPLA$_2$α-deficient mice increased the number of colon tumors at twenty-four weeks after carcinogen exposure relative to wild-type controls, reflected by an increased tumor multiplicity (7.2-fold) and tumor volume (5.5-fold) [48]. The enhanced tumor sensitivity might be explained, in part, by compromised apoptosis, evidenced by the significant reduction (up to 50%) in the number of TUNEL-positive cells observed within the colonic epithelium of heterozygous and nullizygous mice [48]. Interestingly, reduced levels of apoptosis corresponded with diminished production of the cell death mediator, ceramide [48]. A key observation is that enhanced tumorigenesis occurred despite a significant reduction in PGE$_2$ production, even in COX-2 overexpressing tumors.

Lung

The effects of cPLA$_2$α deletion have also been tested in a lung cancer model [52]. cPLA$_2$α-null mice on a genetic background of intermediate sensitivity to lung tumorigenesis (B6/129) were treated with six injections of the lung carcinogen, urethane. Nineteen weeks after carcinogen exposure, cPLA$_2$α-null mice developed 43% fewer tumors compared to their wild-type littermates. Although the steady-state levels of PGE$_2$ and PGI$_2$ in lung tissue from wild-type or cPLA$_2$α-null mice were similar, the levels of both prostaglandins were significantly elevated in tumors harvested from wild-type mice, an increase that was significantly attenuated in cPLA$_2$α-null tumors [52]. The authors conclude that cPLA$_2$α may play a role in lung carcinogenesis that is mediated through the production of prostaglandins [52], an outcome that is consistent with earlier studies using Apc^{Min} and Apc$^{\Delta 716}$ compound mutant mice [46].

4. cPLA$_2$α and apoptosis

A number of recent studies have attempted to determine how cPLA$_2$α activation influences apoptotic signaling [53]. These studies have generated data that are consistent with a mechanism in which AA derived from cPLA$_2$α is cytotoxic. Upon addition of exogenous AA to cells in culture, a range of apoptotic responses are observed including alterations in mitochondrial membrane integrity, caspase activation and DNA fragmentation [54]. It has further been demonstrated that AA can directly activate the sphingomyelinase (SMase)-ceramide pathway, which has also been associated with cell death [55]. However, the relative contribution of endogenous AA, or its downstream metabolites, to cell death remains obscure. For example, the 15-LOX product, 15-HETE, and several cytochrome P450 metabolic products, including 12-EET and 19-HETE, can induce apoptosis when applied directly to cells. The apoptotic effects of these cytochrome P450-derived eicosanoids have been described recently in an excellent review by Cederbaum and Caro [56] and thus will not be further discussed. Instead, we will focus primarily on potential mechanisms by which the activation of cPLA$_2$α and the resulting accumulation of intracellular AA promote cellular apoptosis.

TNF-α

The cytotoxic effects elicited by the pro-inflammatory cytokine, tumor necrosis factor-α (TNF-α), require the induction of cPLA$_2$α gene expression and its functional activation, leading to AA release [57]. Additional evidence for the role of cPLA$_2$α in the biological actions of TNF-α has been obtained with the use of pharmacologic or genetic inhibition [58-61]. In a recent study by Dong et al. [29], inhibition of cPLA$_2$α activity by arachidonyl trifluoromethyl ketone (AACOCF$_3$) in young adult mouse colon cells (YAMC) was found to protect colonocytes from TNF-α-induced apoptosis [29]. In a subsequent study, Dong et al. [31] produced a dose-

dependent inhibition in both the levels and functional activity of $cPLA_2\alpha$ in a human colon tumor cell line (HT-29) with the use of antisense oligonucleotides. As predicted, treatment of HT-29 cells with TNF-α was less effective in activating caspase-3 when $cPLA_2\alpha$ expression was blocked, indicating that its activity is required for signaling by TNF-α. These results are consistent with findings in other tumor cell lines, including those derived from breast, skin and leukemia cells [59,61].

Accumulation of free AA

The intracellular levels of AA are regulated through distinct and non-overlapping mechanisms. In resting cells, low concentrations of AA are tightly maintained by basal levels of rapid catabolism, and also by membrane phospholipid recycling *via* the actions of the arachidonyl-CoA transferase (CoA-T) (Lands cycle) [62]. In addition, esterification of AA by fatty acid CoA ligase (FACL) 4 also contributes to the maintenance of low AA levels [63]. On the other hand, stimulus-induced release of AA by $cPLA_2\alpha$ results in its rapid metabolism by induced COX-2, thereby limiting the intracellular AA pools [62]. In fact, the chemopreventive benefits of the NSAIDs, including the COX-2 selective inhibitors, may result, in part, from their ability to cause a buildup of AA [64].

Several recent studies have attempted to establish the importance of the Lands cycle in apoptosis. For example, pharmacological inhibition of CoA-T and CoA-independent transacylase activity resulted in a buildup of AA in leukemia cells followed rapidly by cell death [65-67]. Interestingly, Perez *et al.* [66] reported that in phagocytes, pharmacological inhibition of COXs or LOXs did not enhance the rate of apoptosis induced by inhibition of the Lands cycle, suggesting that AA-derived eicosanoid metabolites may not be directly involved in this process. Furthermore, in a recent study by Chalimoniuk *et al.* [68], nitric oxide stimulation of neuronal cells caused the activation of $cPLA_2\alpha$ with a concomitant reduction in CoA-T activity. This in turn resulted in inhibition of the Lands cycle [68]. These results suggest that reactive oxygen species induced by nitric oxide are capable of elevating intracellular AA through distinct and non-overlapping mechanisms [68].

A recent report by the Prescott laboratory [69] shows that overexpression of FACL4 and COX-2 synergistically inhibit apoptosis in colon cancer cells by reducing free AA. In addition, increased FACL4 expression was highly correlated with well (or moderately) differentiated adenocarcinomas, suggesting the possibility that selective FACL4 inhibitors may be useful for colon cancer chemoprevention [69]. In a related study, triacsin C was used to inhibit FACL4 in human embryonic kidney cells (HEK293). Apoptosis induced by the addition of exogenous AA or inhibition of COX-2 was amplified by treatment with the FACL4 inhibitor [69]. Additional studies using human lung carcinoma cells have demonstrated a synergistic reduction in cell viability upon treatment with a combination of inhibitors targeted against FACL4, the COXs and the Lands cycle, providing further evidence that intracellular accumulation of AA contributes to cell death [65].

Role of AA in mitochondrial-mediated apoptosis

Emerging evidence has shown that elevated intracellular AA can induce cell death *via* the mitochondrial-mediated apoptosis pathway [54]. Specifically, AA has been shown to affect mitochondrial membrane permeabilization (MMP) [54]. While MMP may involve either the inner or outer mitochondrial membranes, the outcome is always associated with cell death and release of cytochrome *c* [70]. The Bcl-2 family of proteins are responsible for the integrity of the outer membrane, and the inner mitochondrial membrane is regulated by the redox status of vicinal thiols and the mitochondrial permeability transition pore (PTP) [70]. An extended opening of the PTP has been reported in a rat hepatoma (MH1C1) cell line upon treatment with either TNF-α or exogenous AA, followed by release of cytochrome *c* and apoptosis [71]. Penzo

et al. [72] extended these observations using the Ca^{2+} ionophore (A23187) in MH1C1 cells to directly stimulate the activity of $cPLA_2\alpha$ and its subsequent release of AA. In this study, apoptosis was observed in approximately 30% of cells treated with Ca^{2+} alone, while the addition of the non-specific COX and LOX inhibitors, indomethacin and MK866, respectively, increased apoptosis by up to 2-fold [72,73].

There are other mechanisms by which AA may influence cell death. For example, the anti-apoptotic protein, Bcl-2, is overexpressed in a number of human tumors, and elevated levels of Bcl-2 are associated with resistance to apoptosis [70]. As shown by Sun et al. [74], colon tumor cells that stably overexpress COX-2 were resistant to apoptosis induction by NSAIDs and 5-FU. Interestingly, COX-2 expressing cells had markedly elevated Bcl-2 expression, an effect that occurred independently of PGE_2. These observations provide a mechanism by which some colon tumor cells acquire resistance to apoptosis following treatment with the COX-2 inhibitors, NS-398 and sulindac. Of note, the LOX inhibitors may have a dual function in altering the balance of both anti- and pro-apoptotic proteins. For example, treatment of breast cancer cells with the LOX inhibitors, Rev-5901, baicalein and MK-886, induced mitochondria-mediated apoptosis, in part by reducing the expression level of Bcl-2, while increasing the pro-apoptotic protein, Bax [75]. Similarly, alterations to the Bcl-2/Bax ratio have been reported following treatment of gastric and pancreatic cancer cells with LOX inhibitors [75,76], suggesting that the mechanisms that underlie the anti-tumorigenic properties of this class of molecules may be complex.

Recently, several research groups have undertaken a novel strategy to overcome NSAID-resistance to mitochondrial-mediated (intrinsic) apoptosis by combining NSAID treatment with Fas or TRAIL to stimulate membrane receptor-mediated apoptosis *via* the extrinsic pathway [77,78]. . In one such study, Sinicrope *et al.* [78] demonstrated that forced expression of Bcl-2 in colon cancer cells reduced cytochrome *c* release. This effect was associated with resistance to apoptosis in response to the COX-2 inhibitor, sulindac sulfide. Treatment of these colon tumor cells with HA 14-1, a specific Bcl-2 inhibitor, restored cytochrome *c* release and apoptosis [78]. Moreover, simultaneous treatment with a combination of HA 14-1 and TRAIL markedly enhanced sulindac sulfide-mediated apoptosis [78]. This latter result is important and demonstrates the possibility of exploiting alternative mechanisms for targeting NSAID-resistant tumor cells.

Activation of the SMase-ceramide pathway by AA

The cytotoxicity of AA may also be mediated through its ability to increase ceramide levels. Ceramide, a complex family of lipid molecules that is comprised of a sphingosine base and a fatty acid moiety is a potent inducer of cell death. Ceramide can be synthesized through a *de novo* synthetic pathway that involves the condensation of serine and palmitoyl CoA [79]. Ceramide can also be generated directly from sphingomyelin by the actions of the SMases, a family of proteins consisting of three isoforms (acidic, neutral and alkaline) [79]. The activation of SMases with the resultant production of ceramide has been shown to occur in response to various stimuli, including treatment of cells with either TNF-α or NSAIDs. In an attempt to reveal the mechanism of TNF-α-mediated apoptosis, Jayadev *et al.* [80] identified a correlation between TNF-α-induced accumulation of AA and increased levels of ceramide in human promyelocytic leukemia cells. Furthermore, SMase activation has also been shown to occur in a number of human tumor cell lines, including breast [81], prostate [82], brain [83], and colon [84] following treatment of cells with TNF-α. A recent report by Martin *et al.* [77] demonstrated that an accumulation of AA caused by the COX-2 inhibitor, DuP-697, activated acidic SMase to generate ceramide-enriched caveolae within the plasma membrane outer leaflet in HT-29 colon cancer cells [77]. Subsequently, these caveolae enhanced the clustering of a TRAIL-mediated death-inducing signaling complex [77]. Similarly, Voelkel-Johnson *et al.* [85]

showed that the addition of a membrane permeable, synthetic C_6-ceramide enabled metastatic colon cancer cells to overcome their normal resistance to TRAIL-induced apoptosis, thus establishing a strong association between AA, ceramide and subsequent apoptosis.

Our laboratory and others have also shown that alterations of the SMaseceramide pathway may be associated with tumorigenesis [48,86,87]. For example, as noted above, Ilsley *et al.* [48] established an association between reduced ceramide production and diminished apoptosis in carcinogen-induced colon tumors, a finding that was restricted to cPLA$_2$α-deficient mice. In addition, Wu *et al.* [87] showed that the growth inhibitory effects of omega-3 polyunsaturated fatty acids on breast tumor xenografts were correlated with elevated activity of the neutral SMase isoform. Moreover, reduced activity of the intestinal-specific alkaline SMase has been reported in human colorectal tumors [88,89]. Decreased levels of alkaline SMase activity have also been found in human stool samples collected from CRC patients, suggesting its potential as a novel diagnostic marker for colon cancer [86]. For a detailed discussion of alkaline SMase, see the recent review by Duan [90].

5. Regulation of cPLA$_2$α expression and activity

cPLA$_2$α expression induced by transformed cells

The regulation of cPLA$_2$α is complex, involving both direct transcriptional activation, as well as post-translational modifications. As described above, the levels of cPLA$_2$α are tightly regulated in order to maintain consistent production of AA as well as an optimum balance of eicosanoid metabolites [2]. In tumor cells, however, the transcriptional and post-translational regulation of cPLA$_2$α is often dysregulated, resulting in aberrant production of downstream proliferative signals, including PGE$_2$. This observation is well illustrated by the transcriptional activation of cPLA$_2$α by oncogenic Ras in human lung tumor cells (NSCLC) [23]. As noted earlier, Rasactivating mutations are frequently found in a variety of tumors, including prostate, colon and lung [91]. Oncogenic transformation of Ras results in constitutive activation of the ERK and PI3K/Akt pathways, which are commonly associated with tumor promotion [91]. Blaine et al. [92] have shown that Ras induces expression of cPLA$_2$α in NSCLC cells *via* direct binding of the transcription factors, Sp1 and c-jun, to the cPLA$_2$α promoter [92]. Interestingly, it was shown in human skin-derived carcinoma cells (A431) that the translocation and phosphorylation of cPLA$_2$α could be accomplished by another target of c-jun, epidermal growth factor receptor (EGFR) [93]. Recently, Hassan *et al.* [94] demonstrated in prostate cancer cells that enhanced cPLA$_2$α activation during EGFR signaling provides a source of AA for the synthesis of 5-hydroxyeicosatetraenoic acid (5-HETE) by 5-LOX, which will subsequently increase PKC activity, resulting in cell growth. Moreover, EGFR signaling is also reported to induce COX-2 expression in human epithelial carcinoma cells (A431) [93]. Thus, it can be argued that the coordinated activation of these key pathway enzymes in transformed cells likely accounts for the high levels of PGE$_2$ that are found in these tumors.

The tumor growth factor, TGF-β, has also been shown to enhance cPLA$_2$α-mediated PGE$_2$ production [95]. Although TGF-β induces tumor growth arrest in normal epithelial cells through Smad-mediated gene transcription, transformed cells acquire resistance to the anti-tumorigenic effect of TGF-β [95]. To determine whether this resistance is associated with aberrant production of PGE$_2$, Han et al. [95] tested the effects of TGF-β in a panel of human liver cancer cells. Addition of TGF-β1 resulted in the activation of the Smad2 transcription factor, as well as phosphorylation of cPLA$_2$α [95]. Increased levels of cPLA$_2$α blocked TGF-β-mediated growth inhibition, an effect that was observed by the addition of cPLA$_2$α inhibitor which otherwise induced the growth arrest [95]. Moreover, the inhibition of COX-2, PGE$_2$ receptor 1 (EP1) or PGJ$_2$ receptor, peroxisome proliferator-activated receptor-gamma (PPAR-γ), also restored the anti-tumorigenic effects of TGF-β, invoking multiple prostaglandin-dependent pathways for disruption of tumorigenesis [95].

There is recent evidence that $cPLA_2\alpha$ activity may also be involved in cell-cell contact within endothelial HUVEC cells. In the following study, pharmacological inhibition of $cPLA_2\alpha$ with pyrrolidine-1 was found to inhibit HUVEC proliferation, while exogenously added AA reversed this effect [96]. Interestingly, a confluence-dependent redistribution of $cPLA_2\alpha$ to the distal Golgi was observed, an effect that was associated with inhibition of activity and reduced cell proliferation in the HUVECs. $cPLA_2\alpha$ activity was restored by its cellular relocation to the cytoplasm and perinuclear region in response to mechanical wounding of the confluent monolayers, suggesting a novel role for $cPLA_2\alpha$ in angiogenesis [96].

Transcriptional suppression of $cPLA_2\alpha$

$cPLA_2\alpha$ expression can also be down-regulated at the level of gene transcription. A recent report by D'Orazi *et al*. [97] describes the suppression of $cPLA_2\alpha$ expression by a transcriptional co-repressor, homeodomain-interacting protein kinase-2 (HIPK2). HIPK2 is a unique transcriptional regulator with kinase activity that exhibits anti-tumorigenic properties through its ability to directly activate p53 [98,99]. Silencing of HIPK2 by RNAi in colon cancer cells (RKO) enhanced $cPLA_2\alpha$ expression, and its increased activity was associated with elevated production of PGE_2 [97]. Moreover, RKO cells lacking HIPK2 resulted in enhanced tumor formation in nude mice [97]. Interestingly, it was also shown that an inverse relationship between the levels of HIPK2 and $cPLA_2\alpha$ occurred in colon tumors from FAP patients [97]. This novel observation may provide insight into earlier findings of reduced $cPLA_2\alpha$ expression in a subset of human colon cancers [29,58].

Regulation by AA metabolites

There is also evidence that AA and its metabolites can directly influence the levels of $cPLA_2\alpha$, raising the possibility of a positive feedback mechanism for the production of eicosanoids in transformed cells. As shown by Hughes-Fulford *et al*. [100], AA added exogenously to human prostate tumor cells (PC-3) induced the transcriptional activation of $cPLA_2\alpha$ and COX-2, followed by a subsequent dose-dependent increase in the synthesis of PGE_2 [100]. In addition, treatment of PC-3 cells with a COX-2 inhibitor, flurbiprofen, blocked the up-regulation of both $cPLA_2\alpha$ and COX-2 [100]. Although the latter finding suggests that PGE_2 may be able to induce $cPLA_2\alpha$ expression, the role of PGE_2 in this effect is unknown. In fact, it was shown in mouse lung fibroblasts that treatment of cells with various prostaglandins, including PGE_2, PGJ_2 and PGF_2 induced COX-2, but not $cPLA_2\alpha$, expression [101]. Only a slight increase in the cytokine-induced expression of $cPLA_2\alpha$ was observed by the addition of $PGF_2\alpha$ [101]. These conflicting results suggest that the regulation of $cPLA_2\alpha$ by metabolites of AA may not only be tissue-specific, but also context-specific.

6. Conclusions and future directions

Activation of $cPLA_2\alpha$ is the rate-limiting step in the generation of AA. As such, its catalytic activity plays a central role in a variety of cellular processes, including inflammation, proliferation and apoptosis. As highlighted in this review, alterations in the levels of $cPLA_2\alpha$ expression and functional activity are a common feature in tumors. In fact, because of its critical role in controlling the formation of prostaglandins, $cPLA_2\alpha$ has been proposed as a potential target for cancer chemoprevention, perhaps even as a substitute in some cases for the NSAIDs and COX-2 inhibitors. However, there is still much to learn with respect to how the $cPLA_2\alpha$-AA cascade elicits such opposing cellular responses, ranging from robust cellular proliferation to apoptosis. In tumor tissue, the effects of $cPLA_2\alpha$ remain enigmatic. For example, the expression levels of $cPLA_2\alpha$ in human colorectal tumors are highly variable, and it is not clear under what conditions $cPLA_2\alpha$ activity is associated with cancer-promoting or suppressing effects. Considering these observations, it may be more appropriate to consider $cPLA_2\alpha$ status

as a potential diagnostic marker, around which individualized treatment strategies might be designed.

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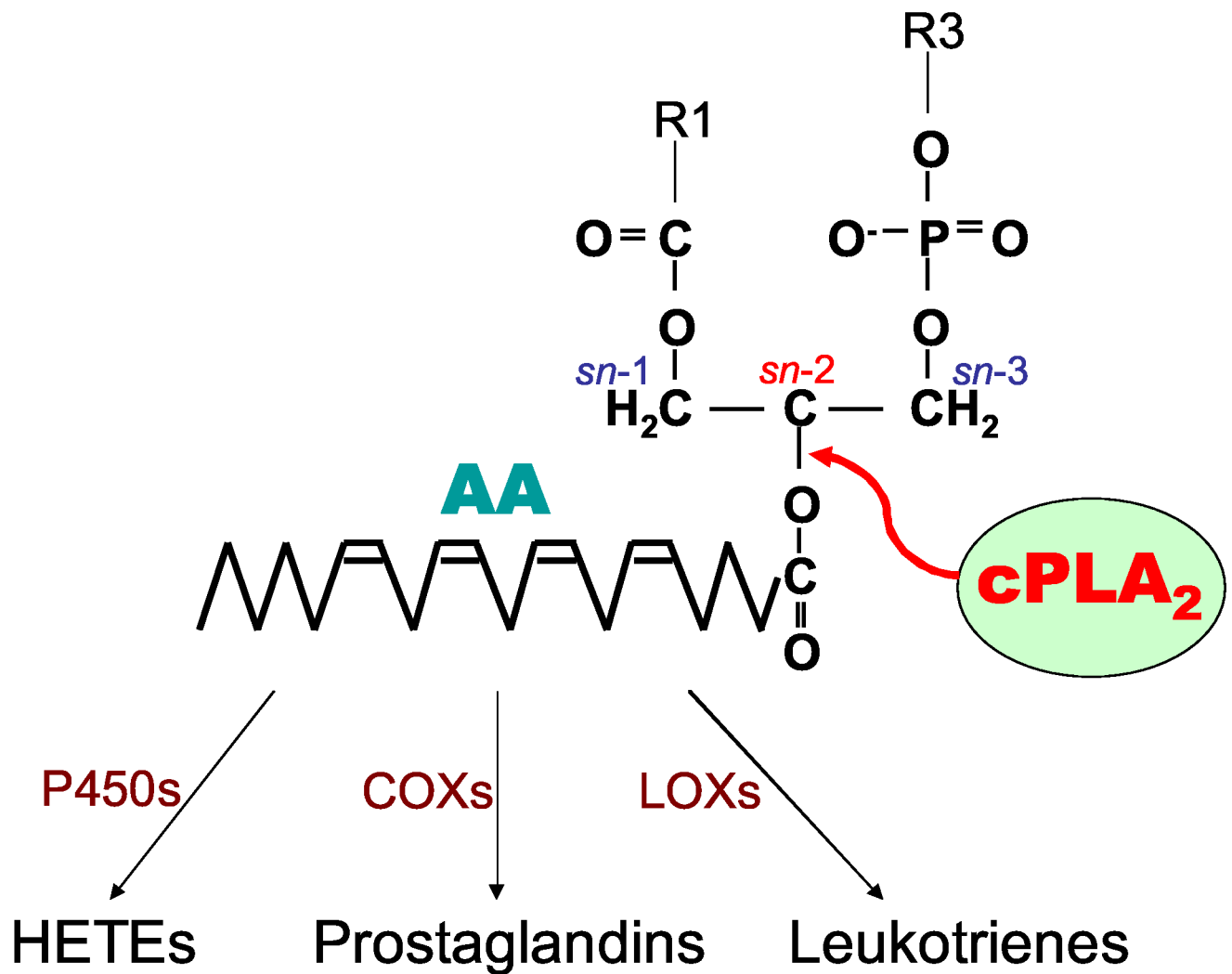
Figure 1.
Catalytic action of cPLA$_2$α at *sn*-2 position of membrane phospholipids. Free AA is metabolized into a variety of eicosanoids including prostaglandins and leukotrines

Table 1

cPLA$_2$α status in human cancers and cell lines

Organ	Tumor type / cell line	cPLA$_2$α status		Reference(s)
		mRNA	Protein	
Bile duct	Cholangiocarcinoma	↑ 5-fold		[20]
Lung	NSCLC		↑	[22]
Small intestine	Adenocarcinoma		↑ 48%	[23]
			↑ 35%	[24]
			↑ 35%	[23]
Colon	Adenocarcionoma		↑ 50%	[25]
			↑ 60%	[26]
			↓ 84.6%	[27]
		↑ 4/5		[28]
	Stromas		-	[29]
Esophagus	Adenocarcinoma		↑ 18%	[16]
Pancreas	BxPC3, Capan2, Cfpac1, L3.6p1		↑ 44%	[30]