RESEARCH COMMUNICATION

Immunohistochemical Expression of Cytosolic Phospholipase A2α in Non-small Cell Lung Carcinoma

Shenbagamoorthy Sundarraj, Soundarapandian Kannan*

Abstract

Purpose: Cytosolic phospholipase A2α is the main target enzyme for the non steroidal anti-inflammatory drugs that have been shown to suppress carcinogenesis in both experimental model and epidemiologic studies. Methods: We examined cPLA2α expression in normal, premalignant bronchial epithelial cells and nonsmall cell lung carcinoma (NSCLC) samples using an immunohistochemical staining technique. Included in the current study were 76 NSCLC samples and 52 bronchial biopsy samples obtained. Results: In the normal bronchial epithelium, cPLA2α expression was found to be completely negative whereas positive cPLA2α expression was limited to a few macrophages, inflammatory cells. There were relatively more cPLA2α positive tumors, as defined by positive staining in >10% of tumor cells 24 of 76 tumors (32%). When tumor types were considered, there were more cPLA2α positive adenocarcinomas compared with squamous cell carcinomas (17 of 36 adenocarcinomas (47%) vs. 6 of 34 squamous cell carcinomas (18%); *P*=0.02). Although smokers tended to have more cPLA2α positive tumors than nonsmokers (23 of 64 tumors in the smokers (36%) vs. 1 of 12 tumors in the nonsmokers (8%); *P*=0.06). Conclusion: The results of the current study suggest that cPLA2α expression may not be a useful intermediate biomarker in bronchial chemoprevention trials. Nevertheless, considering the patterns of cPLA2α expression in tumor cells, cPLA2α expression status may be a useful parameter when designing treatment strategies for a subset of NSCLC patients.

Keywords: Cytosolic phospholipase A2 Alpha - nonsmall cell lung carcinoma - immunohistochemistry

Asian Pacific J Cancer Prev, **11**, 1367-1372

Introduction

Phospholipase A2 (PLA2) enzymes are divided into four classes on the basis of their nucleotide and amino acid sequences, (i) Secretory phospholipase A2 (sPLA2), (ii) cytosolic PLA2α (cPLA2α), (iii) Calcium independent cytosolic PLA2 (iPLA2) and (iv) Platelet activating factor acetylhydrolase (PAF-AH). cPLA2α has been found recently to have a high expression level in many tumor specimens including lung carcinoma (Heasley et al., 1997). The functions of PLA2 are as diverse as their classes and include (i) inflammation, (ii) cell death, (iii) cell growth, (iv) cell signaling, and (v) maintenance of membrane phospholipids. Further the cPLA2 is subdivided into cPLA2α, β, and γ (Song et al., 2006). Of these isoforms cPLA2α has been studied most extensively. cPLA2α is an 85 KDa Serine esterase, which is expressed in a wide range of tissues except lymphocytes (Capper and Marshall, 2001). The gene for human cPLA2α, β, and γ are residing in the chromosomes 1, 15, and 19 respectively (Miyashita et al., 1995).

In the present study we are mainly focusing on cPLA2α because, it has attracted attention as a target for controlling eicosanoid related inflammation and cancer. It is synthesised within the cytosol of the cell in the form of inactive enzyme. When activated, cPLA2α translocated from the cytosol to membranes of (Schievella et al., 1995), such as the golgi, endoplasmic reticulum and nuclear envelope. Earlier reports authentically pointed out that, the translocation of cPLA2α enables interaction between the enzyme and its substrate membrane phospholipids. Further, it hydrolyzes arachidonate at the *Sn*-2 position of phospholipids because membrane phospholipids consist of a glycerol backbone, to which 2 long chain fatty acids are attached at the *Sn*-1 and *Sn*-2 positions and a phosphate containing head group at the *Sn*-3 position. The arachidonic acids located at position *Sn*-2 are found to be highly unsaturated and contain double bonds upto the maximum of five (Dessen, 2000). The cPLA2α enzyme acts upon the specific substrate phosphatidyl choline, and produce rich amount of arachidonic acid (AA), and lysophospholipids (Leslie, 2004).

The release of membrane bound AA initiates a cascade reactions leading to the production of lipid mediators responsible for inflammation (termed eicosanoids). These lipid mediators include Prostaglandins (PGs),

*Department of Zoology, Proteomics and Molecular Cell Biology Lab, School of Life Sciences, Bharathiar University, India. *For correspondence : sk_protein@buc.edu.in*

Leukotrienes (LTs), and Hydroxyeicosatetraenoic acid (HETEs) (Dessen, 2000). Hence cPLA2α has attracted attention as a target for controlling eicosanoid related inflammation and cancer. There are three serine residues Ser^{505}, Ser^{515}, and Ser^{727} of cPLA2α that are phosphorylated by mitogen activated protein kinases (MAPKs), Ca^{2+}/ calmodulin dependent protein kinase II (CaMK II) and mitogen activated protein interaction kinase (MNK I) respectively (Lin et al., 1993, Hefner et al., 2000). Phosphorylation of cPLA2α on Ser^{505} is dependent on the phosphorylation at Ser^{515} elicited by Ca MK II and both sites of phosphorylation are required for AA release (Muthalif et al., 2001). The current study was initiated to examine the potential utility of cPLA2α expression as an intermediate biomarker in lung carcinoma chemoprevention trials. To this end, we examined the expression of cPLA2α enzyme in normal and nonsmall cell lung carcinoma (NSCLC) cells.

Materials and Methods

Patient Selection

Included in the current study are NSCLC tumor samples obtained from 76 patients who underwent surgery at Cancer Research Institute with associated clinical data. These cases were selected according to the availability of paraffin wax embedded tumor tissue blocks with a confirmed diagnosis of NSCLC, as classified by the world Health Organization Criteria (WHO, 1981). No patients had received preoperative chemotherapy or radiotherapy. There were 52 men and 24 women with a range of age 34-78 years. Thirty six patients had adenocarcinoma, 34 patients had squamous cell carcinoma and 6 patients had large cell carcinoma. There were 39 patients with stage I disease, 21 patients with stage II disease, and 16 patients with stage III disease, disease stage was determined according the revised International system for staging lung cancer (Mountain, 1997). In addition, we included in the current study a total of 52 bronchial biopsy samples that were taken from 15 healthy smokers, as an initial screening for a previously reported bronchial chemoprevention trial (Lee et al., 1994).

Immunohistochemistry

Human tumor xenograft samples were formalin fixed, paraffin embedded tissue, sections cut at 4-5μm thickness, deparafinized, and micro-waved for 4x5 min in 0.01 mM citric acid monohydrate (pH 6.0). The slides were first immersed in 80 mM hydrogen peroxide in methanol for 30 min and then in blocking solution (0.01 M Tris, 0.1 M $MgCl_2$, 0.5% Tween20, 1% BSA, and 1:65 horse, rabbit and goat serum for 20 min depending on the primary antibody source) to block endogenous peroxidase activity and nonspecific binding sites, respectively. Tissue sections were incubated with three primary antibodies *viz* cPLA2α (N Terminal), cPLA2α (C Terminal) and Phospho cPLA2α (Ser^{505}) in a dilution of 1:50 in the blocking solution at 4°C over night. The sections were thereafter treated with secondary antibodies *viz* peroxidase Mouse IgG, peroxidase Goat IgG and peroxidase Rabbit IgG in a dilution of 1:200 (Genei Pvt. Ltd., Bangalore) for 1 hour, followed by the secondary antibody binding sites were finally visualized by streptavidin horseradish peroxidase complex solution, for 10 min and 3,3'- diaminobenzidine at room temperature. The solutions were counter stained with Harris's haematoxylin and then dehydrated and mounted.

Negative controls either in the absence of primary antibody or replaced with non-immuno rabbit IgG were included. These controls always showed no color development while positive controls demonstrated appropriate levels of immuolabelling.

Evaluation of Immunostaining

Slides were examined using a light microscope (10x objective lens) (Leica, Microsystems, UK) and lung tumor epithelium and normal alveolar epithelium were identified. Immunoreactivity was scored according to both its intensity and its distribution throughout the tissue observed within the field of view. Staining intensity was scored as 1 (no staining), 2 (Weak), 3 (medium), 4 (Strong), 5 (very strong). Grade was scored as 1 (<1%), 2 (1-10%), 3 (10-30%), 4 (30-50%), 5 (>50%) according to the percentages of positively stained tumor cells. For the evaluation of the relations between cPLA2α expression and other parameters, those cases showing the respective antigen expression in >10% of the tumor cells were considered to be positive.

Statistical Analysis

Statistical analysis was performed using the statistical software SPSS (13.0). Statistics were used to summarize patient characteristics and pathologic evaluation of the tumor and the bronchial biopsy specimens. Comparisons between the groups were made by the Fisher exact test and chi-square test when appropriate. All *P* values were derived from two sided statistical tests and a *P* value <0.05 was considered significant.

Results

cPLA2α Expression in Normal lung cells

Expression of cPLA2α varied from diffuse cytoplasmic to distinct perinuclear and cytoplasmic staining. Positive expression of cPLA2α was limited to a few macrophages, inflammatory cells and fibroblasts. Bronchial epithelium, blood vessels, vascular smooth muscle, which was included in the resected tumor sections, also demonstrated negative staining for cPLA2α. Bronchial biopsy samples from healthy smokers, which contained areas of normal bronchial epithelia in 52 samples all, were negative for cPLA2α expression (Figure 1).

cPLA2α Expression in NSCLC Cells

cPLA2α expression was relatively more prevalent than other enzyme in the current series. Overall, 32 of the 76 tumor samples (42%) exhibited positive immunostaining for cPLA2α in <1% of tumor cells, 31 of the 76 tumor samples (41%) in >10% of tumor cells, and 13 of the 76 tumor samples (17%) in >30% of tumor cells (Table 1). Again, the distribution pattern of cPLA2α positive cells was heterogeneous and patchy. However, in a few

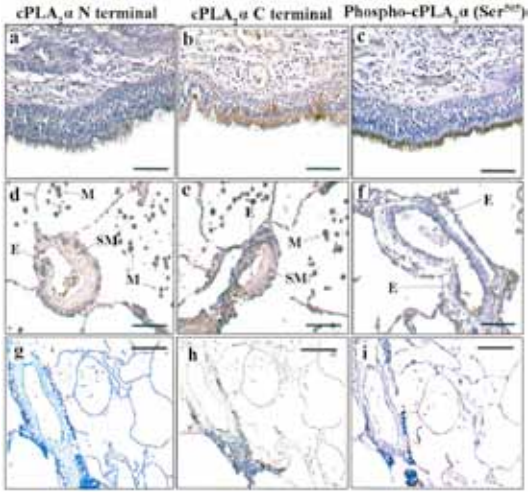

Figure 1. Immunolabelling of Endothelium and Vascular Smooth Muscle, Bronchial Epithelium and Normal Lung Cells (a, b, c) Negative cPLA2α Staining in Bronchial Epithelium, (d, e, f) Endothelium and Vascular Smooth Muscle, (g, h, i) Normal Lung Cells. Haematoxylin and Eosin Stained Section Immunohistochemistry Study Using Three Different cPLA2α Primary Antibodies Namely cPLA2α (N Terminal), cPLA2α (C Terminal) and Phospho-cPLA2α (Ser505). The scale bar 100μm

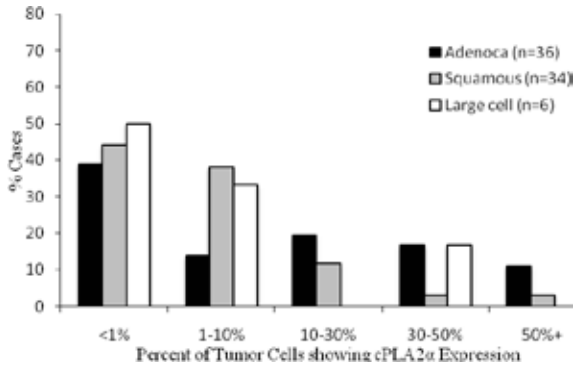

Figure 2. Percent Distribution of Nonsmall Cell Lung Carcinoma Cases Based on Histology that Demonstrated Cytosolic Phospholipase A2α expression in tumor cells

cases, intense cPLA2α staining was dispersed diffusely throughout the entire tumor cell nest, most often in less differentiated adenocarcinoma. Each of the three primary antibodies continued to demonstrate specific cellular staining patterns in keeping with their hypothesized antigen specific targets. Those specimens labeled with cPLA2α (N terminal) primary antibody showed cytosolic staining, those treated with phospho- cPLA2α (Ser505) primary antibody exhibited perinuclear staining with low levels of cytosolic staining whilst, those treated with cPLA2α (C terminal) primary antibody demonstrated staining throughout the cells encompassing both cytosol and nucleus. The incidence of cPLA2α positive tumor cells was common in adenocarcinoma. When a >10% positivity rate was used as the cutoff value, 17 of 36 adenocarcinoma (47%) demonstrated cPLA2α expression compared with 6 of 34 squamous cell carcinomas (18%), a difference that was statistically significant (P=0.02). In contrast

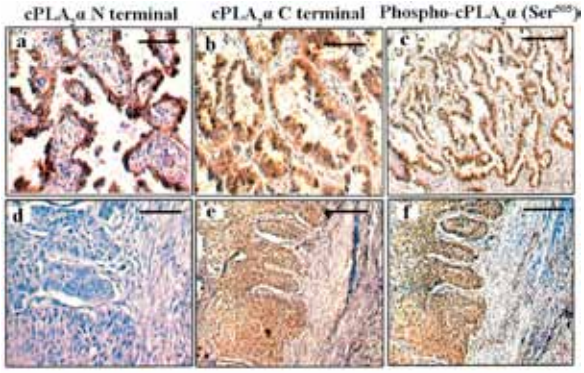

Figure 3. Photomicrographs of cPLA2α Expression in Adenocarcinoma and Squamous Carcinoma. Representative Sections from Each Tumor Type are Shown a, b, c) Strongly Stained Clinical Lung Adenocarcinoma Specimens Demonstrating High cPLA2α Expression, d, e, f) Strongly Stained Clinical lung Squamous Cell Carcinoma Demonstrating High cPLA2α Expression by Using Harris's Haematoxylin 100μm

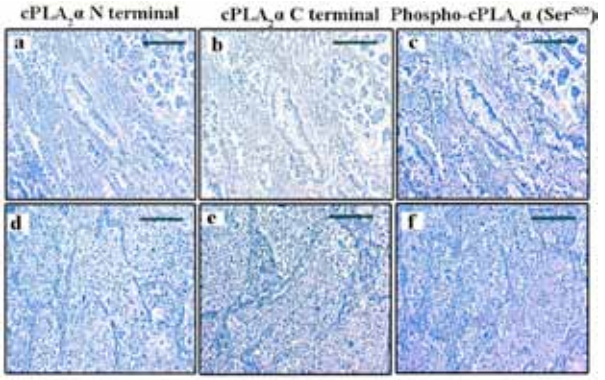

Figure 4. Photomicrographs of cPLA2α Expression in Adenocarcinoma and Squamous Carcinoma. Representative Sections from Each Tumor Type are Shown a, b, c) Weak cPLA2α Staining in the Area of Well Differentiated Adenocarcinoma and (d, e, f) Weak Staining in Squamous Cell Carcinoma by Using Harris's Haematoxylin 100μm

to cPLA2α expression in adenocarcinomas were found to demonstrate more cPLA2α positive tumor cells than squamous cell carcinomas (Figure 2). Adenocarcinoma and squamous carcinoma tumor samples stained with high intensity for cPLA2α which confirmed the overexpression (Figure 3). Although there was no statistically significant difference among the adenocarcinoma based on the overall histologic grading, we noticed that within the same tumor, a well differentiated area tended to demonstrate less cPLA2α expression than moderate or poorly differentiated area (Figure 4). It is interesting to note that none of the 14 well differentiated squamous cell carcinoma demonstrated cPLA2α expression in >30% of the tumor cells (Table 1).

Relation between cPLA2α Expression in Tumor Cells with Clinicopathologic Parameters

We then examined the cPLA2α expression and the major clinicopathologic features of prognostic significance (Table 2). Other than the association between cPLA2α

Table 1. cPLA2α Expression in NSCLC Cells According to Cell Type and Histological Grading

Major cell type and differentiation grading	cPLA2α expression in tumor cells					
	<1%	1-10%	10-30%	30-50%	≥50%	Total
Adenocarcinoma	14	5		7	6	
	4	36				
Well differentiated	4	2		3	4	
	2	15				
Moderate differentiated	8	2		2	1	
	1	14				
Poorly differentiated	2	1		2	1	
	1	7				
Squamous cell carcinoma	15	13	4	1	1	34
Well differentiated	8	6		1	0	
	0	15				
Moderate differentiated	5	5		2	1	
	0	13				
Poor differentiated	2	2		1	0	
	1	6				
Large cell, undifferentiated	3	2		0	1	
	0	6				
Total	32	20	11	8	5	76

cPLA2α: Cytosolic phospholipase A2α; NSCLC: nonsmall cell lung carcinoma; SCC, Squamous cell carcinoma

Table 2. cPLA2α Expression and Clinicopathologic Parameters

Parameters	No. patients	cPLA2α expression No. (%)	P value
Total studies	76	24 (32%)	-
Gender			
Male	52	14 (27%)	0.20
Female	24	10 (42%)	
Histology			
Adenocarcinoma	36	17 (47%)	0.02
Squamous	34	6 (18%)	
Large cell	6	1 (17%)	
T Classification			
T1	18	8 (44%)	0.36
T2	46	12 (26%)	
T3-T4	12	4 (33%)	
N Classification			
N0	47	18 (38%)	0.11
N1	16	5 (31%)	
N2	13	1 (8%)	
Disease Stage			
I	39	14 (36%)	0.63
II	21	5 (24%)	
III	16	5 (31%)	
Smoking Status			
Non smoker	12	1 (8%)	0.06
Smoker	64	23 (36%)	

expression and adenocarcinomas, as described earlier, there was no significant relation found cPLA2α expression and other prognostically significant parameters, such as lymph stage, TNM classification, tumor stage and smoking status. Although nonsmoker appeared to have fewer cPLA2α positive tumors than smokers (1 of 12 cases (8%) vs. 23 of 64 cases (36%); *P*=0.06) the differences were not statistically significant.

Relation between cPLA2α in Tumor Cells and Smoking History

Because cPLA2α is readily inducible by an array of substances, including inflammatory mediators and mitogens (Karin, 2006), and adenocarcinomas were found to have grater cPLA2α expression than squamous cell carcinomas, we further examined the relation between smoking and expression of cPLA2α after stratifying the tumor by cell type (Table 3). In contrast, smokers tended to have more cPLA2α positive adenocarcinomas compared with nonsmokers (16 of 29 cases (55%) vs. 1 of 7 cases (14%), P=0.052 by the fisher exact tests), the same in squamous carcinoma (6 of 29 cases (21%), there is no cPLA2α expression was found in non smoker) and the large cell lung carcinoma (1 of 6 cases (17%). These results suggest that cPLA2α may play an important role in smoking related carcinogenesis of adenocarcinomas.

Table 3. cPLA2α Expression in NSCLC Cells According to Cell Type and Smoking History

Major cell type and smoking status	cPLA2α expression in tumor cells					
	<1%	1-10%	10-30%	30-50%	≥50%	Total
Adenocarcinoma	15	4	7	6	4	36
Non smoker	6	0	0	1	0	7
Smoker	9	4	7	5	4	29
Squamous cell carcinoma	18	10	1	4	1	34
Non smoker	3	2	0	0	0	5
Smoker	15	8	1	4	1	29
Large cell carcinoma	3	2	0	1	0	6
Total	36	16	8	11	5	76

Discussion

In this study we characterized the main isoforms of cPLA2α expressed in Bronchial epithelia and NSCLC tissues. cPLA2α has been proposed to play a significant role in airway inflammatory diseases and cancer, as cPLA2α is significantly elevated in lung tissues in response to multiple pathological stimuli (Nagase et al., 2003). The primary function of cPLA2α in lung tissues is considered to be the generation of Arachidonic acid from membrane phospholipids for the synthesis of eicosanoids (Schaloske and Dennis, 2006). Since

multiple eicosanoids have divergent functions during lung inflammation (Nagase et al., 2000, Uozumi et al., 1997), cPLA2α serves as a key enzyme in mediating many aspect of the lung cancer formation. In the current study, we examined the expression of cPLA2α enzymes in normal and premalignant bronchial epithelial and NSCLC tissue samples. The most important finding was that the bronchial biopsy samples obtained from healthy smokers were completely negative for cPLA2α expression.

In the studies that used immunohistochemistry, some authors demonstrate a diminished cytoplasmic PLA2α staining in tumor cells compared to normal epithelium (Wu et al., 2002). Other report an overexpression of cytosolic PLA2 in tumor cells in up to 35% of the colorectal cancers but this overexpression is then most often weak or moderate (Wendum et al., 2003). The results suggest that normal adult human bronchial epithelial cells do not express cPLA2α when they are in normal physiologic condition. Similarly, cPLA2α expression was not evident in normal rat bronchial epithelial cells. Alternations in the levels and activity of cPLA2α have been associated with cancer pathogenesis. Overexpression has been associated with cholangiocarcinoma (Dong et al., 2003), hepatoma (Han et al., 2002) and nonsmall cell lung carcinoma (Pawliczak et al., 2002). Expression of cPLA2α is increased by pro-inflammatory cytokinese and growth factors, and repressed by glucocorticoids. Expression is induced in smooth muscle cells by PDGF-BB and thrombin via signal transducers and activators of transcription (STAT)-3 (Ghosh et al., 2006). Proliferation of prostate cancer is also depending on the action of cPLA2α (Sved et al., 2004), colorectal cancer has also been reported to overexpress cPLA2α (Soydan et al., 1997). cPLA2α expression is increased in several human cancer including colorectal, small bowel (Wendum et al., 2005), and lung (Kawamoto et al., 1995). Recently Wang et al., 2003 reported cPLA2α gene expression in human pre-implantation embryos.

In the current study, we observed that adenocarcinomas exhibited grater cPLA2α expression than squamous cell carcinomas where as squamous cell carcinomas tend to have more cPLA2α negative tumor cells than adenocarcinomas. Nevertheless, a clear picture emerges based on the results of the current study and those from other investigators. First, adenocarcinomas appear to have greater cPLA2α expression than squamous cell carcinomas whereas cPLA2α expression is more common, although less prevalent in squamous cell carcinomas. This observation further supports the theory that adenocarcinomas are biologically different from squamous cell carcinomas. Furthermore, the cPLA2α positive tumors may have unique biologic and clinical features (although not yet clearly elucidated) that are different from those of cPLA2α negative tumors. cPLA2α expression status may be a useful parameter when designing treatment strategies for a subset of patients with NSCLC. In Barrett's esophagus, however, cPLA2α mRNA expression was found in only 18% of adenocarcinomas, and an inverse association was reported between cPLA2α expression and depth of tumor infiltration, vascular invasion and perineural invasiveness (Lagorce-Pages et al., 2004). Furthermore, constitutively active *Ras* was sufficient to induce the expression of both cPLA2α as well as COX-2 in normal lung epithelial cells (Van Putten et al., 2001), providing a potential mechanism for the high levels of PGE2 often found in this form of lung cancer.

With regards to the relations between smoking and cPLA2α expression, we found a trend toward greater cPLA2α expression in adenocarcinomas diagnosed in smokers. Alterations in the levels and functional activity of cPLA2α have also been associated with cancer pathogenesis. Overexpression of cPLA2α has been reported in a variety of human cancers and tumor cell lines such as cholangiocarcinomas (SG231) and nonsmall cell lung carcinoma (NSCLC) (Wu et al., 2002, Han et al., 2002).

Several studies suggest that cPLA2α can mediate cancer cell growth and death in human cancer cell lines (Pirianov et al., 1999). In one of the earliest studies to examined the levels of cPLA2α in surgically excised colon tumors and matched normal tissue. Using Western analysis, they reported increased levels of cPLA2α in a subset of tumors (6 of 17) with a concomitant increase in functional activity (Soydan et al., 1996). The same group also examined cPLA2α levels in human stomach tumors but found no increase, even when COX-2 levels were highly induced (Soydan et al., 1997). Osterstrom et al., 2002 examined 42 primary colorectal cancers for expression of cPLA2α using DNA dot-blots. Using Semi-quantitative RT-PCR, Dong et al., 2003 found an opposite result in five human colorectal cancers, wherein cPLA2α expression was reduced despite increased COX-2 expression in 4/5 tumors. In this study the cPLA2α expression data was confirmed by Immunohistochemical staining (IHC) of tumor tissue and adjacent normal epithelium. These data revealed intense perinuclear staining of cPLA2α within the normal colonic epithelium, a result that is in agreement with an earlier study in CHO cells showing subcellular localization of activated cPLA2α within the endoplasmic reticular membrane and nuclear envelop (Schievella et al., 1995). This difference in results may be due in part to the differences in the antibodies used for those studies. Although Wendum et al., 2005 used cPLA2α mAb (Santa Cruz Biotechnology, Santa Cruz, CA). In contrast, we used the three different cPLA2α antibodies such as cPLA2α (N Terminal), cPLA2α (C Terminal) and Phospho cPLA2α (Ser^{505}) from Santa Cruz Biotechnology (Santa Cruz, USA).

The findings of the current study examining the expression of cPLA2α in NSCLC demonstrate that cPLA2α are present in a small subset of NSCLC tumors. Thus the results of the present work put forward a new enzyme (cPLA2α) targeted cancer therapy in future. In this contact, non human trials would be performed to determine cPLA2α inhibition and effective strategy for the treatment of cancer associated with abnormal cPLA2α function and regulation.

Acknowledgements

The Authors are very much thankful to all faculty members of the Department of Zoology, Bharathiar

University for their constant encouragement and timely help. The corresponding author gratefully acknowledges the funding agencies UGC and DST-FIST Govt. of India extended to carry out part of this work.

References

Capper EA, Marshall LA (2001). Mammalian phospholipases A2: mediators of inflammation, proliferation and apoptosis. *Prog Lipid Res*, **40**, 167-97.

Dessen A (2000). Structure and mechanism of human cytosolic phospholipase A2. *Biochim Biophys Acta*, **1488**, 40-7.

Dong M, Johnson M, Rezaie A, et al (2003). Inverse association between phospholipase A2 and COX-2 expression during mouse colon tumorigenesis. *Carcinogenesis*, **24**, 307-15.

Ghosh M, Tucker DE, Burchett SA, et al (2006). Properties of the group IV phospholipase A2 family. *Prog Lipid Res*, **45**, 487-510.

Han C, Demetris AJ, Michalopoulos G, et al (2002). 85-kDa cPLA2 plays a critical role in PPAR-mediated gene transcription in human hepatoma cells. *Am J Physiol Gastrointestinal Liver Physiol*, **282**, 586-97.

Heasley LE, Thaler S, Nicks M, et al (1997). Induction of cytosolic phospholipase A2 by oncogenic Ras in human non-small cell lung cancer. *J Biol Chem*, **272**, 14501-4.

Hefner Y, Borsch-Haubold AG, Murakami M, et al (2000). Serine 727 phosphorylation and activation of cytosolic phospholipase A2 by MNK1 related protein kinases. *J Biol Chem*, **275**, 37542-51.

Karin M, Lawrence T, Nizet V (2006). Innate immunity gone away: Limking microbial infections to chronic inflammation and cancer. *Cell*, **124**, 823-35.

Kawamoto S, Shoji M, Setoguchi Y, et al (1995). Molecular cloning of the 31 kDa cytosolic phospholipase A2, as an antigen recognized by the lung cancer-specific human monoclonal antibody, AE6F4. *Cytotechnology*, **17**, 103-8.

Lagorce-Pages C, Paraf F, Wendum D, et al (2004). Expression of inflammatory secretory phospholipase A2 and cytosolic phospholipase A2 in premalignant and malignant Barrett's oesophagus. *Virchows Arch*, **444**, 426-35.

Lee JS, Lippman SM, Benner SE, et al (1994). A randomized placebo controlled trial of isotretinoin in chemoprevention of bronchial squamous metaplasia. *J Clin Oncol*, **12**, 937-45.

Leslie CC (2004). Regulation of arachidonic acid availability for eicosanoid production. *Biochem Cell Biol*, **82**, 1-17.

Lin LL, Wartmann M, Lin AY, et al (1993). cPLA2 is phosphorylation and activated by MAP kinase. *Cell*, **72**, 269-78.

Miyashita A, Crystal RG, Hay JG (1995). Identification of a 27 bp 52-flanking region element responsible for the low level constitutive expression of the human cytosolic phospholipase A2 gene. *Nucleic Acids Res*, **23**, 293-300.

Mountain CF (1997). Revision in the international system for staging lung cancer. *Chest*, **111**, 1710-17.

Muthalif MM, Hefner Y, Canaan S, et al (2001). Functional interaction of calcium-/ calmodulin-dependent protein kinase II and cytosolic phospholipase A2. *J Biol Chem*, **276**, 39653-60.

Nagase T, Uozumi N, Aoki-Nagase T, et al (2003). A potential inhibitor of cytosolic phospholipase A2, arachidonyl trifluromethyl ketone, attenuates LPS induced lung injury in mice. *Am J Physiol Lung Cell Mol Physiol*, **284**, 720-6.

Nagase T, Uozumi N, Ishii S, et al (2000). Acute lung injury by sepsis and acid aspiration: A key role for cytosolic phospholipase A2. *Nat Immunol*, **1**, 42-6.

Osterstrom A, Dimberg J, Fransen K, et al (2002). Expression of cytosolic and group X secretary phospholipase A2 genes in human colorectal adenocarcinomas. *Cancer Lett*, **182**, 175-82.

Pawliczak R, Han C, Huang X-L, et al (2002). 85-kDa cytosolic phospholipase A2 mediates peroxisome proliferator-activated receptor gamma activation in human lung epithelial cells. *J Biol Chem*, **277**, 33153-63.

Pirianov G, Danielsson C, Carlberg C, et al (1999). Potentiation by vitamin D analogs of TNFα and ceramide induced apoptosis in MCF 7cells is associated with activation of cPLA2. *Cell Death Differ*, **6**, 890-901.

Schaloske, Dennis (2006). The phospholipase A2 superfamily and its group numbering system. *Biochim Biophys Acta* **1761**, 1246-59.

Schievella AR, Regier MK, Smith WL, et al (1995). Calcium-mediated translocation of cytosolic phospholipase A2 to the nuclear envelope and endoplasmic reticulum. *J Biol Chem*, **270**, 30749-54.

Song H, Ramanadham S, Bao S, et al (2006). Effects of biological oxidants on the catalytic activity and structure of group VIA phospholipase A2. *Biochemistry*, **45**, 6392-406.

Soydan AS, Tavares IA, Weech PK, et al (1996). High molecular weight phospholipase A2 and fatty acid in human colon tumours and associated normal tissue. *Eur J Cancer*, **32**, 1781-7.

Soydan AS, Tavares IA, Weech PK, et al (1997). High molecular weight phospholipase A2: its occurrence and quantification in human colon cancer and normal mucosa. *Adv Exp Med Biol*, **400**, 31-7.

Sved P, Scott KF, McLeod D, et al (2004). Oncogenic action of secreted phospholipase A2 in prostate cancer. *Cancer Res*, **64**, 6934-40.

Uozumi N, Kume K, Nagase T, et al (1997). Role of cytosolic phospholipase A2 in allergic response and parturition. *Nature*, **390**, 618-22.

Van Putten V, Refaat Z, Dessev C, et al (2001). Induction of cytosolic phospholipase A2 by oncogenic Ras is mediated through the JNK and ERK pathways in rat epithelial cells. *J Biol Chem*, **276**, 1226-32.

Wang XH, Yan GT, Wang LH, et al (2003). The mediating role of cPLA2 in IL-1 β and IL-6 release in LPS-induced HeLa cells. *Cell Biochem Funct*, **22**, 41-4.

Wendum D, Comperat E, Boelle PY, et al (2005). Cytoplasmic phospholipase A2 alpha overexpression in stromal cells is correlated with angiogenesis in human colorectal cancer. *Mod Pathol*, **18**, 212-20.

Wendum D, Svrcek M, Rigau V, et al (2003). COX-2, inflammatory Secreted PLA2 and cytoplasmic PLA2 protein expression in small bowel adenocarcinomas compared with colorectal adenocarcinomas. *Mod Pathol*, **16**, 130-6.

World Health Organization (1981). World health organization histological typing of lung tumors. International histologic classification of tumors, geneva: World health organization, 1981, 1.

Wu T, Han C, Lunz 3rd JG, et al (2002). Involvement of 85-kd cytosolic phospholipase A2 and cyclooxygenase-2 in the proliferation of human cholangiocarcinoma cells. *Hepatology*, **36**, 363-73.