Cytosolic phospholipase A\textsubscript{2} (cPLA\textsubscript{2}) IVA as a potential signature molecule in cigarette smoke condensate induced pathologies in alveolar epithelial lineages

Subodh K. Yadav\textsuperscript{1,3*}, Sanjeev K. Sharma\textsuperscript{1}, Abdullah Farooque\textsuperscript{2}, Gaurav Kaushik\textsuperscript{1,4}, Balwinder Kaur\textsuperscript{1}, Chander M. Pathak\textsuperscript{1}, Bilikere S. Dwarakanath\textsuperscript{2,5} and Krishan L. Khanduja\textsuperscript{1*}

Abstract

Background: Smoking is one of the leading causes of millions of deaths worldwide. During cigarette smoking, most affected and highly exposed cells are the alveolar epithelium and generated oxidative stress in these cells leads to death and damage. Several studies suggested that oxidative stress causes membrane remodeling via Phospholipase A\textsubscript{2}s but in the case of cigarette smokers, mechanistically study is not yet fully defined. In view of present perspective, we evaluated the involvement of cytosolic phospholipase A\textsubscript{2} (cPLA\textsubscript{2}) IVA as therapeutic target in cigarette smoke induced pathologies in transformed type I and type II alveolar epithelial cells.

Methods: Transformed type I (WI26) and type II (A549) alveolar epithelial cells were used for the present study. Cigarette smoke condensate (CSC) was prepared from most commonly used cigarette (Gold Flake with filter) by the Indian population. CSC-induced molecular changes were evaluated through cell viability using MTT assay, reactive oxygen species (ROS) measurement using 2,7 dichlorodihydrofluorescin diacetate (DCFH-DA), cell membrane integrity using fluorescein diacetate (FDA) and ethidium bromide (EtBr) staining, super oxide dismutase (SOD) levels, cPLA\textsubscript{2} activity and molecular involvement of specific cPLA\textsubscript{2}s at selected 24 h time period.

Results: CSC-induced response on both type of epithelial cells shown significantly reduction in cell viability, declined membrane integrity, with differential escalation of ROS levels in the range of 1.5–15 folds and pointedly increased cPLA\textsubscript{2} activity ($p < 0.05$). Likewise, we observed distinction antioxidant potential in these two types of lineages as type I cells had considerably higher SOD levels when compared to type II cells ($p < 0.05$). Further molecular expression of all cPLA\textsubscript{2}s increased significantly in a dose dependent manner, specifically cytosolic phospholipase A\textsubscript{2} IVA with maximum manifestation of 3.8 folds. Interestingly, CSC-induced ROS levels and cPLA\textsubscript{2} s expression were relatively higher in A549 cells as compared to WI26 cells.

Conclusions: The present study indicates that among all cPLA\textsubscript{2}s, specific cPLA\textsubscript{2} IVA are the main enzymes involved in cigarette smoke induced anomalies in type I and type II lung epithelial cells and targeting them holds tremendous possibilities in cigarette smoke induced lung pathologies.

Keywords: Cigarette smoke condensate, Cytosolic phospholipases A\textsubscript{2}, Reactive oxygen species, Inflammation, A549 cells, WI26 cells
Background

Cigarette smoking is leading cause of deaths and projected to cause 8–10 million deaths per year worldwide [1]. It is associated with different types of lung cancer and approximately one third of all cancer death [1–3]. Currently more than 370 billion cigarettes are being consumed by smokers globally and it has been projected that more than 30 % of the people will be smokers by 2030 [2]. On the other hand it has been expected that rate of smoking will be reached 70 % in developing countries [1]. It has been documented that single puff of cigarette smoke contains $10^{17}$ oxidant molecules out of which $10^{15}$ are reactive oxygen species/ reactive nitrogen species. Moreover, these ROS/RNS are known to be one of the causative factors in various lung pathologies including cancer and chronic obstructive pulmonary disease (COPD) [4–6]. During cigarette smoking, the most affected and highly exposed cells are the alveolar epithelium which is lined by type-I (~90–95 %) and type-II (~5–10 %) epithelial cells. In addition, damage and death of epithelial cells induced by cigarette smoke exposure can mostly be accounted for by an increased in oxidant stress. Enhanced levels of free radicals/oxidants leads to oxidative stress and initiation of repair processes whether started as part of an inflammatory response or as a response to injury is still not clear in cigarette smokers. In this context, one of the hallmark enzymes are Phospholipase A$_2$S which are responsible for membranes remodeling [7–9] but in the case of cigarette smokers mechanistically study is not yet fully defined.

PLA$_2$S are lipolytic enzymes that catalyze the hydrolysis of acyl-groups at the sn-2 position of glycerophospholipids and produce free fatty acids and lysophospholipids by an interfacial activation catalytic mechanism. To date, at least 26 genes that encode various types of PLA$_2$ proteins with esterase’s activity have been identified in human and are assigned to five different groups. (i) Secretory PLA$_2$s with molecular weight of 14 kDa, (ii) the 85 kDa cytosolic PLA$_2$s, (iii) Ca$^{2+}$ independent PLA$_2$s, (iv) Platelet-activating factor acetyl hydrolase and (v) lysosomal PLA$_2$s. These PLA$_2$s were differentiated on the basis of their sequence, molecular weight, disulfide bonding patterns, requirement for Ca$^{2+}$ to their biochemical characteristics and localization [10–13]. It has been suggested that PLA$_2$ isoforms are involved either in the promotion or in the resolution of inflammation depending upon cell type and generation of eicosanoid [14–16]. In this context cytosolic phospholipase A$_2$s (cPLA$_2$s) are the main enzymes mediating arachidonic acid release and pro-inflammatory eicosanoids production. Moreover these biomolecules are associated with chronic inflammation which is a recognized risk factor for carcinogenesis [17–19]. The overall aim of this study was to investigate the involvement of particular cPLA$_2$ which could be proposed as future therapeutic target during cigarette smoke induced pathologies in alveolar epithelium and results are reported in present paper.

Methods

Materials

WI26 (type-I) lung epithelial cell line was procured from American type culture collection (ATCC), Rockville, MD (USA), (ATCC® CCL-95.1”, https://www.atcc.org/Products/All/CCL-95.1.aspx). A549 (type-II) lung epithelial cell line was procured from National centre for cell science (NCCS), pune, India (http://www.nccs.res.in/CR5.html). PLA$_2$-inhibitors bromoeno lactone (BEL), arachidonyl trifluoroethyl ketone (ATK), bromophenacyl bromide (BPB), DCFH-DA, FDA and other general reagents were purchased from Sigma (St. Louis, MO, U.S.A.). PLA$_2$-inhibitor YM26734 was purchased from Tocris bioscience, Bristol, UK.

Methods

Cell culturing

Human lung epithelial type I (WI26) and type II (A549) cells were maintained in continuous culture at 37 °C temperature and 5 % CO$_2$ in RPMI-1640 medium with 10 % fetal bovine serum (FBS). After every 48 h, depleted medium from the culture flask was replaced with fresh medium. Experimental design is represented as schematic view (Additional file 1: Figure S1).

Cigarette smoke condensate preparation

Cigarette smoke condensate was prepared in our own laboratory according to standardized method of Kaushik 2009 [20], from most commonly used cigarettes (Gold Flake with filter) by the Indian population [21]. In brief smoke from a burning cigarette was sucked into a flask containing acetone with the help of a vacuum pump. Three ways connecting glass joint was used for suction. The rate of airflow was regulated by a valve so that cigarette burns upto bud in approximately 6 min. Acetone was evaporated under vacuum/nitrogen gas and the residue i.e. CSC was used for the experiments. CSC was dissolved in DMSO, in such a way that the final concentration of CSC for 24 h. Before treatment;
medium was replaced with fresh medium. Four h before the end of desired time interval, 20 μl of MTT solution (2.5 mg/ml) was added to each well. After 4 h, resulting formazan crystals were dissolved in 40 μl of lysis buffer. The developed color was read at 540 nm on ELISA reader. The relative viability was calculated as described earlier.

Reactive oxygen species
Levels of intracellular ROS were measured by the shift in fluorescent intensity resulting from oxidation of DCFH-DA fluorescence dye by the method of Wan et al. [23]. In brief, cells (0.5 x 10^5 cells/well) were seeded into 12 well culture plates and allowed to grow overnight. Cells were challenged with CSC for 24 h. Before completion of treatment, cells were incubated with 5 μM DCFH-DA fluorescence dye for 30 min. After completion of treatment, cells were washed, harvested and re-suspended in ice-chilled PBS and analyzed by flow cytometry (FACScan) and shift in fluorescent peak was represented in terms of mean fluorescent intensity (MFI).

Cellular integrity by FDA uptake
Cellular injury was determined by the FDA and ethidium bromide staining method [24]. FDA is an indicator of membrane integrity and cytoplasmic esterase activity. So the cells with intact membranes fluoresce green and cells with damaged membranes fluoresce red. Cells after CSC treatment was incubated with 10 μM FDA and 25 μM of ethidium bromide and was visualized under fluorescent microscope.

Superoxide dismutase activity
Superoxide dismutase activity was estimated as described earlier [25]. In brief 2 x 10^6 cells were plated in 100 mm² culture dishes and allowed to grow for 24 h. After 24 h of CSC treatment, cells were washed, harvested and centrifuged at 200 x g for 10 min at 4°C. Cell pellet was washed with ice-chilled PBS and resuspended in PBS. Cells were lysed by the three freeze–thaw cycles followed by sonication (Sonicator Q700, Qsonica) for 5 min in ice-chilled water. After centrifugation at 800 x g for 10 min at 4°C, the supernatant was assayed for SOD.

PLA₂ assay
The effect of CSC on the PLA₂ activity was measured in the presence/absence of PLA₂-inhibitors by the modified method of price [26]. In brief, samples were freshly diluted in 2 mM HEPES at pH 7.5 and 20 μl of each sample mixture, or buffer mixture as negative control was put in the well of a round bottom 96 well micro plate. 180 μl of an assay mixture at pH 7.5 at 37°C was added to each sample. The plate was immediately read at 600 nm at one minute intervals for five minutes. Immediately after addition of substrate, the blue color of dye turns yellow with pH change due to PLA₂ activity and absorbance decreases.

Reverse transcription-polymerase chain reaction (RT-PCR)
After treatment total RNA from both type of epithelial cells was isolated using the method of Chomczynski et al [27]. Analysis of mRNA levels of cPLA₂ groups IVA, IVB and IVC was done by RT-PCR. In PCR reactions (25 μl) 100 ng cDNA was used as template DNA. The primer sequences for the expression of all the genes were taken from previously published literature [28]. The primer sequences used for this study are given in (Additional file 1: Table S1).

Protein estimation
Protein content of cell lysate was measured in all samples by Bradford method [29]. Bovine Serum Albumin (BSA) was used as a protein standard.

Statistical analysis
Values displayed in the results are mean ± standard deviation of at least three independent experiments carried out in triplicate. P < 0.05 was considered to be statistically significant. One way analysis of variance (ANOVA) and t test were employed using SPSS software to govern statistical significance of the results.

Results
Effect of CSC on cell viability and reactive oxygen species in A549 and WI26 cells
In A549 cells, it was found that cell survival decreased with increasing concentration of CSC from 1 μg/ml to 200 μg/ml and found to be 90.8 %, 84.2 %, 78.4 % and 76.4 % at 50 μg/ml, 100 μg/ml, 150 μg/ml and 200 μg/ml concentrations respectively. Whereas in WI26 cells, no significant change was observed in survival rate up to 100 μg/ml concentration of CSC. Moreover, it was observed that at 150 μg/ml and 200 μg/ml concentrations of CSC, survival rate of WI26 cells decreased to 95.7 % and 62.2 % (p < 0.05) respectively (Fig. 1).

Effects of CSC at various concentrations on ROS formation in the two cell lines are shown in Fig. 2. It was found that in A549 cells, even 1 μg CSC/ml increased the formation of ROS to 3 fold, which was further increased to 3.95 fold, 7.69 fold, 12.1 fold, 14.1 fold and 16.5 fold at 10 μg/ml, 50 μg/ml, 100 μg/ml, 150 μg/ml and 200 μg/ml concentrations respectively (Fig. 2a). In contrast to A549 cells, in WI26 cells CSC treatment increased the ROS formation to 1.21 fold, 1.58 fold, 2.26 fold, 2.73 fold and 3.56 fold at 10 μg/ml, 50 μg/ml, 100 μg/ml, 150 μg/ml and 200 μg/ml concentrations (Fig. 2b).
Cells morphology
Since the morphology of two types of cells used in the present study were highly affected (Additional file 1: Figure S2) at higher concentrations (150 μg/ml and 200 μg/ml), these concentrations were not used in the next part of the study. Among all other lower concentrations, 50 μg/ml and 100 μg/ml of CSC induced maximum ROS production with optimum cell viability and hence were preferred in most of the other experiments. Time period was selected on the basis of our previous studies and preliminary experiments at different time intervals using ROS measurement and cell survival assay. 24 h time was found to be optimum and was used for current study.

Effect of CSC on cellular integrity in A549 and WI26 cells
FDA uptake assay is an indicator of membrane integrity and cytoplasmic esterase activity. Figure 3 is showing the results of FDA and EtBr uptake in both types of cell lines. There was maximum uptake of FDA with no cellular accumulation of EtBr at control levels in both the cell lines. Accumulation of the fluorescein decreased and uptake of the EtBr increased in a concentration dependent manner in both the cell lines. Result obtained from the assay indicated the membrane integrity was stable at 50 and 100 μg/ml of CSC treatment.

SOD levels in type I and type II epithelial cells
Effect of CSC treatment for 24 h on SOD in A549 and WI26 cells is shown in the Fig. 4. It was found that SOD activity was significantly higher in WI26 cells (0.278 IU/μg protein) as compared to A549 (0.188 IU/μg protein) cells. In the presence of 50 μg/ml of CSC treatment, SOD activity increased significantly in WI26 and A549 cells by 1.68 and 1.49 fold respectively (p < 0.05).

CSC enhanced cPLA2 activity
Effect of CSC induced PLA2 activity in presence/absence of PLA2 inhibitors is shown in Fig. 5. Working PLA2 inhibitors concentration was decided on the basis of cell viability, ROS and apoptosis experiments in the present study (Data not shown). At basal level we observed very low PLA2 activity in both type of lineages as optical density (OD) decreased at every minute interval from 2.88 to 2.63, 2.44, 2.29, 2.11 and 1.95 in A549 cells (Fig. 5a), whereas from 3.13 to 2.95, 2.67, 2.49, 2.2 and 2.01 in WI26 cells. CSC at 50 μg/ml, significantly induced PLA2 activity in both type of cells as OD decreased at every minute interval from 2.57 to 1.5, 0.98, 0.73, 0.59 and 0.52 in A549 cells whereas from 2.80 to 2.17, 1.22, 1.02, 0.88 and 0.75 in WI26 cells (p < 0.05) (Fig. 5b). In presence of cPLA2 specific inhibitor ATK, we observed low CSC-induced PLA2 activity when compared to sPLA2 (YM26734) and iPLA2 (BEL) specific inhibitors in both type of cells. In presence of cPLA2 and sPLA2 + iPLA2 inhibitor, the decrease in OD was from 2.76 to 2.6, 2.41, 2.17, 1.88 and 1.6 (p < 0.05) and from 2.66 to 1.74, 1.17, 0.9, 0.80 and 0.63 respectively in A549 cells; whereas in WI26, OD decreased from 3.04 to 2.71, 2.47, 2.22, 1.93 and 1.70 (p < 0.05) and from 2.81 to 2.27, 1.61, 1.24, 1.02 and 0.94 respectively.
CSC enhanced cPLA$_2$ mRNA expression

Transcriptional modulations of various selected PLA$_2$ groups (IVA, IVB and IVC) were studied at basal level (Fig. 6a) and in presence of CSC as shown in Fig. 6b. Densitometric analysis of all cPLA$_2$ groups was done (Table S2). In A549 cells, mRNA expression of all the cPLA$_2$ groups increased significantly in concentration dependent manner in all CSC treated cells and the most affected group was IVA (3.98 fold) followed by IVC (2.97 fold) and IVB (1.62 fold) ($p < 0.05$). In WI26 cells also, CSC treatment showed significant increase in mRNA expression of cPLA$_2$ groups in a concentration dependent manner and the most prominently induced PLA$_2$ group was IVA (1.95) followed by IVB (1.31) and IVC (1.98) ($p < 0.05$).

Discussion

The lung is the only organ in the entire human architecture which has the greatest exposure to atmospheric oxygen and other environmental toxicants such as cigarette smoke [30, 31]. As we all knew that cigarette smoke is a highly complex mixture having more than 5000 chemicals including high concentration of free radicals and many of its components are known to be carcinogens, co-carcinogens and mutagens [5]. Among all type of cigarettes available in the Indian market, one of the most commonly consumed includes Gold Flake (with filter) [21] and same was used in the current study.

Cigarette smoke-induced chronic inflammation leads to the destruction of alveolar septae, resulted to the loss of elasticity and surface area for gas exchange, known as emphysema [9] and rapidly induces production of ROS thereby impairing endothelial functions [32]. The mechanisms leading to these changes after exposure of cigarette smoke were not yet completely understood in the two types of alveolar epithelial cells. However increased level of free radicals/oxidants in epithelial cells due to cigarette smoke leads to oxidative stress which is considered to be one of the major factors responsible for cell damage and death [31, 33, 34]. Using two lineages of epithelial cell lines, we showed that exposure of CSC leads to decrease in cell viability, increase in ROS production, loosed membrane integrity and altered morphology in a dose dependent manner at 24 h time period. All these observed parameters are well known hallmarks of inflammation and oxidative stress. Optimum time period and different doses of CSC were already reported by our group's previously and same has been used in the present study [20]. In the continuation of previous study, we observed that CSC-induced ROS formation was found to be increased in concentration dependent manner in both WI26 (type I) cells as well as in A549 (type II) cells.
However, the ROS formation was higher in A549 cells in comparison of WI26 cells. One of the possible reasons of such difference seems to be due to the difference in antioxidant status in two types of cells. To find out the reason behind, we investigated SOD levels in both type of cells and the results obtained from the study clearly indicate that WI26 cells had higher SOD activity in comparison of A549 cells alone and in combination with CSC treatment, its justify difference of ROS level finding among two types of lineages. Our finding shows that type II (A549) cells are

![Fig. 5](image)

**Fig. 5** PLA₂ activity at 50 μg/ml of CSC concentration alone or in combinations with PLA₂ isoforms specific inhibitors at 24 h of treatment time in A549 cells (a) and WI26 cells (b). The decrease in absorbance is directly proportional to PLA₂ activity. The results are expressed as mean ± SD of three separate experiments. *p < 0.05. *CSC 50 μg/ml activity response compared with their respective control at every minutes interval. #CSC 50 μg/ml in combination with cPLA₂ specific inhibitor (ATK) activity response compared with their respective CSC 50 μg/ml alone at every minutes interval

![Fig. 6](image)

**Fig. 6** mRNA expression of different cPLA₂ groups at constitutive level (a) and in presence of different concentrations of CSC (b) in A549 and WI26 cells. β-actin was used as an internal control for normalization of mRNA levels of cPLA₂ groups
comparatively more sensitive than type I (WI26) cells in terms of cell viability, higher ROS production as well as lower SOD levels. Moreover, type-I cells are highly exposed to airway insults and have been described as terminally differentiated cells, suggesting that they are incapable of cell division and cannot change their phenotypic expression. On the other hand, type-II epithelial cells are considered as progenitor cells which proliferate to re-epithelialize the damaged alveolar surfaces and then they transform into the type-I epithelial cells. In these distinctive circumstances, type-II epithelial cells may be considered to show characteristics of stem cells mechanisms in alveolar repair [35–37] and seems to be very sensitive in contrast to type-I epithelial cells as observed in the present study.

PLA₂S are the important molecules involved in the remodeling of the membrane lipids and also in modulation of cell signaling which contributes to either the promotion or the resolution of inflammation during various lung pathologies [38, 39]. Moreover PLA₂ activity in cancer tissues found to be much higher than normal cells [40]. In this direction, we examined the PLA₂ activity in both type of lineages. In our observations, we found increased PLA₂ activity after exposure of CSC. Our results are in correlation with another recently described study in which increased PLA₂ activity has been reported after cigarette smoke exposure [41]. PLA₂S have various isoforms but cPLA₂S seems to play crucial role in CSC-induced inflammatory conditions. Results obtained from the present study indicated maximum decrease in cPLA₂S activity in both type of cells by using commercially available isoform specific PLA₂ inhibitors. It is well documented that cPLA₂S have key regulatory roles in the invasive migration, proliferation, and capillary-like tubule formation of vascular endothelial cells as well as in tumor angiogenesis in lung cancer (mouse models) [42]. Our study is well supported by another recent research in which involvement of cPLA₂ has been reported in asthmatic and COPD cases [43]. Moreover, several cPLA₂ isoform specific inhibitors are commercially available and are in clinical trials for various inflammatory diseases and has been discussed in the review by Victoria [44]. The results of CSC simulated lung epithelial cell lineage treated with cPLA₂ specific inhibitor such as ATK may also support the role of cPLA₂ in lung pathologies.

Furthermore, to find out the role of specific cPLA₂, we also characterized the comparative transcriptional level expression of cPLA₂S at constitutive level and it was observed that all cPLA₂S expressed in human lung epithelial type I (WI26) and type II (A549) cells. In presence of cigarette smoke condensate there was an increase in all cPLA₂ groups but most predominantly induced group was PLA₂ IVA in two different lineages of cells. In best of our knowledge, we are first group to report maximum expression of PLA₂ IVA among all cPLA₂S. The involvement of PLA₂ IVA is already documented in several diseases and it has been used as a target molecule [45]. In this context, targeting group IVA may built-up new prospects in CSC-induced lung pathologies.

CSC-induced difference in the expression level of cPLA₂S seems to play crucial role during lung pathologies. In this prospective, we also observed that CSC-induced cPLA₂S expression and ROS levels were comparatively higher in A549 cells when compared to WI26 cells. Our finding clearly advocates that CSC-induced expression level of cPLA₂ group’s seemed to be dependent on ROS levels generated. Our observation is supported by another study which suggested that cigarette smoke activates cPLA₂ expression through NADPH oxidase/ROS in human tracheal smooth muscle cells [8].

The role of specific cPLA₂S in cigarette smoking induced lung pathologies remains elusive due to combine effect of cigarette smoke constituents, smaller to larger extent expression of all isoforms and distinct functions attributable to each isoform. Still PLA₂ IVA had maximum expression in both lineages and can be of maximum interest in CSC induced inflammatory diseases. Now a days combine strategies are required to deal with such inflammatory situations. Firstly, dietary intake of biological molecules which are rich source of antioxidants and have anti-inflammatory effects [46]. Secondly, along with healthy diet use of cPLA₂S specific analogs especially for PLA₂ IVA may be a novel effective therapy in cigarette smoke related lung pathologies.

Conclusion
In conclusion, it is worth mentioning; here the present study indicates involvement of specific cPLA₂ IVA as a potential biomolecule candidate during cigarette smoke induced oxidative stress in type I and type II alveolar epithelial cells and strategies to target them may be key approach in cigarette smoke induced lung pathologies.

Additional file

Additional file 1: Supplementary data. Table S1. Primer Sequences of various genes. Table S2. CSC-induced mRNA expression of various cPLA₂ groups in A-549 and WI-26 Cells. The values (mean ± SD of three different experiments) are in folds of control values. Figure S1. Schematic presentation of proposed plan of experimental design. Figure S2. Effect of CSC treatment at concentrations’ of 150 μg/ml (b) and 200 μg/ml (c) on cell morphology in lung epithelial type II (A-549) cells and type I (WI-26) cells. Figure 2a shows the normal morphology in two types of the cells (Magnification 5x). (DOCX 1098 kb)

Acknowledgments
Financial assistance from the Council of Scientific and Industrial Research (CSIR), New Delhi, India is gratefully acknowledged. We are also thankful to INMAS, DRDO New Delhi, INDIA for providing lab facilities for flow cytometry based experiments.
Funding
Financial assistance from the Council of Scientific and Industrial Research (CSIR), New Delhi, India is gratefully acknowledged. Funding body have no role in the design of the study and collection, analysis, and interpretation of data and in the writing the manuscript.

Availability of data and materials
Detail description of observations is mentioned in the result section. Other observations are reported in the supplementary section of the manuscript.

Authors’ contributions
SKY: Designing and executed the experiments as well as written the manuscript; SKS: Helped in performing the experiments; AF: Executed the ROS assay, GK: Performed the cell toxicity assay; BK: Performed SOD assay; CMP: Supervised and analyzed the data; BSD: Designed the ROS experiments and provided laboratory facilities; KLK: Designed and supervised the whole experiments. All authors read and approved the final manuscript.

Competing interests
The authors declare that they have no competing interests.

Consent for publication
Not applicable.

Ethics approval and consent to participate
Not applicable.

Include a statement on ethics approval and consent (even where the need for approval was waived)
Not applicable.

Include the name of the ethics committee that approved the study and the committee’s reference number if appropriate
Not applicable.

Author details
1Department of Biophysics, PGIMER Chandigarh, Chandigarh 160012, India.
2Institute of Nuclear Medicine and Allied Sciences, New Delhi, India. *Present address: Department of CSIC, PGIMER Chandigarh, Chandigarh 160012, India.
3Present address: Surgery, School of Medicine, KU Medical Center (KUMC), Kansas City, KS 66160, USA. *Present address: Central Research Facility, Sri Ramachandra University, Porur, Chennai 600116, India.

Received: 10 March 2016 Accepted: 3 August 2016
Published online: 15 August 2016

References
1. Ezzati M, Lopez AD. Estimates of global mortality attributable to smoking in 2000. Lancet. 2003;362:847–52.
2. (WHO) WHO report on the global tobacco epidemic, WHO Health Rep. Geneva: WHO; 2008.
3. Stewart SL, Cardinez CJ, Richardson LC, Norman L, Kaufmann R, Surveill Summ. 2008;57:1–18.
4. Diana JN. Tobacco smoking and nutrition. Ann N Y Acad Sci. 1993;686:1–16.
5. Puyor WA, Stone K. Oxidants in cigarette smoke. Radicals, hydrogen peroxide, peroxynitrite, and peroxynitrite. Ann N Y Acad Sci. 1993;686:12–27. discussion 27–18.
6. Shishodia S, Potdar P, Gariola CG, Aggarwal BB. Curcumin (diferuloylmethane) down-regulates cigarette smoke-induced NF-kappaB activation through inhibition of IkappaBalpha kinase in human lung epithelial cells: correlation with suppression of COX-2, MMP-9 and cyclin D1. Carcinogenesis. 2003;24:1269–79.
7. Thompson AB, Robbins RA, Romberger DJ, Sisson JH, Spurzem JR, Teschner H, Bernard SL. Immunological functions of the pulmonary epithelium. Eur Respir J. 1995;8:127–49.
8. Cheng SE, Luo SF, Jou MJ, Lin CC, Kou YR, Lee IT, Hsieh HL, Yang CM. Cigarette smoke extract induces cytosolic phospholipase A2 expression via NADPH oxidase, MAPKs, AP-1, and NF-kappaB in human tracheal smooth muscle cells. Free Radic Biol Med. 2009;46:948–60.
9. Rice-Evans C, Burdon R. Free radical-liptid interactions and their pathological consequences. Prog Lipid Res. 1993;32:71–110.
10. Katsioulis E, Nakos G, Lekka ME. Phospholipase A2 subclasses in acute respiratory distress syndrome: Biochim Biophys Acta. 1792;2009:941–53.
11. Schaloske RH, Dennis EA. The phospholipase A2 superfamily and its group numbering system. Biochim Biophys Acta. 1761;2006:1246–59.
12. Burke JE, Dennis EA. Phospholipase A2 structure/function, mechanism, and signaling. J Lipid Res. 2009;50(Suppl):S237–42.
13. Burke JE, Dennis EA. Phospholipase A2 biochemistry. Cardiovasc Drugs Ther. 2009;23:49–59.
14. Murakami M, Kambe T, Shimbara S, Kudo I. Functional coupling between various phospholipase A2s and cyclooxygenates in immediate and delayed prostanoid biosynthetic pathways. J Biol Chem. 1999;274:103–15.
15. Subbaramaiah K, Zakim D, Weksler BB, Dannenberg AJ. Inhibition of cyclooxygenates: a novel approach to cancer prevention. Proc Soc Exp Biol Med. 1997;216:201–10.
16. Sevastou I, Kaffe E, Mouratis MA, Ailinisi V. Lysoglycerophospholipids in chronic inflammatory disorders: the PLA2/LPC and ATX/LPA axes. Biochim Biophys Acta. 1831;2013:42–60.
17. Sommerfelt RM, Feuerherm AJ, Jones K, Johansen B. Cytosolic phospholipase A2 regulates TNF-induced production of joint destructive effectors in synoviocytes. PLoS One. 2013;8:e83555.
18. 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.
19. 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;271:1505–13.
20. Kaushik G, Kaushik T, Khudha S, Pathak CM, Khudha KL. Cigarette smoke condensate promotes cell proliferation through disturbance in cellular redox homeostasis of transformed lung epithelial type-II cells. Cancer Lett. 2008;270:120–31.
21. Ator A, Reddy KS, Stigler MH, Perry CL. Associations between tobacco marketing and use among urban youth in India. Ann J Health Behav. 2008;39:283–94.
22. Avis IM, Mitt J, Boyle T, Vos MD, Moody T, Treston AM, Martinez A, Mulshine JL. Growth control of lung cancer by interruption of 5-lipoxygenase-mediated growth factor signaling. J Clin Invest. 1996;98:806–13.
23. Wu CN, Myung E, Lau BH. An automated micro-fluorometric assay for monitoring oxidative burst activity of phagocytes. J Immunol Methods. 1995;185:181–8.
24. Yang H, Acker J, Chen A, McGann L. In situ assessment of cell viability. Cell Transplant. 1998;7:443–51.
25. Kono Y. Generation of superoxide radical during autoxidation of hydroxylamine and an assay for superoxide dismutase. Arch Biochem Biophys. 1978;186:189–95.
26. Price 3rd JA. A colorimetric assay for measuring phospholipase A2 degradation of phosphatidylcholine at physiological pH. J Biochem Biophys Methods. 2007;70:441–4.
27. Chomczynski P, Sacchi N. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem. 1987;162:156–9.
28. Lindholm J, Ljungman AG, Lindahl M, Tagesson C. Expression of members of the phospholipase A2 family of enzymes in human nasal mucosa. Eur Respir J. 2001;18:130–8.
29. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem. 1976;72:248–54.
30. Hoyle GW, Brody AR. Gene expression in rodent model of environmental lung disease. Ann N Y Acad Sci. 1996;796:162–72.
31. Biswas SK, Rahman I. Environmental toxicity, redox signaling and lung inflammation: the role of glutathione. Mol Aspects Med. 2009;30:60–76.
32. Michael CA, McKenna MT, Begg S, Tomijima N, Majmudar M, Bulzacchelli MT, Ebrahim S, Ezzati M, Salomon JA, Kreiser JG, et al. The burden of disease and injury in the United States 1996. Popul Health Metr. 2006;4:11.
33. Petecchia L, Sabatini F, Varesio L, Camoirano A, Usai C, Pezzolo A, Rossi GA. Blocking respiratory distress syndrome. Biochim Biophys Acta. 1792;2009:941–53.
34. Gupta MP, Khanduja KL, Sharma RR. Effect of cigarette smoke inhalation on antioxidant enzymes and lipid peroxidation in the rat. Toxicol Lett. 1988;41:107–14.
35. Crapo JD, Barry BE, Foscue HA, Shelburne J. Structural and biochemical changes in rat lungs occurring during exposures to lethal and adaptive doses of oxygen. Am Rev Respir Dis. 1980;122:123–43.
36. Williams MC. Alveolar type I cells: molecular phenotype and development. Annu Rev Physiol. 2003;65:669–95.
37. Beck-Schimmer B, Schimmer RC, Pasch T. The airway compartment: chambers of secrets. News Physiol Sci. 2004;19:129–32.
38. Hurley BP, McCormick BA. Multiple roles of phospholipase A2 during lung infection and inflammation. Infect Immun. 2008;76:2259–72.
39. Yarla NS, Satyakumar K, Srinivasu D, DSVGK K, Aliev G, Dharmapuri G, Putta GRSS, Jagarlapoodi S, Bheeram V, Sadu SP, Duddukuri GR. Phospholipase A2: A Potential Therapeutic Target in Inflammation and Cancer (In silico, In vitro, In vivo and Clinical Approach). J Cancer Sci Ther. 2015;7:8.
40. Scott KF, Sainovic M, Hein J, Nixdorf S, Galletts T, Liauw W, de Souza P, Dong Q, Graham GG, Russell PJ. Emerging roles for phospholipase A2 enzymes in cancer. Biochimie. 2010;92:601–10.
41. Agarwal AR, Yin F, Cadenas E. Short-term cigarette smoke exposure leads to metabolic alterations in lung alveolar cells. Am J Respir Cell Mol Biol. 2014;51:284–93.
42. Linkous AG, Yazlovitskaya EM, Hallahan DE. Cytosolic phospholipase A2 and lysophospholipids in tumor angiogenesis. J Natl Cancer Inst. 2010;102:1398–412.
43. Pniewska E, Pawliczak R. The involvement of phospholipases A2 in asthma and chronic obstructive pulmonary disease. Mediators Inflamm. 2013;2013:793505.
44. Magnioli V, Kokotos G. Phospholipase A2 inhibitors as potential therapeutic agents for the treatment of inflammatory diseases. Expert Opin Ther Pat. 2010;20:1–18.
45. Melton AY, Munoz NM, Lambertino A, Boetticher E, Learoyd J, Zhu X, Jeff AR. Phosphodiesterase 4 inhibition of beta2-integrin adhesion caused by leukotriene B4 and TNF-alpha in human neutrophils. Eur J Respir. 2006;28:920–8.
46. Ciccone MM, Cortese F, Gesualdo M, Carbonara S, Zito A, Ricci G, De Pascalis F, Scocchitano P, Riccioni G. Dietary intake of carotenoids and their antioxidant and anti-inflammatory effects in cardiovascular care. Mediators Inflamm. 2013;2013:782137.