ANTICANCER RESEARCH *41*: 211-218 (2021)
doi:10.21873/anticanres.14767

# Extracellular Acidity-mediated Expression of cPLA2γ Confers Resistance in Gastric Cancer Cells

SUNG-CHUL LIM[1*], TAE-BUM LEE[2*], BYUNG-SOO KANG[1] and SONG IY HAN[2]

[1]*Department of Pathology, College of Medicine, Chosun University, Gwangju, Republic of Korea;*
[2]*Division of Premedical Science, College of Medicine, Chosun University, Gwangju, Republic of Korea*

**Abstract.** *Background/Aim: Extracellular acidity, a characteristic of solid tumors, has been proposed to be a critical factor for aggravating tumor malignancy and conferring resistance to therapeutics. Recently, acidity has been implicated in inflammatory responses, which are mediated through active lipid metabolites in various human tissues. In the present study, we investigated whether acidity can affect lipid-mediated signaling, and found that phospholipase A2 ($PLA_2$) activity increased at acidic pH in SNU601 and AGS gastric carcinoma cell lines. Materials and Methods: To identify the $PLA_2$ isoform that is responsible for the acidity-induced activity, we assessed mRNA levels of $cPLA_2$ isotypes through real-time qPCR, and protein levels through immunoblot assay in cells cultured in acidic medium. Results: It was found that acidic pH conditions markedly elevated the $PLA_2\gamma$ expression. A gene interference study using specific siRNA of $cPLA_2\gamma$ suggested that expression of $cPLA_2\gamma$ in acidic culture conditions may be associated with protection of cancer cells in acidic environment, as shown by cell viability and clonogenic assays. In addition, expression of $cPLA_2\gamma$ appeared to confer cell resistance to anticancer drugs under acidic pH conditions. Conclusion: Acidity-induced $cPLA_2\gamma$ expression may exert protective effects by imparting resistance to the gastric cancer cells under acidic environment.*

Gastric cancer (GC) is a frequently occurring malignancy and one of the major causes of cancer-related mortality worldwide (1). Although the development of surgical operation has brought quite successful results, there is still a large number of patients diagnosed with GC at advanced stages.

Recently, metabolic parameters of tumor tissues, such as extracellular acidity have been proposed to be critical in tumor malignancy and resistance to therapeutic agents (2, 3). Extracellular acidity is a characteristic feature of solid tumor as tumor cells undergo active aerobic and anaerobic glycolysis, resulting in accumulation of $H^+$ and lactic acid (4). Such acidosis has been reported to confer an ineffective therapeutic environment leading to selection of more resistant and aggressive characteristics (5, 6). However, the mechanisms by which acidic pH microenvironment induces resistance in cancer cells are not well understood.

Various studies have shown that biologically active lipids, such as eicosanoids play an essential role in the control of various pathological processes associated with cancer. These lipid metabolites are synthesized through a complex enzymatic pathway, beginning with the release of arachidonic acid. Arachidonic acid generation is catalyzed by phospholipase A2 ($PLA_2$) enzyme family through the hydrolysis of glycerophospholipids (7). So far, more than 30 different forms of $PLA_2$ enzymes have been identified and classified into four main categories: secreted $sPLA_2$s, cytosolic $cPLA_2$s, calcium-independent $iPLA_2$s, and platelet activating factor (PAF) acetyl hydrolase/oxidized lipid lipoprotein associated (Lp) $PLA_2$s. Among the known isoforms, intracellular $PLA_2$s in the group IV family ($cPLA_2$s) are most studied and have been suggested to play a role in various physiological and pathological processes (8, 9). The group IV $PLA_2$ family comprises of six members, including 4A, 4B, 4C, 4D, 4E, and 4F that are commonly referred to as $cPLA_2$ α, β, γ, σ, ε, and ζ, respectively (10). The most extensively studied group IV $PLA_2$ enzyme is $cPLA_2\alpha$, that plays crucial roles in the production of inflammatory lipid mediators with the help of COX and is overexpressed in multiple human cancers (11). Another member of group IV $PLA_2$, $cPLA_2\gamma$ also modulates phospholipid remodeling in the cells (12), therefore, it may be linked to the COX pathway to produce prostanoids (7). However, $cPLA_2\gamma$ shows several structural and functional differences from $cPLA_2\alpha$ indicating a distinct physiological role. $cPLA_2\gamma$ is constitutively associated with the membrane,

*These Authors contributed equally to this study.

*Correspondence to:* Professor Song Iy Han, Division of Premedical Science, College of Medicine, Chosun University, Gwangju 61452, Republic of Korea. Tel: +82 622306194, e-mail: sihan@chosun.ac.kr

*Key Words:* Acidity, resistance, $cPLA_2\gamma$, gastric cancer.

and is calcium-independent unlike cPLA$_2$α (13, 14). It is predominantly expressed in the cardiac and skeletal muscle in humans, and associated with mitochondria, ER and Golgi. Although the physiological significance or regulatory mechanism of cPLA$_2$γ is not well studied, recent studies have shown that cPLA$_2$γ plays roles in chemotaxis and invasion in breast cancer cells, and is involved in lipid droplet biogenesis and hepatitis C virus infection in hepatocytes. Previously, we demonstrated that acidic condition increased malignancy of GC cells (15). In the present study, we investigated whether PLA$_2$ is related with cell viability under acidic environment that is unfavorable for survival.

## Materials and Methods

*Cell culture and acidic pH conditions*. SNU-601 and AGS human GC cells were obtained from the Korean Cell Line Bank (Seoul, Republic of Korea). Cells were maintained in RPMI 1640 medium (Invitrogen, Carlsbad, CA, USA) supplemented with 10% (v/v) fetal bovine serum (FBS) and 1% Penicillin-Streptomycin at 37˚C in an atmosphere containing 5% $CO_2$. For experiments investigating the effect of acidic condition on GC cells, control medium was prepared by RPMI medium (pH 7.4) containing 1% FBS, and acidic pH medium was prepared by adding 5M HCl to the control medium to adjust to pH 6.4 or pH 6.0. Then, cells were incubated in pH 7.4 control medium or acidic pH-adjusted medium for 48 h or 72 h. Unless specified, chemicals were purchased from Calbiochem (San Diego, CA, USA).

*Cell viability assays*. The EZ-cytox viability assay was performed following the manufacturer's protocol. Briefly, cells were plated in wells of a 24-well plate at a density of 5~8×10$^4$ cells/well, cultured for 24 h, and then incubated in the normal pH or acidic pH RPMI medium for 48 h. The EZ-cytox solution (Daeillab, Seoul, Republic of Korea) was added to the wells and incubated at 37˚C in a $CO_2$ incubator for 2 h. Subsequently, the plates were read using an enzyme-linked immunosorbent assay plate reader at 450 nm. The absorbance of the non-treated cells was set as 100% and cell survival was expressed as a percentage of this value.

*Western blot analysis*. Treated cells were lysed in whole-cell lysis buffer (50 mM Hepes, 150 mM NaCl, 1% Triton X-100, 5 mM EGTA, and protease inhibitor cocktail). Equal amounts of protein were electrophoretically separated using SDS-PAGE gel (10-12%) and transferred to a nitrocellulose membrane using the standard protocol. Antibodies were used to probe for c PLA$_2$α (Cell signaling Technology, Danvers, MA, USA), JMJD7-cPLA$_2$β (abcam, Cambridge, MA, USA), cPLA$_2$γ (Novus Biologicals, Littleton, CO, USA), cPLA$_2$σ (Novus Biologicals), cPLA$_2$ζ (abcam), and α-tubulin (santa cruz Biotechnology, Santa Cruz, CA, USA). Signals were acquired using an Image Station 4000MM image analyzer (Kodak, Rochester, NY, USA).

*Real-time reverse transcription-polymerase chain reaction*. Real-time PCR was performed using the Light Cycler 2.0 (Roche, Basel, Switzerland). The reactions were prepared using the Fast Start DNA Master SYBR Green I Kit (Roche) according to the manufacturer's protocol. The cDNA was amplified using gene-specific primers. Primers for *PLA2G4C* (P321848) were purchased from Bioneer. Primers for other genes were designed as follows: for β-actin, 5'-GACTATGACTTAGTTGCGTTA-3' and 5'-GCCTTCATACATCTCAAGTTG-3', for *PLA2G4A*, 5'-TGCATTTCTTAATGGGTGTCT-3' and 5'-TCATCACTGTCCGAGCTATC-3', for *JMJD7-PLA2G4B*, 5'-CAGGAGCTGAGTATTCGC-3' and 5'-CCTCAGTCCTGCTTCTTT-3' for *PLA2G4D*, 5'-CTTTGTGGACCTGTGGG-3' and 5'-GTGTCTCCAGATTGTTCTCTT-3' for *PLA2G4F*, 5'-CCTGTACCAGGAGGAGA-3' and 5'-CAACCTCATAGGGCGTG-3'. PCR was performed at 95˚C for 10 min, followed by 45 cycles of 95˚C for 15 s, 60˚C for 5 s, and 72˚C for 7 s. Data were analyzed using Light Cycler software version 4.0 (Roche). The $2^{\Delta\Delta Ct}$ method was used for analysis of relative gene expression.

*PLA$_2$ activity assay*. PLA$_2$ activity was measured using cPLA$_2$ activity assay kit according to the manufacturer's protocol. Briefly, SNU601 cells were lysed following incubation in normal or acidic pH medium, and total PLA$_2$ activity was measured by incubating the samples with a substrate, arachidonoyl thio-PC, for 1 h at room temperature in the assay buffer. The reactions were stopped by adding dithiobis nitrobenzoic acid (DTNB)/EGTA. The absorbances were measured at a wavelength of 405 nm. Activity was represented as a fold increase.

*RNA interference (RNAi)*. For the RNAi experiment, siRNAs of PLA2G4C (Gene ID:5321) and a scrambled siRNA control were purchased from Bioneer (Daejeon, Republic of Korea). SNU601 cells were individually transfected with siRNA oligonucleotides using an Amaxa™ Transfection System (Basel, Switzerland) and grown for 48 h in the normal pH or acidic pH medium.

*Clonogenic assays*. Cells incubated in normal or acidic pH medium for 24 h were trypsinized, washed, and re-plated (2,000 cells/60 mm dish). After an incubation of 14 days in a 37˚C/5% $CO_2$ incubator, colonies were fixed and stained using crystal violet.

*Caspase-3 activity assays*. Caspase-3 activity assay was carried out using the caspase-3/CPP32 colorimetric assay kit (K-106-100, BioVision, Milpitas, CA, USA), according to the manufacturer's protocol. Briefly, 200 μg of protein lysates in a 50-μl volume was mixed with a reaction buffer, mixed with DEVD-pNA substrate, incubated for 90 min, and the absorbance at 405 nm measured. A fold increase in the activity was determined by comparing the results of the treated samples with the level of the untreated control.

*Statistical analysis*. All numerical data are presented as mean±SE of three independent experiments. For statistical analysis, student's *t*-test was used for simple comparisons, and one-way ANOVA with Tukey's test was used for multiple comparison test. A *p*-value of 0.05 or less was considered statistically significant.

## Results

*Acidic culture conditions increased PLA$_2$ activity in GC cells*. In our previous study, we observed that the gastric cancer cells that were cultured in acidic conditions increased the expression of COX proteins (15). COX is involved in lipid metabolite-mediated signaling pathway. Lipid metabolites generated through the PLA$_2$-mediated arachidonic acid/COX pathway play critical roles in tumor progression and malignancy, and extracellular acidity is implicated in various

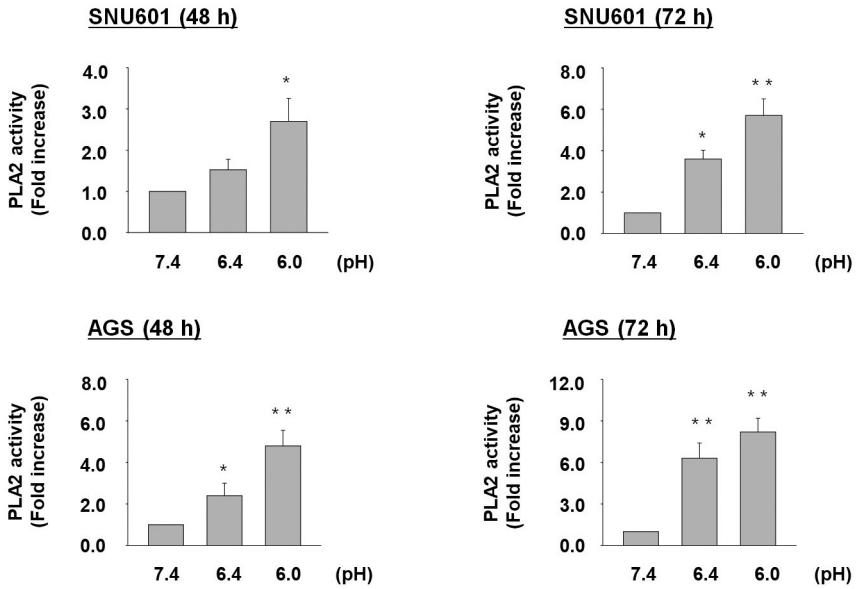


Figure 1. *Acidic culture conditions increase PLA$_2$ activity. SNU601 and AGS cells exposed to normal (pH 7.4) or acidic (pH 6.4 and pH 6.0) medium for 48 h or 72 h, were analyzed for PLA$_2$ activity. *p<0.05, **p<0.01 vs. pH 7.4.*

malignant properties of tumors. Therefore, we questioned whether extracellular acidity can also affect the upstream lipid-mediated signaling that is linked to COX pathway. Initially, we examined if acidic conditions can activate the PLA$_2$ enzyme that generates arachidonic acid, an essential lipid mediator that serves as a substrate for COX enzyme and initiates important lipid-mediated signaling. To assess PLA$_2$ activity, SNU601 and AGS gastric cancer cells were exposed to normal (pH 7.4) and acidic (pH 6.4 and pH 6.0) medium for 48 h and 72 h, respectively and the activity of PLA$_2$ was determined. As shown in Figure 1, exposure to acidic pH showed increased PLA$_2$ activities in both gastric carcinoma cells. Therefore, PLA$_2$ enzymes appear to be activated or up-regulated under acidic pH conditions in these cells.

*Acidic pH conditions induced PLA$_2$γ expression*. Since acidic pH conditions increased the PLA$_2$ activity in GC cells, we explored whether the acidity influences the expression of cellular PLA$_2$ enzymes. At first, we examined the mRNA expression levels of cPLA$_2$ isotypes in SNU601 and AGS cells that were cultured at normal and acidic pH medium for 72 h by performing real-time PCR analysis. Although cPLA$_2$α is known to be the most important member of cPLA$_2$ for eicosanoid generation, the mRNA level of *PLA2G4A* gene coding cPLA$_2$α was rarely altered under acidic conditions (Figure 2A and F). We also examined the expression of other cPLA$_2$ isoforms under acidic culture conditions. The mRNA level of *JMJD7-PLA2G4B* read-through which was previously reported to be overexpressed in several human cancer tissues, was not increased in the present study (Figure 2B and G). *PLA2G4D* and *PLA2G4F* mRNA levels were not altered in acidic culture environment (Figure 2D, E, I and J). The level of *PLA2G4E* was not detected in these conditions. However, the *PLA2G4C* level increased remarkably with an increase in acidity (Figure 2C and H). Consistently, acidic pH culture increased the cPLA$_2$γ protein level, however, the protein levels of other members were either not altered or undetected in immunoblotting (Figure 2K and L). This result indicates that acidic pH conditions increase the expression of cPLA$_2$γ in both SNU601 and AGS GC cells.

*Increased level of cPLA$_2$γ under acidic conditions is associated with cell viability*. To understand the role of cPLA$_2$γ overexpression in acidic culture conditions, we performed gene interference analysis. We transfected SNU601 cells using siRNA, specifically targeting the PLA2G4C gene. Thereafter, cell viability was assessed under normal and acidic pH conditions by performing EZ-cytox assay. Silencing of PLA2G4C had a minor effect on the viability of SNU601 cells under normal culture medium, however, it significantly decreased the survival rate of the cells upon exposure to acidic (pH 6.4 and pH 6.0) culture medium (Figure 3A-C). Moreover, silencing of PLA2G4C reduced colony forming ability under acidic conditions, and not under normal pH conditions (Figure 3D). The silencing efficiency of the PLA2G4C siRNAs was confirmed by the reduced cPLA$_2$γ protein levels under acidic pH culture conditions (Figure 3E). Therefore, an increased expression

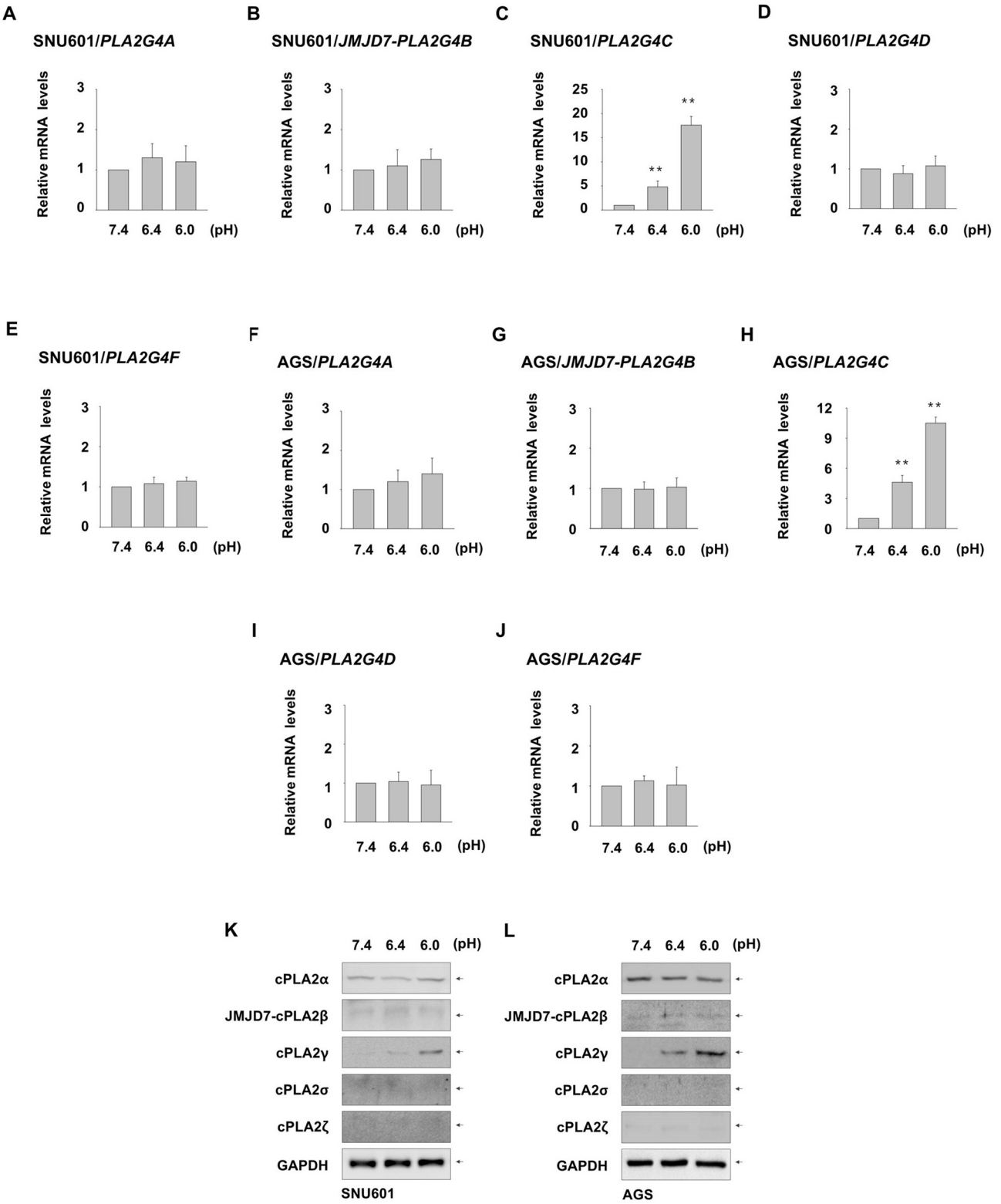


Figure 2. *Extracellular acidity increases mRNA and protein levels of cPLA$_2\gamma$. SNU601 (A-E, K) and AGS (F-J, L) cells maintained in normal (pH 7.4) or acidic (pH 6.4 and pH 6.0) medium were analyzed for mRNA expression of PLA2G4A (A and F), JMJD7-PLA2G4B (B and G), PLA2G4C (C and H), PLA2G4D (D and I), and PLA2G4F (E and J) by real-time PCR, or protein expression by immunoblotting using antibodies against cPLA$_2\alpha$, JMJD7-cPLA$_2\beta$, cPLA$_2\gamma$, cPLA$_2\sigma$, and cPLA$_2\zeta$ (K and L). **p<0.01 vs. pH 7.4.*

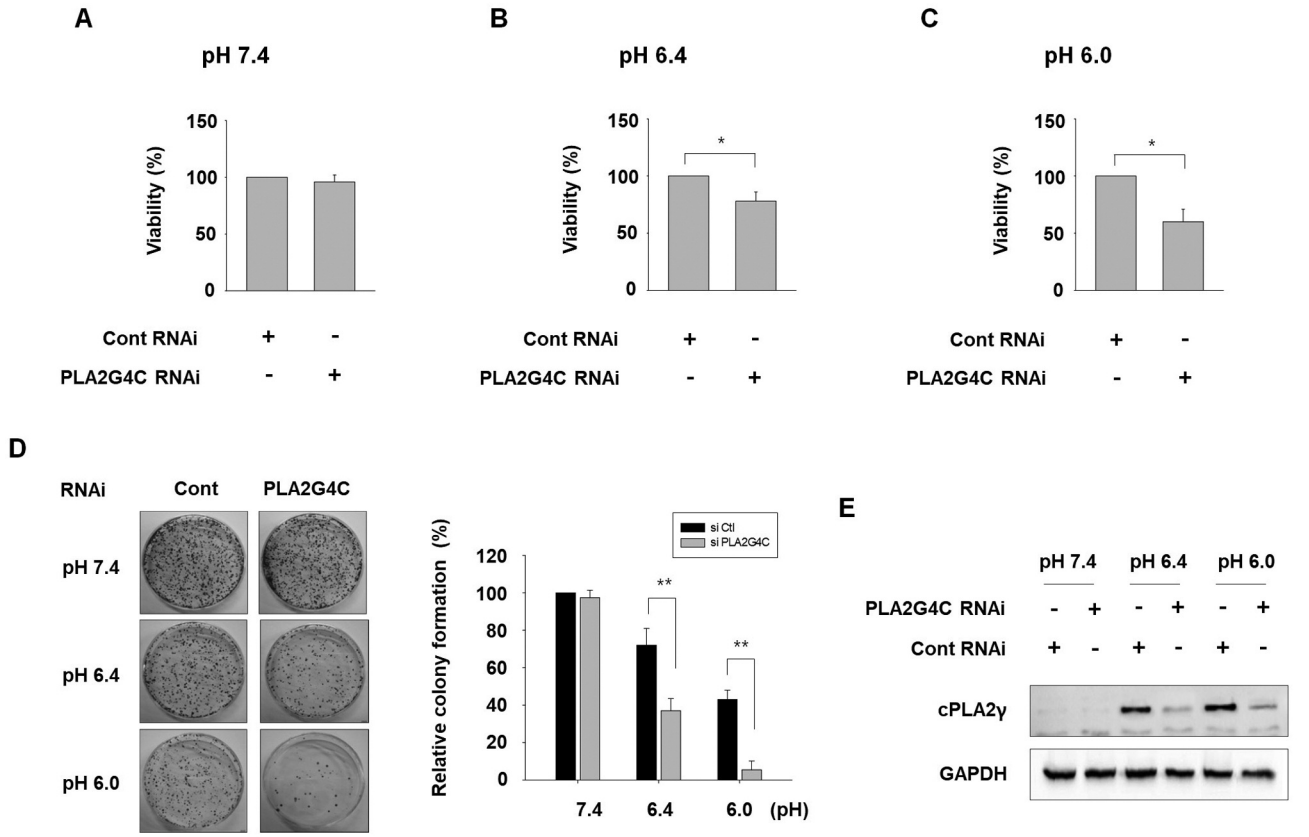


Figure 3. *Silencing of cPLA$_2\gamma$ reduced cell viability and clonogenicity under acidic culture conditions. (A-C) SNU601 cells were transiently transfected with scrambled small interfering RNA (Cont RNAi) or PLA2G4C RNAi, and exposed to the normal (A) or acidic growth medium (B, C) for 48 h. Cells were harvested and mRNA expression of the genes encoding cPLA$_2\gamma$ was analyzed through real-time PCR, or subjected to immunoblot analysis to confirm the silencing effect of PLA2G4C RNAi (E). (D) SNU601 cells were transiently transfected with scrambled small interfering RNA (Cont RNAi) or PLA2G4C RNAi, and incubated in the indicated pH medium for 24 h. Subsequently, cells (2,000) were then re-plated on 60-mm dishes and cultured to detect colony formation. Colonies were stained and counted at 2 weeks post-incubation. *p<0.05, **p<0.01.*

of cPLA$_2\gamma$ may play a role in protecting SNU601 cells from the cytotoxic effect triggered by the acidic environment.

*Elevated levels of cPLA$_2\gamma$ are involved in acidity-mediated resistance to anticancer drugs*. Acidic microenvironment has been implicated in the resistance of cancers to chemotherapeutic treatments (5, 16). Since acidic pH increased the expression of cPLA$_2\gamma$ that was involved in cell viability under acidic culture conditions, we examined whether cPLA$_2\gamma$ induction is also related to acidity-mediated resistance to chemotherapeutics. To examine this, the cells with silenced PLA2G4C gene were exposed to the anticancer drug, doxorubicin under normal pH (pH 7.4) or acidic pH (pH 6.0) medium for 24 h or 48 h, and cytotoxicity was detected at each time point of incubation. As shown in our previous study (17), sensitivity of cells to doxorubicin was much lower in acidic pH conditions than in normal pH conditions (Figure 4A-C). However, the knockdown of PLA2G4C gene increased the sensitivity to doxorubicin in acidic culture medium as assessed by cytotoxicity assay (Figure 4A and B). In addition, silencing of PLA2G4C gene significantly elevated doxorubicin-induced caspase-3 activity under acidic pH conditions (Figure 4C). The silencing efficiency of the PLA2G4C siRNAs was visualized by reduced cPLA$_2\gamma$ protein levels under acidic pH culture conditions (Figure 4D). Thus, an increase in cPLA$_2\gamma$ expression appears to be involved in acidity-mediated resistance to anticancer drugs by reducing apoptosis of tumor cells.

## Discussion

Extracellular acidity confers a harsh tumor environment that inhibits the progression of cell cycle and stimulates apoptotic signaling. Among heterogeneous tumor cell populations, certain cells can adapt to this harsh environment through up-regulation of the defense system. Thereby, the cells that withstand this crisis will continue to proliferate to fill the space created by

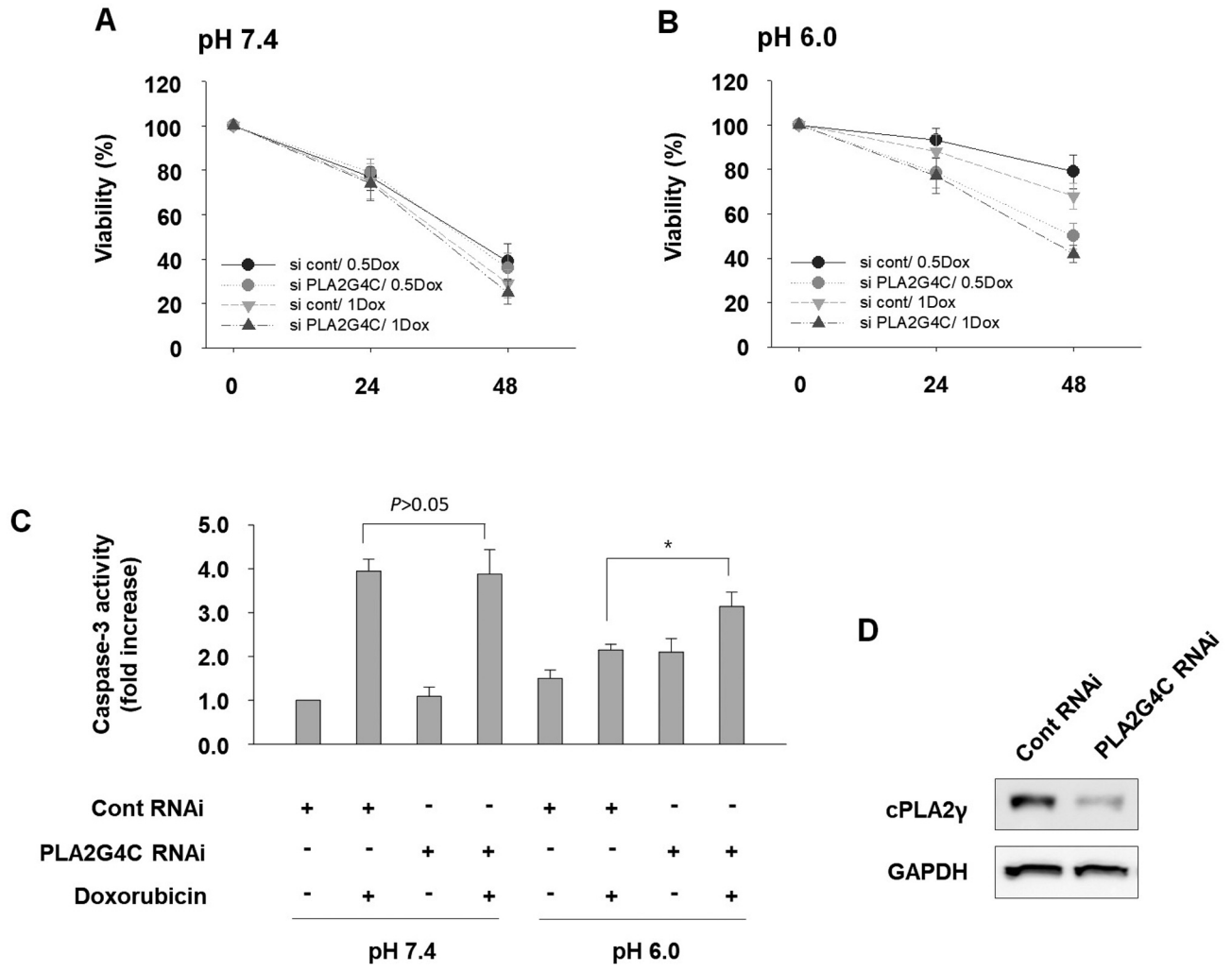


Figure 4. *Expression of cPLA$_2$γ is associated with acidity-mediated drug resistance. SNU601 cells were transiently transfected with scrambled small interfering RNA (Cont RNAi) or PLA2G4C RNAi, and exposed to the normal (pH 7.4) (A) or acidic (pH 6.0) (B) medium containing 0.5 μM or 1 μM doxorubicin for the indicated time points. Then cell viability was assessed through the EZ-cytox assay. (C) SNU601 cells transiently transfected with scrambled small interfering RNA (Cont RNAi) or PLA2G4C RNAi were incubated in the normal or acidic medium with or without 1 μM doxorubicin for 48 h, and cells were harvested and subjected to caspase-3 activity assay. (D) SNU601 cells transiently transfected with scrambled small interfering RNA (Cont RNAi) or PLA2G4C RNAi were incubated in the acidic (pH 6.0) medium for 48 h and subjected to immunoblot analysis to confirm the silencing effect. *p<0.05.*

dead cells. Therefore, the acidic microenvironment is implicated in the selection of more resistant and malignant traits of the tumor. Consistent with this, we previously observed that gastric cancer cells exposed to acidic pH culture conditions were more invasive and resistant to chemotherapeutic drugs compared to cells that were cultured in normal pH (15, 17).

Resistance of cancer cell is related to multiple factors and among them, cellular defense system may be one of the important therapeutic targets. Herein, we identified a specific defense modulator stimulated in acidic environment. It is notable that the acidic microenvironment is implicated in the activation of inflammatory responses in various cell types, including immune cells, fibroblasts, and endothelial cells (18-20). Moreover, chronic inflammation is closely associated with tumor progression.

Lipid metabolism-associated pathways have been shown to play a crucial role in induction of cancer progression under metabolic stress (21). In addition, we previously observed that acidic culture environment increased the expression of COX proteins, which are activated by $PLA_2$-mediated arachidonic acid signaling. Therefore, we questioned whether extracellular acidity can also affect the upstream lipid-mediated signaling, such as $PLA_2$ pathway. Indeed, critical role $cPLA_2$ is implicated in pulmonary tumor metastasis (22).

In the present study, we explored the possible effects of acidic pH on the activation of upstream lipid signaling mediator; PLA$_2$ activity was found to increase in response to acidity. Moreover, investigation of the levels of the cytosolic PLA$_2$ isoform showed a significant increase in cPLA$_2$γ in response to acidic culture conditions.

When we examined the role of cPLA$_2$γ using the gene silencing analysis, it seemed to play protective roles under acidic pH environment as indicated by decrease in cell viability and clonogenicity. Furthermore, elevation of cPLA$_2$γ was linked to drug sensitivity under acidic pH conditions, and consequently, the cellular response to doxorubicin was increased in PLA$_2$γ-silenced cells. However, knockdown of cPLA$_2$γ had no effect on viability and drug-responses in the cells at normal pH. This might be attributed to the fact that the cells had low expression of cPLA$_2$γ at normal pH conditions.

cPLA$_2$γ was shown to possess similar catalytic activity as cPLA$_2$α because the catalytic domain is conserved in both enzymes (9). Hence, cPLA$_2$γ may contribute to the biosynthesis of lipid metabolites through arachidonic acid generation, thereby participating in various malignant phenotypes of cancer. This is in agreement with our results that showed that acidity-mediated cPLA$_2$γ expression contributes to malignant phenotypes, such as increase in cell survival and resistance to stressful stimuli. Moreover, the enzymatic analyses of cPLA$_2$γ by various studies have shown that it possesses lysophospholipase and transacylation activities in addition to phospholipase A2 activity (23, 24). Therefore, cPLA$_2$γ may be able to contribute to lipid signaling through lysophospholipase and transacylation activities. The exact role of cPLA$_2$γ is still not understood, however, a positive association with cancer has been suggested in recent studies. Suppression of cPLA$_2$γ through gene silencing-induced apoptosis of rat mammary tumor cells by NFkB/lipocalin 2 pathway (25), and SNP of cPLA$_2$γ was linked to a worse prognosis in patients with CRC (26).

Nevertheless, cPLA$_2$γ has a distinct regulatory mechanism from cPLA$_2$α because of several differences in structure, distribution, and regulation. cPLA$_2$α is ubiquitously expressed in mammalian tissues, however, the human cPLA$_2$γ mRNA is predominantly found in cardiac and skeletal muscle, and to a lesser extent in brain (14). In addition, lack of the regulatory phosphorylation sites and C2 domain are the structural features of cPLA$_2$γ that are important for association to the membrane and critical for activity of cPLA$_2$α. Another obvious difference in regulatory mechanism is that cPLA$_2$γ is calcium-independent, while other members of cytosolic PLA$_2$s are all calcium-dependent. The calcium independence of this enzyme appears to be an important feature to be used in acidic environments because extracellular acidity increases large fluxes in calcium concentrations (27). Conclusively, based on the findings of the present study, we suggest that cPLA$_2$γ could be an important therapeutic target for resistant cancer cells under acidic environment. Moreover, further study of the mechanism of cPLA$_2$γ expression and activation may provide an effective strategy to overcome extracellular acidity-mediated cancer resistance.

## Conflicts of Interest

The Authors declare no conflicts of interest.

## Authors' Contributions

Conceptualization, SIH, TBL and BSK; methodology and writing, SCL and SIH; review and editing, all Authors.

## Acknowledgements

This research was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (NRF-2015R1D1A1 A01060533 and NRF-2018R1D1A1B07046430). We would like to thank Dr. Mi-Rae Lee and Dr. Hyoin Hwang for their excellent technical assistance.

## References

1 Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA and Jemal A: Global cancer statistics 2018: Globocan estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin *68(6)*: 394-424, 2018. PMID: 30207593. DOI: 10.3322/caac.21492

2 Justus CR, Dong L and Yang LV: Acidic tumor microenvironment and ph-sensing g protein-coupled receptors. Front Physiol *4:* 354, 2013. PMID: 24367336. DOI: 10.3389/fphys.2013.00354

3 Brisson L, Reshkin SJ, Gore J and Roger S: Ph regulators in invadosomal functioning: Proton delivery for matrix tasting. Eur J Cell Biol *91(11-12)*: 847-860, 2012. PMID: 22673002. DOI: 10.1016/j.ejcb.2012.04.004

4 Tannock IF and Rotin D: Acid ph in tumors and its potential for therapeutic exploitation. Cancer Res *49(16)*: 4373-4384, 1989. PMID: 2545340.

5 Taylor S, Spugnini EP, Assaraf YG, Azzarito T, Rauch C and Fais S: Microenvironment acidity as a major determinant of tumor chemoresistance: Proton pump inhibitors (ppis) as a novel therapeutic approach. Drug Resist Updat *23*: 69-78, 2015. PMID: 26341193. DOI: 10.1016/j.drup.2015.08.004

6 Raghunand N and Gillies RJ: Ph and drug resistance in tumors. Drug Resist Updat *3(1)*: 39-47, 2000. PMID: 11498364. DOI: 10.1054/drup.2000.0119

7 Funk CD: Prostaglandins and leukotrienes: Advances in eicosanoid biology. Science *294(5548)*: 1871-1875, 2001. PMID: 11729303. DOI: 10.1126/science.294.5548.1871

8 Burke JE and Dennis EA: Phospholipase a2 structure/function, mechanism, and signaling. J Lipid Res *50 Suppl*: S237-242, 2009. PMID: 19011112. DOI: 10.1194/jlr.R800033-JLR200

9 Murakami M, Masuda S and Kudo I: Arachidonate release and prostaglandin production by group ivc phospholipase a2 (cytosolic phospholipase a2gamma). Biochem J *372(Pt 3)*: 695-702, 2003. PMID: 12611587. DOI: 10.1042/BJ20030061

10 Ohto T, Uozumi N, Hirabayashi T and Shimizu T: Identification of novel cytosolic phospholipase a(2)s, murine cpla(2){delta}, {epsilon}, and {zeta}, which form a gene cluster with cpla(2){beta}. J Biol Chem *280(26)*: 24576-24583, 2005. PMID: 15866882. DOI: 10.1074/jbc.M413711200

11 Tosato G, Segarra M and Salvucci O: Cytosolic phospholipase a2{alpha} and cancer: A role in tumor angiogenesis. J Natl Cancer Inst *102(18)*: 1377-1379, 2010. PMID: 20729476. DOI: 10.1093/jnci/djq324

12 Stewart A, Ghosh M, Spencer DM and Leslie CC: Enzymatic properties of human cytosolic phospholipase a(2)gamma. J Biol Chem *277(33)*: 29526-29536, 2002. PMID: 12039969. DOI: 10.1074/jbc.M204856200

13 Pickard RT, Strifler BA, Kramer RM and Sharp JD: Molecular cloning of two new human paralogs of 85-kda cytosolic phospholipase a2. J Biol Chem *274(13)*: 8823-8831, 1999. PMID: 10085124. DOI: 10.1074/jbc.274.13.8823

14 Underwood KW, Song C, Kriz RW, Chang XJ, Knopf JL and Lin LL: A novel calcium-independent phospholipase a2, cpla2-gamma, that is prenylated and contains homology to cpla2. J Biol Chem *273(34)*: 21926-21932, 1998. PMID: 9705332. DOI: 10.1074/jbc.273.34.21926

15 Lim SC, Hwang H and Han SI: Ellagic acid inhibits extracellular acidity-induced invasiveness and expression of cox1, cox2, snail, twist 1, and c-myc in gastric carcinoma cells. Nutrients *11(12)*, 2019. PMID: 31835645. DOI: 10.3390/nu11123023

16 Thews O, Gassner B, Kelleher DK, Schwerdt G and Gekle M: Impact of hypoxic and acidic extracellular conditions on cytotoxicity of chemotherapeutic drugs. Adv Exp Med Biol *599:* 155-161, 2007. PMID: 17727260. DOI: 10.1007/978-0-387-71764-7_21

17 Hong R and Han SI: Extracellular acidity enhances tumor necrosis factor-related apoptosis-inducing ligand (trail)-mediated apoptosis *via* dr5 in gastric cancer cells. Korean J Physiol Pharmacol *22(5)*: 513-523, 2018. PMID: 30181698. DOI: 10.4196/kjpp.2018.22.5.513

18 Riemann A, Ihling A, Thomas J, Schneider B, Thews O and Gekle M: Acidic environment activates inflammatory programs in fibroblasts *via* a camp-mapk pathway. Biochim Biophys Acta *1853(2)*: 299-307, 2015. PMID: 25461841. DOI: 10.1016/j.bbamcr.2014.11.022

19 Dong L, Li Z, Leffler NR, Asch AS, Chi JT and Yang LV: Acidosis activation of the proton-sensing gpr4 receptor stimulates vascular endothelial cell inflammatory responses revealed by transcriptome analysis. PLoS One *8(4)*: e61991, 2013. PMID: 23613998. DOI: 10.1371/journal.pone.0061991

20 Etulain J, Negrotto S, Carestia A, Pozner RG, Romaniuk MA, D'Atri LP, Klement GL and Schattner M: Acidosis downregulates platelet haemostatic functions and promotes neutrophil proinflammatory responses mediated by platelets. Thromb Haemost *107(1)*: 99-110, 2012. PMID: 22159527. DOI: 10.1160/TH11-06-0443

21 Neagu M, Constantin C, Popescu ID, Zipeto D, Tzanakakis G, Nikitovic D, Fenga C, Stratakis CA, Spandidos DA and Tsatsakis AM: Inflammation and metabolism in cancer cell-mitochondria key player. Front Oncol *9*: 348, 2019. PMID: 31139559. DOI: 10.3389/fonc.2019.00348

22 Jeong WC, Kim KJ, Ju HW, Back HK, Kim HK, Im SY and Lee HK: Cytoplasmic phospholipase a2 metabolites play a critical role in pulmonary tumor metastasis in mice. Anticancer Res *30(9)*: 3421-3427, 2010. PMID: 20944117.

23 Yamashita A, Tanaka K, Kamata R, Kumazawa T, Suzuki N, Koga H, Waku K and Sugiura T: Subcellular localization and lysophospholipase/transacylation activities of human group ivc phospholipase a2 (cpla2gamma). Biochim Biophys Acta *1791(10)*: 1011-1022, 2009. PMID: 19501189. DOI: 10.1016/j.bbalip.2009.05.008

24 Asai K, Hirabayashi T, Houjou T, Uozumi N, Taguchi R and Shimizu T: Human group ivc phospholipase a2 (cpla2gamma). Roles in the membrane remodeling and activation induced by oxidative stress. J Biol Chem *278(10)*: 8809-8814, 2003. PMID: 12502717. DOI: 10.1074/jbc.M212117200

25 Nanashima N, Yamada T, Shimizu T and Tsuchida S: Deletion of phospholipase a2 group ivc induces apoptosis in rat mammary tumour cells by the nuclear factor-kappab/lipocalin 2 pathway. Biochem J *469(2)*: 315-324, 2015. PMID: 26013918. DOI: 10.1042/BJ20150064

26 Olsen RS, Andersson RE, Zar N, Lofgren S, Wagsater D, Matussek A and Dimberg J: Prognostic significance of pla2g4c gene polymorphism in patients with stage ii colorectal cancer. Acta Oncol *55(4)*: 474-479, 2016. PMID: 26364726. DOI: 10.3109/0284186X.2015.1073350

27 Noguchi F, Inui S, Fedele C, Shackleton M and Itami S: Calcium-dependent enhancement by extracellular acidity of the cytotoxicity of mitochondrial inhibitors against melanoma. Mol Cancer Ther *16(5)*: 936-947, 2017. PMID: 28223427. DOI: 10.1158/1535-7163.MCT-15-0235

*Received November 13, 2020*
*Revised December 1, 2020*
*Accepted December 2, 2020*