Secretory Phospholipase A_2 Mediates Human Esophageal Adenocarcinoma Cell Growth and Proliferation *via* ERK 1/2 Pathway

MIRAL R. SADARIA, JESSICA A. YU, XIANZHONG MENG, DAVID A. FULLERTON, T. BRETT REECE and MICHAEL J. WEYANT

Division of Cardiothoracic Surgery, Department of Surgery, University of Colorado Anschutz Medical Campus, Aurora, CO, U.S.A.

Abstract. *Background/Aim: Secretory phospholipase-A_2 ($sPLA_2$) mediates growth and proliferation of human esophageal adenocarcinoma cells (HEAC). Two major molecular pathways of cancer growth regulation include extracellular signal-regulated kinase 1/2 (ERK 1/2) and protein kinase-B (AKT). Phospholipase enzymes have been demonstrated to significantly influence these two growth regulating pathways in other tumor cells. We hypothesize $sPLA_2$ to mediate HEAC growth control through ERK 1/2 and AKT. Materials and Methods: Verified HEAC (FLO-1), treated with either the mitogen-activated protein kinase kinase selective inhibitor (PD98059) or with group IIa $sPLA_2$-specific inhibitor, were assessed for ERK1/2 phosphorylation and AKT activation after tumor necrosis factor-alpha stimulation, as well as for viability, proliferation, and apoptosis responses. Results: PD98059 significantly inhibited ERK 1/2 activation, reduced cell viability, and reduced proliferation ($p<0.05$). $sPLA_2$ inhibition attenuated ERK 1/2 activation ($p<0.01$) but had no effect on AKT activation or apoptosis. Conclusion: Specific inhibition of group IIa $sPLA_2$ directly reduces HEAC viability and proliferation by attenuating ERK 1/2 activation with no effect on AKT activation or apoptosis. This may indicate that the mechanism of action of $sPLA_2$ is mainly the induction of growth arrest.*

Esophageal adenocarcinoma is an aggressive malignancy with an incidence that is drastically rising compared to other malignancies in the United States. In certain populations, the incidence has increased greater than 800% over the past three decades (1, 2). Despite significant study of both systemic and local therapeutics, the disease continues to be characterized by its poor prognosis (3). Improved survival necessitates early diagnosis; however, most patients present late in the disease process with uncontrolled tumor growth and metastatic disease (4, 5). Given the majority of patients present with advanced disease and the bleak prognosis associated with the diagnosis of esophageal adneocarcinoma, understanding the mechanisms of tumor cell survival and growth is critical in order to more effectively treat the disease.

The mitogen-activated protein kinase (MAPK) cascade known as extracellular signal-regulated kinase 1/2 (ERK 1/2) and the protein kinase-B cascade known as AKT have been demonstrated to be regulators of cell growth and survival in Barrett's esophagus and human esophageal adenocarcinoma cells (HEAC) (6). The ERK 1/2 pathway is important in controlling cell-cycle re-entry by affecting G_1 to S cell-cycle progression, and the AKT pathway is primarily responsible for cell survival signaling by preventing apoptosis (7). However, recent data suggests that these pathways both contribute to HEAC proliferation and cell survival (6, 8).

Secretory phospholipase A_2 ($sPLA_2$), a sub-class of phospholipase A_2 enzymes, catalyzes the hydrolysis of membrane phospholipids, producing various inflammatory mediators, such as prostaglandins, leukotrienes, and thromboxane (9, 10). We previously identified $sPLA_2$ as a key mediator in the development of esophageal hyperplasia in the setting of gastroesophageal reflux *in vivo* (11, 12). More recently, we demonstrated that $sPLA_2$ regulates the growth and metastatic potential of HEAC *in vitro*. Group IIa *$sPLA_2$* gene overexpression enhanced esophageal cancer cell growth, while gene knockdown reduced growth (13). Furthermore, treatment with the specific group IIa $sPLA_2$ inhibitor significantly attenuated growth of the tumor cells (13) as well as their metastatic potential, indicated by a reduction in intercellular

Correspondence to: Michael J. Weyant, MD, Assistant Professor of Surgery, University of Colorado Anschutz Medical Campus, Division of Cardiothoracic Surgery, Department of Surgery, Academic Office One, Room 6602, 12631 East 17th Avenue, C310, Aurora, CO 80045, U.S.A. Tel: +1 3037242800, Fax: +1 3037242806, e-mail: Michael.Weyant@UCDenver.edu

Key Words: Secretory phospholipase A2, esophageal adenocarcinoma, growth, proliferation, ERK 1/2.

adhesion molecule-1 expression, which is a marker of the tumor's metastatic potential (14). In human astrocytoma cells, $sPLA_2$ mediates tumor cell growth by activating the major intracellular signaling cascades of ERK 1/2 and AKT (15). We, therefore, hypothesized that the $sPLA_2$ enzyme may mediate growth control of HEAC, as defined by cell viability, proliferation, and apoptosis, through the ERK 1/2 and AKT pathways.

Materials and Methods

Cell line and drug preparation. FLO-1 cells, a verified human esophageal adenocarcinoma cell line (University of Michigan, Ann Arbor, MI, USA), were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum (FBS), penicillin (100 units/ml), streptomycin (100 μg/ml), and fungizone (1.8 μg/ml). Cells were cultured incubated at 37˚C in a humidified atmosphere of 95% air and 5% CO_2.

2'-Amino-3'-methoxyflavone (EMD Chemicals Inc., Gibbstown, NJ, USA), also known as PD98059, a selective inhibitor of mitogen-activated protein kinase kinase (MEK 1/2), was dissolved in dimethyl sulfoxide (DMSO). 5-(4-Benzyloxyphenyl)-4S-(7-phenylheptanoylamino) pentanoic acid (Sigma-Aldrich, St. Louis, MO, USA), a specific inhibitor of group IIa $sPLA_2$, was also dissolved in DMSO. Prior to each experiment, the stock solution of each drug was diluted in serum-reduced medium (0.5% FBS) such that the concentration of DMSO never exceeded 0.5% in PD98059 experiments and 0.03% in $sPLA_2$ inhibitor experiments.

Recombinant human tumor necrosis factor-α (TNF-α) (Sigma-Aldrich) was reconstituted in sterile-filtered distilled water for a final concentration of 100 ng/μl. This was aliquoted appropriately and stored at −20˚C until future use. At the time of use, the stock solution was thawed and dissolved in phosphate-buffered saline to a working concentration of 4 ng/μl.

Cell treatment. FLO-1 cells were cultured in full growth medium (10% FBS) at the following densities: 3×10^5 cells per well (6-well plate) for 48 hours for immunoblotting; 1×10^4 cells per well (96-well plate) for 48 hours for cytotoxicity, viability, and proliferation assays; and 5×10^5 cells per well (6-well plate) for 48 hours for apoptosis assay (13, 14, 16). The cells were serum-reduced for the subsequent 24 h and then treated with either vehicle control (DMSO), PD98059 (5 μM, 10 μM, or 25 μM), or $sPLA_2$ inhibitor (5 μM, 10 μM, or 15 μM). All wells treated with PD98059 and $sPLA_2$ inhibitor contained equivalent final DMSO concentrations as the vehicle control. In experiments verifying the inhibitory effect of PD98059 on ERK 1/2 activation, cells were stimulated one hour after PD98059 treatment with 20 ng/ml of TNF-α and collected 30 minutes after TNF-α stimulation for immunoblotting. In experiments evaluating ERK 1/2 or AKT activation, cells were stimulated one hour after $sPLA_2$ inhibitor treatment with 20 ng/ml of TNF-α (14) and collected 30 minutes after TNF-α stimulation for immunoblotting. In experiments evaluating cytotoxicity, cell viability, and proliferation, the assays were started 12 hours after treatment with PD98059. In experiments evaluating apoptosis, the assays were started 12 hours after treatment with $sPLA_2$ inhibitor.

Immunoblotting. Immunoblotting was used to detect activated ERK 1/2 (1:1000 rabbit anti-human total ERK 1/2 antibody, and 1:1000 rabbit anti-human phospho-ERK 1/2 antibody; Cell Signaling Technology, Inc., Danvers, MA, USA) and activated AKT (1:1000 rabbit anti-human total AKT antibody, and 1:500 rabbit anti-human phospho-AKT antibody; Cell Signaling Technology, Inc.) using glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as previously described (14).

Cell viability and proliferation quantification after PD98059 treatment. To verify PD98059 treatment had no cytotoxic effects on the FLO-1 HEAC, a lactate dehydrogenase cytotoxicity assay (Cayman Chemical Company, Ann Arbor, MI, USA) was performed on the treatment media as previously described (16). None of the concentrations of PD98059 used in this study (5 μM, 10 μM, or 25 μM) had cytotoxic effects on the cells (16).

After completing the 12-hour PD98059 treatment in 96-well plates, cellular viability was quantified using a commercially available 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) assay (Roche Diagnostics Corporation, Indianapolis, IN, USA) according to the manufacturer's instructions (13, 16).

To assess for changes in cellular proliferation, a 5-bromo-2'-deoxyuridine (BrdU) assay was performed 12 hours after PD98059 treatment according to the manufacturer's instructions (Roche Diagnostics Corporation). This assay quantifies *de novo* DNA synthesis as a measure of proliferation (13, 16).

Apoptosis quantification after group IIa $sPLA_2$ inhibitor treatment. Apoptosis was quantified using a commercially available fluorescein isothiocyanate-conjugated annexin V apoptosis detection kit (BD Biosciences, San Jose, CA, USA). Twelve hours after $sPLA_2$ inhibitor treatment, the samples were prepared according to the manufacturer's instructions, and the assay was run as previously described (16).

Statistical analysis. For immunoblotting results, the phosphorylated and total band densities of ERK 1/2 and AKT were normalized to that for GAPDH on their respective membranes by dividing the density of the primary band by the density of the GAPDH band, accounting for any variation in protein loading. The normalized phosphorylated ERK 1/2 and AKT density ratios were then divided by the normalized total ERK 1/2 and AKT density ratios. This adjusted unit was then normalized to that for vehicle control on the same experimental plate, giving the vehicle control an adjusted unit value of 1.0. By normalizing to the vehicle control, results were comparable between experiments, allowing for statistical analysis.

The lactate dehydrogenase cytotoxicity, cell viability (MTT), and proliferation (BrdU) assays were performed in triplicate wells for each experiment. The absorbance value from each well was normalized to that for the vehicle control, giving the vehicle control an adjusted unit value of 1.0. This allowed for comparison of results between experiments and for statistical analysis.

The total percentage of cells in each sample undergoing both early and late apoptosis, as indicated by positive annexin V staining, was used for statistical analysis.

Data are presented as the mean±standard error. Statistical analysis was performed using ANOVA with Fisher's least significant difference post-hoc test (StatView by SAS Institute Inc., Cary, NC, USA). For all statistical comparisons, a p-value <0.05 was considered significant.

Results

Verification of the inhibitory effect of PD98059 on ERK 1/2 activation in FLO-1 HEAC. After treatment of FLO-1 cells

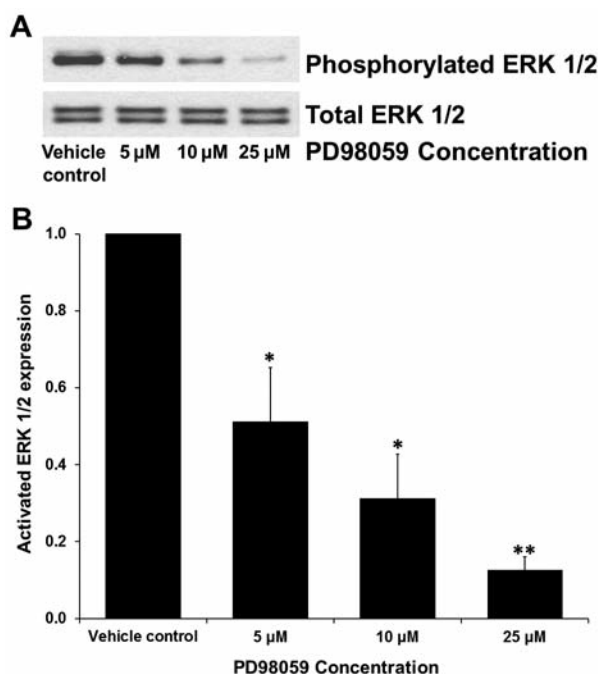

Figure 1. *Extracellular signal-regulated kinase 1/2 (ERK 1/2) activation in human esophageal adenocarcinoma cells (HEAC) (FLO-1) attenuated by mitogen-activated protein kinase kinase selective inhibitor (PD98059). A representative western blot demonstrates dose-dependent attenuation of ERK 1/2 activation in FLO-1 cells when treated with PD98059 (A). ERK 1/2 activation was attenuated at all concentrations, verifying the inhibitory effect of PD98059 on ERK 1/2 activation in FLO-1 cells (B). *p<0.005 compared to vehicle control, **p<0.01 compared to vehicle control and 5 µM, n=5.*

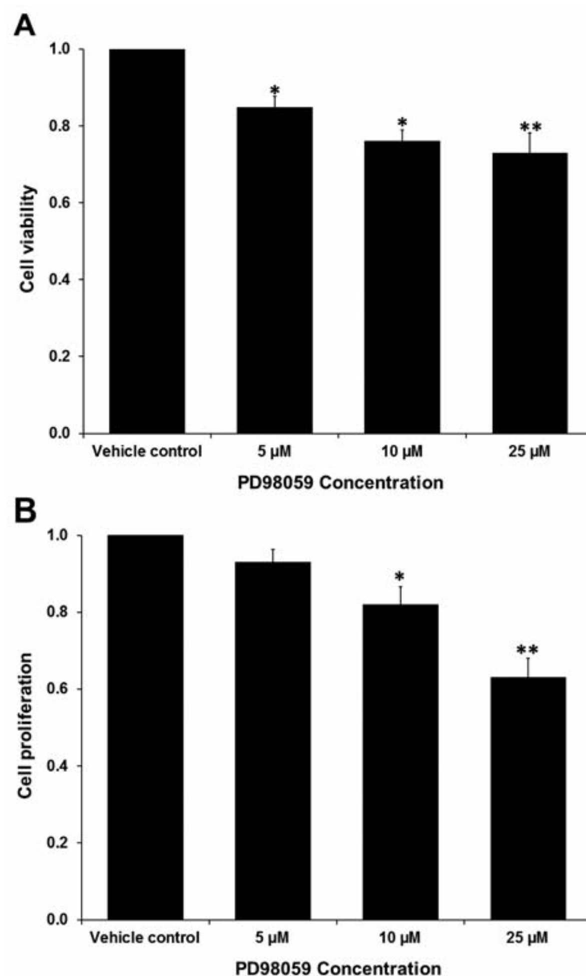

Figure 2. *Cell viability and proliferation of human esophageal adenocarcinoma cells (HEAC) (FLO-1) attenuated by mitogen-activated protein kinase kinase selective inhibitor (PD98059) therapy. PD98059 treatment attenuated FLO-1 cell viability at all concentrations (A); *p<0.01 compared to vehicle control, **p<0.05 compared to vehicle control and 5 µM, n=5. PD98059 treatment attenuated FLO-1 cell proliferation at 10 µM and 25 µM (B); *p<0.005 compared to vehicle control, **p<0.005 compared to vehicle control and all concentrations, n=5.*

with PD98059, immunoblotting demonstrated an attenuation in ERK 1/2 activation by 48.9%, 68.8%, and 87.5% at the 5 µM, 10 µM, and 25 µM concentrations, respectively, compared to the vehicle control (p<0.005) (Figure 1).

Cell viability and proliferation attenuated by PD98059 treatment of FLO-1 HEAC. PD98059 did not cause any cell necrosis at any of the concentrations used in this study, as determined by the lactate dehydrogenase cytotoxicity assay (data not shown).

PD98059 treatment attenuated cell viability by 15.2%, 24.0%, and 27.1% at the 5 µM, 10 µM, and 25 µM concentrations, respectively, compared to the vehicle control (p<0.01) (Figure 2A).

PD98059 treatment attenuated cell proliferation by 18.0% and 37.0% at the 10 µM and 25 µM concentrations, respectively, compared to the vehicle control (p<0.005) (Figure 2B).

ERK 1/2 activation attenuated by group IIa $sPLA_2$ inhibition in FLO-1 HEAC. In FLO-1 HEAC, ERK 1/2 phosphorylation peaked 30 min after TNF-α administration (data not shown). After treatment of FLO-1 cells with specific group IIa $sPLA_2$ inhibitor, immunoblotting demonstrated an attenuation in ERK 1/2 activation by 31.2% and 39.6% at the 10 µM and 15 µM concentrations, respectively, compared to the vehicle control (p<0.01) (Figure 3).

AKT activation after group IIa $sPLA_2$ inhibition in FLO-1 HEAC. In FLO-1 HEAC, AKT phosphorylation peaked 30 min after TNF-α administration (data not shown). Treatment of FLO-1 cells with increasing concentrations of specific group IIa $sPLA_2$ inhibitor demonstrated no significant change in AKT activation *via* immunoblotting, compared to the vehicle control

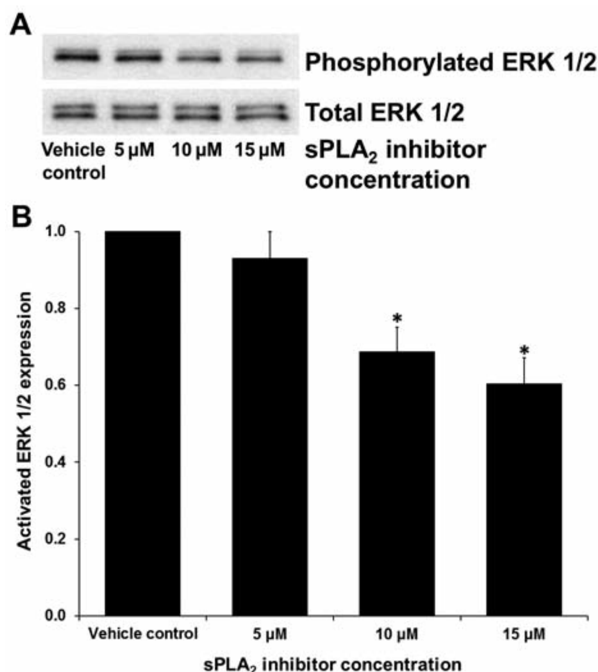

Figure 3. *Extracellular signal-regulated kinase 1/2 (ERK 1/2) activation in human esophageal adenocarcinoma cells (HEAC) (FLO-1) attenuated by inhibition of group IIa secretory phospholipase-A_2 ($sPLA_2$). A representative western blot demonstrates a dose-dependent attenuation of ERK 1/2 activation in FLO-1 cells when treated with specific group IIa $sPLA_2$ inhibitor (A). ERK 1/2 activation was significantly attenuated at 10 µM and 15 µM (B). *$p<0.01$ compared to vehicle control and 5 µM, n=5.*

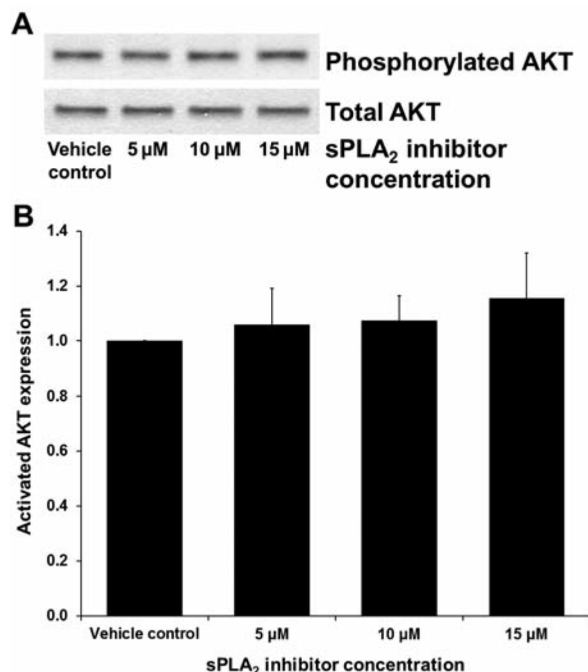

Figure 4. *Protein kinase B (AKT) activation in human esophageal adenocarcinoma cells (HEAC) (FLO-1) after inhibition of group IIa secretory phospholipase-A_2 ($sPLA_2$). A representative western blot demonstrates no change in AKT activation in FLO-1 cells treated with specific group IIa $sPLA_2$ inhibitor (A and B) (n=5).*

(Figure 4).

Apoptosis of FLO-1 HEAC after group IIa $sPLA_2$ inhibition. Treatment of FLO-1 HEAC with increasing concentrations of specific group IIa $sPLA_2$ inhibitor demonstrated no significant change in the total percentage of cells undergoing early and late apoptosis combined, compared to the vehicle control (Figure 5).

Discussion

The aim of this study was to elucidate the molecular mechanism by which the $sPLA_2$ enzyme regulates the observed growth and proliferation attenuation of HEAC *in vitro* by examining the ERK 1/2 and AKT pathways. As previously demonstrated, treatment with the specific group IIa $sPLA_2$ inhibitor significantly attenuated growth and proliferation of the tumor cells (13). The results of this study demonstrate that treatment with the known inhibitor of ERK1/2 activation, PD98059, clearly results in attenuation of ERK 1/2 activation, cell viability, and proliferation of FLO-1 HEAC cells. Furthermore, inhibition of $sPLA_2$ in these cells significantly attenuated ERK 1/2 activation, which suggests that the effects we observed with $sPLA_2$ inhibition are mediated similarly through ERK. In addition, treatment with the $sPLA_2$ inhibitor had no effect on AKT activation or apoptosis. These observations clearly illustrate a role of ERK in the growth-regulating mechanism of $sPLA_2$.

$sPLA_2$ catalyzes the hydrolysis of membrane phospholipids to generate free fatty acids and lysophospholipids, such as arachidonic acid, the rate-limiting step in eicosanoid production (9, 10). The up-regulation of $sPLA_2$ has been implicated as a pathogenic factor in a variety of inflammatory processes, as well as in multiple human cancer types, such as prostate, ovarian, gastric, intestinal, lung, and esophageal cancer (9, 10, 13, 14). We previously identified $sPLA_2$ as a key mediator in the development of esophageal hyperplasia in the setting of gastroesophageal reflux *in vivo* (11, 12). Recently, we demonstrated that $sPLA_2$ regulates the growth and proliferation of HEAC *in vitro* (13). However, we had yet to elucidate the molecular mechanism by which $sPLA_2$ has this growth influence. In other types of malignant cells, $sPLA_2$ has been demonstrated to mediate tumor cell growth without affecting cell survival by activating the major intracellular signaling cascades, such as serine/threonine-specific protein kinases known as Rapidly accelerated fibrosarcoma (RAF)/MEK/ ERK and phosphatidylinositol 3-kinase (PI3K)/AKT (15).

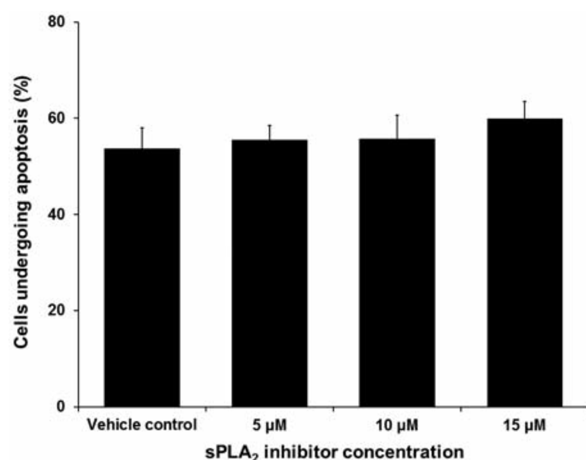

Figure 5. *Apoptosis of human esophageal adenocarcinoma cells (HEAC) (FLO-1) after inhibition of group IIa secretory phospholipase-A_2 ($sPLA_2$). Treatment of FLO-1 cells with increasing doses of specific group IIa $sPLA_2$ inhibitor exhibited no significant impact in the total percentage of FLO-1 cells undergoing early and late apoptosis combined (n=5).*

Furthermore, other drug therapies inhibit proliferation and induce apoptosis in HEAC by inhibiting the farnesylation of small GTPases known as Rat Sarcoma (RAS) and inhibiting the ERK and AKT signaling pathways (6). Given the available data regarding $sPLA_2$ and the ability of the major intracellular signaling cascades ERK and AKT to regulate tumor cell growth and cell survival, we hypothesized that inhibition of $sPLA_2$ may inhibit cell viability and proliferation while inducing apoptosis through the ERK 1/2 and AKT pathways.

While many intracellular signaling pathways are associated with tumor growth and survival, recent studies indicate all three of the MAPK cascades (ERK, p38 MAPK, and c-Jun NH2-terminal kinase), as well as the protein kinase B (AKT) cascade, to be essential in transducing signals, promoting cell growth and survival in esophageal adenocarcinoma cells (6, 17). Amongst the MAPK cascades, however, the ERK 1/2 pathway is one of the most de-regulated in human cancer. Classically, the ERK 1/2 pathway had been identified as being important in G_1 to S cell-cycle progression, leading to a general perception that it controls cell-cycle re-entry (7, 18). Recently, this pathway has also been implicated in modulating other critical cellular responses, such as malignant transformation and apoptosis by the post-translational phosphorylation of apoptotic-regulatory molecules including Bcl-XL/Bcl-2-associated death promoter (BAD), Bcl-2 homology domain 3-only protein (BIM), myeloid cell leukemia-1 (MCL-1), caspase-9, and more controversially the pro-survival B cell leukemia-2 (BCL-2) protein family (7, 17, 19). While pre-clinical data demonstrate MEK-ERK inhibitors to suppress tumor tissues *in vitro*, clinical trials only demonstrated a modest effect, likely attributable to the concentration of inhibitor for tumor cytotoxicity being unknown, alternative pathways compensating for the effects of the MEK inhibitors, and alterations in multiple signaling pathways in cancer (19, 20). Nonetheless, these MEK-ERK inhibitors have exceptional safety profiles and have demonstrated positive anticancer effects when used in combination with radiation therapy, chemotherapy, and/or other targeted therapies *in vivo* for various types of cancer (20).

The PI3K-AKT pathway is the other major pathway regulating essential cellular functions, such as survival and proliferation, frequently altered in human cancer (8). Traditionally, AKT was identified as an oncogene promoting cell survival by preventing apoptosis, but now it is apparent that AKT can also affect proliferation by regulating proteins involved in the cell-cycle machinery (7, 8). The PI3K-AKT pathway is often abnormally activated in cancer, leading to increased cell survival, proliferation, angiogenesis, and metastasis in tumor cells. Furthermore, aberrant activation of this pathway is associated with resistance to cancer therapies, as well as poor prognosis, for a variety of tumor types, such as pancreatic, gastric, breast, non-small cell lung cancer, and others (8). Similar to MEK-ERK inhibitors, pre-clinical data indicate AKT inhibitors have antiproliferative effects in many tumor cell lines and sensitize tumor cells to radiation and chemotherapy, but clinical trials using PI3K-AKT pathway inhibitors as monotherapy lacked an objective response to therapy. However, combining these the PI3K-AKT pathway inhibitors with other anticancer drugs or therapy have shown synergistic effects and has been promising in the treatment of various types of cancer (8).

Cellular oncogenes are often aberrantly regulated in human cancer. Many of these oncogenes serve to activate the RAF/MEK/ERK and PI3K/AKT pathways, both of which play essential roles in cellular proliferation and apoptosis as previously described (20). RAS, a small GTP-binding protein, is a molecule upstream of both the RAF/MEK/ERK and PI3K/AKT signaling pathways. Nearly 30% of human carcinomas demonstrate amplification of *RAS* proto-oncogenes and mutations activating RAS proteins. Four RAS proteins, Ha-RAS, N-RAS, Ki-RAS 4A, and Ki RAS 4B, exist with each of these exhibiting varying potency to activate the RAF/MEK/ERK and PI3K/AKT cascades. For instance, Ki-RAS is able to induce the RAF/MEK/ERK pathway more strongly than Ha-RAS, but Ha-RAS is a stronger inducer of the PI3K/AKT pathway. Of these four RAS proteins, Ki-RAS is the more commonly mutated isoform in human cancer (19). In addition to RAS, B-RAF has been identified as a frequent mutation in various types of cancer, such as melanoma, papillary thyroid cancer, colorectal cancer, and ovarian cancer (19). The individual contribution of the RAF/MEK/ERK and PI3K/AKT pathways to tumor cell growth and survival are ultimately difficult to determine since both are intimately linked and a great deal of cross-talk exists between the two pathways. Although both pathways can be

regulated by RAS, AKT can negatively regulate RAF activity to affect both cell survival and proliferation. Hence, activation of PI3K/AKT could result in suppression of RAF/MEK/ERK, while mutation of B-RAF or RAF-1 would only activate the RAF/MEK/ERK pathway (19).

In summary, the results of the present study demonstrate that treatment of HEAC with PD98059, an inhibitor of ERK 1/2 activation, clearly decreased cell viability and proliferation of these cells and treatment with $sPLA_2$ inhibitor significantly attenuated ERK 1/2 activation, directly linking the effects of $sPLA_2$ inhibition in FLO-1 cells to the ERK 1/2 pathway. Furthermore, treatment with $sPLA_2$ inhibitor had no significant effect on AKT activation or cell survival, demonstrating no major role of the AKT pathway on the mechanism of action of $sPLA_2$. Although this study demonstrates that inhibition of $sPLA_2$ and inhibition of RAF/MEK/ERK pathway have a clear association between their effects on human esophageal adenocarcinoma cell growth and proliferation, this study is limited in determining what role the RAF/MEK/ERK and PI3K/AKT intracellular signaling pathways play individually. Much of this limitation comes from their intimate relationship and the amount of cross-talk between the two pathways.

Nonetheless, these data demonstrate the importance of ERK 1/2 in the ability of the $sPLA_2$ enzyme to mediate the growth control of esophageal adenocarcinoma cells, as defined by cell viability and proliferation. These data demonstrate that $sPLA_2$ may be functioning through the RAF/MEK/ERK pathway, and an inhibition of $sPLA_2$ has the potential to act similar to an MEK/ERK inhibitor and in conjunction with standard chemotherapeutic regimens, in the treatment of esophageal adenocarcinoma. These findings help to illustrate the mechanism of $sPLA_2$-related growth control and continue to highlight this molecule as a target for therapeutic and chemopreventive interventions of esophageal adenocarcinoma.

Acknowledgements

We would like to thank Dr. David Beer (Department of Surgery, University of Michigan, Ann Arbor, MI, USA) for his generous gift of the FLO-1 human esophageal adenocarcinoma cell line.

References

1 Kubo A and Corley DA: Marked regional variation in adenocarcinomas of the esophagus and the gastric cardia in the United States. Cancer *95*: 2096-2102, 2002.

2 Brown LM, Devesa SS and Chow WH: Incidence of adenocarcinoma of the esophagus among white Americans by sex, stage, and age. J Natl Cancer Inst *100*: 1184-1187, 2008.

3 Jankowski JA, Perry I and Harrison RF: Gastro-oesophageal cancer: Death at the junction. BMJ *321*: 463-464, 2000.

4 di Pietro M, Peters CJ and Fitzgerald RC: Clinical puzzle: Barrett's oesophagus. Dis Model Mech *1*: 26-31, 2008.

5 Jankowski JA, Harrison RF, Perry I, Balkwill F and Tselepis C: Barrett's metaplasia. Lancet *356*: 2079-2085, 2000.

6 Ogunwobi OO and Beales IL: Statins inhibit proliferation and induce apoptosis in Barrett's esophageal adenocarcinoma cells. Am J Gastroenterol *103*: 825-837, 2008.

7 Balmanno K and Cook SJ: Tumour cell survival signaling by the ERK 1/2 pathway. Cell Death Diff *16*: 368-377, 2009.

8 Calvo E, Bolós V and Grande E: Multiple roles and therapeutic implications of Akt signaling in cancer. Onco Targets and Ther *2*: 135-150, 2009.

9 Fijneman RJ and Cormier RT: The roles of $sPLA_2$-IIA (Pla2g2a) in cancer of the small and large intestine. Front Biosci *13*: 4144-4174, 2008.

10 Granata F, Balestrieri B, Petraroli A, Giannattasio G, Marone G and Triggiani M: Secretory phospholipase A_2 as multivalent mediators of inflammatory and allergic disorders. Int Arch Allergy Immunol 131: 153-163, 2003.

11 Mauchley D, Meng X, Babu A, Gamboni-Robertson F, Yang X, Banerjee A, Fullerton DA and Weyant MJ: Gastroduodenal reflux induces group IIa secretory phospholipase A_2 expression and activity in murine esophagus. Dis Esophagus 23: 430-436, 2010.

12 Babu A, Meng X, Banerjee AM, Gamboni-Robertson F, Cleveland JC, Damle S, Fullerton DA and Weyant MJ: Secretory phospholipase A_2 is required to produce histologic changes associated with gastroduodenal reflux in a murine model. J Thorac Cardiovasc Surg 135: 1220-1227, 2008.

13 Mauchley D, Meng X, Johnson T, Fullerton DA and Weyant MJ: Modulation of growth in human esophageal adenocarcinoma cells by group IIa secretory phospholipase A2. J Thorac Cardiovasc Surg 139: 591-599, 2010.

14 Sadaria MR, Meng X, Fullerton DA, Reece TB, Shah RR, Grover FL and Weyant MJ: Secretory phospholipase A_2 inhibition attenuates intercellular adhesion molecule-1 expression in human esophageal adenocarcinoma cells. Ann Thor Surg 91: 1539-1545, 2011.

15 Martín R, Hernández M, Ibeas E, Fuentes L, Salicio V, Arnés M and Nieto ML: Secreted phospholipase A2-IIA modulates key regulators of proliferation on astrocytoma cells. J Neurochem 111: 988-999, 2009.

16 Sadaria MR, Reppert AE, Yu JA, Meng X, Fullerton DA, Reece TB and Weyant MJ: Statin therapy attenuates growth and malignant potential of human esophageal adenocarcinoma cells. J Thorac Cardiovasc Surg 142: 1152-1160, 2010.

17 Santarpia L, Lippman SM and El-Naggar AK: Targeting the MAPK-RAS-RAF signaling pathway in cancer therapy. Expert Opin Ther Targets 16: 103-119, 2012.

18 Meloche S and Pouysségur J: The ERK 1/2 mitogen-activated kinase pathway as a master regulator of the G_1- to S-phase transition. Oncogene 26: 3227-3239, 2007.

19 McCubrey JA, Steelman LS, Chappell WH, Abrams SL, Wong EW, Chang F, Lehmann B, Terrian DM, Milella M, Tafuri A, Stivala F, Libra M, Basecke J, Evangelisti C, Martelli AM and Franklin RA: Roles of the Raf/MEK/ERK pathway in cell growth, malignant transformation and drug resistance. Biochim Biophys Acta 1773: 1263-1284, 2007.

20 McCubrey JA, Steelman LS, Abrams SL, Chappell WH, Russo S, Ove R, Milella M, Tafuri A, Lunghi P, Bonati A, Stivala F, Nicoletti F, Libra M, Martelli AM, Montalto G and Cervello M: Emerging MEK inhibitors. Expert Opin Emerg Drugs 15: 203-223, 2010.

Received January 11, 2013
Revised February 20, 2013
Accepted February 21, 2013