Transcriptome Analysis of Piperlongumine-Treated Human Pancreatic Cancer Cells Reveals Involvement of Oxidative Stress and Endoplasmic Reticulum Stress Pathways

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ABSTRACT Piperlongumine (PL), an alkaloid obtained from long peppers, displays antitumorigenic properties for a variety of human cell- and animal-based models. The aim of this study was to identify the underlying molecular mechanisms for PL anticancer effects on human pancreatic cancer cells. RNA sequencing (RNA-seq) was used to identify the effects of PL on the transcriptome of MIA PaCa-2 human pancreatic cancer cells. PL treatment of pancreatic cancer cells resulted in differential expression of 683 mRNA transcripts with known protein functions, 351 of which were upregulated and 332 of which were downregulated compared to control-treated cells. Transcripts associated with oxidative stress, endoplasmic reticulum (ER) stress, and unfolded protein response pathways were significantly overexpressed with PL treatment. Reverse transcription-quantitative polymerase chain reaction and western blotting were used to validate the RNA-seq results, which included upregulation of HO-1, IRE1 α , cytochrome c, and ASNS. The results provide key insight into the mechanisms by which PL alters cancer cell physiology and identify that activation of oxidative stress and ER stress pathways is a critical avenue for PL anticancer effects.

KEY WORDS: • ER stress • oxidative stress • pancreatic cancer • piperlongumine • RNA sequencing • transcriptomics

INTRODUCTION

PANCREATIC CANCER IS the fourth leading cause of cancer-related deaths in both men and women in the United States. The median overall 5-year survival rate of pancreatic cancer is extremely poor at less than 7%.¹ The standard chemotherapeutic agent used to treat metastatic pancreatic cancer has been gemcitabine (GEM), a cytidine analog that inhibits DNA synthesis and DNA repair.² However, GEM only produces a modest survival benefit (6–8 months) for patients with pancreatic cancer.^{3,4} More recently, the combination chemotherapy consisting of oxaliplatin, irinotecan, fluorouracil, and leucovorin (FOLFIRINOX) has shown a better response than GEM for advanced pancreatic cancer patients.⁵ However, FOLFIRINOX results in greater toxicity than GEM and still only results in a median overall survival of about 11 months.⁵ Novel therapeutic agents are critically needed to help fight this deadly cancer, which is projected to be the second leading cause of cancer death by the year 2030.⁶

We recently demonstrated that piperlongumine (PL), an alkaloid present in the fruits and roots of the long pepper (*Piper longum*) plant, inhibits the growth of both *KRAS* mutant and wild-type human pancreatic cancer cell lines both *in vitro* and *in vivo* through a reactive oxygen species

(ROS)–dependent mechanism that leads to DNA damage and cell death.⁷ Previous studies have shown that PL selectively kills a variety of cancer cell lines with minimal death for normal cells by elevating ROS levels and inducing apoptosis.⁸ Additionally, PL has been shown to cause cell cycle arrest,⁹ induce autophagy,¹⁰ and inhibit migration,¹¹ adhesion, and invasion.¹² While multiple studies have reported the cancer-specific cytotoxicity of PL, the gene expression profile and pathways associated with PL-mediated anticancer effects have not been fully elucidated, particularly for pancreatic cancer.

RNA sequencing (RNA-seq) is an unbiased sequencing tool that allows transcriptome profiling to detect gene expression changes in a cell or tissue sample. In this study, we used RNA-seq as an approach to identify the mechanisms by which PL induces antitumor effects in a human pancreatic cancer cell line, MIA PaCa-2.

MATERIALS AND METHODS

Cell culture and treatment

MIA PaCa-2 human pancreatic cancer cells were obtained from the ATCC (Manassas, VA, USA) and cultured at 37°C with 5% carbon dioxide in Dulbecco's modified Eagle's medium (Thermo Fisher Scientific, Waltham, MA, USA) supplemented with 10% fetal bovine serum (Atlanta Biologicals, Lawrenceville, GA, USA). Cells were subcultured by enzymatic digestion with 0.25% trypsin/1 mM EDTA solution (Thermo Fisher Scientific) when they

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reached ~70% confluency. PL (INDOFINE Chemical Company, Hillsborough, NJ, USA) was dissolved in 100% DMSO at a stock concentration of 10 mM and then diluted in water to a working concentration. The cells were seeded on 100×20 mm dishes (Greiner Bio-One, Monroe, NC, USA) at 5×10^5 cells/dish and incubated at 37°C with 5% carbon dioxide. Twenty-four hours later, they were treated with 10 μ M PL or with 0.1% DMSO (vehicle control) for 6 h. The time point and concentration were chosen based on our previous study reporting the induction of ROS in these cells.⁷ The experiment was conducted in triplicate.

RNA sequencing

Following PL treatment, total RNA was extracted using a Fisher SurePrep Kit (Thermo Fisher Scientific) according to the manufacturer's instructions. RNA was quantified using a NanoDrop (Thermo Fisher Scientific). Three micrograms of total RNA per sample was sent for sequencing to the University of Minnesota Genomics Center. All the samples passed quality control with an RNA integrity number >8. Six barcoded libraries were created, which were pooled together and sequenced in one full lane of the flow cell on an Illumina HiSeq 2500. Single-end reads of 50 base pair (bp), which are sufficient to reliably identify mRNA expression differences relative to paired-end longer reads,¹³ were obtained.

TopHat¹⁴ was used to map reads to the reference genome (Build 38 and Ensembl 78) for each sample (max mismatches 15 and maximum insertion and deletion lengths 18). Cufflinks¹⁵ were used to assemble the transcripts from each library with multiread count (-u) and upper quartile normalization (-N). The transcripts from each of these libraries were combined together using cuffmerge. Cuffdiff was used to identify differentially expressed transcripts using the following cutoff criteria:

- (1) $\log_2$ fold change ≥ 1 or < -1 ,
- (2) q -value false discovery rate < 0.01 , and
- (3) fragments per kilobase of exon per million fragments mapped (FPKM) value ≥ 1 .

Reverse transcription-quantitative polymerase chain reaction

cDNA was synthesized using 100 ng total RNA and the qScript cDNA Synthesis Kit (Quanta Biosciences, Gaithersburg, MD, USA). Primers were designed using Primer Express software (version 2.0; Applied Biosystems, Foster City, CA) and synthesized by Integrated DNA Technologies (Coralville, IA, USA). Primer sequences are listed in Table 1. Steady-state mRNA levels were determined for the cDNAs by real-time polymerase chain reaction (PCR) using the PerfeCTa SYBR Green FastMix Kit (Quanta Biosciences). The cycling parameters were 95°C for 10 min, followed by 40 cycles of 95°C for 30 sec and 60°C for 1 min, and a dissociation program that included 95°C for 1 min, 55°C for 30 sec, and 95°C for 30 sec ramping up at 0.2°C/sec. One distinct peak was observed for all primer sets. The fold change in mRNA expression was calculated by comparing the 18S rRNA-normalized threshold cycle numbers (C_T) in the PL-treated cancer cells with those in the DMSO-treated cancer cells. Duplicate wells were run for each experiment, and the experiments were performed in triplicate.

SDS-PAGE and western blotting

MIA PaCa-2 cells were treated for 0, 6, 12, or 24 h with 10 μ M PL or 0.1% DMSO. Cell pellets were lysed using an SDS lysis buffer (Cell Signaling Technologies, Danvers, MA, USA) containing protease and phosphatase inhibitors (Roche, Indianapolis, IN, USA). Samples were briefly sonicated to dissociate cell membranes. Mitochondrial and cytosolic fractions were isolated using a mitochondria isolation kit (Thermo Fisher Scientific). Twenty-five micrograms of total protein isolated from PL- or DMSO-treated MIA PaCa-2 cells was separated on 10% SDS-polyacrylamide gels at 100 V for 1 h. Proteins were transferred to PVDF membranes at 100 V for 70 min at 4°C. Blots were then probed overnight at 4°C with the primary antibodies IRE1 α , HO-1, GAPDH (Cell Signaling Technologies), ASNS (Protein Tech, Chicago, IL, USA), and cytochrome c (BD Biosciences, San Jose, CA, USA). The next day, the blots were rinsed with 1× TBS-tween

TABLE 1. PRIMER SEQUENCES USED FOR MEASURING mRNA EXPRESSION VIA REVERSE TRANSCRIPTION-QUANTITATIVE POLYMERASE CHAIN REACTION

Gene	Forward sequence	Reverse sequence
ABCB10	5'-CCA GTG TGG CTG AGA TCC AA-3'	5'-GGA AGG GTC ATC AGC ACC AT-3'
ASNS	5'-CAC TCC GCG ACT CCC TTT T-3'	5'-ACC ATT TCC ACG GAT GCA A-3'
ATF4	5'-CGT TGC CAT GAT CCC TCA GT-3'	5'-GCT GAG GAG ACC CCA GAT AGG-3'
BCL2L2	5'-TCA GCC CAA CAA CGC TTC A-3'	5'-CAC AGT GCA GCC CCA AAG A-3'
HERPUD1	5'-AAA CCA GCC TGC CAA TCA GA-3'	5'-CTT CCA CAA TAG GGC CAC CTT-3'
IRE1 α	5'-GAA GAC GTC ATT GCA CGT GAA TT-3'	5'-AGG TCC TGA ATT TAC GCA GGT-3'
NLRC5	5'-CCA GCA GAC AGG CTA TGC TTT-3'	5'-CTG TAC CCA GCG GGA ATG G-3'
OSGIN1	5'-GAT GCT GTA CCC CGA GTA CCA-3'	5'-GCC TGG CAG TCT TCC TTG AAG-3'
PARP9	5'-CTG CCA GGG ACA TCC GTT AA-3'	5'-AGG TTT CAG GGC TGG AGA CA-3'
TNFRSF10A	5'-AAC AAA ACC GGG CGA GAT G-3'	5'-GAG CTC AAC AAG TGG TCC TCA A-3'
TRIB3	5'-AAG AAG CGG TTG GAG TTG GA-3'	5'-GCA CGA TCT GGA GCA GTA GGT-3'
XBPI	5'-CTG AGT CCG AAT CAG GTG CAG-3'	5'-ATC CAT GGG GAG ATG TTC TGG-3'

FIG. 1. Volcano plot of differentially expressed genes in PL-treated MIA PaCa-2 cells. The vertical axis is the $\log_2$ fold change between control- and PL-treated cells. The $-\log_{10}(q\text{-value})$ is plotted on the horizontal axis. Each transcript is represented by one point on the graph. Points to the right of the dotted gray line indicate sequences that pass the $q < 0.01$ significance threshold. The two horizontal rows represented by black boxes in the chart are the genes for which expression was only detected in either the control- or PL-treated cells, and the $\log_2$ fold change values were artificially set to ± 0.5 (instead of $\pm \infty$) as appropriate. PL, piperlongumine.

(0.1%) and probed with anti-rabbit or anti-mouse secondary antibodies (Jackson ImmunoResearch, West Grove, PA, USA) for 1 h at room temperature. The western blots were developed using SuperSignal West Pico Chemiluminescent Substrate (Thermo Fisher Scientific), and the images were captured using the MultiImage™ Light Cabinet (Alpha Innotech; San Leandro, CA, USA). Densitometry analysis was performed using ImageJ software. IRE1 α , HO-1, ASNS, and cytochrome c protein levels were normalized to GAPDH expression. Immunoblots were performed in triplicate, and the images in the figures represent one typical replicate.

Software

Gene-annotation enrichment analysis was completed using the functional annotation clustering tool of DAVID Bioinformatics Resources 6.7 using differentially expressed genes.¹⁶ Pathway analysis was completed with the Reactome pathway analysis tool (Reactome V51).¹⁷

RESULTS

PL treatment changes the gene expression profile in MIA PaCa-2 cells

RNA-seq was used to determine the effects of 10 μ M PL on the transcriptome of MIA PaCa-2 cells following 6 h of

treatment or vehicle control. A total of 143 million reads of 50 bp in length were obtained for six samples (three replicates each of control- and PL-treated cells). Approximately 88% of the reads explicitly mapped to a unique region of the human genome. Of this 88%, $\sim 30\%$ of the reads mapped to multiple locations of the genome. A total of 33,572 transcripts were differentially expressed in PL-treated cells compared to control (Fig. 1). A cutoff criterion as mentioned in the “Materials and Methods” section was used to generate significantly different transcripts ($q\text{-value} < 0.01$) in PL-treated cells. Seven protein-coding genes with known function were exclusively expressed, whereas 4 protein-coding genes were suppressed in PL-treated cells. Importantly, 683 protein-coding genes were differentially expressed with PL treatment, of which 351 were upregulated and 332 were downregulated (Supplementary Table S1 and Figs. S1–S3; Supplementary Data are available online at www.liebertpub.com/jmf).

PL-treated MIA PaCa-2 cells are highly enriched with transcription and cell death–associated gene categories

To illustrate the changes in cell physiology associated with PL treatment in MIA PaCa-2 cells, gene ontology enrichment analysis was performed using DAVID software. Genes associated with the transcription category were significantly enriched ($P = 6.30E-10$), suggesting a gene

FIG. 2. Gene ontology enrichment analysis for differentially expressed transcripts in PL-treated MIA PaCa-2 cells. The enrichment score represents the negative logarithm of the P value associated with the gene ontology terms for the differentially expressed transcripts. The top 15 annotation clusters obtained from the DAVID functional annotation tool are listed.

TABLE 2. PATHWAY AFFECTED BY PIPERLONGUMINE TREATMENT OF MIA PaCa-2 CELLS

	Entities found, n	Entities total, n	Entities P value
Pathways altered in PL-treated cells			
Unfolded protein response	21	140	.005273
HSF1-dependent transactivation	11	56	.006255
PERK regulates gene expression	9	38	.004027
ATF4 activates genes	9	32	.001299
Pathways expressed only in PL-treated cells			
DNA replication initiation	1	6	.006009
Telomere C-strand synthesis initiation	1	6	.006009
Pathways suppressed in PL-treated cells			
Mitochondrial ABC transporters	1	4	.002007
NLRP3 inflammasome	1	13	.006511
Inflammasomes	1	21	.010502

PL, piperlongumine.

regulatory role for PL. In addition, genes related to cell death, apoptosis, and negative regulation of growth were also significantly enriched with PL treatment. The RAS association category was enhanced in the *KRAS* mutant MIA PaCa-2 cells. Other categories with high significance ($P < .01$) included membrane-enclosed lumen, negative regulation of biosynthetic process, zinc finger (C2H2 like), RNA biosynthetic process, BTB/POZ fold, zinc ion binding, heat shock protein binding, and collagen (Fig. 2).

PL activates the endoplasmic reticulum stress-associated pathway

To further investigate the relationships among genes regulated by PL in MIA PaCa-2 cells, pathway analysis was completed using Reactome software. This software contains a large database of human pathways spanning signaling cascades and metabolic and cellular processes. PL treatment resulted in upregulation of 21 genes associated with the unfolded protein response, 11 genes associated with heat shock transcription factor 1 (HSF1), and 9 genes involved in PERK- or ATF4-regulated gene expression (Table 2). Interestingly, PL treatment resulted in expression of 2 genes whose ex-

pression was not detected in control cells and included pathways involved with DNA replication initiation and telomere C-strand synthesis. Furthermore, PL treatment resulted in silencing of three entities associated with mitochondrial ABC transporters and inflammasomes. These results suggest that PL activates a cellular stress response pathway that is primarily associated with the endoplasmic reticulum.

Reverse transcription-quantitative polymerase chain reaction results validate the RNA-seq results in MIA PaCa-2 cells

To validate the gene expression data obtained from RNA-seq, reverse transcription-quantitative polymerase chain reaction (RT-qPCR) was performed. Eight upregulated genes were selected based on high expression (*OSGIN1*) and involvement in the ER stress pathway (*TRIB3*, *ATF4*, *TNFRSF10A*, *ASNS*, *IRE1 α* , *HERPUD1*, and *XBPI*). RT-qPCR confirmed the significant increase in mRNA expression that was observed by RNA-seq after 6 h of PL treatment (Table 3). Furthermore, four genes, which were downregulated by PL treatment, were also validated by RT-qPCR to be downregulated (Table 3). A time course was performed to

TABLE 3. PIPERLONGUMINE-MEDIATED DIFFERENTIALLY EXPRESSED GENES VALIDATED BY BOTH RNA SEQUENCING AND RT-qPCR

Gene name	Gene symbol	RNA-seq	RT-qPCR
		Log ₂ fold change	Log ₂ fold change
Oxidative stress-induced growth inhibitor 1	<i>OSGIN1</i>	4.11	3.29
Tribbles homolog 3 (drosophila)	<i>TRIB3</i>	2.30	1.53
Activating transcription factor 4	<i>ATF4</i>	1.60	0.97
Tumor necrosis factor receptor superfamily, member 10b	<i>TNFRSF10A</i>	1.28	0.45
Asparagine synthetase	<i>ASNS</i>	1.33	0.83
Endoplasmic reticulum to nucleus signaling 1	<i>IRE1α</i>	1.44	1.04
X-box binding protein 1	<i>XBPI</i>	1.56	3.24
Homocysteine-inducible, endoplasmic reticulum stress-inducible Ubiquitin-like domain member 1	<i>HERPUD1</i>	2.25	1.60
ATP-binding cassette, subfamily B (MDR/TAP), member 10	<i>ABCB10</i>	—	−0.37
NLR family, CARD domain containing 5	<i>NLRC5</i>	—	−0.24
Poly(ADP-ribose) polymerase family, member 9	<i>PARP9</i>	−1.08	−0.40
BCL2-like 2	<i>BCL2L2</i>	−1.30	−0.56

RNA-seq, RNA sequencing; RT-qPCR, reverse transcription-quantitative polymerase chain reaction.

FIG. 3. qRT-PCR results to validate RNA-seq results. The log₂ mRNA expression fold change for upregulated *TRIB3*, *OSGIN1*, *IRE1α*, *ASNS*, *ATF4*, *TNFR*, *XBP1*, and *HERPUD1* and downregulated *NLRC5*, *ABCB10*, *PARP9*, and *BCL2L2* genes is shown for PL-treated cells compared to the vehicle-treated control cells. MIA PaCa-2 cells were treated with 0 or 10 μM PL for 3, 6, or 12 h, and mRNA expression of genes identified with RNA-seq was assessed by reverse transcription-quantitative polymerase chain reaction (RT-qPCR). 18S rRNA expression in the control- and PL-treated cells was used to normalize the data. Statistically significant differences ($P < .05$) from DMSO-treated cells are denoted by *. Error bars represent 95% confidence intervals for three replicate experiments. RNA-seq, RNA sequencing.

evaluate the selected genes' expression values at 3, 6, and 12 h in PL- versus control-treated MIA PaCa-2 cells. A significant elevation in mRNA expression at 3 and 12 h was observed in the majority of the upregulated genes, which is consistent with the results obtained after 6 h of PL treatment (Fig. 3). Furthermore, a significant decrease in mRNA expression at 12 h, but not 3 h, was observed in downregulated genes (Fig. 3).

PL enhances IRE1α, HO-1, ASNS, and cytochrome c protein expression in MIA PaCa-2 cells

To determine the correlation between mRNA and protein expression and validate the involvement of ER stress-related genes in PL-mediated antitumor effects, western blotting was performed for a select group of proteins. MIA PaCa-2 cells were treated with 0 or 10 μM PL for 6, 12, and 24 h and assessed for IRE1α, HO-1, ASNS, and cytochrome c protein expression. We found that 10 μM PL significantly enhanced IRE1α, ASNS, and cytochrome c protein expression after 24 h. A significant increase in HO-1 protein expression was also observed after 12 h of PL treatment (Fig. 4).

DISCUSSION

Chemopreventive agents obtained from pepper plants have demonstrated potent anticancer activity in cell cultures¹⁸ and animal models.^{19,20} Most notably, the pepper-derived bioactive agent PL has shown cancer-killing potential for a variety of cancer types.⁸ Therefore, studies that identify the molecular targets and anticancer effects of PL will provide critical information to help maximize its potential for clinical use. In this study, our goal was to more fully understand the underlying mechanisms mediating PL-induced pancreatic cancer cell death. While we recently showed that PL inhibits pancreatic cancer progression in a mouse model by elevating ROS and enhancing DNA damage,⁷ the specific events

FIG. 4. PL increases IRE1α, HO-1, ASNS, and cytochrome c protein expression. Western blot analysis (A) and quantitative densitometry (B) of protein expression in MIA PaCa-2 cells treated with 10 μM PL for 6, 12, and 24 h. Densitometry shows the results of PL treatment on IRE1α, HO1, ASNS, and cytochrome c expression normalized to GAPDH in three replicate experiments ± standard deviation. Statistically significant differences ($P < .05$) from DMSO-treated cells are denoted by *.

leading to DNA damage and cell death have not been previously identified.

In this study, we used RNA-seq to identify 683 genes with known functions that were differentially expressed in PL-treated MIA PaCa-2 pancreatic cancer cells. MIA PaCa-2 cells show similar sensitivity toward PL as other commonly studied pancreatic cancer cell lines PANC-1 and BxPC3.⁷ The concentration (10 μ M) and duration (6 h) of PL treatment that were selected for this study do not cause significant toxicity toward healthy or cancer cells (unpublished observation). However, it is clear from the results presented in this study that significant changes in gene expression are initiated within hours of PL exposure that eventually lead to cancer cell death. The effects of PL on gene expression in normal pancreatic ductal epithelium have not been studied and should also be explored.

Genes related to cell death and apoptosis were highly enriched, which is consistent with numerous reports on PL anticancer effects.^{21–23} Among them, the *GADD 45* family of genes, which is involved in the regulation of growth, DNA repair, and induction of apoptosis,²⁴ was upregulated in response to PL treatment. PL also modulated the expression of many cell signaling molecules, such as mitogen-activated protein kinases in the pancreatic cancer cells, which is in support of previous studies showing that PL modulates cell signaling pathways to lead to enhanced cancer cell death.^{23,25,26}

Not surprisingly, the RNA-seq data revealed that PL triggers an oxidative stress response in pancreatic cancer cells. What was interesting was the identification of new genes likely involved in this response. *OKL38*, also known as oxidative stress-induced growth inhibitor 1 (*OSGIN1*), was upregulated 17-fold in PL-treated cells. *OKL38* is a tumor suppressor gene that inhibits tumor cell growth, and its expression is reduced or altered in cancer cell lines and tumor tissues.^{27,28} *OKL38* has been shown to interact with the p53 protein and induce formation of megamitochondria, increase cellular ROS levels, and release cytochrome c from the mitochondria in U2OS cells.²⁹ It is possible that PL activates an *OKL38*-mediated apoptotic pathway by increasing p53 and cytoplasmic cytochrome c protein expression in pancreatic cancer cells (Fig. 5). Similarly, we observed a greater than 75-fold upregulation of heme oxygenase-1 (*HMOX-1*) expression in PL-treated cells. *HMOX-1* is known for its antioxidant and cytoprotective effects under normal physiological conditions. However, *HMOX-1* can induce cancer cell apoptosis and inhibit tumor cell motility.^{30,31} Recently, *HMOX-1* expression was shown to be elevated by PL in breast cancer cells and a determining factor in the differential response of normal breast cells to PL.³² Interestingly, PL silenced ATP-binding cassette subfamily B, member 10 (*ABCB10*) expression. Recent evidence suggests that *ABCB10*, a regulator of heme synthesis, helps protect against oxidative stress *in vivo* by peptide export and/or other molecules specifically regulating antioxidant function and/or avoiding mitochondrial oxidative damage.^{33,34} Therefore, PL may elevate oxidative stress in pancreatic cancer cells by downregulating *ABCB10*.

FIG. 5. A hypothetical model for PL-mediated MIA PaCa-2 pancreatic cancer cell death. Genes with an *arrow* represent those up-regulated by PL at the mRNA or protein level as assessed by qRT-PCR or western blot, respectively. The model suggests that PL works through oxidative stress- and endoplasmic reticulum (ER) stress-related pathways to trigger cancer cell death.

Disturbances in cellular redox regulation and generation of ROS elicit a protective response in the ER known as the unfolded protein response (UPR) that aims to restore ER homeostasis.³⁵ However, under conditions of prolonged/severe stress, the ER triggers cell death pathways.^{36–38} We observed an activation of the ER stress response gene *IRE1 α* , a transmembrane ER sensor, which cleaves and activates *XBP1* in PL-treated MIA PaCa-2 cells. PL also elevated *ATF4*, *ASNS*, *HERPUD1*, *TRIB3*, and *TNFRSF10A* UPR mRNA expression. The transcription factor ATF4 is known to activate the C/EBP homologous protein (CHOP) transcription, which is required for ER stress-mediated apoptosis both *in vitro* and *in vivo*.^{39,40} Kim *et al.* reported that PL caused an ROS-mediated increase in ER stress and unfolded protein response markers (CHOP and p-eIF2 α) and apoptosis in sphere-cultured high-grade glioma cells.⁴¹ Similarly, Jin *et al.* demonstrated the role of ROS in PL-mediated CHOP activation leading to apoptosis in breast cancer cells.⁴² Taken together, these data suggest that ROS-mediated ER stress induction with PL treatment is consistent across a variety of tumor cell lines. Further studies to overexpress or knockdown ER stress and UPR genes are needed to identify the functional role in PL-mediated cancer cell death.

In summary, this study shows that PL alters the expression of numerous genes in human pancreatic cancer cells,

which may explain, in part, the therapeutic potential for PL that has been reported in the literature. Specifically, our study demonstrates that PL alters the expression of genes that play important roles in the regulation of endoplasmic reticulum and oxidative stress pathways. Further studies evaluating the mechanistic response of noncancerous pancreatic cells to PL and a better understanding of how PL might be used alongside existing chemotherapies are required to consider it as a potential anticancer agent for clinical trials.

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AUTHOR DISCLOSURE STATEMENT

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