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RESEARCH ARTICLE

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Lucidone inhibits autophagy and MDR1 via HMGB1/RAGE/PI3K/Akt signaling pathway in pancreatic cancer cells

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Abstract

Gemcitabine (GEM) drug resistance remains a difficult challenge in pancreatic ductal adenocarcinoma (PDAC) treatment. Therefore, identifying a safe and effective treatment strategy for PDAC is urgent. Lucidone is a natural compound extracted from the fruits of *Lindera erythrocarpa* Makino. However, the role of lucidone in PDAC inhibition remains unclear. In addition, high-mobility group box 1 (HMGB1) and receptor for advanced glycation end products (RAGE) are involved in multidrug resistance protein 1 (MDR1) regulation and GEM resistance. Thus, this study aimed to explore the function of lucidone in tumor cytotoxicity and chemosensitivity through the suppression of RAGE-initiated signaling in PDAC cells. The data showed that lucidone significantly promoted apoptotic cell death and inhibited the expression of autophagic proteins (Atg5, Beclin-1, LC3-II, and Vps34) and MDR1 by inhibiting the HMGB1/RAGE/PI3K/Akt axis in both MIA Paca-2 cells and MIA Paca-2^{GEMR} cells (GEM-resistant cells). Notably, convincing data were also obtained in experiments involving RAGE-specific siRNA transfection. In addition, remarkable cell proliferation was observed after treatment with lucidone combined with GEM, particularly in MIA Paca-2^{GEMR} cells, indicating that lucidone treatment enhanced chemosensitivity. Collectively, this study provided the underlying mechanism by which lucidone treatment inhibited HMGB1/RAGE-initiated PI3K/Akt/MDR1 signaling and consequently enhanced chemosensitivity in PDAC.

KEYWORDS
autophagy, gemcitabine, lucidone, MDR1, pancreatic ductal adenocarcinoma

1 | INTRODUCTION

Pancreatic ductal adenocarcinoma (PDAC) is known for its poor prognosis and poor 5-year survival rate (9%) (Siegel, Miller, & Jemal, 2019). Researchers predict that by 2030, PDAC will become a major leading cause of cancer-related mortality, following lung cancer (Rahib et al., 2014). Surgical resection remains the best curative treatment after the early diagnosis of PDAC. Unfortunately, fewer than 20% of patients with early-stage PDAC have resectable tumors, and even after successful resection, the median overall survival (OS) is estimated to be 20–22 months (Fogel et al., 2017). As a result, surgical resection is often combined with chemotherapy. Many PDAC patients exhibit resistance to gemcitabine (GEM, the standard first-line chemotherapy drug for PDAC treatment) and consequently experience treatment failure (Binenbaum, Na'ara, & Gil, 2015). Dysregulation of the participated proteins involved in GEM metabolism pathways or high expression of drug efflux pumps such as ATP-binding cassette (ABC) transporters are the main mechanism of GEM resistance (Binenbaum

Abbreviations: ATG5, autophagy gene 5; Bax, Bcl2-associated x protein; Bcl-xL, B-cell lymphoma-extra large; GEM, gemcitabine; HMGB1, high-mobility group box 1; LC3-II, microtubule-associated protein light chain 3-II; MDR1, multidrug resistance protein 1; PDAC, pancreatic ductal adenocarcinoma; RAGE, receptor for advanced glycation end products; siRNA, small interfering RNA.; Vps34, vacuolar protein sorting 34.

Sheng-Yi Chen and Yi-Hao Hsu contributed equally to this work.

et al., 2015). Therefore, finding novel effective, and safe treatment options targeting the GEM metabolism pathway or drug transporters is required.

Lucidone, a natural cyclopentenedione analog, is extracted from the fruits of *Lindera erythrocarpa* Makino, which is widely distributed in Asia, such as in China, Japan, Korea, and Taiwan (Kumar, Yang, Chu, Chang, & Wang, 2010). Previous studies showed the antiinflammatory effects of lucidone via the inhibition of the lipopolysaccharide (LPS)-induced production of inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) via NF-κB and MAPK signal transduction in murine RAW264.7 macrophage cells (Senthil Kumar & Wang, 2009; Wang et al., 2008). In addition, the hepatoprotective effect of lucidone treatment upregulates heme oxygenase-1 (HO-1) and nuclear factor erythroid 2-related factor 2 (Nrf2) expression in ethanol-treated HepG2 cells (Senthil Kumar, Liao, Xiao, Gokila Vani, & Wang, 2012). Moreover, lucidone promotes antioxidative, antiinflammatory, and wound healing processes by activating the Nrf2/HO-1, PI3K/AKT, and Wnt/β-catenin signaling pathways, respectively (Kumar et al., 2013; Yang, Tsai, Korivi, Chang, & Hseu, 2017). It has not only focused on the antioxidative or antiinflammatory effects, but also observed positive results on anticancer research. The antitumor capacity of methyllucidone (similar structure to lucidone) affects diverse human carcinoma cell lines, such as colorectal cancer cells (HCT-116 and SW620 cells), lung adenocarcinoma (NCI-H23 and A549 cells), breast adenocarcinoma (MDA-MB-231 cells), and prostate carcinoma cells (DU145 cells), by inducing apoptosis and inhibiting the activity of farnesyl protein transferase (FPTase) (Oh et al., 2005). To date, the effect of lucidone on PDAC growth remains unknown.

Cell cycle progression and apoptosis are the critical intracellular degradation mechanisms responsible for the effects of chemotherapy (Vancsik, Forika, Balogh, Kiss, & Krenacs, 2019). The receptor for advanced glycation end products (RAGE) has been linked to sustained autophagy, limited apoptosis, and increased PDAC chemoresistance and cell survival (Kang et al., 2010; Shahab et al., 2018). In addition, RAGE expression is highly correlated with increased levels of autophagy and acts as an "autophagic switch" in PDAC in vivo and in vitro (Kang et al., 2012). Recently, we found that RAGE upregulates the multidrug resistance protein 1 (MDR1) levels in a GEM-resistant pancreatic carcinoma cell line (denoted MIA Paca-2^{GEMR} cells) (Hsu, Chen, Wang, Lin, & Yen, 2020; Lan, Chen, Kuo, Lu, & Yen, 2019; Lin, Chen, Lu, Lin, & Yen, 2020). In this study, we aimed to evaluate the effects of lucidone in enhancing chemotherapy efficacy through underlying MDR1-associated mechanisms in PDAC cells.

2 | MATERIALS AND METHODS

2.1 | Chemicals

Lucidone (purity > 99%) was kindly provided by Professor Sheng-Yang Wang (National Chung Hsing University, Taiwan) (Kumar et al., 2010). GEM was purchased from Sigma-Aldrich (St. Louis, MO).

2.2 | Cell maintenance

The human pancreatic cancer cell line MIA Paca-2 was purchased from the Bioresource Collection and Research Center (Hsinchu, Taiwan). The MIA Paca-2^{GEMR} cell line was established by gradually increasing GEM concentrations for tolerance of 0.5 μM GEM and maintained by incubation with 0.5 μM GEM to keep the stability (Lan et al., 2019). Cells were cultured in DMEM (high glucose) medium supplemented with 10% fetal bovine serum (FBS) and 2.5% horse serum at 37°C in a humidified atmosphere containing 5% CO_2. The cell passage number of MIA Paca-2 and MIA Paca-2^{GEMR} cells from 5 to 20 was used in this study.

2.3 | Cell viability and cell cycle analysis

The cells (2×10^4 cells/well) were plated in a 96-well culture plate and incubated in a CO_2 incubator overnight. After lucidone (1–50 μM) treatment for 48 or 72 hr, cell viability and cell cycle progression were evaluated by MTT analysis and propidium iodide (PI) staining, respectively (Lan et al., 2019).

2.4 | RAGE-specific siRNA transfection

Transfection was performed according to the methods described in a previous report (Lan et al., 2019). Briefly, cells (1×10^5 cells/ml) were cultured in 6-well culture plate overnight and then treated with 25 nM negative control (nontargeting) or RAGE-specific siRNA for 24 hr. The knock-down efficiency was evaluated by protein analysis.

2.5 | Western blotting assay

Western blotting was carried out following the methods described in a previous report (Lan et al., 2019). Briefly, cells (6×10^4 cells/ml) were treatment with lucidone (25 and 50 μM) for 72 hr, then the apoptosis-related proteins (Bax and Bcl-xL), autophagy-related protein (Atg5, Beclin-1, LC3 II, and Vps34), PI3K/Akt pathway, drug resistance-related protein (RAGE, HMGB1, and MDR1) were measured by Western blot. The protein levels were quantified by a BioSpectrum® 810 Imaging System (UVP, Upland, CA).

2.6 | Extracellular HMGB1 measurement

The levels of extracellular HMGB1 in the culture supernatants of cells treated with or without lucidone were assessed by an ELISA kit (Cell Signaling Technology, Beverley, MA) according to the manufacturers' instructions.

2.7 | Statistical analysis

All experiments were repeated three independent times. The results were analyzed by Student's *t*-test using SPSS 20 statistical software (IBM Corporation, Armonk, NY) and are shown as the mean ± SD.

3 | RESULTS

3.1 | Lucidone inhibited PDAC cell proliferation

To assess the effects of lucidone on tumor cell proliferation, MIA Paca-2 and GEM-resistant cells were incubated with various concentrations of lucidone for 48 and 72 hr, respectively. As shown in Figure 1a, treatment of MIA Paca-2 cells with 50 μM lucidone for 48 or 72 hr significantly decreased cell survival but did not reach the half-maximal inhibitory concentration. Surprisingly, lucidone dramatically inhibited MIA Paca-2^{GEMR} cell proliferation in a time- and concentration-dependent manner (Figure 1b). The IC_{50} value of the treatment MIA Paca-2^{GEMR} cells with lucidone for 72 hr was 38.99 μM. Accordingly, lucidone concentrations of 25 and 50 μM were selected in subsequent experiments. In addition, after 50 μM lucidone treatment, the MIA Paca-2^{GEMR} cell morphology became rounder than that of the untreated cells (Figure 1c). To further evaluate cell cycle regulation, cells were treated with lucidone and then evaluated by propidium iodide (PI) staining. Unexpectedly, cell cycle progression was not significantly altered in either cell line (Figure 2), indicating that lucidone-induced anticancer cytotoxicity may occur through other mechanisms.

3.2 | Lucidone induced apoptosis but not autophagy in PDAC cell lines

Because the cell cycle modulation was not a significant obstacle by lucidone treatment, we then focused on apoptosis-associated protein expression. The results demonstrated that lucidone decreased the levels of antiapoptotic Bcl-xL and increased the ratio of Bax (proapoptotic)/Bcl-xL in MIA Paca-2 cells (Figure 3a–d). Moreover, a stronger effect on Bax protein upregulation and Bcl-xL protein downregulation by lucidone treatment was observed in GEM-resistant cells in a dose-dependent manner (Figure 3e–h). In addition, the benefits of autophagy in enhancing chemosensitivity are still controversial. Here, we investigated whether autophagy was yet another cell death pathway induced by lucidone treatment. Our results showed that the autophagy-associated protein levels were significantly inhibited in both cell lines subjected to lucidone treatment (Figure 4). Vps34 is also known as class III PI3K and is essential for the process of autophagy (Jaber et al., 2012). We further confirmed the effect of lucidone on the Vps34 protein levels in these two cell lines. As shown

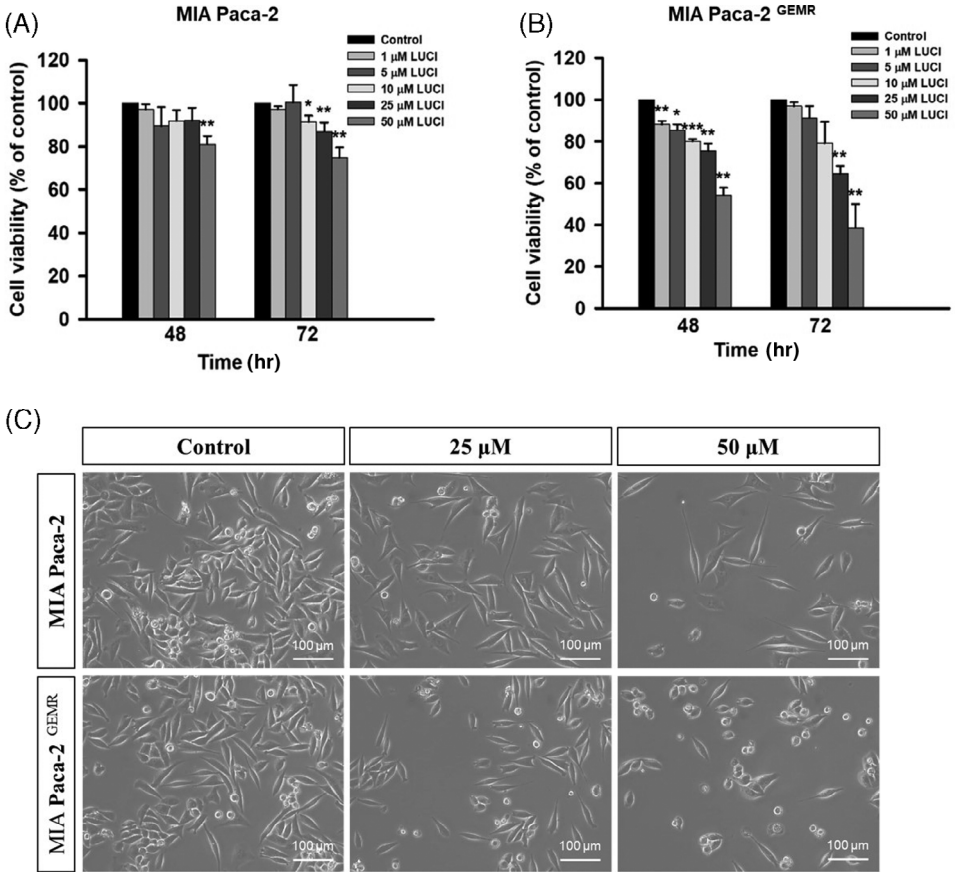
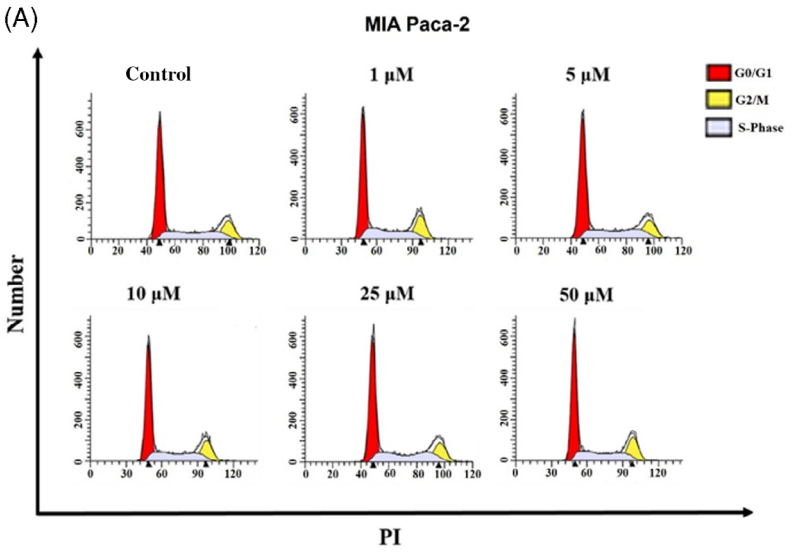
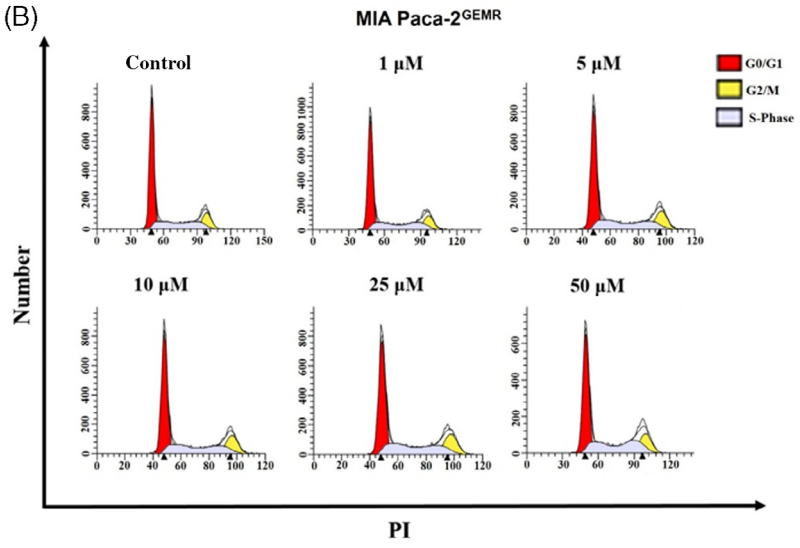


FIGURE 1 Cell viability analysis. (a) MIA Paca-2 and (b) GEM-resistant cells were treated with lucidone and analyzed using the MTT assay. The cell morphology of (c) both cell lines was observed by phase-contrast microscopy after lucidone incubation (All data are expressed as the mean ± SD. The experiments were performed in triplicate. *$p < .05$; **$p < .01$; ***$p < .001$ vs the untreated control)



Lucidone (μM)	Cell cycle analysis (%)		
	G0/G1	S	G2/M
Control	56.06 ± 2.77	28.24 ± 1.69	15.70 ± 1.73
1	54.35 ± 3.11	29.18 ± 1.50	16.48 ± 2.26
5	54.26 ± 1.57	29.53 ± 0.71	15.78 ± 1.98
10	53.72 ± 2.30	29.84 ± 1.62	16.44 ± 1.65
25	53.07 ± 1.55	31.87 ± 0.76*	15.06 ± 0.95
50	52.45 ± 2.15	30.45 ± 3.47	17.10 ± 2.09



Lucidone (μM)	Cell cycle analysis (%)		
	G0/G1	S	G2/M
Control	59.33 ± 3.85	26.73 ± 2.59	13.93 ± 1.49
1	58.45 ± 3.75	28.85 ± 3.33	12.70 ± 1.04
5	56.33 ± 5.33	29.29 ± 3.20	14.38 ± 2.14
10	56.68 ± 4.16	29.36 ± 2.19	13.96 ± 2.28
25	53.79 ± 3.83	30.50 ± 2.54	15.70 ± 1.63
50	51.13 ± 2.85*	31.47 ± 2.37	17.40 ± 2.84

FIGURE 2 Cell cycle analysis. (a) MIA Paca-2 and (b) GEM-resistant cells were treated with lucidone, and then, cell cycle determination was performed using flow cytometry with PI staining. Asterisks indicate *p* values less than .05 and are considered statistically significant compared to the untreated control (All data are expressed as the mean ± SD. The experiments were performed in triplicate)

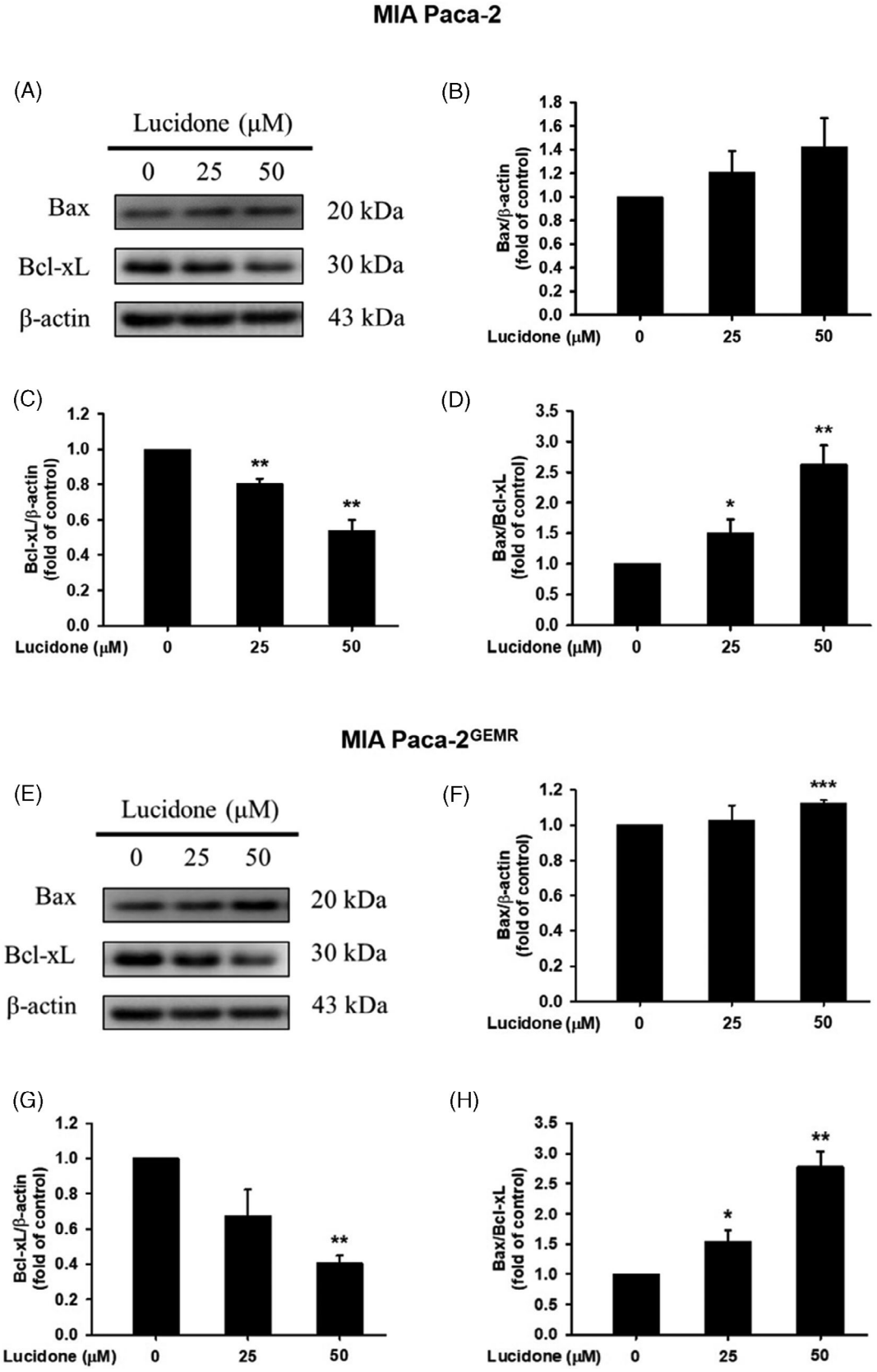


FIGURE 3 Apoptotic protein expression. Cells were treated with lucidone for 72 hr. The protein levels of Bax and Bcl-xL in (a) MIA Paca-2 and (e) GEM-resistant cells. The quantification results of (b,f) Bax, (c,g) Bcl-xL, and (d,h) the Bax/Bcl-xL ratio (All data are expressed as the mean ± SD. The experiments were performed in triplicate. *$p < .05$; **$p < .01$; ***$p < .001$ vs the untreated control)

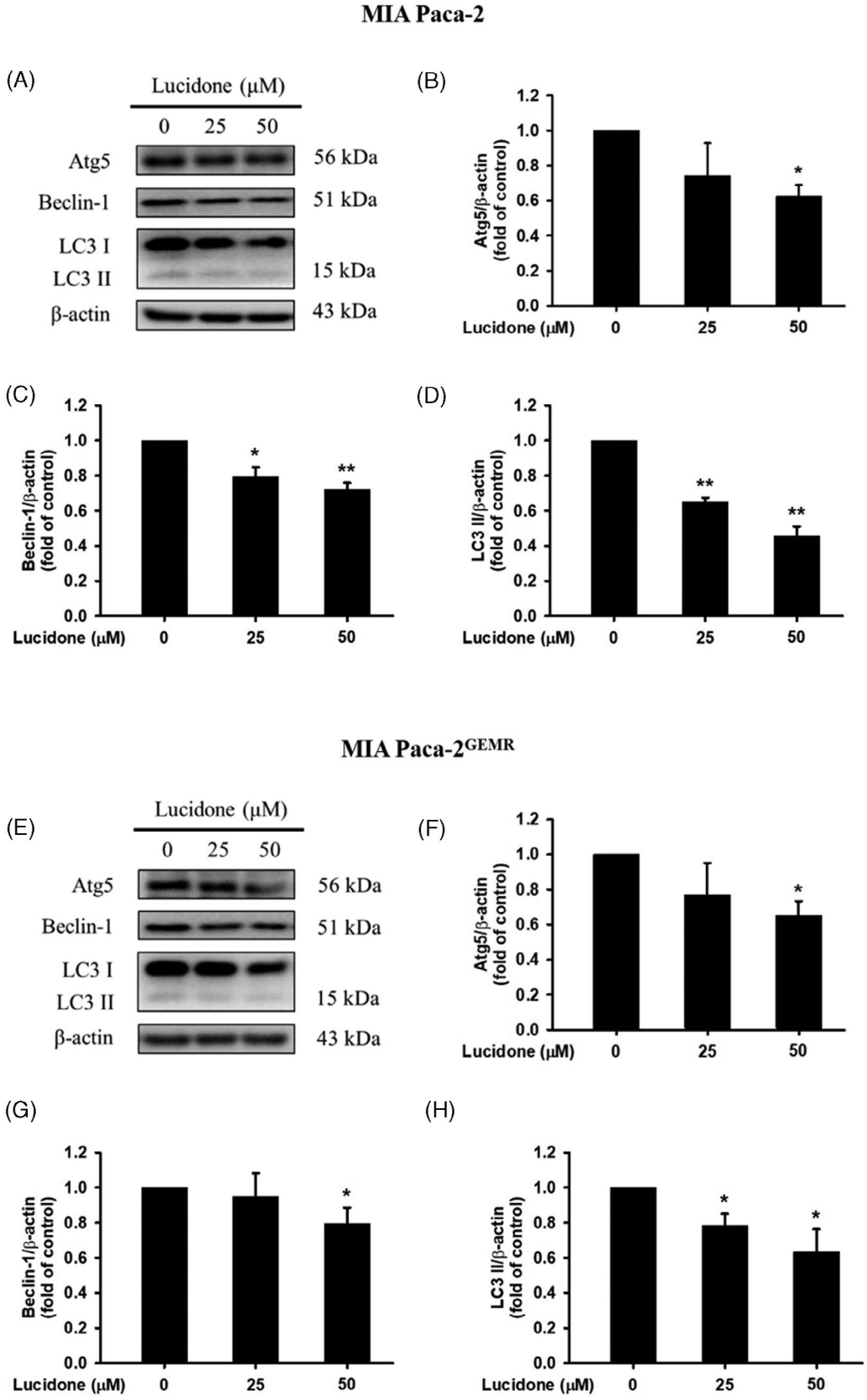


FIGURE 4 Autophagic protein expression. Cells were treated with lucidone for 72 hr. The protein levels of Atg5, Beclin-1, and LC3 II in (a) MIA Paca-2 and (e) GEM-resistant cells. The quantification results of (b,f) Atg5, (c,g) Beclin-1, and (d,h) LC3 II (All data are expressed as the mean ± SD. The experiments were performed in triplicate. *$p < .05$; **$p < .01$ vs the untreated control)

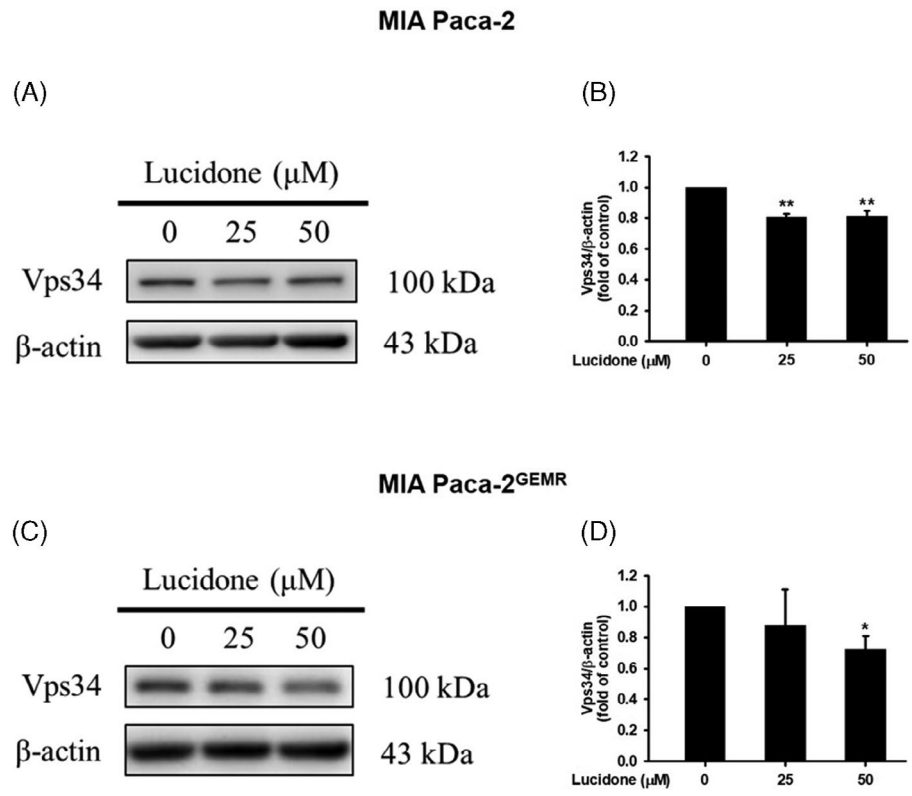


FIGURE 5 Vps34 protein expression. Cells were treated with lucidone for 72 hr. The protein levels of Vps34 and quantification results in (a,b) MIA Paca-2 and (c,d) GEM-resistant cells (All data are expressed as the mean ± SD. The experiments were performed in triplicate. $^{*}p < .05$; $^{**}p < .01$ vs the untreated control)

in Figure 5, Vps34 expression was significantly downregulated by 50 μM lucidone treatment in both cell lines compared to untreated cells.

3.3 | Apoptosis induction was accompanied by HMGB1-RAGE cascade inhibition

The signaling pathways initiated by HMGB1/RAGE engagement and the PI3K/AKT axis have been demonstrated to promote cell proliferation and survival (Cheng et al., 2017; He et al., 2018). Our recent results demonstrated that RAGE expression is elevated in GEM-resistant cells, whereas cell death and chemosensitivity enhanced after the inhibition of the RAGE/PI3K/AKT/mTOR cascades in both cell lines (Lan et al., 2019). These results indicated that RAGE-initiated signaling may play a crucial role in PDAC chemoresistance. In addition, HMGB1 coordinates cellular responses through RAGE, which plays an important role in enhancing cell survival and chemoresistance in cancers (Huang et al., 2018; Yin et al., 2017). To validate this phenomenon, we assessed the HMGB1, RAGE, PI3K, and AKT protein levels after treatment of both cell lines with lucidone. The results showed that the levels of both the endogenous and secreted forms of HMGB1 in MIA Paca-2 cells were significantly decreased after incubation with 50 μM lucidone for 72 hr (Figure 6a–e). Similarly, the protein levels of HMGB1 and RAGE were markedly inhibited in GEM-resistant cells subjected to lucidone incubation (Figure 7a–e). In addition, the activity of the kinases PI3K and AKT was decreased in both cell lines after lucidone treatment for 72 hr (Figures 6f–j and 7f–j, respectively).

3.4 | Activation of the PI3K/Akt signaling pathway was initiated by RAGE

Previous evidence showed that RAGE upregulates the expression of MDR1 in MIA Paca-2^{GEMR} cells and xenograft mouse models (Li, Chen, Weng, & Yen, 2021). Next, we investigated MDR1 expression under conditions of RAGE inhibition. Knocking down the RAGE levels or treatment with lucidone effectively prevented PI3K and Akt phosphorylation and led to the inhibition of MDR1 protein expression compared to transfection with siControl (Figure 8). Moreover, the effects of siRAGE transfection or lucidone treatment on PI3K/Akt and MDR1 inhibition were observed in GEM-resistant cells and compared with the effects of nonspecific siRNA transfection (Figure 9). Interestingly, lucidone treatment and siRAGE transfection of GEM-resistant cells dramatically suppressed the expression levels of RAGE, p-AKT, and MDR1 compared with those observe in untreated cells (Figure 9a,b,f,g).

3.5 | Lucidone treatment enhanced GEM chemosensitivity in PDAC cells

To determine the relevance of lucidone-modulated chemosensitivity in PDAC cells, we assessed the MDR1 protein

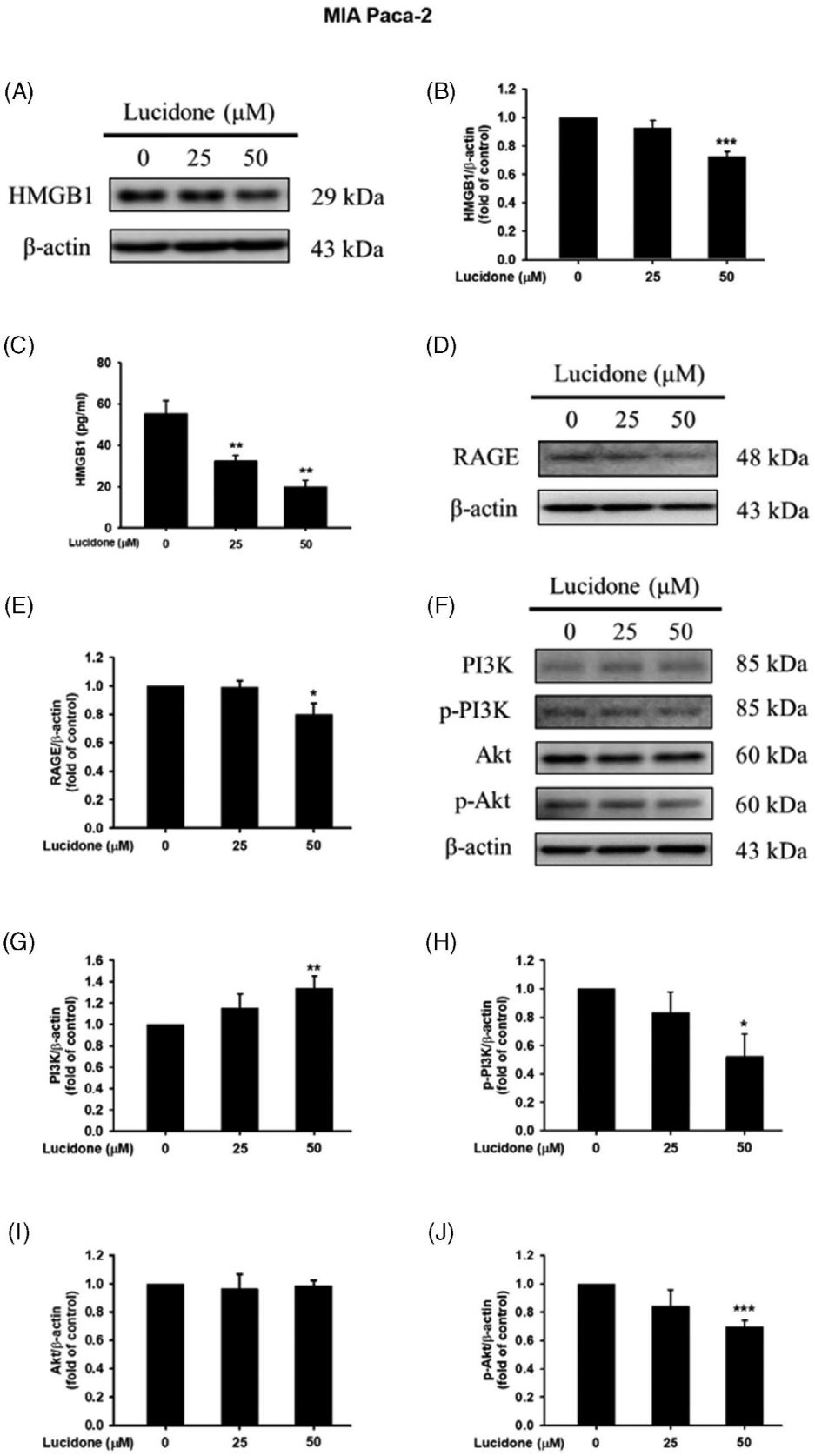


FIGURE 6 HMGB1, RAGE, PI3K, and Akt protein expressions in MIA Paca-2 cells. Cells were treated with lucidone for 72 hr. The protein expression and quantification results of (a,b) intracellular HMGB1, (c) extracellular HMGB1, (d,e) RAGE, and (f–j) PI3K/Akt were analyzed by Western blotting (All data are expressed as the mean ± SD. The experiments were performed in triplicate. $^{*}p < .05$; $^{**}p < .01$; $^{***}p < .001$ vs the untreated control)

levels in both cell lines. Treatment with 50 μM lucidone significantly reduced MDR1 protein expression in both cell lines (Figure 10a–b,d–e). Furthermore, lucidone-induced cytotoxicity was explored in GEM-treated cells. Treatment with 0.5 μM GEM significantly inhibited the growth of MIA Paca-2 cells compared to untreated cells (Figure 10c). In addition, an inhibitory effect on cell

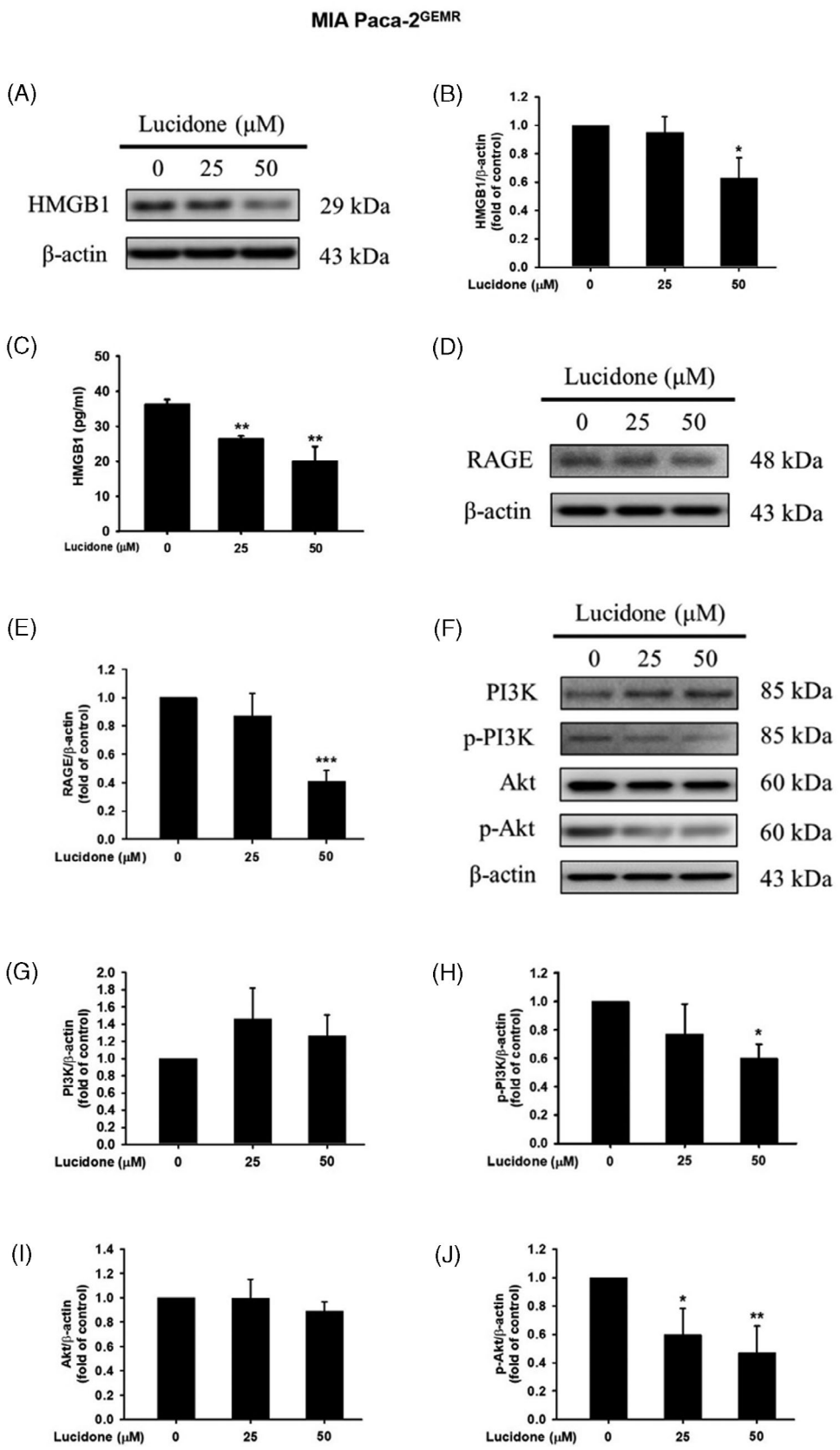


FIGURE 7 HMGB1, RAGE, PI3K, and Akt protein expression in MIA Paca-2^{GEMR} cells. Cells were treated with lucidone for 72 hr. The protein expression and quantification results of (a,b) intracellular HMGB1, (c) extracellular HMGB1, (d,e) RAGE, and (f–j) PI3K/Akt were analyzed by Western blotting (All data are expressed as the mean ± SD. The experiments were performed in triplicate. $^{*}p < .05$; $^{**}p < .01$; $^{***}p < .001$ vs the untreated control)

proliferation was also observed in 50 μM lucidone-treated MIA Paca-2 cells (Figure 10c). In addition, pretreatment with 50 μM lucidone significantly increased the cytotoxicity in GEM-incubated cells (Figure 10c). As anticipated, cell viability was not affected by GEM treatment in GEM-resistant cells (Figure 10f). Lucidone treatment effectively inhibited cell proliferation in a dose-dependent

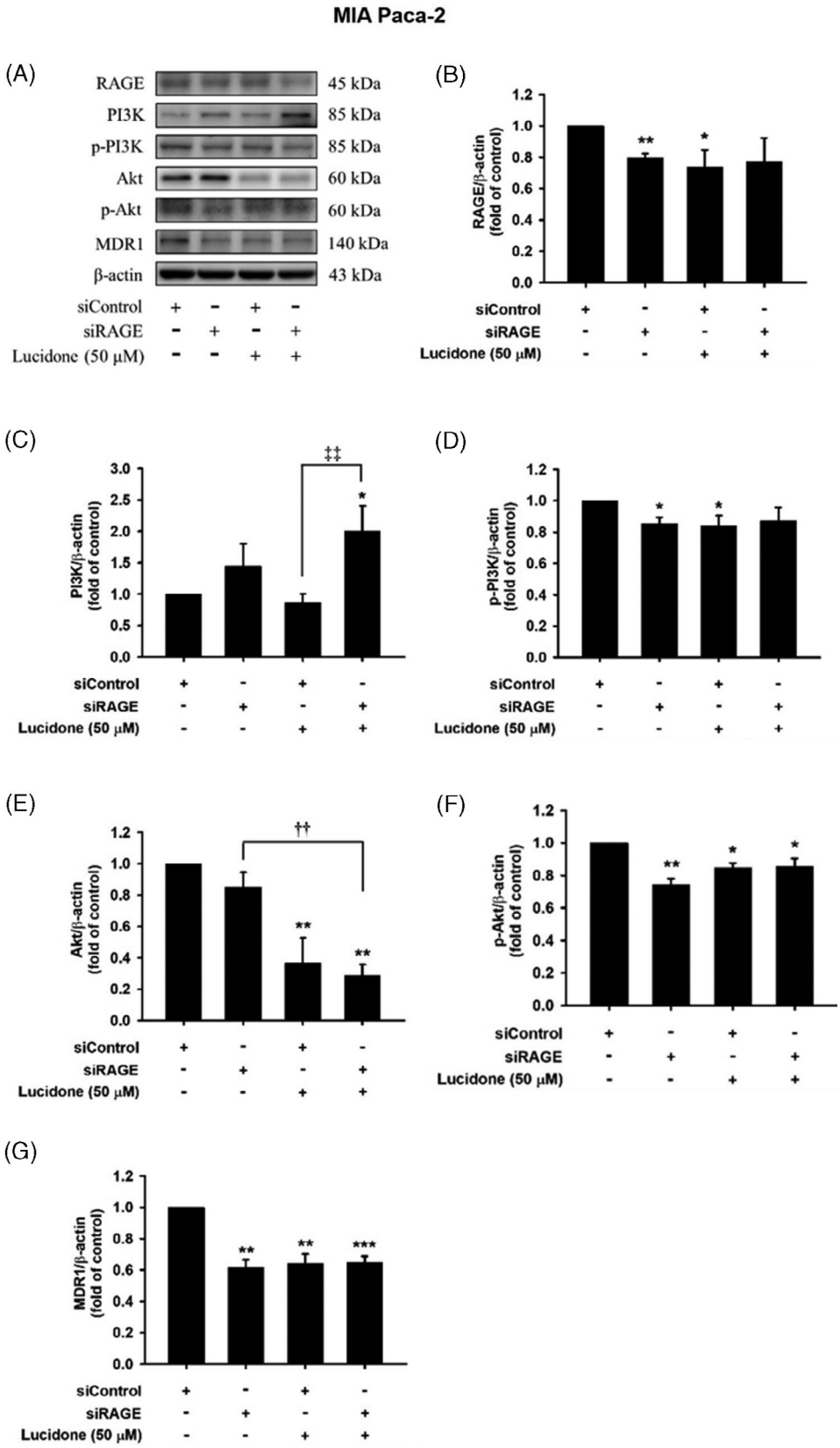


FIGURE 8 RAGE cascade protein expression in siRAGE MIA Paca-2 cells. siRAGE cells were treated with lucidone for 72 hr. (a) Protein expression and (b–g) quantification results of siRAGE MIA Paca-2 cells. (*p < .05, **p < .01 and ***p < .001 vs the untreated control siRNA cells). The values were considered significant for lucidone-treated siRAGE cells compared to siControl MIA Paca-2 cells ($^{\dagger}p$ < .05 and $^{\dagger\dagger}p$ < .01). The values were considered significant for lucidone-treated siRAGE cells compared to lucidone-treated siControl MIA Paca-2 cells ($^{\ddagger\ddagger}p$ < .01). All data are expressed as the mean ± SD. The experiments were performed in triplicate

manner in contrast to the proliferation of untreated GEM-resistant cells (Figure 10f). In addition, pretreatment with lucidone markedly enhanced cytotoxicity in GEM-treated MIA Paca-2^{GEMR} cells compared to GEM treatment alone (Figure 10f).

4 | DISCUSSION

GEM enhances proapoptotic pathway activation and results in DNA damage in cancers (Binenbaum et al., 2015). However, approximately

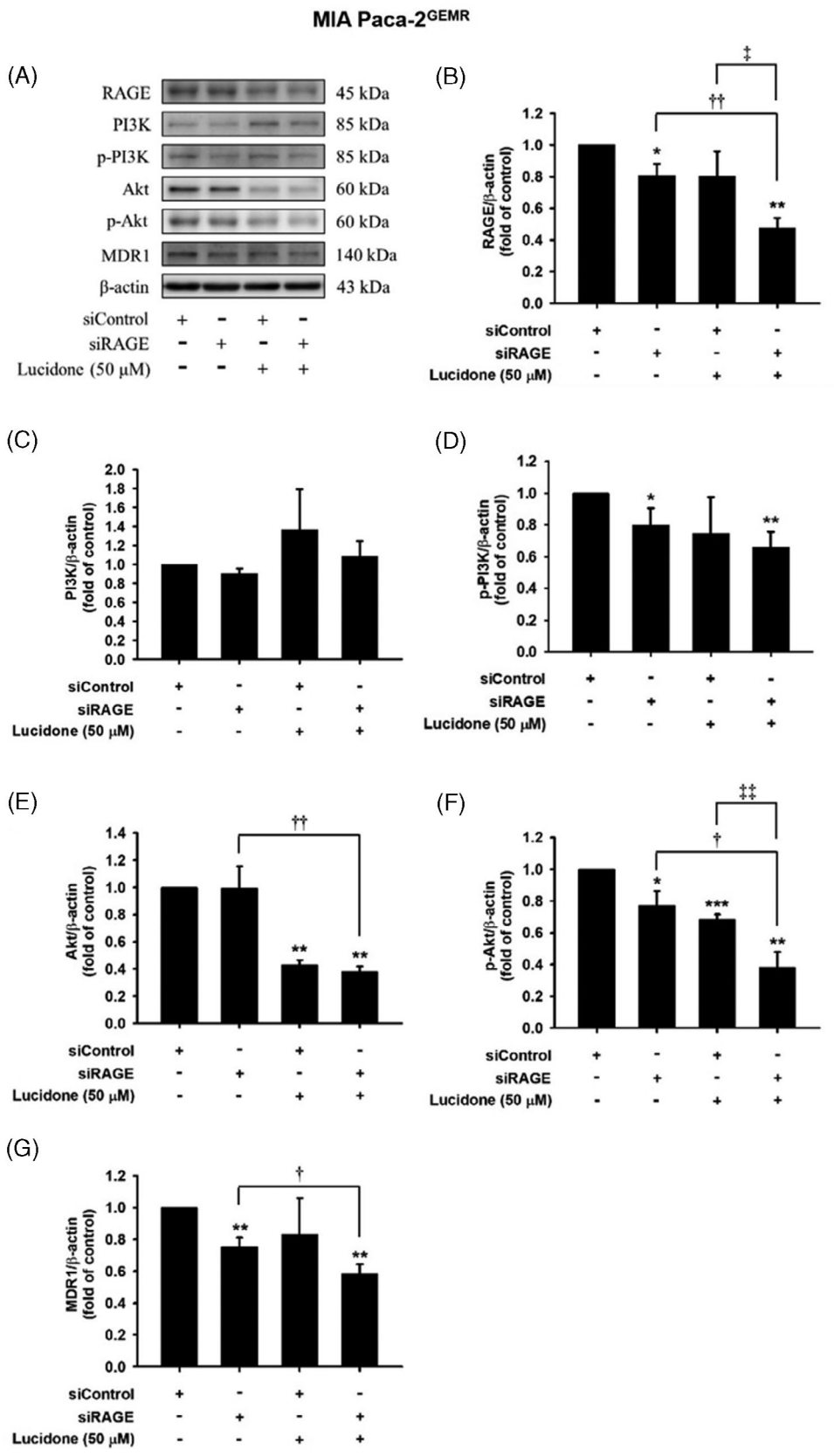


FIGURE 9 RAGE cascade protein expression in siRAGE MIA Paca-2^{GEMR} cells. siRAGE cells treated with lucidone for 72 hr. (a) Protein expression and (b–g) quantification results in siRAGE MIA Paca-2^{GEMR} cells. (*p < .05, **p < .01 and ***p < .001 vs the untreated control siRNA cells). The values were considered significant for lucidone-treated siRAGE cells compared to siControl MIA Paca-2^{GEMR} cells ($^{\dagger}p$ < .05 and $^{\dagger\dagger}p$ < .01). The values were considered significant for lucidone-treated siRAGE cells compared to lucidone-treated siControl MIA Paca-2 cells ($^{\ddagger}p$ < .05 and $^{\ddagger\ddagger}p$ < .01). All data are expressed as the mean ± SD. The experiments were performed in triplicate

75% of PDAC patients exhibit GEM resistance, which exacerbates the reduction in the survival rate of PDAC patients (Binenbaum et al., 2015). Although clinically used combination strategies such as FOLFIRINOX and nab-paclitaxel with GEM for pancreatic cancer patient treatment, challenges of safety, and multiple drug resistance still exist (Jain & Bhardwaj, 2021). The above evidence indicated that

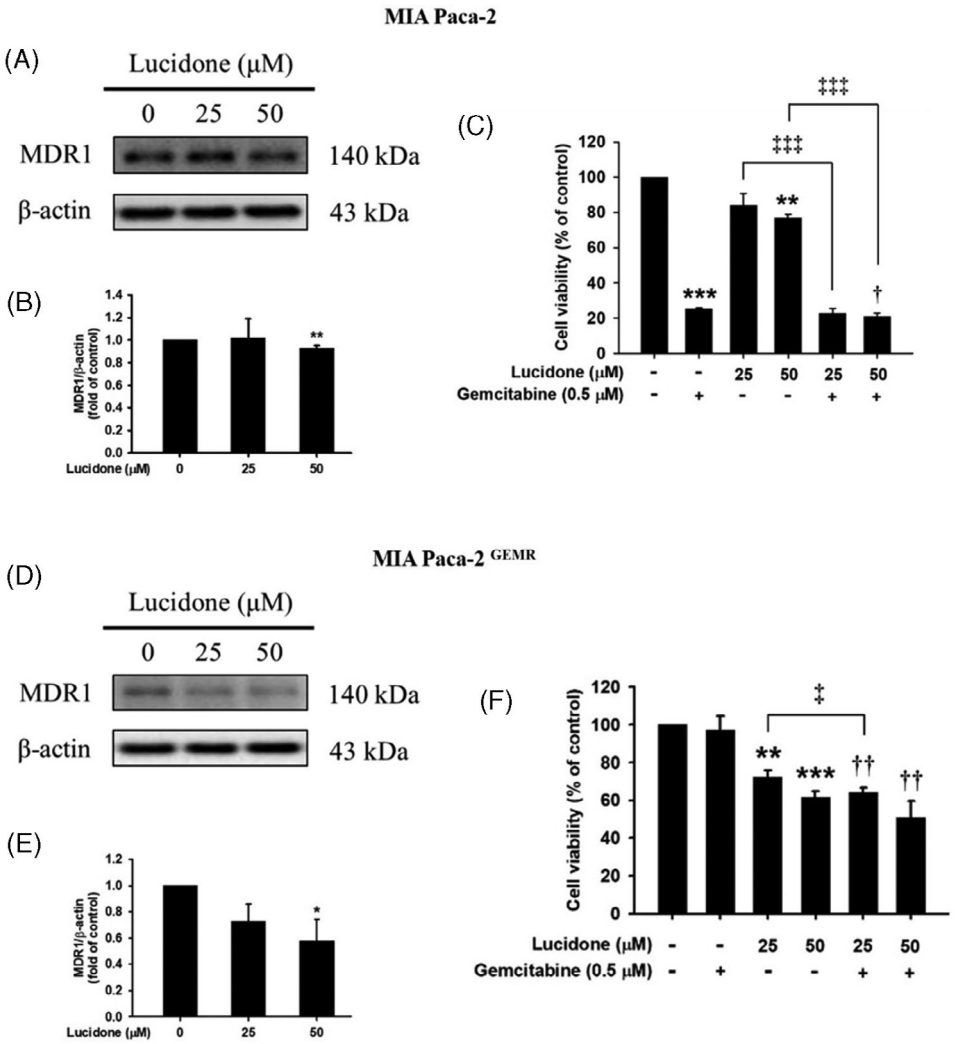


FIGURE 10 Lucidone enhanced chemosensitivity. Cells were treated with lucidone for 72 hr. MDR1 protein expression in (a,b) MIA Paca-2 cells and (d,e) MIA Paca-2^{GEMR} cells. Cells were pretreated with lucidone for 48 hr and then treated with or without 0.5 μM GEM for another 72 hr. The cell viability of (c) MIA Paca-2 cells and (f) MIA Paca-2^{GEMR} cells was analyzed (**p < .01, ***p < .001 vs the untreated control; $^{\dagger}p$ < .05, $^{\dagger\dagger}p$ < .01 vs the gemcitabine group; $^{\ddagger}p$ < .05, $^{\ddagger\ddagger}p$ < .001 vs the lucidone group). All data are expressed as the mean ± SD. The experiments were performed in triplicate

FOLFIRINOX and nab-paclitaxel are not suitable to be the positive control compared with natural compound treatment. Until now, the positive control for gemcitabine chemosensitivity enhancement remains a significant limitation. Thus, positive control was excluded in this study. Despite numerous studies demonstrating the disadvantageous effects of GEM resistance, an effective and safe treatment strategy is lacking. In this study, we showed that the combination of lucidone and GEM suppressed pancreatic cancer cell proliferation by reducing autophagy and facilitating apoptosis through the HMGB1/RAGE/PI3K/Akt signaling pathway.

Although autophagy regulation is coordinated with cell division, the mechanism remains unclear. However, accumulating evidence suggests that autophagy and cell cycle progression are reciprocal regulators (Zheng, He, Kitazato, & Wang, 2019). Our current evidence also shows that sub-G1 stage cell cycle arrest consequently triggers autophagy via the inhibition of PI3K/AKT/mTOR signal transduction (Lan et al., 2019). Unexpectedly, the results of the present study showed that cell cycle progression was not altered and autophagy-associated protein expression was decreased by lucidone treatment. These results indicated that cell cycle regulation may not regulate autophagy in PDAC cells treated with lucidone. Consistently, a previous review article demonstrated that cell cycle progression and autophagy can be independently regulated by common signaling pathways (Neufeld, 2012). Here, we found that inhibition of HMGB1-RAGE by lucidone treatment initiated the PI3K/Akt signaling pathway, consequently inhibiting autophagy and enhancing apoptosis.

Autophagy is closely associated with chemotherapy resistance and is well documented to contribute to cell survival (Katheder et al., 2017; Levy, Towers, & Thorburn, 2017). Previous literature has demonstrated that sustaining autophagy and limiting apoptosis results in increased cancer chemoresistance and tumor growth (Yu et al., 2017). HMGB1 and RAGE play a crucial role in promoting pancreatic carcinogenesis and tumor progression (Kang et al., 2014). Interference with HMGB1 significantly inhibits cell autophagy, increases cell apoptosis, and enhances the sensitivity of lung adenocarcinoma SPC-A1 and H1299 cell lines to docetaxel chemotherapy drugs (Pan et al., 2014). Moreover, HMGB1-RAGE is an autophagy effector that increases the Beclin-1-Vps34 interaction and regulates autophagosome formation, therefore inhibiting antiapoptotic processes and chemotherapy resistance (Funderburk, Wang, & Yue, 2010; Kang, Tang, Loze, & Zeh, 2011). Convincing evidence in

our study has established that lucidone treatment significantly attenuated both the intracellular and extracellular protein levels of HMGB1, RAGE, Beclin-1, and Vps34, which induced apoptosis, reduced autophagy, and improved chemosensitivity in GEM-resistant cells.

Although an anticancer growth effect of lucidone treatment was observed, the underlying mechanism remains unclear. To date, an article has shown that lucidone causes G2/M phase arrest and apoptotic cytotoxicity by suppressing PI3K/Akt/NF-κB signal transduction in ovarian cancer cells (Yoon et al., 2020). PI3K/Akt is the core survival pathway and is frequently hyperactivated in cancers (Yang, Polley, & Lipkowitz, 2016). Inhibition of PI3K/Akt signaling activation may improve the clinical outcomes of chemotherapy. In agreement with previous evidence, these results demonstrated that lucidone significantly inhibited PI3K, Akt, and MDR1 expression and enhanced chemosensitivity in GEM-resistant cells subjected to lucidone plus GEM treatment. Importantly, silencing RAGE expression significantly reduced the activation of the PI3K/Akt signaling pathway and the protein expression of MDR1, which provided compelling evidence not only for lucidone treatment but also for RAGE-specific siRNA transfection of cells.

Previous evidence demonstrated that HMGB1 promotes chemoresistance in various cancers such as osteosarcoma, leukemia, breast cancer, human hepatocellular carcinoma, and nasopharyngeal carcinoma through mediating autophagy (Huang et al., 2012; Kong, Xu, Xu, Fang, & Xu, 2015; Li, et al., 2021; Liu et al., 2019; Zhu et al., 2020). For example, targeting the transformation of autophagic complexes via HMGB1 inhibition enhances the chemosensitivity in osteosarcoma (Li, Wang, Chen, Liu, & Yang, 2014), hepatocellular carcinoma (Li, et al., 2021), breast cancer (Liu et al., 2019), and leukemia (Kong et al., 2015). In addition, RAGE involved in chemoresistance is also documented. Growing evidence suggests that RAGE plays a crucial role in the development of chemoresistance in human prostate tumors (Zhou et al., 2015), colorectal cancer (Huang et al., 2018), and gastric cancer (Nishiguchi et al., 2019). Recently, we also demonstrated that ursolic acid restores GEM sensitivity through the RAGE/NF-κB/MDR1 axis in pancreatic cancer cells and the xenograft mouse model (Li, et al., 2021). Consistent with this possibility, lucidone may raise chemosensitivity through downregulation of RAGE/HMGB1-initiated signaling pathways in other cancers.

Collectively, these results demonstrated that the natural compound lucidone inhibited the HMGB1/RAGE/PI3K/Akt signaling pathway and promoted apoptosis, consequently reducing autophagy in GEM-resistant cells. Enhanced effects of inhibited MDR1 protein expression and increased cytotoxicity occurred with lucidone combined with RAGE gene silencing or GEM treatment, indicating that lucidone could be a novel treatment option in PDAC.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data used to support the findings of this study are included within the article.

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