Int J Clin Exp Med 2016;9(11):21048-21056
www.ijcem.com /ISSN:1940-5901/IJCEM0024468

Original Article

Lycorine induces apoptosis in human pancreatic cancer cell line PANC-1 via ROS-mediated inactivation of the PI3K/Akt/mTOR signaling pathway

Qing Liu[1,2], Sanyuan Hu[1], Yongming Zhang[2], Guangyong Zhang[1], Shaozhuang Liu[1]

[1]*Department of General Surgery, Qilu Hospital of Shandong University, No 107 Wenhuaxi Road, Jinan 250012, China;* [2]*Department of Hepatobiliary Surgery, Yantaishan Hospital, No. 91 Jiefang Road, Yantai 264001, China*

Received January 19, 2016; Accepted May 4, 2016; Epub November 15, 2016; Published November 30, 2016

Abstract: Lycorine, a main phenanthridine Amaryllidaceae alkaloid, has shown wide ranging pharmacological activities. This study aimed to investigate the antitumor activity of lycorine against pancreatic cancer and to elucidate the underlying mechanisms. Human pancreatic cancer cell line PANC-1 was treated with lycorine. Cell viability was monitored by MTT assay. The expression of related proteins was identified by western blot analysis. Antitumor activity of lycorine *in vivo* was assessed in BALB/c athymic nude mice. The results demonstrated that lycorine induced apoptosis in PANC-1 cells, which was supported by PARP cleavage, activation of caspase-3 and caspase-9 as well as down-regulation of Bcl-2, upregulation of Bax and Bax/Bcl-2 ratio. Meanwhile, lycorine treatment decreased the phosphorylation of Akt and mTOR, while LY294002 pretreatment enhanced lycorine-triggered dephosphorylation of Akt, cell apoptosis and cleavage of PARP. In addition, lyocrine induced ROS generation, pretreatment of cells with ROS scavenger NAC reduced ROS generation, rescued cell from apoptosis, reversed Akt suppression and PARP cleavage. These results demonstrated that lycorine induced apoptosis in PANC-1 cells via ROS-mediated PI3K/Akt/mTOR signaling pathway. *In vivo*, lycorine effectively inhibited tumor growth in xenografts model in BALB/c mice without obviously additional toxicity. Lycorine may be a promising option in the treatment of pancreatic cancer.

Keywords: Lycorine, pancreatic cancer, ROS, PI3K/Akt/mTOR, apoptosis

Introduction

Pancreatic cancer is one of the most aggressive tumors of the digestive system with poor response to current therapeutics [1, 2]. Due to a largely asymptomatic progress of the disease, only 10-15% of the patients have a tumor localized to the pancreas at the time of diagnosis allowing of a potentially curative resection [3]. Besides surgical therapy, the major therapies for pancreatic cancer treatment include chemotherapy, radiotherapy, immunotherapy, and vaccine therapy [4, 5]. However, the therapeutic outcome of pancreatic cancer is disappointing in clinic owing to the resistance to cytotoxic chemotherapeutic agents and/or radiotherapy, the treatment of pancreatic cancer remains a major challenge [6]. Therefore, there is an urgent need of therapeutic strategies for pancreatic cancer treatment.

Emerging evidence has shown that naturally occurring products from medicinal plants or diets is a fertile source of potential cancer chemotherapeutic and chemoprevention agents, and they have received great attention because they are considered to be safe and to reduce the risk of mutagenicity in normal cells [7]. Lycorine, an alkaloid extracted from amaryllis plants, has shown to exhibit various pharmacological effects, including anti-inflammatory activities, anti-malarial properties, emetic actions, anti-virus activities [8, 9]. Recent studies have focused on the potential antitumor activity of lycorine. Numerous evidence have demonstrated that lycorine could suppress the growth of various caner cells, including poliovirus-infected Hela cells [10], leukemia cell [11-13], mammary cancer MM46 cells [14], human anaplastic oligodendroglioma Hs683 cells and mouse melanoma B16F10 cells [15]. Interestingly,

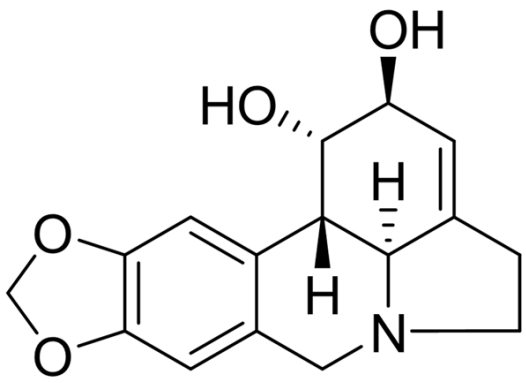

Figure 1. Chemical structures of lycorine.

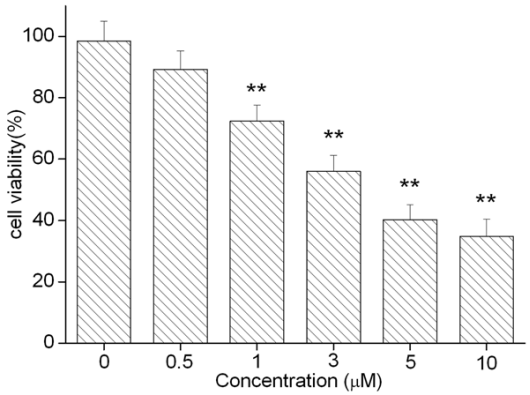

Figure 2. Effects of lycorine on the cell viability of PANC-1 cells. Cells were treated with 0 μM, 0.5 μM, 1 μM, 3 μM, 5 μM and 10 μM lyocrine for 24 h and the cell viability was monitored by MTT assay. Data were represented as means ± SD. $^{**}P < 0.01$ vs. the control.

lyocorine displays significantly higher anti-proliferative activities in tumor cells than in normal cells [16]. Up to now, whether lycorine can inhibit the proliferation of human pancreatic cancer cell line PANC-1 is still unknown. In this study, we investigated the anticancer activity of lycorine in PANC-1 cells and explored the potential mechanisms.

Materials and methods

Chemicals and antibodies

Lycorine (purity > 98%) was purchased from Sigma Chemical Co. (St. Louis, MO, USA) and its chemical structure is shown in **Figure 1**. Lycorine was dissolved in dimethyl sulfoxide (DMSO; Sigma) as a stock solution and diluted in serum-free medium until needed. LY294002 and N-acetyl-cysteine (NAC) were purchased from Sigma (St. Louis, MO, USA). Z-VAD-FMK (Z-VAD), anti-cytochrome *c*, anti-Bax and anti-Bcl-2 were obtained from Abcam (Cambridge, MA, USA). Anti-Akt, anti-phosphorylated Akt, anti-mTOR, anti-phosphorylated-mTOR, anti-caspase-3, anti-caspase-8, anti-caspase-9, anti-poly (ADP-ribose) polymerase (PARP), anti-β-actin, goat anti-rabbit IgG (H&L)-HRP secondary antibody were purchased from Cell Signaling Technology (Danvers, MA, USA). All reagents were prepared and used as recommended by their suppliers.

Cell line and cell culture

Human pancreatic cancer cell line PANC-1 was obtained from American Type Culture Collection (Manassas, VA, USA) and cultured in Dulbecco's modified Eagle's medium (DMEM, Gibco, NY, USA) supplemented with 10% heat-inactivated fetal bovine serum, 100 U/ml of penicillin and 100 μg/ml of streptomycin. The cells were maintained in a humidified incubator containing 5% CO_2 in air at 37°C.

Cell viability assay

The viability of cell treated with lycorine was determined by MTT assay. Exponentially growing PANC-1 cells were suspended at a final concentration of 1×10^4 cell/well and cultured in a 96-well flat-bottomed microplate. After 12 hour's incubation, the cells were treated with 0 μM, 0.5 μM, 1 μM, 3 μM, 5 μM and 10 μM lycorine for 24 h. Subsequently, the drug containing medium was removed and replaced by fresh medium. MTT solution was added to each well and cells were then incubated for 4 h at 37°C. The culture media were then replaced with 150 μL DMSO. After gentle mixing, the absorbance was measured at 570 nm on a Bio-Rad Model 680 microplated reader (Bio-RadLaboratories, Hercules, CA, USA).

Western blot analysis

Collected cells were lysed immediately in RIPA buffer (20 mm Tris, 2.5 mm EDTA, 1% Triton X-100, 1% deoxycholate, 0.1% SDS, 40 mm NaF, 10 mm $Na_4P_2O_7$, and 1 mm phenylmethylsulphonyl fluoride) supplemented with a protease inhibitor cocktail (Calbiochem, San Diego, CA, USA). Cytosolic and mitochondrial protein extracts were isolated using the cytochrome *c*

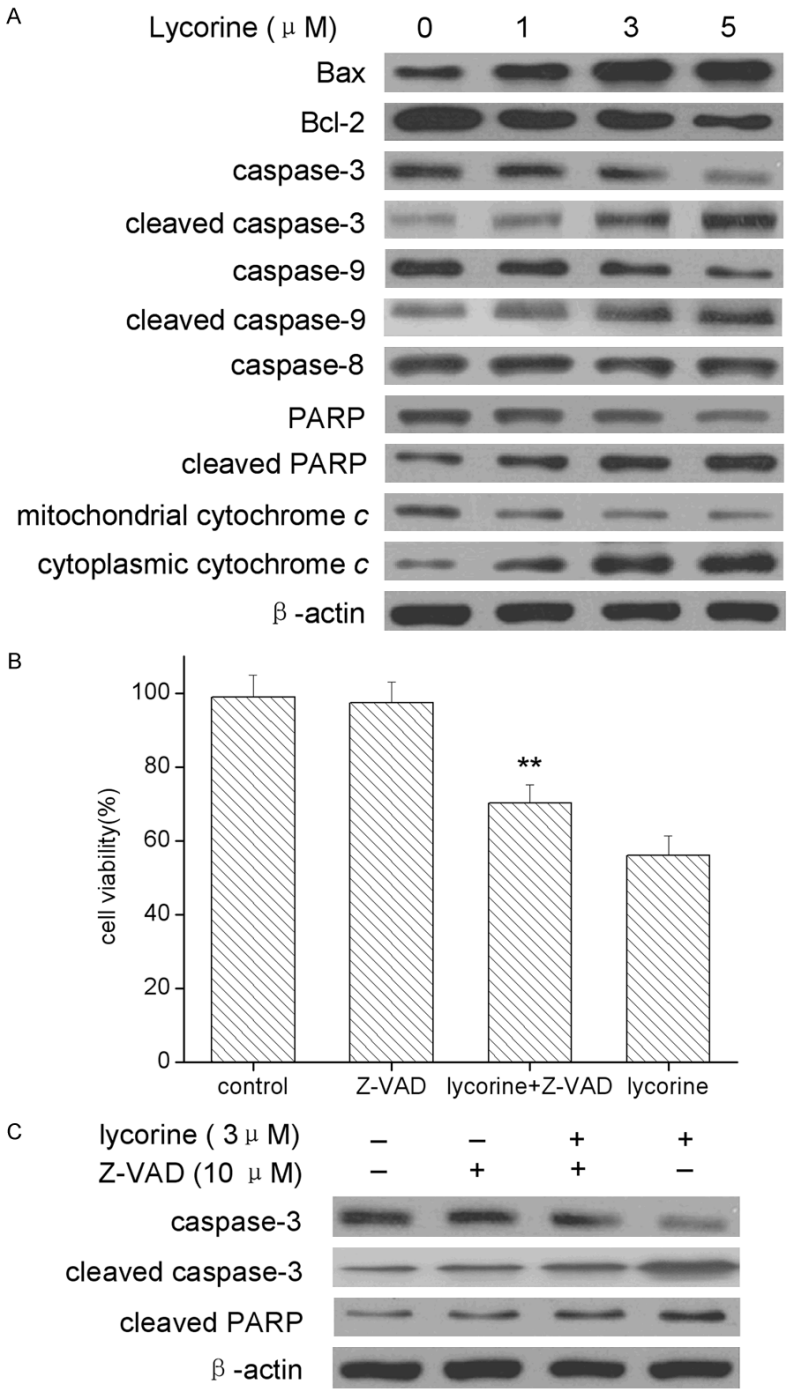

Figure 3. Lycorine induced apoptosis in PANC-1 cells. (A) PANC-1 cells were treated with 0 μM, 1 μM and 3 μM lycorine for 24 h and the expression of apoptosis related proteins was determined by western blot assay. (B and C) Cells were pretreated with 10 μM Z-VAD for 1 h, then treated PANC-1 cells with 3 μM lycorine for 24 h. The cell viability (B) and cleavage of caspase-3 and PARP (C) were detected. Data were represented as means ± SD. $^{**}P < 0.01$ vs. cells treated with lycorine alone.

Releasing Apoptosis Assay kit (Millipore, Germany) according to the manufacturer's instruction. The nuclear protein extracts were isolated using the Nuclear Extraction kit from Activate Motif (Carlsbad, USA). Protein concentration was determined using Micro BCA kit (Beyotime Biotechnology, Haimen, China). Proteins were separated by SDS-PAGE, and transferred onto polyvinylidene difluoride (PVDF) membranes (Millipore, Bedford, MA, USA). After being blocked with 5% non-fat milk, blots were incubated with primary antibodies overnight at 4°C, and then exerted to incubation with the corresponding secondary antibody at room temperature for 2 h. Immunoreactive bands were then developed using an enhanced chemiluminescence reagent (Pierce, Rockford, IL, USA) according to the manufacturer's instruction. The bands were quantitatively analyzed using the public domain NIH Image software (open source Image J software available at http://rsb.info.nih.gov/ij/).

ROS assay

Intracellular ROS generation was measured by using the fluorescent dye 2', 7'-dichlorofluorescein diacetate (DCFH-DA; Sigma, USA) as a substrate. After being pretreated with 0 μM, 1 μM, 3 μM and 5 μM lycorine for 24 h, PANC-1 cells were incubated with DCFH-DA for 30 min at 37°C in dark. After loading, cells were rinsed with PBS and DCF fluorescence was measured using the FlexStation II 384 fluorometric imaging plate reader (Molecular Devices, Sunnyvale, CA, USA) with excitation wavelength of 488 nm and an emission wavelength of 525 nm.

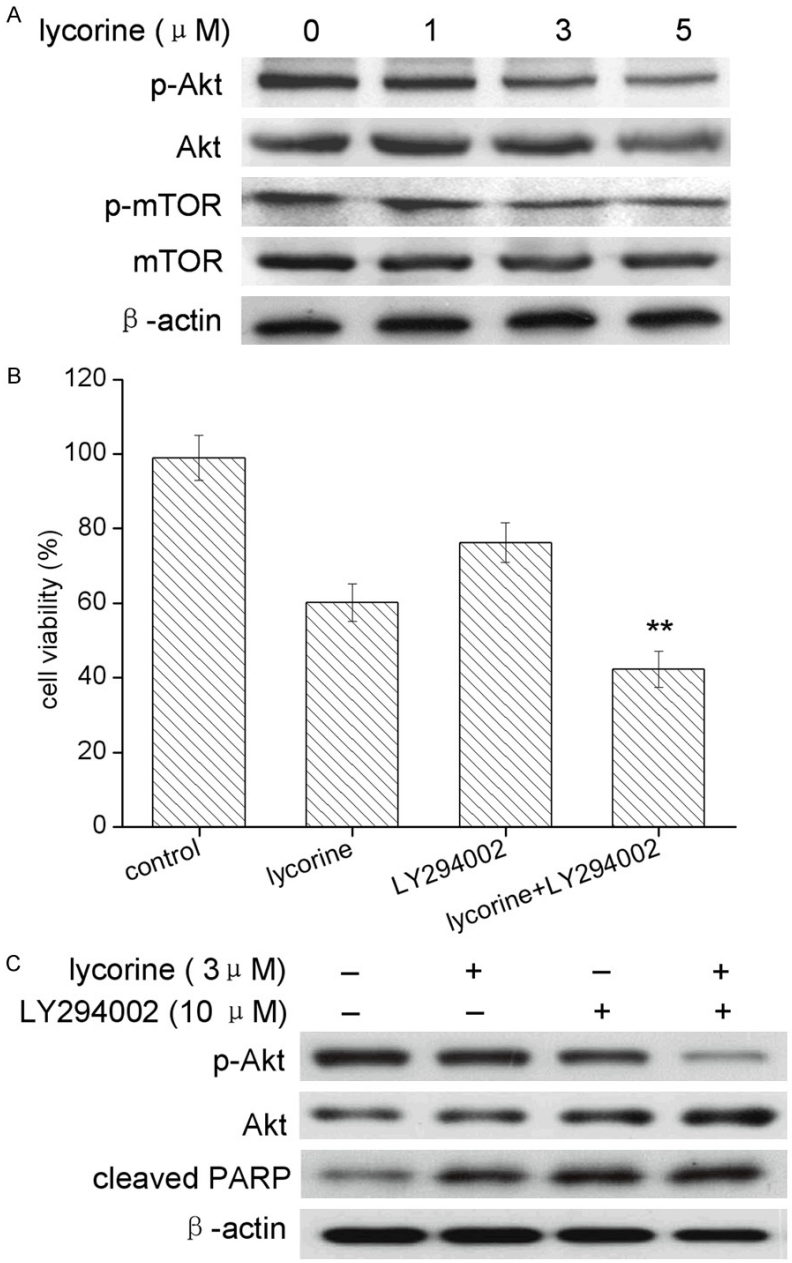

Figure 4. Lycorine inactivated the PI3K/Akt/mTOR signaling pathway in PANC-1 cells. (A) PANC-1 cells were treated with 0 μM, 1 μM and 3 μM lycorine for 24 h and the expression of p-Akt, Akt, p-mTOR and m-TOR was determined by western blot assay. (B and C) Cells were pretreated with LY294002 for 1 h, then treated PANC-1 cells with 3 μM lycorine for 24 h. The cell viability (B) and the expression of p-Akt, Akt and PARP (C) were detected. Data were represented as means ± SD. $^{**}P < 0.01$ vs. cells treated with lycorine alone.

Antitumor activity in vivo

Animal experiments were conducted in accordance with the Bioethics Committee guidelines in Yantaishan Hospital. Six-week-old BALB/c athymic nude mice obtained from Shanghai SLAC Laboratory Animal Co. Ltd (Shanghai, China) were established as a xenograft tumor model of PANC-1. PANC-1 cells (5×10^6 cells) were inoculated subcutaneously into the dorsal area of the mice. When the tumors grew to approximately 100 mm^2, the mice were randomly assigned into 3 groups (n = 8, saline control group, low dose lycorine group and high dose lycorine group) and injected intraperitoneally with the following treatments: the same volume saline, 10 mg/kg/2 day lycorine and 20 mg/kg/2 day lycorine, respectively. Tumor growth was measured with a microcaliper every 3 days throughout the experiment. Animals were sacrificed at 21 days after cancer cells inoculation. The implanted sarcomas were separated and weighed. Tumor volume was calculated as the following: volume = $d^2 \times D/2$, where d and D were the shortest and the longest diameters, respectively.

Statistical analysis

Data were presented as means ± SD. The SPSS19.0 software package was used to perform all statistical analysis. Comparisons between two group were using the Student's test and between multiple group using ANOVA analysis. The level of statistical significance was set at $P < 0.05$.

Results

Lycorine inhibited proliferation of PANC-1 cells

To determine the appropriate inhibitory concentrations of lycorine, PANC-1 cells were treated with different concentrations (0~10 μM) of

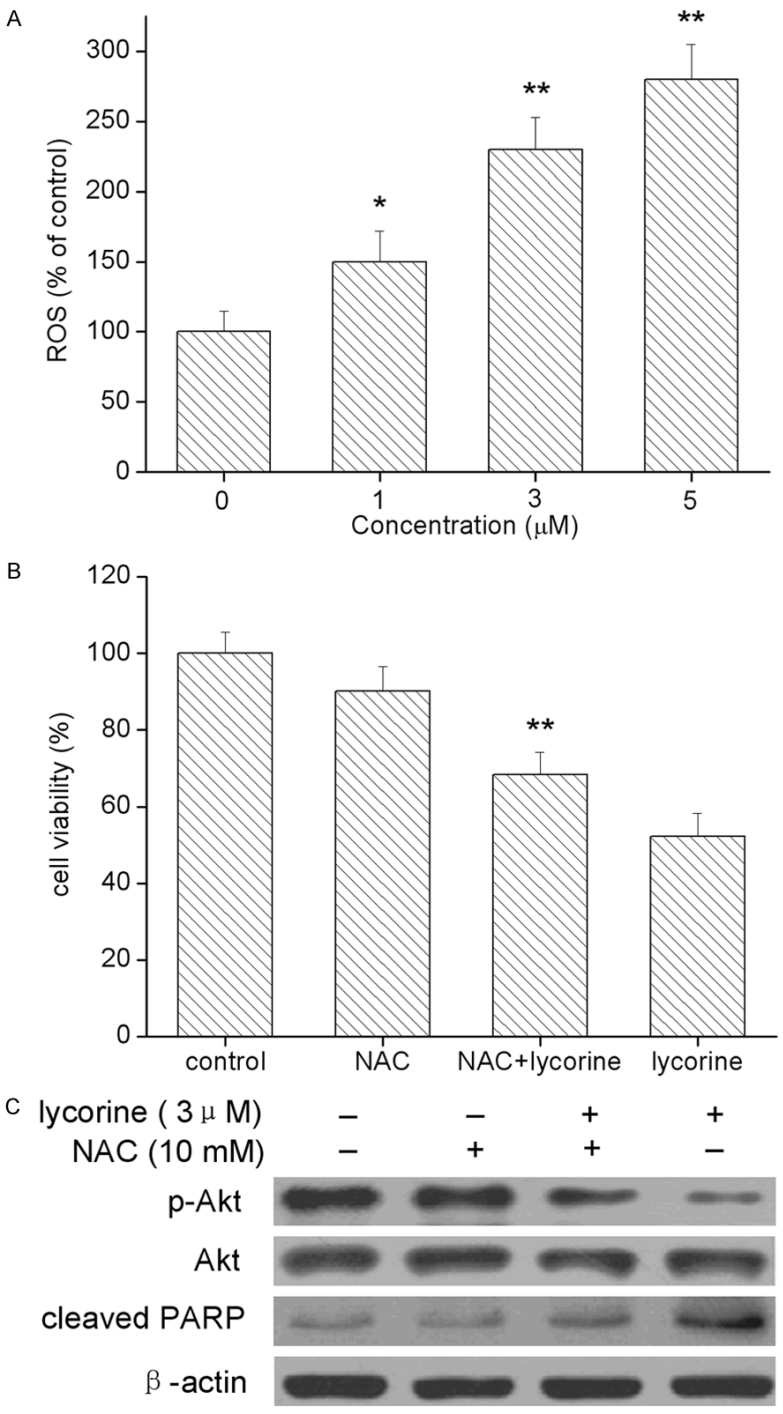

Figure 5. Lycorine induced apoptosis via the generation of ROS in PANC-1 cells. (A) PANC-1 cells were treated with 0 μM, 1 μM and 3 μM lycorine for 24 h, the generation of ROS was determined. Data were represented as means ± SD. **P* < 0.05 and ***P* < 0.01 vs. control cells. (B and C) Cells were pretreated with 10 mM NAC for 1 h, then treated PANC-1 cells with 3 μM lycorine for 24 h. The cell viability (B) and the expression of p-Akt, Akt and cleavage of PARP (C) were detected. ***P* < 0.01 vs. cells treated with lycorine alone.

lycorine for 24 h and the viability of cells were assessed by MTT assay. As demonstrated in **Figure 2**, lycorine inhibited the viability of PANC-1 cells in a dose-dependent manner and the 50% inhibitory concentration (IC_{50}) value was 3~5 μM ($P < 0.05$ for the lycorine-treated group compared with the control). Therefore, we chose lycorine at concentrations of 0, 1 μM, 3 μM and 5 μM for the subsequent experiments.

Lycorine induced apoptosis of PANC-1 cells

As illustrated in **Figure 3A**, lycorine induced clearly down-regulations of anti-apoptotic proteins Bcl-2, and upregulation of proapoptotic protein Bax, indicating the apoptosis-promoting effect of lycorine was partly attributed to up-regulating Bax/Bcl-2 protein ratio. In addition, exposure of cells to different concentrations of lycorine induced cleavage of caspase-3, caspase-9 and PARP. Lycorine treatment also triggered increasing release of cytochrome *c* from the mitochondria of PANC-1 cells without affecting caspase-8, suggesting that the intrinsic apoptosis (mitochondrial apoptosis) instead of extrinsic apoptosis was induced. To ascertain if caspase-dependent apoptosis was induced, the pan-caspase inhibitor z-VAD was used. As demonstrated in **Figure 3B** and **3C**, pre-treatment with z-VAD significantly attenuated cell apoptosis induced by lycorine treatment and suppressed the cleavage of caspase-3 and PARP, implying lycorine induced caspase-dependent apoptosis in PANC-1 cells.

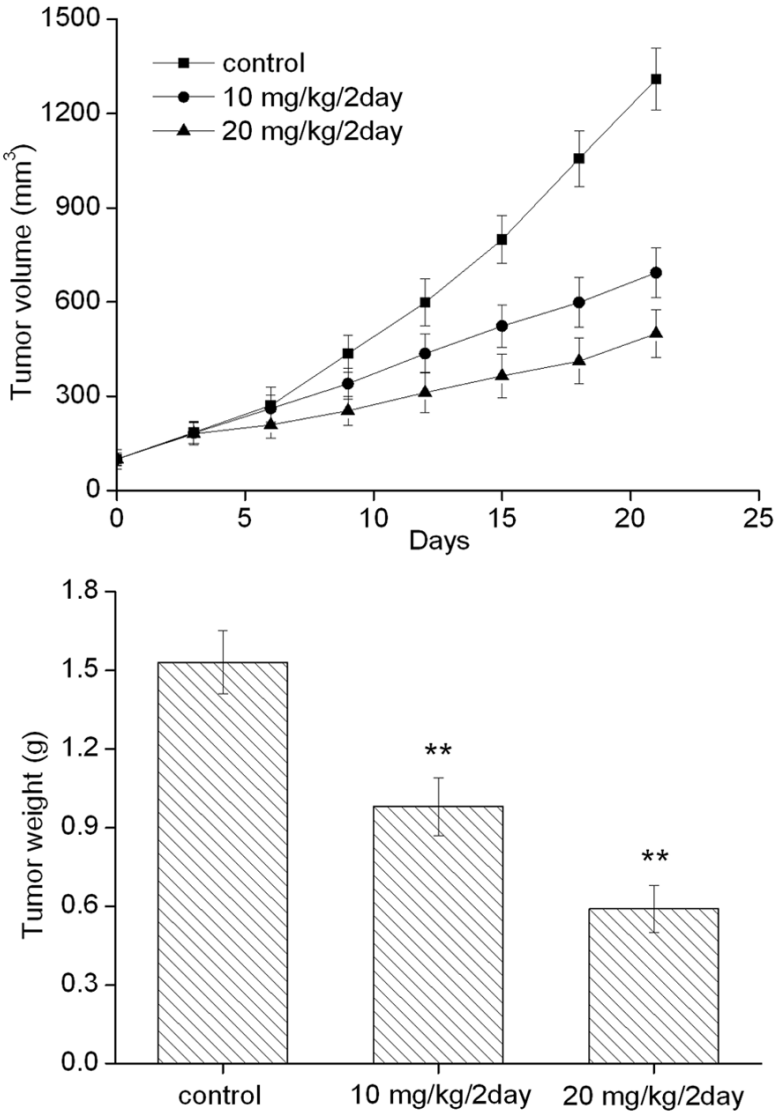

Figure 6. Lycorine showed antitumor activity on PANC-1 tumor xenografts. Tumor volume of PANC-1 was measured on the indicated days. Tumor weight was measured on the final day. Data are expressed as means ± SD, n = 8. $^{**}P < 0.01$ compared with control group.

Lycorine suppressed the PI3K/Akt/mTOR pathway in PANC-1 cells

As demonstrated in **Figure 4A**, lycorine administration decreased phosphorylation of Akt, mTOR in a dose-dependent manner. To further confirm the role of PI3K/Akt/mTOR pathway in lycorine treatment-induced apoptosis, cells were pretreated with PI3K kinase inhibitor LY294002. As manifested in **Figure 4B** and **4C**, pretreatment of LY294002 further promoted lycorine-induced the dephosphorylation of Akt, cell death and cleavage of PARP, suggesting that lycorine-induced apoptosis was mediated through the PI3K/Akt/mTOR pathway.

Lycorine elevated the level of ROS in PANC-1 cells

ROS involved in the regulation of cell apoptosis if generated in excessive amounts [17]. Intracellular ROS generation plays crucial role in the apoptosis of cancer cells triggered by various anticancer agents [18]. Recently, ROS was demonstrated to be related to apoptosis induced by antineoplastic drugs and may be upstream of PI3K/Akt/mTOR signaling pathways [19]. In this study, we investigated whether excessive generation of ROS was involved in lycorine induced apoptosis. As demonstrated in **Figure 5A**, lycorine treatment elevated the generation of ROS in a dose-dependent manner. Furthermore, pretreatment of cells with the ROS scavenger NAC not only abrogated lycorine induced ROS generation but also rescued cell from lycorine-induced apoptosis. In addition, pretreatment with NAC resulted in the reversal of Akt inhibition and PARP cleavage induced by lycorine (**Figure 5B** and **5C**). Collectively, lycorine-induced apoptosis and inactivation of PI3K/Akt/mTOR signaling pathway was partly modulated by ROS.

Lycorine demonstrated antitumor activity in vivo

In order to translate the results reported above *in vivo*, a xenograft model with subcutaneously implanted PANC-1 cells was established. As demonstrated in **Figure 6**, lycorine significantly reduced tumor volume in mice compared with

the control group. Meanwhile, mean tumor weight in mice treated with lycorine was lower than that in control group, implying that lycorine possesses potent antitumor activity. Meanwhile, the body weight of mice treated with lycorine has not significantly different from those in the control group, which was consistent with the notion that lycorine preferably targets tumor cells and thus exhibited little toxicity (Supplemental Table 1).

Discussion

Pancreatic cancer is a common cancer of the digestive system and is a leading cause of morbidity and mortality worldwide. Since toxicity and limited efficacy are common disadvantages of the antitumor drugs, identification of the new agents with low toxicity is very useful change for treatment cancer. Lycorine is a natural anti-tumor alkaloid extracted from Amaryllidaceae and has various biological effects on malignant cells. In the present study, we found that lycorine demonstrated the antitumor effects *in vivo* and *in vitro* by inducing apoptosis in pancreatic cancer cell line PANC-1.

Apoptosis is a programmed cell death mechanism that can be driven by two major apoptotic pathways: the cell death receptor-mediated extrinsic pathway and the mitochondrial-mediated intrinsic pathway [20]. Caspases, a family of cysteine proteases, play an important role in the induction of apoptosis through cleavage of different substrates [21, 22]. The mitochondrial mediated apoptotic pathway causes cytochrome *c* release from mitochondria, and the cleavage of executioner caspase-3, ultimately resulting in chromatin condensation, DNA fragmentation and the formation of apoptotic bodies [23]. Our results demonstrated that lycorine treatment released cytochrome *c* from mitochondria into cytoplasm as well as activated and induced cleavage of caspase-3, caspase-9 and PARP, but not caspase-8, indicating an activation of the mitochondrial-mediated intrinsic pathway. Bcl-2 family members located on the mitochondrial membrane are involved in the regulation of intrinsic apoptosis pathway by altering the permeability of the mitochondrial membrane and triggering the release of cytochrome *c* [24]. To further confirm the involvement of the mitochondrial pathway in lycorine induced apoptotic death, the expression of Bax and Bcl-2 were evaluated. These results indicated that upregulation of Bax and Bax/Bcl-2 ratio as well as downregulation Bcl-2 and may be involved in the release of cytochrome *c* from mitochondria into cytosol after lycorine treatment.

The PI3K/Akt/mTOR signaling pathway is the major pathway that regulates cell proliferation, differentiation, apoptosis, and chemotherapy resistance of tumor cells and is one of the most commonly deregulated pathways in cancer [25]. The constitutive activation of this pathway is considered to be corrected with aggravated clinical chemoresistance and poor prognosis for pancreatic cancer patients [26]. Suppression of the PI3K/Akt/mTOR signaling pathway has been extensively investigated for anticancer therapy [27]. The key molecules involved in PI3K/Akt/mTOR pathway include PI3K, PTEN, Akt and mTOR [28]. Akt is an evolutionarily conserved serine/threonine kinase, located mainly in the cytoplasm in resting status. The activated protein p-Akt is translocated into cytoplasm or nucleus, where it will further phosphorylate a series of substrates that may modulate mechanisms including protein synthesis and gene transcription and thus regulate the survival and apoptosis of cells [25, 29]. mTOR is a serine/threonine kinase of the PI3K kinase family and is considered to be a key effector of PI3K/AKT/mTOR pathway, which involves in regulating cell proliferation, survival and angiogenesis [28, 30]. Akt and mTOR are two key kinases in the PI3K downstream of PI3K/Akt/mTOR signaling pathway and often abnormally activated in many tumors [31, 32]. In the present study, we found that lycorine treatment decreased the phosphorylation of Akt and mTOR. However, pretreatment cells with LY294002 enhanced lycorine-triggered the dephosphorylation of Akt, cell apoptosis and cleavage of PARP, indicating that lycorine-induced apoptosis was regulated via the PI3K/Akt/mTOR pathway.

ROS is an important secondary messenger and normally exists in all cells in balance with biochemical antioxidants. Appropriate ROS levels are pivotal for cell survival, while excessive ROS could oxidize the mitochondrial pores, disrupt the mitochondrial membrane potential leading to cytochrome c release, and finally lead to cell death [33, 34]. The mitochondrial apoptotic signaling pathway has been described as an

important downstream event mediated by ROS in apoptotic cell death [35]. In the present study, lycorine administration increased ROS generation, while pretreatment of cells with ROS scavenger NAC reduced ROS generation, rescued cell from apoptosis, reversed Akt suppression and PARP cleavage induced by lyocorine. Taken together, these results indicated that lycorine treatment-induced apoptosis and inhibition of PI3K/Akt/mTOR signaling pathway were at least partly mediated by generation of ROS.

Conclusion

In summary, the current study demonstrated that lycorine exerts potent antitumor activity both in vitro and in vivo against pancreatic cancer and the underlying mechanisms of lycorine induced apoptosis may be through ROS generation to modulate the PI3K/Akt/mTOR signaling pathway. These results support the notion that lycorine could facilitate development of an effective strategy for pancreatic cancer.

Disclosure of conflict of interest

None.

Address correspondence to: Sanyuan Hu, Department of General Surgery, Qilu Hospital of Shandong University, No. 107 Wenhuaxi Road, Jinan 250012, China. Tel: 0531-82169114; E-mail: husy0120@163.com

References

[1] Li D, Xie K, Wolff R and Abbruzzese JL. Pancreatic cancer. Lancet 2004; 363: 1049-1057.

[2] Shi SQ and Cao HM. Shikonin promotes autophagy in BXPC-3 human pancreatic cancer cells through the PI3K/Akt signaling pathway. Oncol Lett 2014; 8: 1087-1089.

[3] Arlt A, Gehrz A, Müerköster S, Vorndamm J, Kruse ML, Fölsch UR and Schäfer H. Role of NF-κB and Akt/PI3K in the resistance of pancreatic carcinoma cell lines against gemcitabine-induced cell death. Oncogene 2003; 22: 3243-3251.

[4] Sabater L, Calvete J, Aparisi L, Cánovas R, Muñoz E, Añón R, Roselló S, Rodríguez E, Camps B, Alfonso R, Sala C, Sastre J, Cervantes A and Lledó S. Pancreatic and periampullary tumors: morbidity, mortality, functional results and long-term survival. Cir Esp 2009; 86: 159-166.

[5] Arlt A, Gehrz A, Müerköster S, Vorndamm J, Kruse ML, Fölsch UR and Schäfer H. Role of NF-κB and Akt/PI3K in the resistance of pancreatic carcinoma cell lines against gemcitabine-induced cell death. Oncogene 2003; 22: 3243-3251.

[6] Wang F, Li H, Yan XG, Zhou ZW, Yi ZG, He ZX, Pan ST, Yang YX, Wang ZZ, Zhang X, Yang T, Qiu JX and Zhou SF. Alisertib induces cell cycle arrest and autophagy and suppresses epithelial-to-mesenchymal transition involving PI3K/Akt/mTORand sirtuin 1-mediated signaling pathways in human pancreatic cancer cells. Drug Des Devel Ther 2015; 9: 575-601.

[7] Shrivastava S, Jeengar MK, Reddy VS, Reddy GB and Naidu VG. Anticancer effect of celastrol on human triple negative breast cancer: Possible involvement of oxidative stress, mitochondrial dysfunction, apoptosis and PI3K/Akt pathways. Exp Mol Pathol 2015; 98: 313-327.

[8] Berkov S, Codina C, Viladomat F and Bastida J. Alkaloids from galanthus nivalis. Phytochemistry 2007; 68: 1791-1798.

[9] Li SY, Chen C, Zhang HQ, Guo HY, Wang H, Wang L, Zhang X, Hua SN, Yu J, Xiao PG, Li RS and Tan X. Identification of natural compounds with antiviral activities against SARS-associated coronavirus. Antiviral Res 2005; 67: 18-23.

[10] Vrijsen R, Vanden Berghe DA, Vlietinck AJ and Boeye A. Lycorine: a eukaryotic termination inhibitor? J Biol Chem 1986; 261: 505-507.

[11] Li L, Dai HJ, Ye M, Wang SL, Xiao XJ, Zheng J, Chen HY, Luo YH and Liu J. Lycorine induces cell-cycle arrest in the G0/G1 phase in K562 cells via HDAC inhibition. Cancer Cell Int 2012; 12: 49.

[12] Liu J, Hu WX, He LF, Mao Y and Li Y. Effects of lycorine on HL-60 cells via arresting cell cycle and inducing apoptosis. FEBS Lett 2004; 578: 245-250.

[13] Liu J, Hu JL, Shi BW, He Y and Hu WX. Up-regulation of p21 and TNF-α is mediated in lycorine-induced death of HL-60 cells. Cancer Cell Int 2010; 10: 25.

[14] Yui S, Mikami M, Kitahara M and Yamazaki M. The inhibitory effect of lycorine on tumor cell apoptosis induced by polymorphonuclear leukocyte-derived calprotectin. Immunopharmacology 1998; 40: 151-162.

[15] Lamoral-Theys D, Andolfi A, Van Goietsenoven G, Cimmino A, Le Calve B, Wauthoz N, Megalizzi V, Gras T, Bruyere C, Dubois J, Mathieu, V, Kornienko A, Kiss R and Evidente A. Lycorine, the main phenanthridine amaryllidaceae alkaloid, exhibits significant antitumor activity in cancer cells that display resistance to proapoptotic stimuli: an investigation of structure-activity relationship and mechanistic insight. J Med Chem 2009; 52: 6244-6256.

[16] Liu XS, Jiang J, Jiao XY, Wu YE, Lin JH and Cai YM. Lycorine induces apoptosis and downregu-

lation of MCL-1 in human leukemia cells. Cancer Lett 2009; 274: 16-24.
[17] Li X, Zhu F, Jiang J, Sun C, Wang X, Shen M, Tian R, Shi C, Xu M, Peng F, Guo X, Wang M and Qin R. Synergistic antitumor activity of withaferin A combined with oxaliplatin triggers reactive oxygen species-mediated inactivation of the PI3K/AKT pathway in human pancreatic cancer cells. Cancer Lett 2015; 375: 219-230.
[18] Nogueira V and Hay N. Molecular pathways: reactive oxygen species homeostasis in cancer cells and implications for cancer therapy. Clin Cancer Res 2013; 19: 4309-4314.
[19] Zhang L, Wang H, Xu J, Zhu J and Ding K. Inhibition of cathepsin S induces autophagy and apoptosis in human glioblastoma cell lines through ROS-mediated PI3K/AKT/mTOR/p70-S6K and JNK signaling pathways. Toxicol Lett 2014; 228: 248-259.
[20] Ghobrial IM, Witzig TE and Adjei AA. Targeting apoptosis pathways in cancer therapy. CA Cancer J Clin 2005; 55: 178-194.
[21] Ghavami S, Hashemi M, Ande SR, Yeganeh B, Xiao W, Eshraghi M, Bus CJ, Kadkhoda K, Wiechec E, Halayko AJ and Los M. Apoptosis and cancer: mutations within caspase genes. J Med Genet 2009; 46: 497-510.
[22] Philchenkov A. Caspases: potential targets for regulating cell death. J Cell Mol Med 2004; 8: 432-444.
[23] Shrivastava S, Jeengar MK, Reddy VS, Reddy GB and Naidu VG. Anticancer effect of celastrol on human triple negative breast cancer: Possible involvement of oxidative stress, mitochondrial dysfunction, apoptosis and PI3K/Akt pathways. Exp Mol Pathol 2015; 98: 313-327.
[24] Wang TE, Wang YK, Jin J, Xu BL and Chen XG. A novel derivative of quinazoline, WYK431 induces G2/M phase arrest and apoptosis in human gastric cancer BGC823 cells through the PI3K/Akt pathway. Int J Oncol 2014; 45: 771-781.
[25] Yu GP, Huang B, Chen GQ and Mi YD. Phosphatidylethanolamine-binding protein 4 promotes lung cancer cells proliferation and invasion via PI3K/Akt/mTOR axis. J Thorac Dis 2015; 7: 1806-1816.
[26] Mu GG, Zhang LL, Li HY, Liao Y and Yu HG. Thymoquinone pretreatment overcomes the insensitivity and potentiates the antitumor effect of gemcitabine through abrogation of Notch1, PI3K/Akt/mTOR regulated signaling pathways in pancreatic cancer. Dig Dis Sci 2015; 60: 1067-1080.
[27] Li H, Hu J, Wu S, Wang L, Cao X, Zhang X, Dai B, Cao M, Shao R, Zhang R, Majidi M, Ji L, Heymach JV, Wang M, Pan S, Minna J, Mehran RJ, Swisher SG, Roth JA and Fang B. Auranofin-mediated inhibition of PI3K/AKT/mTOR axis and anticancer activity in non-small cell lung cancer cells. Oncotarget 2016; 7: 3548-58.
[28] Zhang L, Wu J, Ling MT, Zhao L and Zhao KN. The role of the PI3K/Akt/mTOR signalling pathway in human cancers induced by infection with human papillomaviruses. Mol Cancer 2015; 14: 87.
[29] Guo H, German P, Bai S, Barnes S, Guo W, Qi X, Lou H, Liang J, Jonasch E, Mills GB and Ding Z. The PI3K/Akt pathway and renal Cell carcinoma. J Genet Genomics 2015; 42: 343-353.
[30] Lee DF, Kuo HP, Chen CT, Hsu JM, Chou CK, Wei Y, Sun HL, Li LY, Ping B, Huang WC, He X, Hung JY, Lai CC, Ding Q, Su JL, Yang JY, Sahin AA, Hortobagyi GN, Tsai FJ, Tsai CH, Hung MC. IKK beta suppression of TSC1 links inflammation and tumor angiogenesis via the mTOR pathway. Cell 2007; 130: 440-455.
[31] Song BQ, Chi Y, Li X, Du WJ, Han ZB, Tian JJ, Li JJ, Chen F, Wu HH, Han LX, Lu SH, Zheng YZ and Han ZC. Inhibition of Notch signaling promotes the adipogenic differentiation of mesenchymal stem cells through autophagy activation and PTENPI3K/AKT/mTOR pathway. Cell Physiol Biochem 2015; 36: 1991-2002.
[32] Xing XF, Zhang LH, Wen XZ, Wang XH, Cheng XJ, Du H, Hu Y, Li L, Dong B, Li ZY and Ji JF. PP242 suppresses cell proliferation, metastasis, and angiogenesis of gastric cancer through inhibition of the PI3K/AKT/mTOR pathway. Anticancer Drugs 2014; 25: 1129-1140.
[33] Zamzami N, Marchetti P, Castedo M, Decaudin D, Macho A, Hirsch T, Susin SA, Petit PX, Mignotte B and Kroemer G. Sequential reduction of mitochondrial transmembrane potential and generation of reactive oxygen species in early programmed cell death. J Exp Med 1995; 182: 367-377.
[34] Zhao Y, Wang X, Sun Y, Zhou Y, Yin Y, Ding Y, Li Z, Guo Q and Lu N. LYG-202 exerts antitumor effect on PI3K/Akt signaling pathway in human breast cancer cells. Apoptosis 2015; 20: 1253-1269.
[35] Ma Q, Fang H, Shang W, Liu L, Xu Z, Ye T, Wang X, Zheng M, Chen Q and Cheng H. Superoxide flashes: early mitochondrial signals for oxidative stress-induced apoptosis. J Biol Chem 2011; 286: 27573-27581.

Supplemental Table 1. Body weight of mice treated with or without lycorine

Group	Body weight (g)	
	Initial	Final
Control	18.08 ± 0.79	24.89 ± 2.46
Lycorine (10 mg/kg)	18.19 ± 0.62	23.78 ± 3.12
Lycorine (20 mg/kg)	18.91 ± 0.83	21.49 ± 3.56

Data are mean ± SD for 8 mice.