Int J Clin Exp Med 2020;13(11):8429-8437
www.ijcem.com /ISSN:1940-5901/IJCEM0116921

Original Article

Polyphyllin A suppresses growth and promotes apoptosis by partially decreasing CIP2A expression in lymphoma cell lines

Liang Zhang[1,2*], Yangyang Liu[1,2*], Yuan Si[1,2,3], Yunhe Chen[1], Dan Cheng[1], Lan Jin[1,3], Ying Liu[1,2,3], Yang Guo[1,2,3]

[1]Institute of Basic Medical Sciences, Hubei University of Medicine, Shiyan, Hubei, China; [2]Hubei Key Laboratory of Wudang Local Chinese Medicine Research and Institute of Medicinal Chemistry, Hubei University of Medicine, Shiyan, Hubei, China; [3]Biomedical Research Institute, Hubei University of Medicine, Shiyan, Hubei, China. [*]Equal contributors.

Received June 24, 2020; Accepted September 8, 2020; Epub November 15, 2020; Published November 30, 2020

Abstract: Abnormally high expression of CIP2A (cancerous inhibitor of protein phosphatase 2A) causes poor prognosis and promotes the malignant growth and development of a variety of solid and hematological tumors. Polyphyllin A (PPA) is a bioactive compound isolated from the *Paris polyphylla* that has inhibitory effects on several cancers. However, there are few reports on the effects of PPA on human lymphoma cells. Here, we determined the inhibitory effects of PPA on lymphoma cells by CCK-8 and cell counting assays. Flow cytometry and western blot assays were performed to assess apoptosis. Our data indicated that PPA inhibited growth and promoted apoptosis in the Raji and Jurkat lymphoma cell lines. PPA significantly downregulated CIP2A expression. PPA increased protein phosphatase 2A activity and decreased Akt phosphorylation by reducing CIP2A. Furthermore, low CIP2A expression promoted PPA-induced lymphoma cell growth inhibition and apoptosis, while CIP2A overexpression antagonized these effects. These data suggested that PPA suppresses the growth and promotes the apoptosis of lymphoma cells via the CIP2A/PP2A/Akt signaling pathways. Therefore, PPA is expected to become a potential anticancer drug for the treatment of lymphoma.

Keywords: Polyphyllin A, lymphoma, CIP2A, apoptosis

Introduction

Malignant lymphoma is a large heterogeneous tumor. Lymphoma occurs in the lymph nodes, but because of the distribution of the lymphatic system throughout the body, lymphoma is a systemic disease that can affect almost all the tissues and organs in the body [1]. In 2018, there were approximately 589,530 new lymphoma cases and 274,891 lymphoma-related deaths worldwide [2]. Nearly 90% of lymphoma cases originate from B cells, but lymphoma can also be derived from T cells or natural killer cells [3]. Currently, chemotherapy, radiotherapy, peripheral blood stem cell transplantation, rituximab and targeted therapy are used to treat lymphoma. However, there are still some more aggressive types of lymphoma with lower survival rates [4]. Therefore, the discovery of new effective treatments is necessary to extend the survival time of lymphoma patients.

Cancerous inhibitor of protein phosphatase 2A (CIP2A) is a known oncogene that inhibits the phosphatase activity of protein phosphatase 2A (PP2A) [5]. CIP2A is abundantly expressed in many solid malignancies and is associated with tumorigenesis, chemotherapy and apoptosis resistance, and poor prognosis [6-9]. We previously reported that abnormally high expression of CIP2A is also related to poor prognosis in acute myeloid leukemia and multiple myeloma [10, 11]. In 2013, Lilja L. et al. reported upregulated expression of CIP2A in B cell lymphoma [12]. The molecular biological function of CIP2A and the underlying mechanisms in lymphoma remain unclear. Therefore, it is urgent to conduct in-depth studies to explore the underlying mechanisms of CIP2A activation in lymphoma cells.

Natural products derived from traditional Chinese herbal medicines are still an important

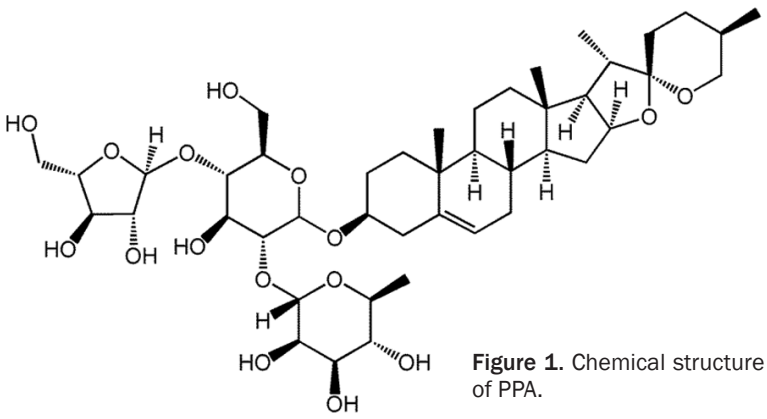

Figure 1. Chemical structure of PPA.

resource for oncology drug discovery. The *Paris polyphylla* is a member of the Liliaceae family and is a traditional Chinese herbal medicine. *P. polyphylla* has been used to treat infectious diseases and tumors [13]. This species is widely distributed in China's Yunnan Province and the Shennongjia National Nature Reserve. The root of *P. polyphylla,* called Chonglou (Rhizoma Paridis), has been used to treat sore throat, mumps, snake bites, hemostasis, and malignancies [14, 15]. One of the main active ingredients of Choulou, namely polyphyllin A (PPA, **Figure 1**), exerts anticancer effects in several tumor types [16-18]. This article aims to explore the toxicity of PPA, and the mechanism, in human lymphoma cells.

Material and methods

Ethics statement

All the studies were performed according to the protocol approved by Hubei University of Medicine (Institutional Review Board No.: 2019-TH-052). All the peripheral blood samples were collected after written informed consent was obtained from healthy volunteers.

Reagents

PPA (purity ≥ 95%) was purchased from Yuanye Bio-Technology Co., Ltd. (Shanghai, China). The compound was dissolved in DMSO at a stock solution of 4 mM.

Cell culture

The human B lymphoma Raji and Jurkat cell lines, and the T cell leukemia Molt-4 cell line were obtained from ATCC (Manassas, VA, USA). The cells were cultured in Dulbecco's RPMI 1640 medium (Gibco BRL, Grand Island, NY, USA) supplemented with 10% or 15% FBS (Gibco BRL) and antibiotics.

Peripheral blood mononuclear cells (PBMCs)

PBMCs were isolated from the peripheral blood of 3 healthy volunteers by Ficoll-Hipaque density sedimentation.

Cell viability and morphology

Cells were seeded in 96-well plates at 1×10^4 cells/well, pre-cultured for 4 h, and then treated with PPA. Cytotoxicity was detected by the CCK-8 assay. Cell viability was detected by trypan blue dye exclusion. Cell morphology was detected by Wright-Giemsa staining.

Apoptosis assay

Cells were harvested and stained with an AV-FITC kit (BD Biosciences, San Jose, CA, USA), and apoptosis was detected by flow cytometry using a BD FACScanto II flow cytometer (Becton Dickinson, San Jose, CA) [16, 19].

Western blot

Cells were collected and lysed with RIPA buffer. The protein lysates were isolated by SDS-PAGE. The gel was then transferred to a PVDF membrane (Millipore, Kenilworth, NJ, USA). The primary antibodies used were anti-CIP2A (1:500 dilution; catalog no. sc-80662), anti-phospho-Akt (S473) (1:500 dilution; catalog no. sc-7985), anti-Akt (1:500 dilution; catalog no. sc-8312) (Santa Cruz Biotechnology, Santa Cruz, CA, USA), anti-caspase-3 (1:1000 dilution; catalog no. 9662), anti-PARP (1:1000 dilution; catalog no. 9542), anti-PP2A (1:1000 dilution; catalog no. 2038) (Cell Signaling Technology, Danvers, MA, USA), and anti-GAPDH (1:5000 dilution; catalog no. M20006; Abmart, Shanghai, China). The blots were then washed, and incubated with a horseradish peroxidase (HRP)-conjugated secondary antibody (1:10000 dilution; catalog no. E030120-01 (rabbit) and E030110-01 (mouse); EarthOx, LLC, San Francisco, CA, USA) at room temperature for 1.5 h. Detection was performed using the SuperSignal® West Pico Trial kit (Pierce Biotechnology, Inc., Rockford, IL, USA). The defined sections of the

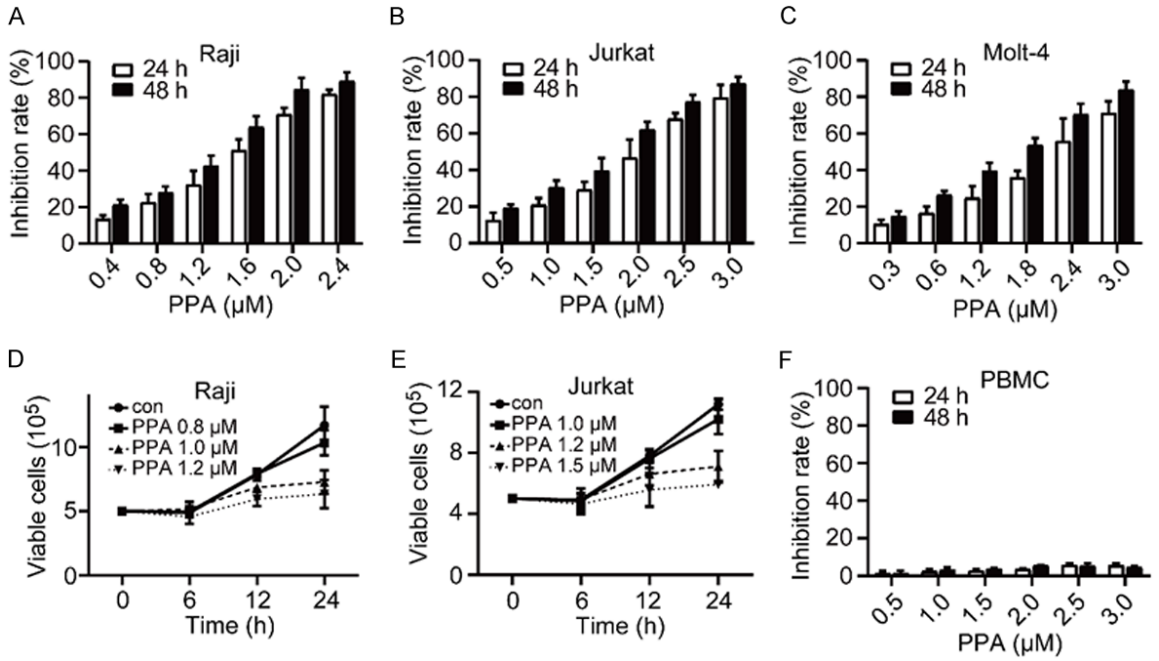

Figure 2. PPA inhibits the growth of lymphocyte cell lines. A-C. The inhibitory effects of PPA on the Raji, Jurkat, and Molt-4 cell lines were investigated at 24 h and 48 h by the CCK-8 assay. D, E. The effects of PPA on Raji and Jurkat cell growth were investigated by trypan blue exclusion analysis. F. The CCK-8 assay was used to detect the cytotoxicity of PPA on PBMCs of 3 healthy volunteers at 48 h.

film were scanned to capture the images, and quantification was performed using Adobe Photoshop software (CS4, Adobe Systems Incorporated, USA) and ImageJ software (National Institutes of Health, Bethesda, MD, USA).

PP2A activity assay

A PP2A phosphatase assay kit (Upstate, Temecula, CA) was used according to the manufacturer's instructions [20].

RNA interference

siRNA targeting CIP2A (Biomics, Jiangsu, China) was transfected into the cells by using HiPerFect Transfection Reagent (Qiagen). 24 h after transfection, the cells were treated with or without PPA for the indicated times and then harvested for western blot and CCK-8 assays. The siRNA sequences were as follows: 5'-CUGUGGUUGUGUUUGCACUTT-3' (CIP2A siRNA1), 5'-ACCAUUGAUAUCCUUAGAATT-3' (CIP2A siRNA2), 5'-UUCUCCGAACGUGUCACGUTT-3' [negative control (NC) siRNA] [21].

Transfection with overexpression plasmid

The pOTENT-1-CIP2A expression plasmid was purchased from Youbio Co. (Changcha, China). Transfection of the plasmid into Raji and Jurkat cells was carried out using Lipofectamine 3000 transfection reagent (Invitrogen).

Statistical analysis

SPSS 22.0 (IBM Corp., Armonk, NY, USA) and GraphPad Prism 8.0 (GraphPad Software, Inc., La Jolla, CA, USA) were utilized for all the statistical analyses. The results were analyzed using unpaired Student's t-test or one-way analysis of variance followed by Bonferroni posttest. Differences were considered statistically significant when $P < 0.05$. All the experiments were repeated at least three times.

Results

PPA suppresses the growth of lymphoma cell lines

The human lymphocyte cell lines Raji, Jurkat, and Molt-4 were treated with PPA (**Figure 2A-C**). The results indicated that PPA exerted a significant cytotoxic effect on the Raji, Jurkat, and Molt-4 cells, with 24 h IC_{50} values of 1.78 μM, 2.25 μM, and 2.39 μM, respectively. Trypan blue exclusion analysis indicated that PPA reduced the viability of the Raji (**Figure 2D**) and Jurkat (**Figure 2E**) cells. PPA exhibited relatively

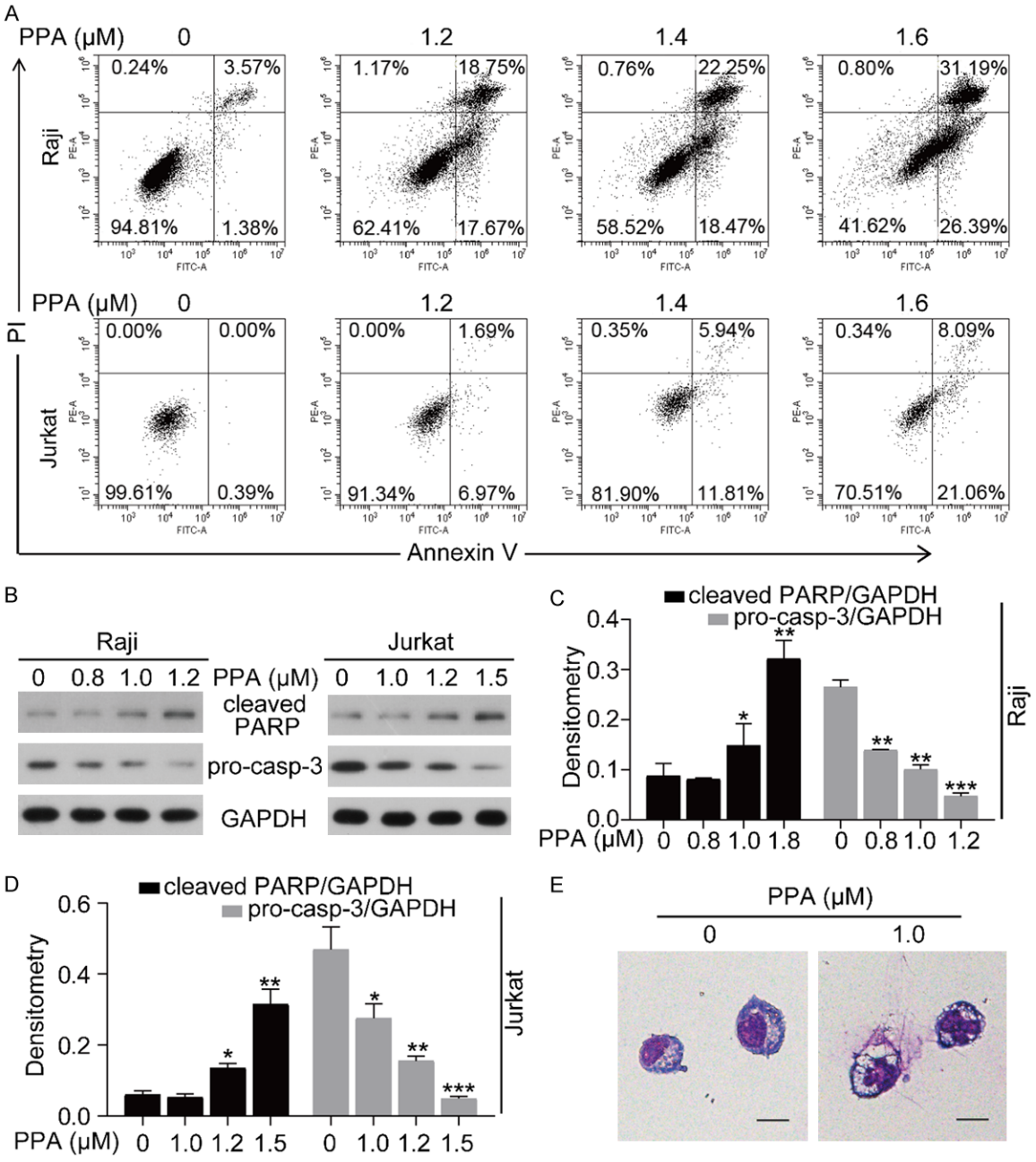

Figure 3. PPA promotes caspase-mediated apoptosis in lymphoma cells. A. Raji or Jurkat cells were treated with PPA for 24 h and then analyzed by annexin V/PI staining and flow cytometry. B-D. Raji or Jurkat cells were treated with PPA for 24 h and then subjected to western blot. E. Raji cells were treated with PPA for 24 h and then stained with Giemsa staining. Scale bar = 100 μm. Each data point represents the mean ± SD of three independent experiments that were analyzed with a T-test. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

low toxicity in normal PBMCs (**Figure 2F**), suggesting that it has a relatively specific inhibitory effect on lymphoma cells.

PPA promotes caspase-mediated apoptosis in lymphoma cell lines

We evaluated whether PPA can induce apoptosis in lymphoma. Apoptotic cells were analyzed using flow cytometry. PPA promoted an increase in the proportion of early apoptotic cells (annexin V^+/PI^-) (**Figure 3A**). After treatment with 1.6 μM PPA, the proportion of early apoptotic Raji cells increased from 1.38% to 26.39%, and the proportion of apoptotic Jurkat cells increased from 0.39% to 21.06%. Furthermore, western blot analysis suggested that PPA significantly decreased the levels of pro-caspase-3 (pro-

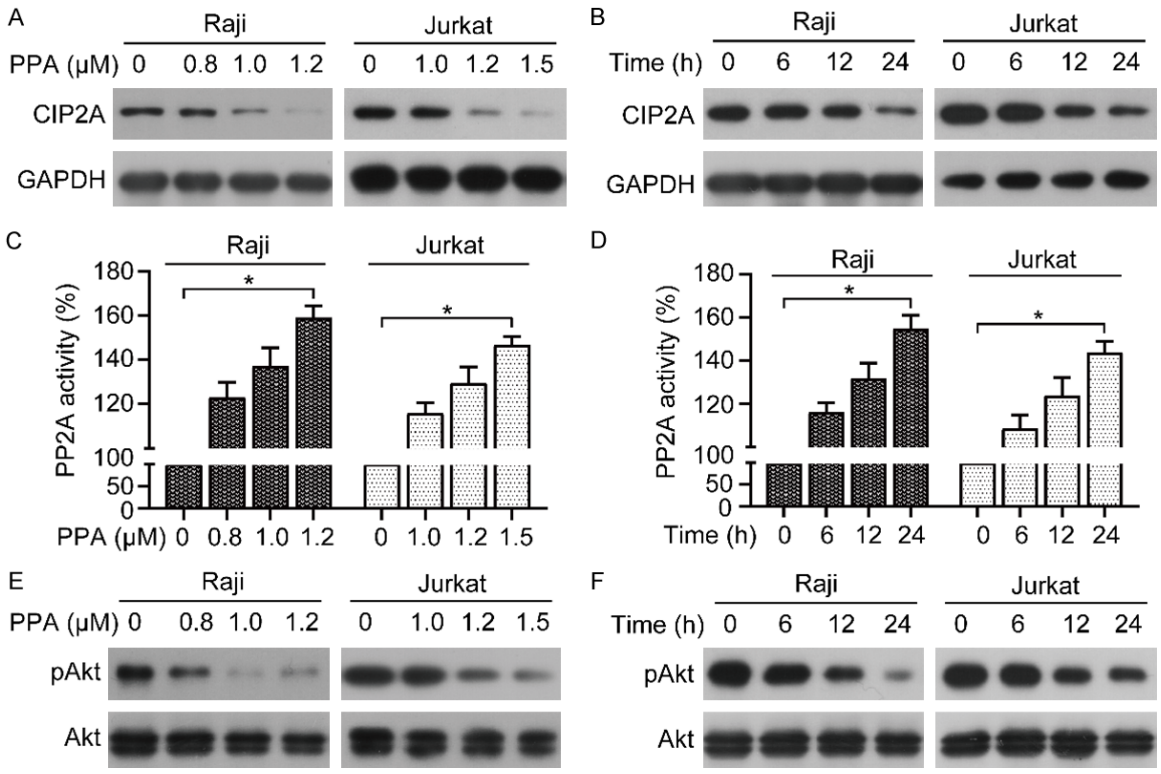

Figure 4. PPA suppresses the CIP2A/PP2A/Akt signaling axis. A. Raji or Jurkat cells were treated with PPA for 24 h, and then, the expression of the indicated proteins were determined via western blot. B. Raji (or Jurkat) cells were treated with 1.0 μM (or 1.2 μM) PPA for the indicated times and then subjected to western blot. C. Raji or Jurkat cells were treated with the indicated doses of PPA for 24 h and subsequently subjected to a PP2A activity assay. D. Raji (or Jurkat) cells were treated with 1.0 μM (or 1.2 μM) PPA for the indicated times and subsequently subjected to a PP2A activity assay. E. Raji or Jurkat cells were treated with PPA for 24 h and then subjected to western blot. F. Raji (or Jurkat) cells were treated with 1.0 μM (or 1.2 μM) PPA for the indicated times, and the cell lysates were analyzed by western blot. Each data point represents the mean ± SD of three independent experiments that were analyzed with a T-test. *, $P < 0.05$.

casp-3) and cleaved PARP in the Raji and Jurkat cells (**Figure 3B-D**). Additionally, significant apoptotic morphological features, such as cell membrane shrinkage, chromatin concentration in the nucleus, and DNA fragmentation, were observed in Raji cells treated with PPA (**Figure 3E**). These results indicated that PPA promoted caspase-mediated apoptosis in lymphoma cell lines.

PPA suppresses CIP2A/PP2A/Akt signal axis in lymphoma cell lines

CIP2A is a well-known oncoprotein in several types of cancer. Several natural antitumor compounds have been reported to downregulate CIP2A expression [9, 22]. We detected whether PPA altered CIP2A expression. With increasing doses, PPA downregulated CIP2A expression in Raji and Jurkat cells. Additionally, we demonstrated that PPA decreased CIP2A expression in a time-dependent manner (**Figure 4A**, **4B**). CIP2A inhibits PP2A by directly binding to it [23]. Next, we showed that PP2A activity in the PPA-treated Raji and Jurkat cells was significantly increased (**Figure 4C**, **4D**). Abnormal inactivation of PP2A leads to activation of its downstream molecule Akt [23]. Furthermore, we showed that PPA reduced the levels of phosphorylated Akt (pAkt) in Raji and Jurkat cells. Moreover, the levels of the total Akt protein changed slightly (**Figure 4E**, **4F**). These results indicated that PPA downregulates CIP2A to reactivate PP2A and suppress Akt phosphorylation in lymphoma cell lines.

Decreased CIP2A expression is necessary for PPA to suppress proliferation and promote apoptosis in lymphoma cell lines

We further examined the effects of low expression of CIP2A on the inhibition of growth and

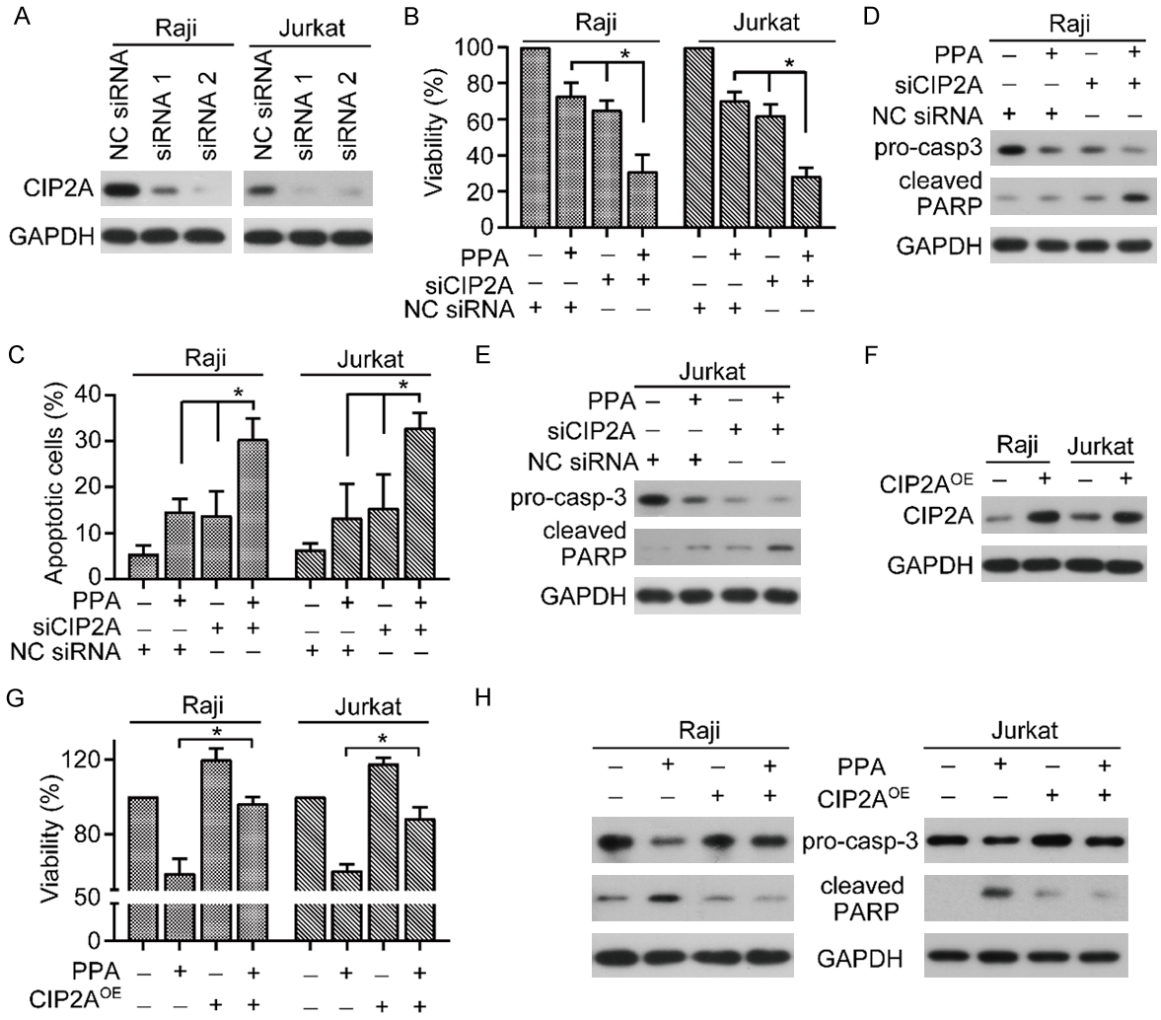

Figure 5. A decrease in CIP2A expression is necessary for PPA to suppress the proliferation and promote the apoptosis of lymphoma cell lines. A. Raji or Jurkat cells were transfected with siRNA for 48 h and then subjected to western blot analysis. B. Raji or Jurkat cells were transfected with siRNA for 48 h and then subjected to a CCK-8 assay. C. Raji or Jurkat cells were transfected with siRNA for 24 h and then subjected to PPA (1.0 μM) treatment for 24 h. Cell apoptosis was examined by annexin V/PI staining and flow cytometry. D, E. Raji or Jurkat cells were transfected with siRNA for 24 h, subsequently subjected to PPA (1.0 μM) treatment for 24 h, and examined by western blot. F. Raji or Jurkat cells were transfected with the pOTENT-1-CIP2A expression plasmid for 48 h and then subjected to western blot analysis. G. Raji or Jurkat cells were transfected with the pOTENT-1-CIP2A expression plasmid for 24 h and then subjected to a CCK-8 assay. H. Raji (or Jurkat) cells were transfected with $CIP2A^{OE}$, subsequently subjected to 1.0 μM PPA (or 1.2 μM) treatment for 24 h and analyzed by western blot. Each data point represents the mean ± SD of three independent experiments that were analyzed with a T-test. *, $P < 0.05$.

induction of apoptosis by PPA. Two siRNAs targeting CIP2A were synthesized, and lymphoma cells transfected with these siRNA exhibited reduced CIP2A expression (**Figure 5A**). We further examined the role of CIP2A in the anti-lymphoma effects of PPA. CIP2A siRNA was transfected into Raji and Jurkat cells before PPA treatment. CCK-8 and western blot assays were used to detect changes in cell proliferation and apoptosis. It is worth noting that low expression of CIP2A promotes the PPA-induced inhibition of proliferation (**Figure 5B**) and activation of apoptosis (**Figure 5C**). Assessment of caspase-3 and PARP expression indicated that PPA reduced the levels of pro-casp-3 and increased the levels of cleaved-PARP in the Raji and Jurkat cells with low CIP2A expression (**Figure 5D**, **5E**). Furthermore, we overexpressed CIP2A in Raji and Jurkat cells and examined its effect on the anti-lymphoma

effects of PPA (**Figure 5F**). The results show that high expression of CIP2A antagonizes the PPA-induced inhibition of proliferation (**Figure 5G**) and activation of apoptosis (**Figure 5H**). In summary, the results described above indicated that inhibition of CIP2A is very important for the therapeutic effect of PPA on lymphoma.

Discussion

It has been reported that PPA has a therapeutic effect on multiple myeloma by inhibiting the β-catenin signaling pathway [24]. The therapeutic effects of PPA on lymphoma cell lines, as well as the underlying mechanisms, are unknown. This research investigation found that PPA has a therapeutic effect on lymphoma cell lines by suppressing the CIP2A/PP2A/Akt signaling axis. This finding suggests that PPA has potential therapeutic effects on patients with lymphoma.

Apoptosis, which is a type I programmed cell death process, is caused by exogenous death receptor signaling or endogenous mitochondrial signaling. It is widely known that inducing the activation of apoptosis in cancer cells is the mechanism of most cancer treatments [25]. Activation of apoptosis is characterized by cleavage of caspase-3 and PARP, release of cytochrome C, and fragmentation of DNA. The present study used flow cytometric assays to assess apoptosis, and these assays indicated that 1.6 μM PPA induced apoptosis in 26.39% and 21.06% of Raji and Jurkat cells, respectively (**Figure 3A**). Increasing the concentration of PPA significantly decreased the levels of procasp-3 and cleaved PARP (**Figure 3B**). Thus, PPA may induce caspase-mediated apoptosis in lymphoma. In addition, Wright-Giemsa staining showed that the nuclear/cytoplasmic ratio of PPA-treated Raji cells was decreased (**Figure 3C**), suggesting that the cells had undergone apoptosis.

Several natural compounds exert therapeutic effects in tumors by targeting the oncoprotein CIP2A [22, 26, 27]. This study found that PPA significantly downregulated the abnormal high expression of CIP2A in lymphomas (**Figure 4A**, **4B**) and attenuated its inhibitory effect on the activity of PP2A, thereby restoring the activity of PP2A. Inactivation of the regulatory subunits of PP2A, abnormal expression of the scaffold subunits and mutations are often observed in a variety of human cancers [28]. Studies have reported that restoring PP2A activity may be a new strategy for treating cancer [29, 30]. Further results showed that PPA decreased the activity of Akt, which is a downstream molecule of the CIP2A/PP2A signaling (**Figure 4E**, **4F**). The constitutive activation of Akt is a key locus in the progression of multiple cancers and multidrug resistance [31]. Therefore, PPA may be a new tumor therapy drug that targets the CIP2A/PP2A/Akt signaling axis. We further demonstrated that low expression of CIP2A can sensitize cells to PPA-induced cytotoxicity and apoptosis activation (**Figure 5B-E**). Conversely, abnormal high expression of CIP2A attenuates PPA-induced cytotoxicity and apoptosis activation (**Figure 5G**, **5H**). Although the knockdown of CIP2A and the therapeutic effect of PPA showed similar results, PPA acted in part through the downregulation of CIP2A. However, even if CIP2A expression is reduced to an undetectable level, PPA can still exert its toxic effect. Similarly, overexpression of CIP2A promoted the growth of lymphoma cells, but PPA displayed a weak but still inhibitory effect on lymphoma cells that overexpressed CIP2A. These data suggested that PPA inhibited lymphoma cell growth and promoted apoptosis by decreasing CIP2A expression and then repressing PP2A/Akt signaling.

This study revealed that PPA suppressed the proliferation and promoted the apoptosis of two lymphoma cell lines. Moreover, PPA decreased CIP2A expression and suppressed the PP2A/Akt signaling cascade to reduce proliferation and promote apoptosis. Overall, PPA is expected to be used for the treatment of lymphoma.

Acknowledgements

This work was supported by grants from the National Natural Science Foundation of China (No. 81702930), the Natural Science Foundation of Hubei Province (No. 2019CFB424), the Principal Investigator Grant of Hubei University of Medicine (Nos. HBMUPI201804 and HBMUPI201806), and the National Training Program of Innovation and Entrepreneurship for Undergraduates (No. 201910929020).

Disclosure of conflict of interest

None.

Address correspondence to: Yang Guo, Institute of Basic Medical Sciences, Hubei University of Medicine, 30 Renmin Road, Shiyan, Hubei, China. Tel: +86-719-8891150; E-mail: guoyang_hbmu@foxmail.com; Ying Liu, Biomedical Research Institute, Hubei University of Medicine, Shiyan, Hubei, China; Institute of Basic Medical Sciences, Hubei University of Medicine, 30 Renmin Road, Shiyan, Hubei, China. Tel: +86-719-8875313; E-mail: ying_liu1002@163.com

References

[1] Mugnaini EN and Ghosh N. Lymphoma. Prim Care 2016; 43: 661-675.
[2] Siegel RL, Miller KD and Jemal A. Cancer statistics, 2018. CA Cancer J Clin 2018; 68: 7-30.
[3] Armitage JO, Gascoyne RD, Lunning MA and Cavalli F. Non-Hodgkin lymphoma. Lancet 2017; 390: 298-310.
[4] Means RT Jr. Lymphoma classification: morphology to molecular. J Investig Med 2020; 68: 319-320.
[5] Junttila MR, Puustinen P, Niemela M, Ahola R, Arnold H, Bottzauw T, Ala-aho R, Nielsen C, Ivaska J, Taya Y, Lu SL, Lin S, Chan EK, Wang XJ, Grenman R, Kast J, Kallunki T, Sears R, Kahari VM and Westermarck J. CIP2A inhibits PP2A in human malignancies. Cell 2007; 130: 51-62.
[6] Liu PF, Xiang YC, Liu XW, Zhang T, Yang R, Chen S, Xu L, Yu QQ, Zhao HZ, Zhang L, Liu Y and Si Y. Cucurbitacin B induces the lysosomal degradation of EGFR and suppresses the CIP2A/PP2A/Akt signaling axis in gefitinib-resistant non-small cell lung cancer. Molecules 2019; 24: 647.
[7] Li S, Feng TT, Guo Y, Yu X, Huang Q, Zhang L, Tang W and Liu Y. Expression of cancerous inhibitor of protein phosphatase 2A in human triple negative breast cancer correlates with tumor survival, invasion and autophagy. Oncol Lett 2016; 12: 5370-5376.
[8] Ji J, Zhen W, Si Y, Ma W, Zheng L, Li C, Zhang Y, Qin S, Zhang T, Liu P, Zheng X and Liu Y. Increase in CIP2A expression is associated with cisplatin chemoresistance in gastric cancer. Cancer Biomark 2018; 21: 307-316.
[9] Jin L, Si Y, Hong X, Liu P, Zhu B, Yu H, Zhao X, Qin S, Xiong M, Liu Y, Luo Z and Guo Y. Ethoxysanguinarine inhibits viability and induces apoptosis of colorectal cancer cells by inhibiting CIP2A. Int J Oncol 2018; 52: 1569-1578.
[10] Ma WJ, Xiang YC, Yang R, Zhang T, Xu JX, Wu YZ, Liu XW, Xiang K, Zhao HZ, Liu Y and Si Y. Cucurbitacin B induces inhibitory effects via the CIP2A/PP2A/C-KIT signaling axis in t(8;21) acute myeloid leukemia. J Pharmacol Sci 2019; 139: 304-310.
[11] Liu X, Cao W, Qin S, Zhang T, Zheng J, Dong Y, Ming P, Cheng Q, Lu Z, Guo Y, Zhang B and Liu Y. Overexpression of CIP2A is associated with poor prognosis in multiple myeloma. Signal Transduct Target Ther 2017; 2: 17013.
[12] Lilja L, Haapasaari KM, Bloigu R, Salonen T, Ristimaki A, Turpeenniemi-Hujanen T and Kuittinen O. Increased expression of CIP2A in aggressive subtypes of B-cell lymphoma. Histopathology 2013; 63: 438-439.
[13] Dong R, Guo J, Zhang Z, Zhou Y and Hua Y. Polyphyllin I inhibits gastric cancer cell proliferation by downregulating the expression of fibroblast activation protein alpha (FAP) and hepatocyte growth factor (HGF) in cancer-associated fibroblasts. Biochem Biophys Res Commun 2018; 497: 1129-1134.
[14] Yang Q, Chen W, Xu Y, Lv X, Zhang M and Jiang H. Polyphyllin I modulates MALAT1/STAT3 signaling to induce apoptosis in gefitinib-resistant non-small cell lung cancer. Toxicol Appl Pharmacol 2018; 356: 1-7.
[15] He J, Yu S, Guo C, Tan L, Song X, Wang M, Wu J, Long Y, Gong D, Zhang R, Cao Z, Li Y and Peng C. Polyphyllin I induces autophagy and cell cycle arrest via inhibiting PDK1/Akt/mTOR signal and downregulating cyclin B1 in human gastric carcinoma HGC-27 cells. Biomed Pharmacother 2019; 117: 109189.
[16] Li GB, Fu RQ, Shen HM, Zhou J, Hu XY, Liu YX, Li YN, Zhang HW, Liu X, Zhang YH, Huang C, Zhang R and Gao N. Polyphyllin I induces mitophagic and apoptotic cell death in human breast cancer cells by increasing mitochondrial PINK1 levels. Oncotarget 2017; 8: 10359-10374.
[17] He H, Zheng L, Sun YP, Zhang GW and Yue ZG. Steroidal saponins from Paris polyphylla suppress adhesion, migration and invasion of human lung cancer A549 cells via down-regulating MMP-2 and MMP-9. Asian Pac J Cancer Prev 2014; 15: 10911-10916.
[18] Yue G, Wei J, Qian X, Yu L, Zou Z, Guan W, Wang H, Shen J and Liu B. Synergistic anticancer effects of polyphyllin I and evodiamine on freshly-removed human gastric tumors. PLoS One 2013; 8: e65164.
[19] Chen W, Qian X, Hu Y, Jin W, Shan Y, Fang X, Sun Y, Yu B, Luo Q and Xu Q. SBF-1 preferentially inhibits growth of highly malignant human liposarcoma cells. J Pharmacol Sci 2018; 138: 271-278.
[20] Zhang Y, Huang P, Liu X, Xiang Y, Zhang T, Wu Y, Xu J, Sun Z, Zhen W, Zhang L, Si Y and Liu Y. Polyphyllin I inhibits growth and invasion of cisplatin-resistant gastric cancer cells by partially inhibiting CIP2A/PP2A/Akt signaling axis. J Pharmacol Sci 2018; 137: 305-312.
[21] Liu Y, Chen XQ, Liang HX, Zhang FX, Zhang B, Jin J, Chen YL, Cheng YX and Zhou GB. Small

compound 6-O-angeloylplenolin induces mitotic arrest and exhibits therapeutic potentials in multiple myeloma. PLoS One 2011; 6: e21930.
[22] Huang Q, Qin S, Yuan X, Zhang L, Ji J, Liu X, Mai W, Zhang Y, Liu P, Sun Z, Zhang J and Liu Y. Arctigenin inhibits triple-negative breast cancers by targeting CIP2A to reactivate protein phosphatase 2A. Oncol Rep 2017; 38: 598-606.
[23] Rincon R, Cristobal I, Zazo S, Arpi O, Menendez S, Manso R, Lluch A, Eroles P, Rovira A, Albanell J, Garcia-Foncillas J, Madoz-Gurpide J and Rojo F. PP2A inhibition determines poor outcome and doxorubicin resistance in early breast cancer and its activation shows promising therapeutic effects. Oncotarget 2015; 6: 4299-4314.
[24] Liang Y, Li X, He X, Qiu X, Jin XL, Zhao XY and Xu RZ. Polyphyllin I induces cell cycle arrest and apoptosis in human myeloma cells via modulating beta-catenin signaling pathway. Eur J Haematol 2016; 97: 371-378.
[25] Wu H, Jeffrey Medeiros L and Young KH. Apoptosis signaling and BCL-2 pathways provide opportunities for novel targeted therapeutic strategies in hematologic malignances. Blood Rev 2018; 32: 8-28.
[26] Liu X, Sun Z, Deng J, Liu J, Ma K, Si Y, Zhang T, Feng T, Liu Y and Tan Y. Polyphyllin I inhibits invasion and epithelial-mesenchymal transition via CIP2A/PP2A/ERK signaling in prostate cancer. Int J Oncol 2018; 53: 1279-1288.
[27] Xiao X, He Z, Cao W, Cai F, Zhang L, Huang Q, Fan C, Duan C, Wang X, Wang J and Liu Y. Oridonin inhibits gefitinib-resistant lung cancer cells by suppressing EGFR/ERK/MMP-12 and CIP2A/Akt signaling pathways. Int J Oncol 2016; 48: 2608-2618.
[28] Seshacharyulu P, Pandey P, Datta K and Batra SK. Phosphatase: PP2A structural importance, regulation and its aberrant expression in cancer. Cancer Lett 2013; 335: 9-18.
[29] Schonthal AH. Role of serine/threonine protein phosphatase 2A in cancer. Cancer Lett 2001; 170: 1-13.
[30] Lv P, Wang Y, Ma J, Wang Z, Li JL, Hong CS, Zhuang Z and Zeng YX. Inhibition of protein phosphatase 2A with a small molecule LB100 radiosensitizes nasopharyngeal carcinoma xenografts by inducing mitotic catastrophe and blocking DNA damage repair. Oncotarget 2014; 5: 7512-7524.
[31] Radisavljevic Z. AKT as locus of cancer multidrug resistance and fragility. J Cell Physiol 2013; 228: 671-674.