Int J Clin Exp Med 2017;10(4):6351-6359
www.ijcem.com /ISSN:1940-5901/IJCEM0043971

Original Article

Salidroside induces cell cycle arrest and apoptosis in human cervical cancer SiHa cells

Xuemei Hu[1*], Yi Li[2*], Fang Wang[1*], Yuejun Tian[1,6], Yingqin Ye[3], Jixue Zhang[4], Hongyu Guo[1], Rongxia He[1], Huixin Li[5#], Zhiping Wang[6#]

[1]Department of Gynecology and Obstetrics, [6]Key Laboratory of Urological Diseases, The Second Hospital of Lanzhou University, Lanzhou, P. R. China; [2]Institute of Systems Biology and TCM Transformation, Gansu Traditional Chinese Medical University, Lanzhou, P. R. China; [3]Health of Women and Child of Hospital, Lanzhou, P. R. China; [4]Health of Women and Child of Hospital, Baiyin, P. R. China; [5]Department of Gynecology and Obstetrics, Zhoupu's Hospital of Pudong District, Shanghai, P. R. China. [*]Equal contributors and co-frst authors. [#]Equal contributors.

Received November 9, 2016; Accepted February 24, 2017; Epub April 15, 2017; Published April 30, 2017

Abstract: Background: Salidroside has potent anti-oxidant, anti-inflammation and anti-tumor activity. However, little is known regarding its effect on cervical cancer cells. This study investigated the effect of different concentrations of salidroside on the viability, cell cycling and apoptosis of cervical cancer SiHa cells and its potential mechanisms. Methods: Cell growth potential was measured by Cell Counting Kit-8 assay and colony formation. Cell cycle distribution was measured by flow cytometry. A light microscope was used to detect the morphology of SiHa cells. Western blot was used to measure the protein expression of the indicated genes. Results: Treatment with different concentrations of salidroside significantly reduced the viability of SiHa cells in a dose- and time-dependent manner. Treatment with salidroside resulted in morphological changes in SiHa cells and induced cell cycle arrest at the G2/M and/or S phase, which was associated with significantly decreased levels of Cyclin B1, Cyclin A and Cyclin-dependent kinase-2 expression but up-regulating P21 expression. Furthermore, treatment with different concentrations of salidroside induced the apoptosis of SiHa cells, and their effects were dose-dependent. Finally, treatment with salidroside enhanced the relative levels of cleaved caspase 3, Bax, and Fas expression but down-regulated the relative levels of BcL-2 and FasL expression in SiHa cells. These results demonstrated that salidroside had potent cytotoxicity against SiHa cells by inducing cell cycle arrest and apoptosis in cervical cancer. Salidroside may be a promising candidate for cervical cancer chemotherapy.

Keywords: Salidroside, cell cycling, apoptosis, cervical cancer SiHa cells

Introduction

Cervical cancer is one of the most common malignant tumors in women. Although the incidence of cervical cancer in developed countries has reduced due to vaccination, cervical cancer remains a serious global threat for woman's health [1]. Cervical cancer at the early stage can currently be treated with surgical resection of the tumor; recurrent and advanced cervical cancers usually require radiotherapy and chemotherapy. However, radiotherapy and chemotherapy usually have severe side effects, which remain an obstacle for cervical cancer interventions [2-4]. Hence, the development and discovery of new regimens with low drug resistance and few adverse effects will be of great significance [5].

Salidroside is a glycoside present in *Rhodiola rosea*. Previous studies showed that salidroside has potent anti-oxidant, anti-inflammation, antiviral and immunomodulation activity [6-9]. Salidroside has cardiovascular and neuronal protective activity and can ameliorate the toxicity of chemotherapy or radiotherapy [10, 11]. Furthermore, salidroside also has potent antitumor effects by inhibiting the growth of bladder, breast, and liver cancer cells in vitro [12-15]. However, whether salidroside can modulate the viability and cell cycling and induce the apoptosis of cervical cancer cells have not been clarified. Furthermore, the molecular mechanisms underlying the action of salidroside in regulating the growth and survival of cervical cancer cells remains unclear.

This study tested the effect of different concentrations of salidroside on the viability, cell cycling and apoptosis of SiHa cells and explored the potential mechanisms underlying the action of salidroside in vitro. Our data indicated that treatment with different concentrations of salidroside significantly reduced the viability and induced cell cycle arrest at the G2/M and/or S phase, leading to apoptosis of SiHa cells in a dose-dependent manner.

Materials and methods

Cell culture

Human cervical cancer SiHa cells were obtained from Shanghai Institute of Cell Biology, Chinese Academic of Science (Shanghai, China). SiHa cells were cultured in DMEM medium (HyClone, Logan, UT, USA) containing 10% heat-inactivated fetal bovine serum (GIBCO, Grand Island, NY, USA) as the complete medium, at 37°C in a humidified atmosphere with 95% air and 5% CO_2.

MTT assay

Salidroside was purchased from Chengdu Biological Development (Chengdu, China). SiHa cells (1×10^4/well) were cultured overnight in complete medium in 96-well plates and treated in triplicate with vehicle as the controls or different concentrations (20-120 μg/ml) of salidroside for 24-72 h. During the last 4-h culture, the cells were exposed to 20 μl of MTT (5 mg/ml, Sigma), and the resulting formazan in each well was dissolved in 100 μl DMSO. The absorbance was measured at 570 nm in a microplate reader (Bio-Rad, Hercules, CA, USA). The inhibitory rate of salidroside on SiHa cell proliferation was calculated as [1- SiHa (experimental wells)/SiHa (control wells)]×100%.

Cell cycle analysis

The effect of salidroside on the cell cycling of SiHa cells was determined by flow cytometry. Briefly, SiHa cells (3×10^5/well) were cultured overnight in complete medium and treated in triplicate with vehicle alone or different concentrations of salidroside for 48 h. The cells were fixed overnight in ice-cold 70% ethanol and digested with DNase-free RNase for 30 min. After being washed, the cells were stained with propidium iodide, and different phases of the cells were analyzed in a Cytomics FC500 flow cytometer (Beckman Coulter, Fullerton, CA, USA).

Morphological evaluation

SiHa cells were treated with or without different concentrations of salidroside for 48 h, and cell morphology was imaged under a light microscope. Additionally, the cells were treated with or without different concentrations of salidroside for 72 h and fixed with 1% glutaraldehyde in PBS for 30 min at room temperature (RM). After washing, the cells were stained with 1 mM Hoechst 33342 for 20 min at 37°C and examined under a fluorescent microscope (ix-81, Olympus, Tokyo, Japan).

Western blot analysis

SiHa cells were treated with or without different concentrations of salidroside for 48 h. After washing, the cells were harvested and lysed in ice-cold lysis buffer (50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 5 mM EDTA, 50 mM NaF, 1% Triton X-100, 1 mM sodium orthovanadate, 1 mM phenylmethanesulfonyl fluoride, 1 mg/ml aprotinin, 2 g/ml pepstatin A, and 2 g/ml leupeptin), followed by centrifugation. The concentrations of the protein lysates were quantified by BCA method. The cell lysate samples were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene difluoride membranes (Millipore, Bedford, MA, USA). The membranes were blocked with 5% non-fat dry milk in TBST saline (20 mM Tris-HCl, pH 7.4, 137 mM NaCl, and 0.1% Tween-20) at RT for 1 h and incubated with primary antibodies against Cdc2 (1:1,000), cyclin B1 (1:1,000), CDK2 (1:1,000), cyclin A (1:1,000), p21 (1:1,000), Bax (1:500), Bcl-2 (1:500), Fas (1:1,000), FasL (1:1,000), cleaved caspase 3 (1:5,000) or control IgG (Santa Cruz Biotech, Santa Cruz, USA) at RM for 3 h. After washing, the bound antibodies were detected with peroxidase-conjugated optimal second antibodies at RM for 1 h and visualized with enhanced chemiluminescent reagent (Bio-Rad, Hercules, CA, USA). The relative levels of interesting protein to the control were determined by densitometric scanning using ImageJ software.

Statistical analysis

Data are expressed as the means ± SD. The differences between groups were determined by analysis of variance. A *P*-value of < 0.05 was considered statistically significant.

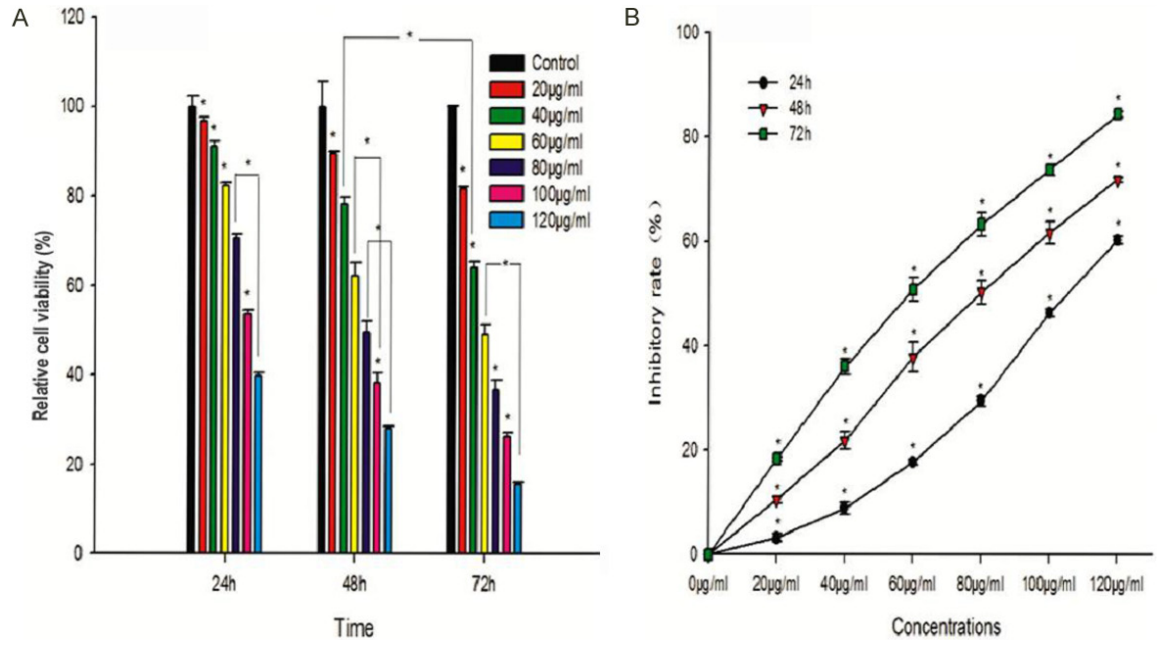

Figure 1. The effect of salidroside on the viability of SiHa cells. SiHa cells were treated in triplicate with or without the indicated concentrations of salidroside for varying periods, and the viability of different groups of cells was determined by MTT assay, followed by calculating the inhibition rates. Data are expressed as the means ± SD of each group from four separate experiments. The differences between groups were determined by analysis of variance. A *P*-value of < 0.05 was considered statistically significant. A. Inhibition rates. B. Percentages of cell viability. The control cells without salidroside treatment were designated as 100%. *P < 0.05 vs. the control group.

Results

Salidroside suppresses SiHa cells viability

Salidroside is a phytochemical and has potent anti-oxidant, anti-inflammation and antitumor activity. To test the effect of salidroside on cervical cancer, Siha cells were treated with or without different concentrations of salidroside for varying periods; their viability was then determined with MTT assays (**Figure 1A**). Inhibition calculations indicated that treatment with 20-120 μg/ml salidroside significantly reduced the viability of SiHa cells and that the effects of different doses of salidroside on inhibiting SiHa cell growth were dose-dependent (**Figure 1B**). Furthermore, treatment with salidroside for a longer period, such as 48 h, further significantly reduced the viability of SiHa cells. Hence, treatment with salidroside inhibited the growth of SiHa cells in a dose- and time-dependent manner.

Salidroside results in morphological changes in SiHa cells

We next tested the impact of treatment with salidroside on the morphology of SiHa cells. SiHa cells were treated with or without different concentrations of salidroside for 48 h, and their morphology was observed under a light microscope. As shown in **Figure 2**, while control cells without salidroside treatment displayed a healthy shuttle type with a few rounded cells, the experimental groups with different concentrations of salidroside had gradually reduced numbers of cells and exhibited an increased body size with less process but obvious retraction, disintegration and necrosis. The morphological changes in the cells treated with different concentrations of salidroside increased in a dose-dependent manner. There were no obvious living cells after treatment with 120 μg/ml salidroside. Thus, treatment with different concentrations of salidroside resulted in morphological changes in SiHa cells in a dose-dependent manner.

Salidroside induces cell cycle arrest at the G2/M and/or S phase in SiHa cells

Cytotoxic drugs are associated with inducing tumor cell cycle arrest. To understand the mechanisms underlying the action of salidroside, SiHa cells were treated with or without dif-

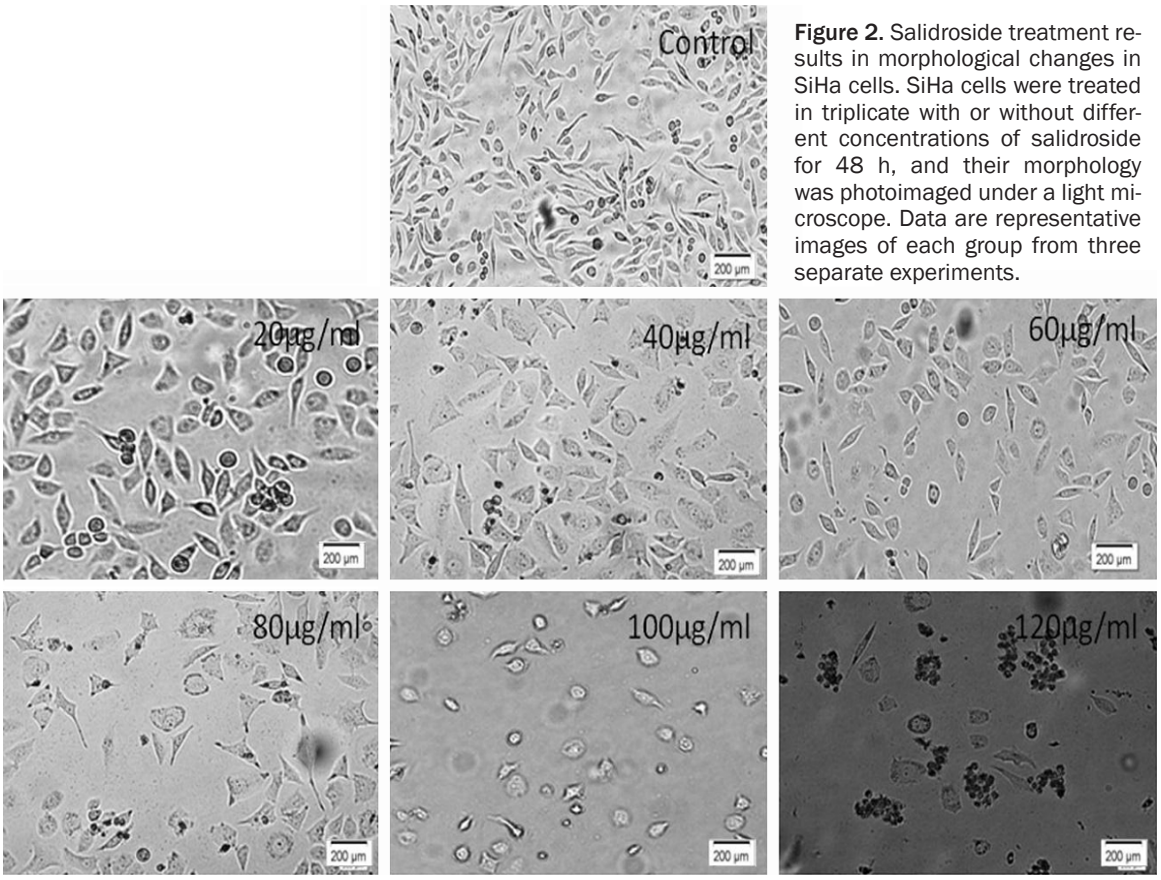

Figure 2. Salidroside treatment results in morphological changes in SiHa cells. SiHa cells were treated in triplicate with or without different concentrations of salidroside for 48 h, and their morphology was photoimaged under a light microscope. Data are representative images of each group from three separate experiments.

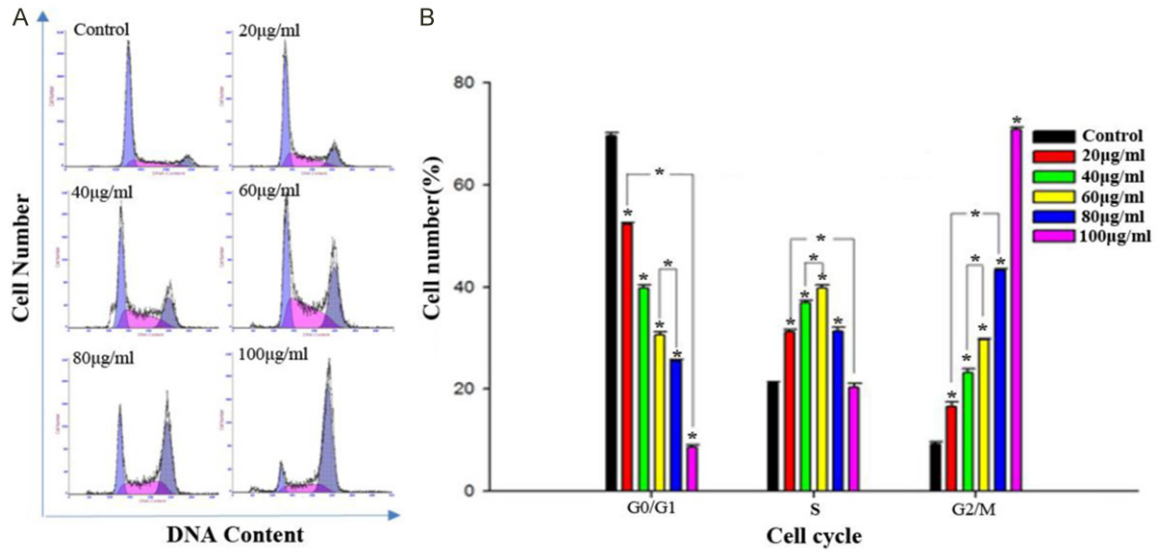

Figure 3. Effect of salidroside on cell cycle E of SiHa cells. SiHa cells were treated in triplicate with or without different concentrations of salidroside for 48 h, and the status of cell cycling of different groups of cells was determined by flow cytometry. Data are representative histograms or expressed as the means (%) ± SD of each group of cells from three separate experiments. A *P*-value of < 0.05 was considered statistically significant. A. Histograms of cell cycling. B. The status of cell cycling in each group of cells. *P < 0.05 vs. the control cells.

ferent concentrations of salidroside for 48, and the cell cycle states of the different groups of cells were analyzed by flow cytometry. As shown in **Figure 3**, treatment with different concentra-

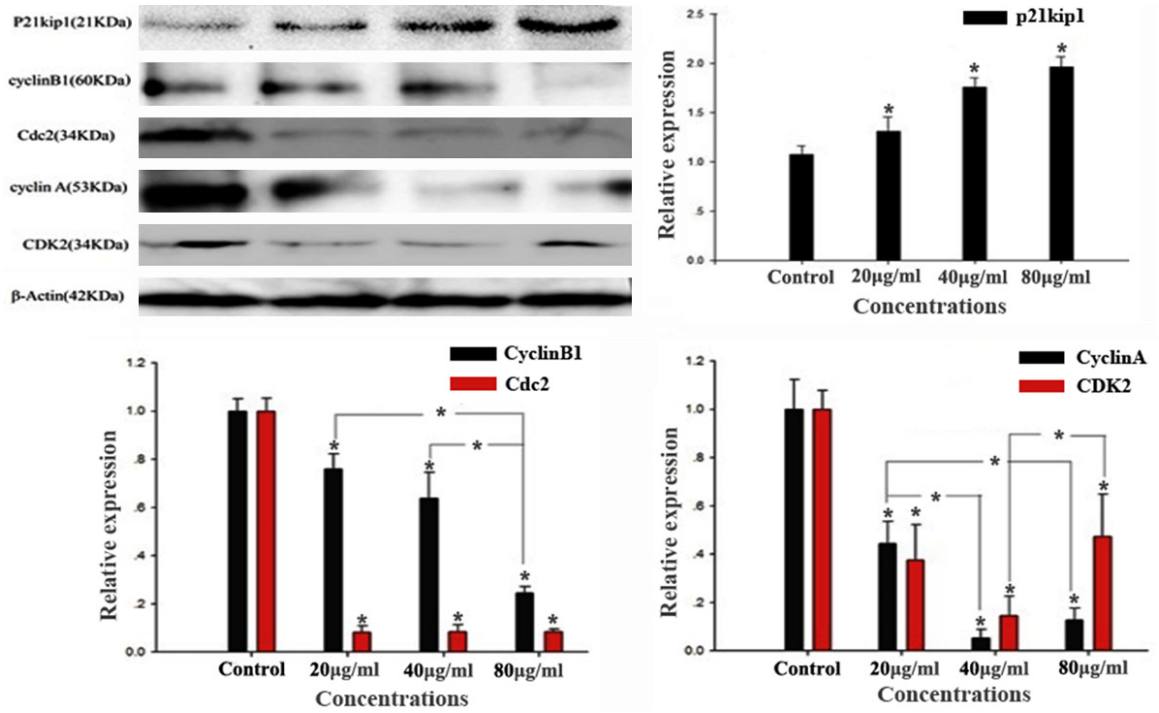

Figure 4. Effect of salidroside on the expression of cell cycle regulators in SiHa cells. SiHa cells were treated in triplicate with or without different concentrations of salidroside for 48 h, and the relative levels of Cdc-2, cyclin B1, cyclin A, CDK2 and P21 expression in the different groups of cells were determined by Western blot assays. Data are representative images or expressed as the means ± SD of each group of cells from three separate experiments. A *P*-value of < 0.05 was considered statistically significant. *P < 0.05 vs. the control cells.

tions of salidroside significantly decreased the percentages of cells at the G0/G1 phase but increased the percentages of cells at the G2/M phase. Additionally, treatment with 20-80 μg/ml of salidroside also increased the percentages of cells at the S phase.

Cell cycling is regulated by kinases and inhibitors. To further understand the action of salidroside, SiHa cells were treated with different concentrations of salidroside for 48 h. The relative levels of Cdc2, cyclin B1, CDK2, cyclin A and P21 expression in different groups of cells were determined by Western blot assays. Compared with the control cells, treatment with salidroside dramatically reduced the relative levels of Cdc2 regardless of varying concentrations of salidroside (**Figure 4**). Treatment with different concentrations of salidroside gradually decreased the relative levels of Cyclin B1, Cyclin A and CDK2 in SiHa cells, with the exception of treatment with 80 μg/ml of salidroside, which elevated the relative levels of CDK2 compared with that in the cells treated with 40 μg/ml of salidroside. Furthermore, treatment with different concentrations of salidroside gradually increased the relative levels of P21 expression in SiHa cells. Collectively, treatment with salidroside induced cell cycle arrest at the S and/or G2/M phases in SiHa cells by modulating the expression of cell cycle regulators.

Salidroside induces the apoptosis of SiHa cells

The induction of cell cycle arrest is associated with triggering apoptosis, particularly for cytotoxic drug-induced arrest at the G2/M phase. Finally, we tested the impact of treatment with salidroside on the apoptosis of SiHa cells. SiHa cells were treated with or without different concentrations of salidroside for 48 h, and the percentages of apoptotic cells were determined by flow cytometry. Treatment with different concentrations of salidroside increased the percentages of apoptotic SiHa cells, and the effects of different concentrations of salidroside on inducing SiHa cell apoptosis were dose-dependent (**Figure 5A**).

Similarly, the cells were stained with Hoechst 33342 and observed under a fluorescent

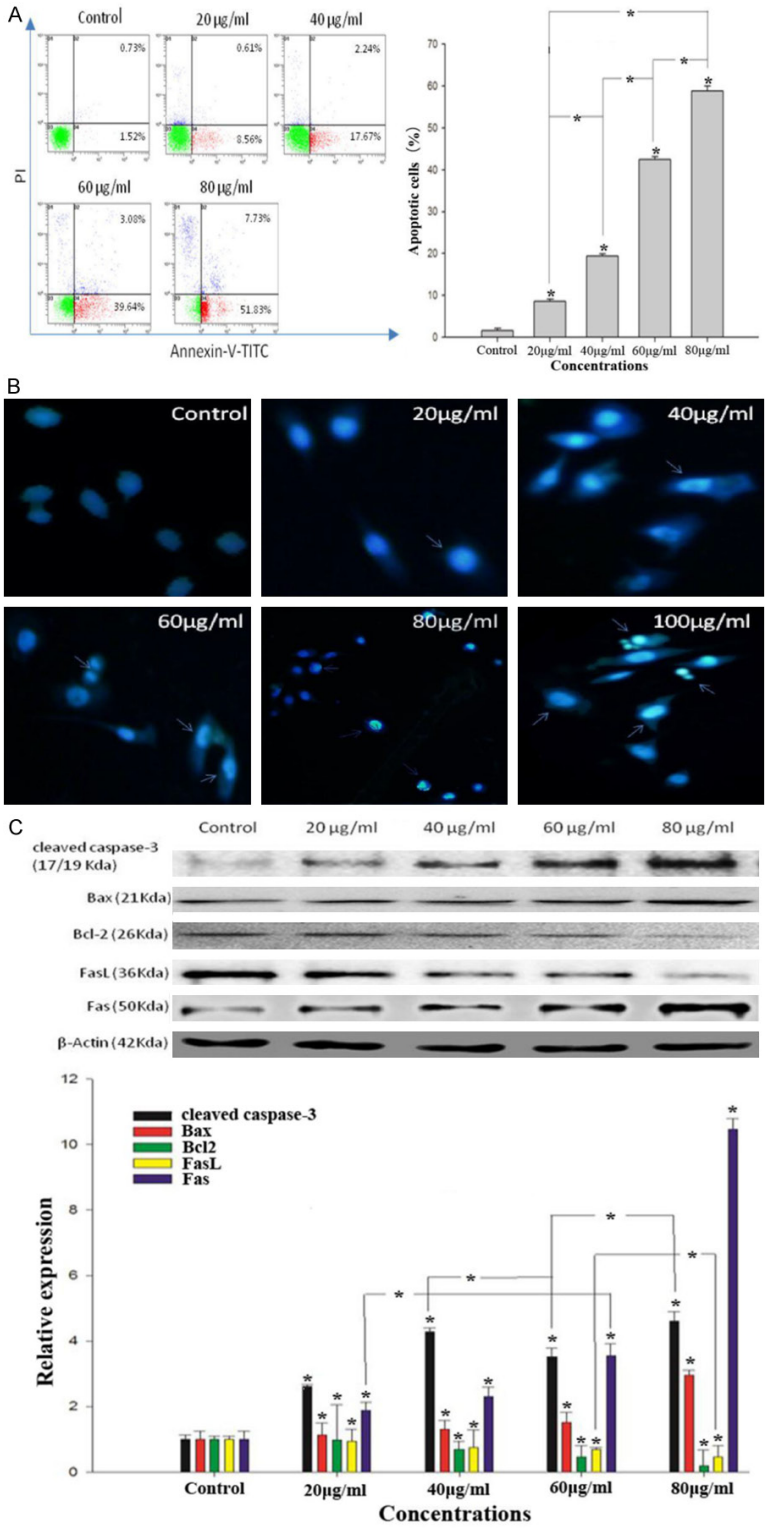

Figure 5. Effect of salidroside on the expression of apoptotic regulators in SiHa cells. A. SiHa cells were treated in triplicate with or without different concentrations of salidroside for 48 h, and the percentages of apoptotic SiHa cells were determined by flow cytometry. B. After treatment with salidroside, the cells stained with Hoechst 33342 and apoptotic SiHa cells were photoimaged under a fluorescent microscope. Data are representative facs charts, photoimages (magnification ×100) or expressed as the mean ± SD of each group of cells from three separate experiments. P < 0.05 vs. the control cells. C. SiHa cells were treated in triplicate with or without different concentrations of salidroside for 48 h, and the relative levels of Bcl-2, Bax, Fas, FasL expression and cleaved caspase 3 were determined by Western blot assays. Data are representative images or expressed as the means ± SD of each group of cells from three separate experiments. A *P*-value of < 0.05 was considered statistically significant. P < 0.05 vs. the control cells.

microscope. Treatment with different concentrations of salidroside resulted in SiHa cell apoptosis (**Figure 5B**). Further Western blot analysis indicated that treatment with salidroside significantly increased the relative levels of cleaved caspase 3, Bax and Fas but decreased the relative levels of BcL-2 and FasL in SiHa cells (**Figure 5C**). The effects of different concentrations of salidroside on modulating apoptosis regulators were dose-dependent. Together, treatment with different concentrations of salidroside induced the apoptosis of SiHa cells in a dose-dependent manner.

Discussion

This study examined the effect of salidroside on the survival, cell cycling and apoptosis of SiHa cells in vitro. We found that salidroside had potent cytotoxicity against cervical cancer SiHa cells and that its effects were dose-dependent. Treatment with different concentrations of salidroside significantly reduced the viability of SiHa cells in a dose- and time-dependent manner. Salidroside treatment modulated morphology and induced cell

cycle arrest at the G2/M and/or S phases and led to apoptosis in SiHa cells. Our data extended previous findings that salidroside has strong anti-tumor activity against several types of cancer cells [12, 13] and demonstrated that natural rhodioloside had excellent anti-tumor activity [16-18]. To the best of our knowledge, this was the first study to demonstrate that salidroside had potent cytotoxicity against cervical cancer cells. These novel findings suggest that salidroside may represent a promising lead in designing new therapeutic regimens for cervical cancer intervention.

Cell cycling is classified into different checkpoints that are regulated by unique factors, such as kinases and specific inhibitors of P27, P21 and P16. CDK2 and cyclin E activation are crucial for S phase and DNA synthesis, while Cdc2, Cyclin A and B1 as well as P21 regulate DNA repair and mitosis at the G2/M phase during cell cycling [19-22]. To understand the molecular mechanisms involved, we analyzed the relative levels of Cdc2, Cyclin A, Cyclin B1, CDK2 and P21 expression in the different groups of cells. We found that treatment with different concentrations of salidroside dramatically reduced the relative levels of Cdc2 and gradually reduced the relative levels of Cyclin B1 but significantly up-regulated P21 expression in SiHa cells in a dose-dependent manner relative to that in the control cells. These, together with the gradually increased percentages of cells at the G2/M phase, clearly indicate that salidroside preferably induced cell cycle arrest at the G2/M phase. Notably, salidroside may interfere with DNA repair and mitosis during the cell cycling, leading to cell cycle arrest at the G2/M phase in SiHa cells. We also found that the relative levels of CDK2 expression in the cells treated with relatively lower concentrations (20-40 μg/ml) of salidroside were significantly lower than that in the cells treated with a relatively higher concentration (80 μg/ml) of salidroside, accompanied by higher percentages of cells at the S phase in Siha cells. These observations suggest that treatment with relatively lower doses of salidroside retarded the process of DNA synthesis while treatment with a relative higher concentration of salidroside may have potent cytotoxicity, which attenuates DNA repair and mitosis in SiHa cells. Previous studies showed that flavopiridol and alkaloid rohitukine can induce cell cycle arrest at the G2/M phase in lung cancer cells by directly inhibiting CDK activation enzymes. Salidroside may have a similar activity. We are interested in further investigating the molecular mechanisms underlying the action of salidroside in regulating cell cycling in cervical cancer cells.

The induction of cell cycle arrest is associated with triggering cell apoptosis, which is based on the balanced expression of pro-apoptotic and anti-apoptotic factors [23]. To further understand the role of salidroside, we examined the relative levels of Bcl-2, Bax, Fas, FasL and cleaved caspase 3 expression in the different groups of SiHa cells. We found that treatment with different concentrations of salidroside gradually decreased the relative levels of Bcl-2 but increased the relative levels of Bax and cleaved caspase 3 expression, leading to an increase in the ratios of pro-apoptotic Bax to anti-apoptotic Bcl-2 expression in SiHa cells. The alternation in the ratios of pro-apoptotic to anti-apoptotic factor expression leads to an altered mitochondrial membrane permeability, the release of cytochrome C into the cytosol and the subsequent activation of caspase 3 [24]. The increased levels of Bax expression suggest that the induction of cell cycle arrest at the G2/M phase by salidroside may trigger apoptosis through the mitochondrial pathway [25]. Furthermore, we found that treatment with different concentrations of salidroside significantly increased the relative levels of Fas expression but that treatment with a relatively higher concentration of salidroside significantly reduced the relative levels of FasL expression in SiHa cells. The increased levels of Fas expression should enhance the sensitivity of SiHa cells to FasL, thereby triggering apoptosis [26-29]. The reduction in the relative levels of FasL expression in the cells treated with a relatively higher concentration of salidroside suggests that salidroside may preferably trigger apoptosis by interfering with cell cycling, rather than through a membrane receptor-mediated apoptosis pathway. Further studies are needed to investigate the precise molecular mechanisms by which salidroside regulates cell apoptosis.

Conclusions

In summary, our data indicated that salidroside had potent cytotoxicity against SiHa cells and that its effects were dose-dependent. Treat-

ment with salidroside induced cell cycle arrest at the G2/M phase and apoptosis of SiHa cells by modulating the expression of cell cycle and apoptosis regulators. These novel findings suggest that salidroside may be valuable for the development of new therapies for cervical cancer and may provide new insights into the pharmacological mechanisms underlying the cytotoxicity of salidroside against cervical cancer cells.

Acknowledgements

This study was supported by a grant from the Traditional Chinese Medicine Scientific Research Funds of Health and Family Planning Committee of Shanghai, (number: 2014.JP024A).

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Zhiping Wang, Department of Urology, Lanzhou University Second Hospital, Chengguan District, Lanzhou 730030, P. R. China. Tel: +86-931-8942498; Fax: +86-931-89-42821; E-mail: wangzplzu@163.com; Dr. Huixin Li, Department of Gynecology and Obstetrics, Zhoupu's Hospital of Pudong District, Shanghai, P. R. China. Tel: +8613976768665, E-mail: lihx14@163.com

References

[1] Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J and Jemal A. Global cancer statistics, 2012. CA Cancer J Clin 2015; 65: 87-108.

[2] Kasamatsu T, Onda T, Sawada M, Kato T, Ikeda S, Sasajima Y and Tsuda H. Radical hysterectomy for FIGO stage I-IIB adenocarcinoma of the uterine cervix. Br J Cancer 2009; 100: 1400-1405.

[3] Wei LC, Wang N, Shi M, Liu JY, Li JP, Zhang Y, Huang YH, Li X and Chen Y. Clinical outcome observation of preoperative concurrent chemoradiotherapy/radiotherapy alone in 174 Chinese patients with local advanced cervical carcinoma. Onco Targets Ther 2013; 6: 67-74.

[4] Li XY, Liu L, Xie XM and Zhou C. The role of raltitrexed/cisplatin with concurrent radiation therapy in treating advanced cervical cancer. Eur Rev Med Pharmacol Sci 2014; 18: 3491-3496.

[5] Li XY, Hu SQ and Xiao L. The cancer-associated fibroblasts and drug resistance. Eur Rev Med Pharmacol Sci 2015; 19: 2112-2119.

[6] Wu T, Zhou H, Jin Z, Bi S, Yang X, Yi D and Liu W. Cardioprotection of salidroside from ischemia/reperfusion injury by increasing N-acetylglucosamine linkage to cellular proteins. Eur J Pharmacol 2009; 613: 93-99.

[7] Xu MC, Shi HM, Gao XF and Wang H. Salidroside attenuates myocardial ischemia-reperfusion injury via PI3K/Akt signaling pathway. J Asian Nat Prod Res 2013; 15: 244-252.

[8] Zhao Y, Ling Y, Zhao J, Yuan Y, Guo Y, Liu Q, Wu B, Ding Z and Yang Y. Synthesis and protective effects of novel salidroside analogues on glucose and serum depletion induced apoptosis in PC12 cells. Arch Pharm (Weinheim) 2013; 346: 300-307.

[9] Zhang JK, Yang L, Meng GL, Yuan Z, Fan J, Li D, Chen JZ, Shi TY, Hu HM, Wei BY, Luo ZJ and Liu J. Protection by salidroside against bone loss via inhibition of oxidative stress and bone-resorbing mediators. PLoS One 2013; 8: e57251.

[10] Zhang H, Shen WS, Gao CH, Deng LC and Shen D. Protective effects of salidroside on epirubicin-induced early left ventricular regional systolic dysfunction in patients with breast cancer. Drugs R D 2012; 12: 101-106.

[11] Goel HC, Bala M, Prasad J, Singh S, Agrawala PK and Swahney RC. Radioprotection by Rhodiola imbricata in mice against whole-body lethal irradiation. J Med Food 2006; 9: 154-160.

[12] Liu Z, Li X, Simoneau AR, Jafari M and Zi X. Rhodiola rosea extracts and salidroside decrease the growth of bladder cancer cell lines via inhibition of the mTOR pathway and induction of autophagy. Mol Carcinog 2012; 51: 257-267.

[13] Hu X, Lin S, Yu D, Qiu S, Zhang X and Mei R. A preliminary study: the anti-proliferation effect of salidroside on different human cancer cell lines. Cell Biol Toxicol 2010; 26: 499-507.

[14] Li MH, Zhang GZ and Wang YS. [Effect of salidroside on salivary adenoid cystic carcinoma cells in vitro]. Hua Xi Kou Qiang Yi Xue Za Zhi 2008; 26: 312-315, 319.

[15] Skopinska-Rozewska E, Malinowski M, Wasiutynski A, Sommer E, Furmanowa M, Mazurkiewicz M and Siwicki AK. The influence of Rhodiola quadrifida 50% hydro-alcoholic extract and salidroside on tumor-induced angiogenesis in mice. Pol J Vet Sci 2008; 11: 97-104.

[16] Tian Z, Chen S, Zhang Y, Huang M, Shi L, Huang F, Fong C, Yang M and Xiao P. The cytotoxicity of naturally occurring styryl lactones. Phytomedicine 2006; 13: 181-186.

[17] Marchetti S, Mazzanti R, Beijnen JH and Schellens JH. Concise review: clinical relevance of drug drug and herb drug interactions mediated by the ABC transporter ABCB1 (MDR1, P-glycoprotein). Oncologist 2007; 12: 927-941.

[18] Wong BY, Nguyen DL, Lin T, Wong HH, Cavalcante A, Greenberg NM, Hausted RP and Zheng J. Chinese medicinal herb Scutellaria barbata modulates apoptosis and cell survival in murine and human prostate cancer cells and tumor development in TRAMP mice. Eur J Cancer Prev 2009; 18: 331-341.
[19] Foijer F, Delzenne-Goette E, Dekker M and Te Riele H. In vivo significance of the G2 restriction point. Cancer Res 2007; 67: 9244-9247.
[20] Chang L, He X, Yu G and Wu Y. Effectiveness of HPV 16 viral load and the E2/E6 ratio for the prediction of cervical cancer risk among Chinese women. J Med Virol 2013; 85: 646-654.
[21] Zeng WJ, Li Y, Fei HL, Cheng XD, Ye F, Lu WG and Xie X. The value of p16ink4a expression by fluorescence in situ hybridization in triage for high risk HPV positive in cervical cancer screening. Gynecol Oncol 2011; 120: 84-88.
[22] Besson A, Dowdy SF and Roberts JM. CDK inhibitors: cell cycle regulators and beyond. Dev Cell 2008; 14: 159-169.
[23] Roninson IB, Broude EV and Chang BD. If not apoptosis, then what? Treatment-induced senescence and mitotic catastrophe in tumor cells. Drug Resistance Updates 2001; 4: 303-313.
[24] Clad A, Reuschenbach M, Weinschenk J, Grote R, Rahmsdorf J and Freudenberg N. Performance of the Aptima high-risk human papillomavirus mRNA assay in a referral population in comparison with Hybrid Capture 2 and cytology. J Clin Microbiol 2011; 49: 1071-1076.
[25] Hung SK, Perry R and Ernst E. The effectiveness and efficacy of Rhodiola rosea L.: a systematic review of randomized clinical trials. Phytomedicine 2011; 18: 235-244.
[26] Chatterjee K, Engelmark M, Gyllensten U, Dandara C, van der Merwe L, Galal U, Hoffman M and Williamson AL. Fas and FasL gene polymorphisms are not associated with cervical cancer but differ among Black and Mixed-ancestry South Africans. BMC Res Notes 2009; 2: 1.
[27] Lee YH, Bae SC and Song GG. Association between the CTLA-4, CD226, FAS polymorphisms and rheumatoid arthritis susceptibility: a meta-analysis. Hum Immunol 2015; 76: 83-89.
[28] Kroon AA, DelRiccio V, Tseu I, Kavanagh BP and Post M. Mechanical ventilation-induced apoptosis in newborn rat lung is mediated via FasL/Fas pathway. Am J Physiol Lung Cell Mol Physiol 2013; 305: L795-L804.
[29] Montes-Berrueta D, Ramirez L, Salmen S and Berrueta L. Fas and FasL expression in leukocytes from chronic granulomatous disease patients. Invest Clin 2012; 53: 157-167.