4SC-202 exerts an anti-tumor effect in cervical cancer by targeting PRLR signaling pathway

Huijuan Zhang¹ · Mingxia Li² · Huiru Sun¹ · Wen Yang³ · Mingxia Ye² · Hua Li⁴ · Yuanguang Meng³

Abstract
The aim of the present study is to investigate whether 4SC-202, a selective class I histone deacetylase inhibitor (HDACi), plays an anti-tumor role in cervical cancer (CC) by targeting prolactin receptor (PRLR). CCK-8 and colony formation assays were used to evaluate the effects of 4SC-202 on the proliferation of CC cells in vitro. Effects of 4SC-202 on the cell cycle distribution and apoptosis in SiHa cells were determined by flow cytometry and western blotting, respectively. Immunofluorescence, western blotting and quantitative real-time polymerase chain reaction (qRT-PCR) were performed to detect the activities of PRLR-related pathways and PRLR expression in CC cells. A xenograft tumor model in nude mice was established to examine effects of 4SC-202 on the tumor growth, apoptosis and PRLR-related pathways in vivo. The biochemical analyzer and H&E staining were used to detect the serum biochemical indexes and organ toxicity. 4SC-202 inhibited the proliferation of CC cells (SiHa, HeLa, and CaSki) in vitro in a time- and dose-dependent manner. SiHa cells were treated with 1 or 5 µM 4SC-202 for 72 h and then subjected to various functional assays. The assays showed that 4SC-202 significantly induced G2/M phase arrest and apoptosis, while inhibiting the activities of PRLR-related pathways and PRLR expression. In addition, 4SC-202 reduced tumor growth and induced apoptosis in vivo. 4SC-202 down-regulated the expression of PRLR and activities of PRLR-related pathways in the mouse model, displayed no effects on serum biochemical indicators and caused no toxicity to mouse organs. This finding suggests that 4SC-202 may serve as a novel therapeutic agent for CC.

Keywords Cervical cancer · 4SC-202 · Prolactin receptor · Proliferation · Apoptosis

Introduction
In recent years, cervical cancer (CC) has become one of the leading malignancies that threaten the health and lives of women worldwide (Olusola et al. 2019). The vast majority of patients with CC are mainly caused by persistent infection with human papillomavirus (HPV) (Ascencio-Cedillo et al. 2015). At present, while regular HPV screening, colposcopy directed biopsy, and HPV vaccination have been routinely used for the prevention of cervical cancer, it remains a high-risk disease for women (Hu and Ma 2018). Although HPV16 and HPV18 have been proven to be the main factors causing cervical cancer (Riera-Leal et al. 2018), some studies have shown that changes in hormone cofactors may be involved in pathogenesis of cervical tumors (zur Hausen 1996). It has recently been demonstrated that prolactin receptor (PRLR) is highly expressed in cervical cancer cell lines (Ascencio-Cedillo et al. 2015; Lopez-Pulido et al. 2013), suggesting that it may be implicated in the cancer development. However, the mechanism underlying role of PRLR in CC awaits further studies.

PRLR, a type I cytokine receptor family protein, promotes cell proliferation and differentiation through mediating the
activity of prolactin (PRL) and triggering intramembrane signal cascades, such as signal transduction and the activators of transcription (STAT) family, extracellular regulated kinase (ERK1/2), and serine threonine kinase (AKT) (Kan et al. 2016; Ramirez De Arellano et al. 2018; Lopez-Pulido et al. 2013). Studies have shown that PRLR is highly expressed in CC tissues (Ramirez De Arellano et al. 2018), and signal transduction and activation of STAT3 promotes anti-apoptotic effect of PRL (Ramirez De Arellano et al. 2015). Lopez-Pulido et al. (Lopez-Pulido et al. 2013) demonstrated that PRL/PRLR signal can be used as an important survival factor for CC. In a previous study, we showed that receptor dimerization is necessary for cytokines to activate signal transduction pathways in cells (Amoutzias et al. 2008). PRLR enhances lysine acetylation by recruiting CREB-binding protein (CBP) to neutralize the positive charge of the lysine side chain, and dimerizing the receptors, thereby facilitating the acquisition of transcription factors to increase transcription (Li et al. 2010; Gallagher et al. 2015). It has been reported that histone deacetylase inhibitors (HDACi) promote PRLR dimerization and subsequent signal transduction, whereas exogenous HDACs inhibit the processes (Li et al. 2010). While several HDACi have been shown to be involved in anti-tumoral immune responses (Li and Seto 2016), the underlying molecular mechanisms have not been fully understood. Domatinostat (4SC-202), a type 1 HDACi (von Tresckow et al. 2019), shows potent anti-tumor activities in various cell lines and preclinical models. 4SC-202 has been found to markedly inhibit proliferation and survival of colorectal cancer (CRC) cells as well as the growth of colorectal tumors in vivo (Huang et al. 2016). Moreover, 4SC-202 was capable of inhibiting the cell viability in urothelial carcinoma (UC) and inducing cell cycle disturbances and cell death (Pinkerneil et al. 2016). Gruber et al. found that 4SC-202 efficiently inhibits Hedgehog (HH)/GLI signaling in SMOi-sensitive and SMOi-resistant settings, while suppressing the growth of basal cell carcinoma (BCC) cells in vivo (Gruber et al. 2018). Given that research on anti-tumor effects of 4SC-202 on cervical cancer and the underlying mechanism is still lacking, we intended to investigate whether 4SC-202 plays an anti-cervical cancer effect through targeting the PRLR signaling pathway.

Materials and methods

Chemicals and reagents

4SC-202 was provided by Lan-jun Biotechnology Co.Ltd (Shanghai, China). Cell Counting Kit-8 (CCK-8) kit and DMSO were purchased from Sigma Aldrich (MO, USA). All cell culture reagents were purchased from Gibco Life Technologies (Grand Island, CA, USA).

Cell culture

Cervical cancer cell lines SiHa, Hela, and CaSki were obtained from the American Type Culture Collection (Manasas, VA, USA). Cells were maintained in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% fetal bovine serum (FBS), penicillin (100 U/ml) and streptomycin (100 μg/ml). Cell culture was conducted in a humidified atmosphere of 5% CO2 at 37 °C. Cells were grown to 80% confluence for further assays. All culture reagents were obtained from Gibco (Carlsbad, CA).

CCK-8 assay

SiHa, Hela, and CaSki cells were plated in 96-well plates and incubated with various concentrations of 4SC-202 (0, 0.01, 0.1, 1, 5, and 10 µM) for a certain period of time (24 h, 48 h, or 72 h). CCK-8 reagent (10 µL) was added and the mixture was incubated for 2 h in the dark. The optical density (OD) value for each well was measured at a wavelength of 450 nm. The half maximal inhibitory concentration (IC50) was calculated by using SPSS software.

Colony formation assay

Colony formation assay was carried out as described previously (Liao et al. 2020). SiHa, Hela, and CaSki cells (5 × 10^5 cells/well) were plated in 6-well plates and incubated with various concentrations of 4SC-202 (0, 1, and 5 µM) for 72 h. Culture media were refreshed every 2 days. The cells were stained with crystal violet, and the colonies were photographed and manually counted on day 10.

Cell cycle analysis and apoptosis detection

For cell cycle analysis, SiHa cells were incubated with various concentrations of 4SC-202 (0, 1, and 5 μM) for 72 h, digested with 0.25% trypsin and resuspended in phosphate-buffered saline (PBS) at a density of 1 × 10^6 cells/mL. Cell cycle distribution was detected by flow cytometry (BD Biosciences, CA, USA) and analyzed using Cell Quest software. For apoptosis detection, the cell suspension was gently mixed with 5 µL Annexin V-FITC and 10 µL PI, followed by an incubation at room temperature for 15 min in...
the dark. Apoptosis rate was determined by using the flow cytometry.

Western blotting

Total proteins were extracted from the cell lines using RIPA lysis buffer (Beyotime Biotechnology, Shanghai, China). Tissue lysates were centrifuged at 14,000 rpm for 5 min at 4 °C, and the supernatants were collected for further analysis. The protein concentration was determined using BCA protein Assay Kit (Beyotime). Fifty µg of total protein were mixed with loading buffer, denatured at 95 °C for 5 min, resolved on a 10% SDS polyacrylamide gels and electrotransferred to PVDF membranes (Bio-RAD, CA). After being blocked with 5% milk and 1% bovine serum albumin solution, the membrane was incubated at 4 °C overnight with the following primary antibodies: anti-Cleaved-caspase3, anti-BCL-2, anti-Bax, anti-p-STAT5a, anti-STAT5a, anti-p-JAK2, anti-JAK2, anti-p-MEK1/2, anti-MEK1/2, anti-p-ERK1/2, anti-ERK1/2, anti-p-AKT, anti-AKT, anti-p-PI3K, anti-PI3K, and anti-GAPDH (Abcam, diluted 1:1000). On the following day, the membrane was washed 3 times with TBST and then incubated with horseradish peroxidase-conjugated secondary antibodies (Abcam, diluted 1:5,000) for 2 h at room temperature. The protein bands were developed with a chemiluminescence detection system (Immobilion, Millipore), and the relative band intensity was analyzed using ImageJ software.

Quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA was isolated from the cell lines using the TRIzol kit (Thermo Fisher Scientific, Inc.). Afterward, total RNA was then reverse transcribed to cDNA by the SuperScript III Reverse Transcriptase Kit (Thermo Fisher Scientific, Inc.) using the manufacturer’s protocol. The gene expression was then detected using an ABI Stepone plus real-time PCR instrument (illumina eco, USA). The 2−ΔΔCq method was used to analyze the relative expression of mRNA. The primer sets are listed in Table 1. Glyceraldehyde-3-phosphate dehydrogenase (GADPH) was used as an internal control. The thermal cycling conditions were 95 °C for 3 min, followed by 45 cycles of 95 °C for 7 s, 57 °C for 10 s and 72 °C for 15 s.

Immunofluorescence

Cultured cells were fixed with 4% paraformaldehyde for 20 min at room temperature. Fixed cells were blocked with 1% BSA at room temperature for 30 min. After the pretreatments, the cells were incubated with the primary antibodies (PRLR:1:20) at 4 °C overnight, followed by an incubation with Alexa Flour-conjugated secondary antibody (Invitrogen) at room temperature for 1 h in the dark. Nuclear DNA was counterstained with 10 ng/ml DAPI at 4 °C for 30 min in the dark. Stained cells were observed and photographed under a fluorescence microscope (200 ×).

Xenograft assay

Five-week-old female C57BL/6 nude mice were obtained from Bioscience (Beijing, China) and housed in a laboratory animal room under standard conditions (12-h light/dark cycle, 20–25 °C, and 60–85% humidity), with ad libitum access to sterilized food and water. The mice were randomly divided into three groups (n = 5 per group): control, 50 mg/kg 4SC-202, and 100 mg/kg 4SC-202. SiHa cells were injected into the upper right flank of 15 nude mice. 4SC-202 treatment was started on day 6 post-injection when the tumors were palpable. 4SC-202 was dissolved in DMSO and administered by gavage every two days. The tumor size was measured every 3 days, and tumor volumes were calculated as follows: (V) = width² × length × 0.5. In the meantime, body weights of the mice were recorded every week. All the animals were euthanized, and the xenografts were weighted after 30 days. Subcutaneous tumors were dissected, removed and kept for immunohistochemical, western blot and qRT-PCR
analyses. Serum levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were measured by AU680 Chemistry System (Beckman Coulter, Brea, CA, USA). Organ tissues including lung, liver, kidney, and heart were removed, sectioned and subjected to H&E staining. Animal experiments were approved by the Ethics Committee of Medical Laboratory Animal Center of Chinese People’s Liberation Army General Hospital and performed in accordance with National Research Council (US) Committee for the Update of the Guide for the Care and Use of Laboratory Animals.

Immunohistochemical analysis

Paraffin-embedded sections were dewaxed with dimethyl benzene and rehydrated in dH₂O. The slides were immersed in citrate buffer (0.01 M, pH 6.0) with high-pressure heating for 2 min. After being blocked with 1% BSA (Sigma, USA) in TBST buffer for 5 min at room temperature, the slides were incubated with primary antibodies against Ki-67 (Proteintech, Wuhan, China) at a dilution of 1:16000 overnight at 4 °C, followed by an incubation with goat anti-rabbit IgG secondary antibody (Thermo Fisher, 1:5000 dilution) and DAB staining. Finally, the slides were counterstained with hematoxylin, dehydrated and sealed with neutral gum. Organ tissues were stained with H&E for histological analysis. The images were captured under a Nikon microscope.

Statistical analysis

Results were presented as the mean ± standard deviation (SD). At least three experimental replicates were evaluated for each sample. The significance of the experimental data was analyzed by using PRISM® GraphPad 8.0 software, one-way or two-way analysis of variance (ANOVA) followed by Tukey’s post-hoc tests. The significance level was set at P < 0.05, and p-values were indicated accordingly.

Results

4SC-202 suppresses the proliferation of cervical cancer cells

To study the effect of 4SC-202 on the proliferation of cervical cancer cells, we performed CCK-8 assay to detect the cell proliferation in selected three human cervical cancer cell lines SiHa, Hela and CaSki. As depicted in Fig. 1 A, 4SC-202 displayed a dose-dependent cytotoxicity against SiHa cells, while SiHa cells treated with 4SC-202 at a concentration of 1–10 µM showed significantly reduced cell viability. Moreover, we observed that while 4SC-202 exerted cytotoxic effect on SiHa cells in a time-dependent manner, the effect was the most obvious at 72 h. Strikingly, 4SC-202 exhibited the similar effect on both Hela (Fig. 1B) and CaSki cells (Fig. 1 C). Among the three cell lines, SiHa cells treated with 4SC-202 showed the most significant decrease in the cell viability (Fig. 1 A). Meanwhile, the IC50 values of 4SC-202 in all three cell lines decreased with increasing length of incubation. Collectively, these data indicated that 4SC-202 had anti-proliferative activity on cervical cancer cells. Besides, we performed colony formation assay and found that colony-forming ability of SiHa cells treated with 4SC-202 was most significantly inhibited (Fig. 1D), indicative of a suppressive effect of 4SC-202 on the growth of cervical cancer cells. The SiHa cells showed greater sensitivity to 4SC-202 than HeLa and CaSki cells. We, therefore, chose SiHa cells for subsequent experiments.

4SC-202 induces cell cycle arrest and apoptosis

Effects of 4SC-202 on the cell cycle distribution of SiHa cells were analyzed by flow cytometry. As illustrated in Fig. 2 A, treatment with 4SC-202 caused cell cycle arrest in SiHa cells. The quantitative analysis revealed a cell cycle arrest at G2/M phase in SiHa cells incubated with 4SC-202. The ratios of G2/M phase in SiHa cells treated with 0, 1 or 5 µM 4SC-202 for 72 h were 21.49 ± 0.81%, 30.01 ± 2.18% and 44.74 ± 1.64%, respectively. These observations demonstrated that 4SC-202 caused cell cycle arrest at G2/M phase in SiHa cells in a dose-dependent manner.

We next performed western blotting to analyze the expression of several apoptosis-related proteins. As shown in Figs. 2B and 4SC-202 down-regulated the expression of BCL-2 (26 kDa), while up-regulating the expression of Cleaved-caspase3 (17 kDa) and Bax (21 kDa). In addition, we evaluated the effects of 4SC-202 on cell apoptosis by flow cytometry and found that 4SC-202 treatment led to a dose-dependent increase in the apoptosis rate of SiHa cells (Fig. 2 C).

4SC-202 reduces the expression of PRLR in cervical cancer cells by inhibiting the PRLR pathway

We further examined the expression of PRLR pathway-related proteins by western blot. As presented in Fig. 3 A-C, compared with the control group, the phosphorylation levels of STAT5a, JAK2, AKT and PI3K were decreased after 4SC-202 treatment of SiHa cells, and the phosphorylation
levels of MEK1/2 and ERK1/2 increased. qRT-PCR showed that 4SC-202 resulted in a significant decrease in STAT5a, AKT and PI3K mRNA expression, while a significant increase in MEK mRNA expression. However, the mRNA expression of JAK2 and ERK1/2 only changed to a certain extent, and there was no significant difference. Moreover, we performed an immunofluorescence assay and qRT-PCR and observed that 4SC-202 significantly down-regulated the expression of PRLR in SiHa cells compared with the control group (Fig. 3E-F). Together, these results indicated that 4SC-202 reduced the expression of PRLR in CC cells through down-regulating PRLR pathway-related proteins.
mice (Fig. 4E), and PRLR mRNA was also significantly inhibited (Fig. 4F). In addition, we observed a consistency in the expression of PRLR pathway related proteins and mRNAs between in vivo and in vitro studies (Fig. 5).

Finally, H&E staining was performed to histologically analyze major organs such as lung, liver, spleen and heart. As shown in Fig. 6 A, no significant histological changes were detected in the tissue sections of mice treated with 4SC-202. We found that 4SC-202 had no significant effects on the serum biochemical function in the nude mice (Fig. 6B).

**Discussion**

HDAC is essential for epigenetic control of cancer gene expression and functions, while being involved in the regulation of cell cycle progression, cell proliferation and differentiation, apoptosis, metastasis as well as angiogenesis (Fu et al. 2016; Li and Seto 2016; Falkenberg and Johnstone 2014). HDACi can restore the homeostasis of cell
Fig. 3 4SC-202 affects the expression of related proteins by inhibiting the PRLR pathway in vitro. (A-C) The total protein expression and phosphorylation levels of PRLR pathway-related proteins STAT5a, JAK2, MEK1/2, ERK1/2, AKT and PI3K were determined by western blot analysis. (D) The relative mRNA expression of STAT5a, JAK2, MEK1/2, ERK1/2, AKT and PI3K were measured by qRT-PCR. (E) Immunofluorescence was performed to detect the expression of PRLR. The immunofluorescence intensity was quantified. Scale bar = 30 μm. (F) The PRLR mRNA expression was determined by qRT-PCR. *P < 0.05, **P < 0.01, ***P < 0.001 vs. the control group.
anti-tumor and anti-apoptotic effects in xenograft mouse model, dramatically decreased the percentage of Ki-67-positive cells and inhibited the PRLR pathway in tumor tissues. Meanwhile, we observed no significant alterations in serum biochemical function and histological morphology of organ tissues in the nude mice treated with 4SC-202, indicating that 4SC-202 was nontoxic in vivo. All these results suggested that 4SC-202 could serve as a potential therapeutic agent for CC.

HDACi can cause cell cycle arrest in G1/S or G2/M phase, highlighting HDACi as a promising target for cancer treatment (Li and Seto 2016). In this study, we showed that 4SC-202 treatment led to cell cycle arrest of SiHa cells in G2/M phase (Fig. 2A). This observation is consistent with the previous study showing a G2/M phase arrest in colorectal cancer (CRC) cell lines and primary human CRC tumor cells following 4SC-202 treatment (Huang et al. 2016). In addition, HDACi can regulate apoptosis in cancer processes of cancer. And HDACi has been applied in some clinical trials (Lu et al. 2016; Iyer and Foss 2015; Lee et al. 2015). DNA methylation and covalent histone modification have been identified as the most important epigenetic changes in cervical cancer (Fang et al. 2014). To date, HDACi such as valproic acid (VPA) (de la Cruz-Hernández et al. 2007), suberoylanilide hydroxamic acid (SAHA) (Zuo 2010), and trichostatin A (TSA) (Liu et al. 2012) have been used as anti-tumor agents in cervical cancer. However, the research on anti-tumor effects of HDACi 4SC-202 on CC is still lacking.

In this study, we showed that 4SC-202, a novel oral class I specific HDACi of benzamide compounds (Henning et al. 2010), can effectively inhibit the growth, proliferation, and cell cycle progression of CC cells and promote the apoptosis. Moreover, we found that 4SC-202 treatment led to inhibition of the PRLR pathway as well as down-regulation of PRLR expression in CC cells. Notably, 4SC-202 displayed anti-tumor and anti-apoptotic effects in xenograft mouse model, dramatically decreased the percentage of Ki-67-positive cells and inhibited the PRLR pathway in tumor tissues. Meanwhile, we observed no significant alterations in serum biochemical function and histological morphology of organ tissues in the nude mice treated with 4SC-202, indicating that 4SC-202 was nontoxic in vivo. All these results suggested that 4SC-202 could serve as a potential therapeutic agent for CC.
PRL had a protective effect on etoposide-induced apoptosis in CC cells (Lopez-Pulido et al. 2013). A study on melanoma cells showed that HDACi mainly suppressed the expression of anti-apoptotic proteins by inhibiting transcription (Galagher et al. 2015). Cytotoxicity of 4SC-202 against CRC cells was enhanced by AKT inhibition or AKT1 knockdown (Huang et al. 2016). Herein, we observed that the expression of PRLR and PRLR-related pathway components (STAT5a, JAK2, MEK1/2, ERK1/2, AKT, and PI3K) was significantly inhibited in SiHa cells treated with 4SC-202 (Fig. 3). Furthermore, 4SC-202 markedly decreased the proliferation of CC cells (SiHa, HeLa, and CaSki) in a dose- and time-dependent manner (Fig. 1). Combined with the results of apoptosis, we speculated that 4SC-202 may promote apoptosis in CC cells by down-regulating the expression of PRLR and inhibiting the PRLR pathway, thereby reducing the cell proliferation.

Previous studies have shown that PRLR signaling pathways play an important role in initiating CC (Ascencio-Cedillo et al. 2015). PRLR dimerization activates intracellular signaling cascades including downstream signaling pathways such as JAK/STAT5a, PI3K/AKT, and ERK1/2, thereby promoting cell proliferation, differentiation and survival (Kan et al. 2016; Gorvin et al. 2019). It has been reported that PRL induced an increased expression of anti-apoptotic genes in CC cell lines by activating the STAT pathway, which was linked to cell survival (Ramirez de Arellano et al. 2015). In a previous study, we found that PRL had a protective effect on etoposide-induced apoptosis in CC cells (Lopez-Pulido et al. 2013). A study on melanoma cells showed that HDACi mainly suppressed the expression of anti-apoptotic proteins by inhibiting transcription (Galagher et al. 2015). Cytotoxicity of 4SC-202 against CRC cells was enhanced by AKT inhibition or AKT1 knockdown (Huang et al. 2016). Herein, we observed that the expression of PRLR and PRLR-related pathway components (STAT5a, JAK2, MEK1/2, ERK1/2, AKT, and PI3K) was significantly inhibited in SiHa cells treated with 4SC-202 (Fig. 3). Furthermore, 4SC-202 markedly decreased the proliferation of CC cells (SiHa, HeLa, and CaSki) in a dose- and time-dependent manner (Fig. 1). Combined with the results of apoptosis, we speculated that 4SC-202 may promote apoptosis in CC cells by down-regulating the expression of PRLR and inhibiting the PRLR pathway, thereby reducing the cell proliferation.

The present study provided evidence that tumor growth was inhibited in the nude mice administered with 4SC-202, while no toxicity was detected in major organs of the mice.

Fig. 5 4SC-202 affects the expression of related proteins by inhibiting the PRLR pathway in vivo. (A-C) The expression levels of PRLR pathway-related proteins were determined by western blot analysis. (D) The relative mRNA expression of STAT5a, JAK2, MEK1/2, ERK1/2, AKT and PI3K were measured by qRT-PCR. *P<0.01, **P<0.01, ***P<0.001 vs. the control group.
In addition, immunohistochemical assays revealed that the expression of Ki67, a nuclear and nucleolar protein related to cell proliferation and severity of cervical lesions (Aslani et al. 2013; Riera-Leal et al. 2018), decreased significantly with the increasing concentration of 4SC-202 in the tumor tissues. The expression of apoptotic protein and PRLR pathway components decreased in the tumor tissues, and 4SC-202 displayed no effect on the levels of ALT and AST in serum. All these findings indicated that 4SC-202 may affect the proliferation and activity of CC cells through inhibiting the expression of PRLR-related pathway components as well as induce cycle phase arrest in vivo and was non-toxic to organs.

In conclusion, the present study showed that 4SC-202 caused cell cycle arrest of CC cells at G2/M phase through regulating the expression of PRLR-related pathway components (STAT5a, JAK2, MEK1/2, ERK1/2, AKT, and PI3K), induced apoptosis and inhibited CC cell proliferation. Moreover, 4SC-202 significantly suppressed the growth of CC in vivo, while exerting no toxicity to major organs in the nude mice. These findings suggest that 4SC-202 may potentially serve as a novel and efficacious therapeutic agent for CC.

Abbreviations
CC  cervical cancer
PRL  prolactin
PRLR  prolactin receptor

HDAC  histone deacetylase
HDACi  histone deacetylase inhibitor
CCK-8  Cell Counting Kit-8
IC50  half maximal inhibitory concentration
GADPH  Glyceraldehyde-3-phosphate dehydrogenase
ALT  alanine aminotransferase
AST  aspartate aminotransferase

Supplementary information The online version contains supplementary material available at https://doi.org/10.1007/s10735-022-10105-6.

Author contribution Y.G.M and H.J.Z conceived and designed the experiments. M.X.L, H.R.S, W.Y, M.X.Y, and H.L performed the experiments and analyzed the data; H.J.Z and M.X.L interpreted the results and wrote the manuscript. Y.G.M contributed to the review of the manuscript. All authors read and approved the final manuscript.

Funding This work was supported by grant from the China Postdoctoral Science Foundation General items (2018M633728).

Data availability All data generated or analyzed during this study are included in this published article.

Declarations

Conflict of interest The authors declare that they have no financial and non-financial conflict of interest.
References

Amoutzias GD, Robertson DL, Van de Peer Y, Oliver SG (2008) Choose your partners: dimerization in eukaryotic transcription factors. Trends Biochem Sci 33:220–229. doi:https://doi.org/10.1016/j.tibs.2008.02.002

Ascencio-Cedillo R, Lopez-Pulido EI, Munoz-Valle JF, Villegas-Sepulveda N, Del Toro-Arreola S, Estrada-Chavez C, Daneri-Navarro A, Franco-Topete R, Perez-Montiel D, Garcia-Carranca A, Pereira-Suarez AL (2015) Prolactin and prolactin receptor expression in cervical intraepithelial neoplasia and cancer. Pathol Oncol Res 21:241–246. doi:https://doi.org/10.1007/s12253-014-9814-6

Aslani FS, Safaei A, Pourjafari M, Mottaham M (2013) Evaluation of Ki67, p16 and CK17 Markers in Differentiating Cervical Intraepithelial Neoplasia and Benign Lesions. Iran J Med Sci 38:15–21

de la Cruz-Hernández E, Pérez-Cárdenas E, Contreras-Paredes A, Cantú D, Mohar A, Lizano M, Dueñas-González A (2007) The effects of DNA methylation and histone deacetylase inhibitors on human papillomavirus early gene expression in cervical cancer, an in vitro and clinical study. Virol J 4:18. doi:https://doi.org/10.1186/1743-422x-4-18

Falkenberg KJ, Johnstone RW (2014) Histone deacetylases and their inhibitors in cancer, neurological diseases and immune disorders. Nat Rev Drug Discov 13:673–691. doi:https://doi.org/10.1038/nrd4360

Fang J, Zhang H, Jin S (2014) Epigenetics and cervical cancer: from pathogenesis to therapy. Tumour Biol 35:5083–5093. doi:https://doi.org/10.1007/s13277-014-1737-z

Fu M, Wan F, Li Z, Zhang F (2016) 4SC-202 activates ASK1-dependent mitochondrial apoptosis pathway to inhibit hepatocellular carcinoma cells. Biochem Biophys Res Commun 471:267–273. doi:https://doi.org/10.1016/j.bbrc.2016.01.030

Gallagher SJ, Tiffen JC, Hersey P (2015) Histone Modifications, Modifiers and Readers in Melanoma Resistance to Targeted and Immune Therapy. Cancers (Basel) 7:1959–1982. doi:https://doi.org/10.3390/cancers7040870

Gorvin CM, Newey PJ, Rogers A, Stokes V, Neville MJ, Lines KE, Ntali G, Lees P, Morrison PI, Singhellakis PN, Malandrinou FC, Karavitaki N, Grossman AB, Karpe F, Thakker RV (2019) Association of prolactin receptor (PRLR) variants with prolactinomas. Hum Mol Genet 28:1023–1037. doi:https://doi.org/10.1093/hmg/ddy396

Gnuber W, Peer E, Elmer DP, Sternberg C, Tesanovic S, Del Burgo P, Ntali G, Grossman AB, Meier K, Kohlhof H, Vitt D, Aberger F (2018) Targeting class I histone deacetylases by the novel small molecule inhibitor 4SC-202 blocks oncogenic hedgehog-GLI signaling and overcomes smoothened inhibitor resistance. Int J Cancer 142:968–975. doi:https://doi.org/10.1002/ijc.31117

Henning SW, Dobhofer R, Kohlhof H, Jankowsky R, Hentsch B (2010) 178 Preclinical characterization of 4SC-202, a novel iso-type specific HDAC inhibitor. Eur J Cancer Suppl 8:61–61

Hu Z, Ma D (2018) The precision prevention and therapy of HPV-related cervical cancer: new concepts and clinical implications. Cancer Med 7:5217–5236. doi:https://doi.org/10.1002/cam4.1501

Huang Z, Wang S, Chen L, Li J, Qin LS, Li D (2016) Pre-clinical characterization of 4SC-202, a novel class I HDAC inhibitor, against colorectal cancer cells. Tumor Biology 37:10257–10267

Iyer SP, Foss FF (2015) Romidepsin for the Treatment of Peripheral T-Cell Lymphoma. Oncologist 20:1084–1091. doi:https://doi.org/10.1634/theoncologist.2015-0043

Kan QE, Su Y, Yang H, Man H (2016) Different intracellular signaling pathways triggered by an anti-prolactin receptor (PRLR) antibody: Implication for a signal-specific PRLR agonist. Int J Biol Macromol 82:892–897. doi:https://doi.org/10.1016/j.ijbiomac.2015.10.068

Lee HZ, Kwitkowski VE, Del Valle PL, Ricci MS, Saber H, Habtemariam BA, Bullock J, Bloomquist E, Li Shen Y, Chen XH, Brown J, Mehrota N, Dorff S, Charlab R, Kane RC, Kaminskas E, Justice R, Farrell AT, Pazdur R (2015) FDA Approval: Belinostat for the Treatment of Patients with Relapsed or Refractory Peripheral T-cell Lymphoma. Clin Cancer Res 21:2666–2670. doi:https://doi.org/10.1158/1078-0432.ccr-14-3119

Li M, Gao JS, Guan Y, Shi X, Hao Z, Ayrapetov MK, Zhe Z, Xu L, Hyun YM, Kim M (2010) Acetylation modulates prolactin receptor dimerization. Proc Natl Acad Sci USA 107:19314–19319

Li Y, Seto E (2016) HDACs and HDAC Inhibitors in Cancer Development and Therapy. Cold Spring Harb Perspect Med 610.1101/cshperspect.a026831

Liao B, Sun Q, Yuan Y, Yin Q, Qiao J, Jiang P (2020) Histone deacetylase inhibitor MGCD0103 causes cell cycle arrest, apoptosis, and autophagy in liver cancer cells. J Cancer 11:1915–1926. doi:https://doi.org/10.7150/jca.34091

Liu N, Zhao LJ, Li XP, Wang JL, Chai GW, Wei LH (2012) Histone deacetylase inhibitors inducing human cervical cancer cell apoptosis by decreasing DNA-methyltransferase 3B. Chin Med J (Engl) 125:3273–3278

Lopez-Pulido EI, Muñoz-Valle JF, Del Toro-Arreola S, Jave-Suárez LF, Bueno-Topete MR, Estrada-Chávez C, Pereira-Suárez AL (2013) High expression of prolactin receptor is associated with cell survival in cervical cancer cells. Cancer Cell Int 13:103. doi:https://doi.org/10.1186/1475-2867-13-103

Lu X, Ning Z, Li Z, Cao H, Wang X (2016) Development of chidamide for peripheral T-cell lymphoma, the first orphan drug approved in China. Intractable & Rare Diseases Research 5:185

Oltuska P, Banerjee HN, Philley JV, Dagsupta S (2019) Human Papilloma Virus-Associated Cervical Cancer and Health Disparities. Cells 801.3390/cells8060622

Pinkernel M, Hoffmann MJ, Kohlhof H, Schulza WA, Niegisch G (2016) Evaluation of the Therapeutic Potential of the Novel Iso-type Specific HDAC Inhibitor 4SC-202 in Urothelial Carcinoma Cell Lines. Target Oncol 11:783–798. doi:https://doi.org/10.1007/s11523-016-0444-7

Ramirez de Arellano A, Lopez-Pulido EI, Martinez-Neri PA, Estrada Chavez C, Gonzalez Lucano R, Fafuris-Morris M, Aguilar-Lemarroy A, Munoz-Valle JF, Pereira-Suarez AL (2015) STAT3 activation is required for the antiapoptotic effects of prolactin in cervical cancer cells. Cancer Cell Int 15:83. doi:https://doi.org/10.1186/s12935-015-0234-9

Ramirez De Arellano A, Riera Leal A, Lopez-Pulido EI, Gonzalez-Lucano LR, Macias Barragan J, Del Toro Arreola S, Garcia-Chagollan M, Palafoux-Sanchez CA, Munoz-Valle JF, Pereira-Suarez AL (2018) A 60 kDa prolactin variant secreted by cervical cancer cells modulates apoptosis and cytokine production. Oncol Rep 39:1253–1260. doi:https://doi.org/10.3892/or.2018.6222

Riera-Leal A, Ramirez De Arellano A, Ramirez-Lopez IG, Lopez-Pulido EI, Davila Rodriguez JR, Macias-Barragan JG, Ortiz-Lazaren BC, Jave-Suarez LF, Artaza-Irigary C, Del Toro Arreola S, Montoya-Buelna M, Munoz-Valle JF, Pereira-Suarez AL (2018) Effects of 60 kDa prolactin and estradiol on metabolism and cell survival in cervical cancer: Coexpression of their hormonal receptors during cancer progression. Oncol Rep 40:3781–3793. doi:https://doi.org/10.3892/or.2018.6743

von Tresckow B, Sayehchi C, Aultzky WE, Goebeler ME, Schwab M, Berez E, Krauss B, Krauss R, Hermann F, Bartz R, Engel A (2019) Phase I Study of domatinostat (4SC-202), a class I histone deacetylase inhibitor in patients with advanced hematological malignancies. Eur J Haematol 102:163–173. doi:https://doi.org/10.1111/ejh.13188

© Springer
Wen X, Lin ZQ, Liu B, Wei YQ (2012) Caspase-mediated programmed cell death pathways as potential therapeutic targets in cancer. Cell Prolif 45:217–224. doi:https://doi.org/10.1111/j.1365-2184.2012.00814.x
Zuo (2010) Synergistic induction of apoptosis in HeLa cells by the proteasome inhibitor bortezomib and histone deacetylase inhibitor SAHA. Molecular Medicine Reports 3
zur Hausen H (1996) Papillomavirus infections—a major cause of human cancers. Biochim Biophys Acta 1288:F55–78. doi:https://doi.org/10.1016/0304-419x(96)00020-0

Publisher’s note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Springer Nature or its licensor holds exclusive rights to this article under a publishing agreement with the author(s) or other rightsholder(s); author self-archiving of the accepted manuscript version of this article is solely governed by the terms of such publishing agreement and applicable law.