Original Paper

Periplocin Extracted from Cortex Periplocae Induced Apoptosis of Gastric Cancer Cells via the ERK1/2-EGR1 Pathway

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Key Words

Periplocin • Apoptosis • Gastric cancer cell • EGR1

Abstract

Background/Aims: Periplocin is extracted from the traditional herbal medicine cortex periplocae, which has been reported to suppress the growth of cancer cells. However, little is known about its effect on gastric cancer cells. **Methods:** Gastric cancer cells were treated with periplocin, and cell viability was assessed using MTS assay. Flow cytometry and TUNEL staining were performed to evaluate apoptosis, and protein expression was examined by western blotting. Microarray analysis was used to screen for changes in related genes. **Results:** We found that periplocin had an inhibitory effect on gastric cancer cell viability in a dose-dependent manner. Periplocin inhibited cell viability via the ERK1/2-EGR1 pathway to induce apoptosis. Periplocin also inhibited the growth of tumor xenografts and induced apoptosis *in vivo*. **Conclusion:** Our results show that periplocin inhibits the proliferation of gastric cancer cells and induces apoptosis *in vitro* and *in vivo*, indicating its potential to be used as an antitumor drug.

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Introduction

Gastric cancer is the fourth most common cancer and the second most common cause of cancer deaths worldwide and is particularly prevalent in Asian countries [1]. Currently, an effective treatment for the disease is not available, and identification of early stage gastric cancer is difficult given that it is often asymptomatic or misdiagnosed [2]. Surgery is the chief prevailing therapy for gastric cancer, but 42.2% of patients with advanced gastric cancer are unable to undergo surgical therapy [3]. While chemotherapy is also an option for treatment

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of gastric cancer, it is likely to have off-target effects that can damage other tissues and organs as well [4]. Therefore, it is imperative to develop novel and effective chemotherapeutic agents that target critical pathways and induce the death of gastric cancer cells.

Cortex periplocae is the dry root of *Periploca sepium* Bge. It is a traditional herbal medicine that contains a variety of compounds, such as sterols, glycosides and esters, and can be used in cardiogenic and diuretic treatments [5]. Itokawa et al., initially found that periplocoside A from cortex periplocae inhibited the proliferation of S180 cancer cells [6]. Recent reports have shown that periplocin extracted from cortex periplocae significantly inhibits the growth of several tumor cells, including colon cancer and lung cancer cells, and promotes the induction of the apoptosis signaling pathway [7, 8]. It also exhibits low toxicity in normal cells. However, the complex mechanism by which periplocin renders its antitumor activity has not been elucidated. While studies have shown that periplocin inhibits the proliferation of colon cancer or esophageal cancer cells by inducing apoptosis, the effects of cortex periplocae in gastric cancer have not been examined yet. Therefore, the purpose of our study was to investigate the effect of cortex periplocae on gastric cancer cells and to determine its molecular mechanism.

Materials and Methods

Cell lines and cell culture

Human gastric cancer cell lines SGC-7901, MGC-803 and BGC-823 and the normal epithelial gastric cell line GES-1 were obtained from the Cellular Biology Institute of the Shanghai Academy of Sciences (Shanghai, China) and cultured in RPMI-1640 medium containing 10% bovine serum, penicillin (100 U/ml) and streptomycin (100 µg/ml) at 37°C in a 5% CO₂ atmosphere.

Chemicals and antibodies

Periplocin (purity ≥ 96%) was obtained from the New Drug Research and Development Center of North China Pharmaceutical Group Corporation (Hebei, China). It was dissolved in DMSO and diluted to 50, 100, 200 ng/ml using RPMI-1640 medium (the final concentration of DMSO < 0.01%). Fetal bovine serum was purchased from Biological Industries (Beit Haemek, Israel). RPMI-1640 medium was purchased from Gibco-BRL (Life Technologies, Paisley, Auckland). Antibodies against Mcl-1, Bid, p-ERK1/2, ERK1/2 and Ki-67 were purchased from Cell Signaling Technology (CST, CA, USA), caspase-3, EGR1 and GAPDH were from Abcam (Cambridge, UK). DeadEnd™ Colorimetric TUNEL System and 3-(4, 5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt (MTS) were purchased from Promega Corporation (Madison, USA). Annexin V FITC Apoptosis Kit was purchased from BD Biosciences (San Diego, CA, USA). SPlink Detection Kits and 3, 3'-diaminobenzidine tetrahydrochloride were purchased from ZSGB-BIO (Beijing, China). siRNA Transfection Reagent Kit was purchased from RIBOBIO (Guangzhou, China).

MTS assay detects periplocin-mediated inhibition of growth in human gastric cancer cell lines (SGC-7901, MGC-803, BGC-823 and GES-1)

Cells in the logarithmic growth phase were plated in 96-well culture plates at 1×10^4 cells/well. Upon treatment of cells with periplocin (0, 50, 100, 200 ng/ml) for 24 h and 48 h, 20 µl of MTS solution was added to each well, followed by a 2 h MTS incubation. Then, the optical densities (ODs) were measured using a microplate reader at 490 nm. The cell growth inhibition rate was calculated using the following formula:

$$1 - \frac{\text{OD}_{\text{experiment}}}{\text{OD}_{\text{control}}}$$

Flow cytometry analysis of apoptosis with Annexin-V/PI double staining

SGC-7901 and MGC-803 cells were treated with periplocin (0, 50, 100, 200 ng/ml) for 24 h. Approximately 5×10^5 cells were harvested and washed twice with phosphate buffer saline (PBS) followed by resuspension in 500 µl of 1X binding buffer. Five microliters Annexin V and 5 µl PI were added to the solution and incubated for 5 minutes at room temperature in the dark. The cells were gently vortexed for flow cytometric analysis.

Western blot analysis

Proteins were extracted with RIPA buffer containing protease inhibitor, and the concentrations were determined using BCA assay. Equal amounts of protein (50 µg) from each sample was separated by 12% sodium dodecyl sulfate polyacrylamide gel (SDS-PAGE) and transferred to a polyvinylidene difluoride (PVDF) membrane (Millipore), which was then incubated for 1 h at room temperature with 5% skim milk. Afterwards, the membranes were incubated overnight with appropriate primary antibodies at 4°C. After washing the membranes three times with tris buffered saline containing Tween (TBST), they were incubated with fluorochrome-labeled secondary anti-rabbit IgG (IRDye 800-LI-COR, Odyssey) for 1 h at room temperature. Membranes were imaged using the Odyssey infrared imaging system (USA, LI-COR). GAPDH was taken as a loading control. Western blot analysis was repeated independently 3 times.

Terminal deoxynucleotidyl transferase (TdT) - mediated dUTP nick end labeling (TUNEL) assay for cell apoptosis

The DeadEnd™ Colorimetric TUNEL System was used to assay cell apoptosis. To detect apoptosis in cultured cells, coverslips were immersed in 4% paraformaldehyde for 25 min, 0.2% Triton X-100 in PBS for 5 min, and 100 µl Equilibration Buffer at room temperature for 5 min, followed by addition of 100 µl TdT reaction mix (98 µl Equilibration Buffer, 1 µl Biotinylated Nucleotide Mix and 1 µl rTdT Enzyme) and incubation at 37°C for 60 min. The coverslips were then immersed in 2 × SSC for 15 min, 0.3% hydrogen peroxide for 3 min, and 100 µl Streptavidin HRP for 30 min, after which 100 µl DAB was added until a light brown background developed. For detection of apoptosis in tissue sections, paraffin-embedded tissue sections were deparaffinized and permeabilized with Proteinase K. The other steps were similar to apoptosis detection in cultured cells. The nuclei of apoptotic cells were stained dark brown. Quantitative analysis was performed blindly by counting the number of TUNEL-positive cells in ten microscopic fields, as described previously. Apoptosis rate % = (apoptotic cells/total cells) %.

Microarray data

Differential gene expression between SGC-7901 cells treated with 100 ng/ml periplocin and untreated cells was analyzed. Total RNA of cells was extracted using TRIzol reagent. The Affymetrix Human Genome U133 Plus 2.0 Array was used to detect gene expression profiles following the manufacturer's instructions. Preliminary data analysis was conducted using the software of the Affymetrix microarray suite. The cutoff criterion for gene calls was at least a 4-fold difference in expression between two compared profiles, and p-values < 0.05 were identified. The microarray data set was submitted to the GEO repository (GSE78211).

Cell rescue

The level of EGR1 in SGC-7901 cells was knocked down using the human-specific EGR1 siRNA Transfection Reagent Kit following the manufacturer's protocol. Effective downregulation of EGR1 in cell lines was verified using RT-PCR. First, SGC-7901 cells were treated with periplocin 18 h. Second, the EGR1 siRNA or negative control with transfection reagents were overlaid on the cells for 6 h at 37°C in a 5% CO₂ atmosphere. Finally, cell viability was measured by MTS assay after transfer of cells into normal growth medium for 24 h.

Tumor xenograft study using athymic nude mice

To determine the *in vivo* chemotherapeutic efficacy of periplocin against gastric cancer tumor xenograft growth, five- to six-week-old male immunodeficient BALB/c mice (n = 12) were obtained from Beijing Weitong Lihua Experimental Animal Technology Co. Ltd (Beijing, China). The animals were housed at the Fourth Hospital of Hebei Medical University Experiment Animal Center. SGC-7901 cells (1 × 10⁵ / 100 µl / mouse) were suspended in PBS and injected subcutaneously into the right flank of each mouse. The mice were randomized into four groups. Group I (n = 3) was treated with 0.9% physiological saline; Group II (n = 3) was treated with 0.04 mg cisplatin; Group III (n = 3) was treated with 0.36 mg periplocin; and Group IV (n = 3) was treated with 0.09 mg periplocin every two days. The tumor sizes in the mice in each group were measured every two days. After 14 days, the mice were sacrificed and the tumors were harvested. The tumor tissues were fixed in formalin to obtain sections for the TUNEL assay and immunohistochemistry.

Immunohistochemistry

Immunohistochemistry was performed to detect the levels of Mcl-1, pro-bid, pro-caspase-3, Ki-67, EGR1 and p-ERK1/2 in tumor tissue. The slides of tumor tissue were deparaffinized with xylene and rehydrated through a series of ethanol concentrations. Antigens were retrieved by boiling under pressure in ethylene di-ammonium tetra acid (EDTA) buffer (pH = 9.0) for 3 minutes. Sections were incubated with 0.3% H₂O₂ for 20 minutes and blocked with goat serum for 45 minutes followed by washing with PBS. Sections were then incubated with primary antibodies of Mcl-1, Bid, caspase-3, Ki-67, EGR1 and p-ERK1/2 (dilution 1:100) overnight at 4°C. The next day, sections were incubated with the secondary antibody for 30 minutes at 37°C followed by incubation with the HRP labeled streptavidin solution for 30 minutes. PBS was used for washing after each step. After visualization of the positive antigen antibody reaction by incubation with 3, 3-diaminobenzidine-tetrachloride (DAB) for 5 minutes, sections were counterstained with hematoxylin and evaluated by light microscopy.

Statistical analysis

SPSS13.0 software was utilized to analyze data, which was expressed as the mean ± SEM. One-way ANOVA test was used to examine the significant differences in the experimental data between the groups. Within the two groups, data were compared using SNK-q. The threshold for significance was defined as $P < 0.05$. All experiments were repeated three times independently.

Results

Periplocin inhibits the cell viability of gastric cancer cells

The effect of periplocin on the viability of gastric cancer cell lines SGC-7901, MGC-803 and BGC-823 was determined using the MTS assay. Cells were treated with different

Fig. 1. Effect of periplocin on the viability of gastric cancer cells. (A) Gastric cancer cells (SGC-7901, MGC-803, BGC-823 and GES-1) were treated with various concentrations of periplocin (0, 50, 100, and 200 ng/ml) for 24 h and 48 h. Cell viability was determined by MTS assays. Data are shown as the mean ± SEM. * $P < 0.05$ compared to the untreated cell group. (B) Cell morphology of gastric cancer cell lines treated with periplocin (0, 50, 100, and 200 ng/ml).

Fig. 2. Periplocin induces apoptosis in gastric cancer cells. Flow cytometry analysis of (A) SGC-7901 cells and (B) MGC-803 cells treated with different concentrations of periplocin for 24 h and examined by Annexin-V/PI double staining. The lower right quadrant indicates the percentage of early apoptotic cells and the upper right quadrant indicates the percentage of late apoptotic cells. * $P < 0.05$ compared to the untreated cell group.

concentrations of periplocin (0, 50, 100, or 200 ng/ml) for 24 h and 48 h. A dose- and time-dependent inhibition of the viability of gastric cancer cells was observed (Fig. 1A). After treatment with periplocin (50, 100, or 200 ng/ml) for 24 h, the cell viability of SGC-7901 and MGC-803 cells ranged from 63.94% to 53.39% ($P < 0.05$) and 66.43% to 37.06% ($P < 0.05$), respectively. A similar effect was also observed in BGC-823 cells. However, periplocin had no effect on GES-1 cells ($P > 0.05$). We also detected dramatically altered cell morphology in cells treated with periplocin (Fig. 1B). The shape of SGC-7901, MGC-803 and BGC-823 cells treated with periplocin appeared round compared to untreated cells. However, no change was observed in GES-1 cells.

Treatment of gastric cancer cells with periplocin induces apoptosis

To investigate whether periplocin induced apoptosis, SGC-7901 and MGC-803 cells were treated with varying doses of periplocin for 24 h, and the number of apoptotic cells was determined by flow cytometry using Annexin V-FITC staining. The lower right and upper right quadrants of the histogram represent early and late stage apoptotic cells, respectively (Fig. 2). Treatment of SGC-7901 and MGC-803 cells with periplocin for 24 h resulted in induction of apoptotic cell death in both cell lines. Apoptotic cells among SGC-7901 and MGC-803 cells after periplocin (50, 100, or 200 ng/ml) treatment ranged from 26.77% to 61.78% ($P < 0.05$) and 20.34% to 42.90% ($P < 0.05$), respectively, compared to only 0.27% and 5.93% in untreated control cells.

Fig. 3. TUNEL assay of gastric cancer cells treated with periplocin. TUNEL staining of (A) SGC-7901 cells and (B) MGC-803 cells treated with different concentrations of periplocin for 24 h. The number of apoptotic cells was increased in the periplocin treated group. * $P < 0.05$ compared to the untreated cell group.

TUNEL assays confirmed that treatment of SGC-7901 and MGC-803 cells with periplocin for 24 h resulted in a significant increase in apoptotic cells (Fig. 3).

Periplocin induces changes in the expression of apoptosis-related proteins

To investigate the potential mechanism involved in periplocin-induced apoptosis, we determined the expression of various apoptosis-related molecules by western blot analysis (Fig. 4). Treatment of SGC-7901 and MGC-803 cells with periplocin (0, 50, 100, or 200 $\mu\text{g/ml}$) for 24 h resulted in a dose-dependent decrease in the expression of anti-apoptotic protein, Mcl-1, while pro-Bid, decreased with increasing doses of periplocin. In addition, the results revealed a gradual increase of cleaved caspase-3 in a dose-dependent manner.

Periplocin was identified as a differential expressed gene by microarray

Next, we performed a differential gene expression analysis between untreated and 100 ng/ml periplocin treated SGC-7901 cells. There were many differentially expressed genes identified by the Affymetrix Human Genome U133 Plus 2.0 Array. The genes that exhibit the most significant fold changes and are related to cancer growth are shown in the Table 1. We verified these results by real-time PCR (Fig. 5A). The results of pathway analysis performed with differentially expressed genes showed that several signals were related to periplocin inhibited proliferation of gastric cancer cells, such as RhoA signaling, GADD45 signaling and p53 signaling (Fig. 8).

Many studies have reported that upregulation of EGR1 expression can inhibit proliferation of breast cancer, non-small cell lung carcinoma and esophageal squamous cell

Fig. 4. Periplocin induces changes in the expression of apoptosis-related proteins. Western blot analysis of pro-bid, Mcl-1, cleaved caspase-3 and p-ERK1/2 protein levels in (A) SGC-7901 cells and (B) MGC-803 cells treated with periplocin for 24 h. * $P < 0.05$ compared to the untreated cell group.

Table 1. Differential gene expression in SGC-7901 cells treated with periplocin compared to untreated cells

Gene	Fold-Change (t vs. c)	Description
ID2	26.4446	inhibitor of DNA binding 2, dominant negative helix-loop-helix
LINC00273	13.1932	long intergenic non-protein coding RNA 273
RN45S	11.0344	45S pre-ribosomal RNA
TMEM27	9.54151	transmembrane protein 27
RN45S	9.18391	45S pre-ribosomal RNA
EGR1	7.65261	early growth response 1
PTGS2	6.66579	prostaglandin-endoperoxide synthase 2
ID2	6.62147	inhibitor of DNA binding 2, dominant negative helix-loop-helix protein
UGCG	6.30983	UDP-glucose ceramide glucosyltransferase
NR4A3	6.173	nuclear receptor subfamily 4, group A, member 3
AREG	5.56132	amphiregulin
DKK1	5.52635	dickkopf 1 homolog (Xenopus laevis)
SRGAP1	5.525	SLIT-ROBO Rho GTPase activating protein 1
ARL5B	5.15782	ADP-ribosylation factor-like 5B
GEM	5.13883	GTP binding protein overexpressed in skeletal muscle
DUSP4	5.1209	dual specificity phosphatase 4
DUSP4	5.06099	dual specificity phosphatase 4
SERPINF5	-9.56029	serpin peptidase inhibitor, clade B (ovalbumin), member 5
KCTD18	-6.99119	potassium channel tetramerisation domain containing 18
DGKA	-5.9721	diacylglycerol kinase, alpha 80kDa
SORBS2	-5.82356	sorbin and SH3 domain containing 2
MEGF6	-5.60072	multiple EGF-like-domains 6
ASB9	-5.46465	ankyrin repeat and SOCS box containing 9
CHD9	-5.42373	chromodomain helicase DNA binding protein 9
LOC728431	-5.25579	uncharacterized LOC728431
NUCKS1	-5.05523	nuclear casein kinase and cyclin-dependent kinase substrate 1

Fig. 5. Periplocin activates p-ERK1/2/EGR1 and suppresses gastric cancer cell viability via EGR1. (A) Real-time PCR analysis of EGR1, H19 and SerpinB5 mRNA levels in SGC-7901 cells treated with periplocin for 24 h. (B) Western blot analysis of EGR1 and p-ERK1/2 protein levels in SGC-7901 and MGC-803 cells treated with periplocin for 24 h. (C) RT-PCR analysis of EGR1 mRNA levels in SGC-7901 and MGC-803 cells transfected with EGR1 siRNA. (D) MTS assay of SGC-7901 and MGC-803 cells transfected with EGR1 siRNA or negative control siRNA and treated with periplocin.

carcinoma cells [9-11]. Our microarray results also showed increased expression of EGR1 in SGC-7901 cells treated with periplocin. Therefore, we examined the role of EGR1 in greater detail to study the mechanism of inhibition of cell viability by periplocin in gastric cancer cells.

Periplocin upregulated the expression of EGR1 and ERK1/2

We evaluated the expression of EGR1 protein in SGC-7901 and MGC-803 cells treated with periplocin by western blot and obtained similar results as the microarray findings (Fig. 5B). ERK1/2 is an upstream regulators of EGR1 [12], so we also examined its expression and activation (phosphorylation) status. The western blot results showed that periplocin also upregulated the expression of p-ERK1/2 (Fig. 5B). Together, these results suggest that periplocin may inhibit the cell viability of gastric cancer cells by the ERK1/2-EGR1 pathway.

Knockdown of EGR1 could rescue the cell viability of gastric cancer cells treated with periplocin

To more closely examine the role of EGR1 in periplocin suppression of gastric cancer cell viability, we performed knockdown of EGR1 using siRNA transfection. Effective downregulation of EGR1 in cell lines was verified using RT-PCR (Fig. 5C). MTS assay analysis

Fig. 6. Effects of periplocin on gastric tumors in nude mice. (A) Representative images from each treatment group are presented. The sizes of the tumor xenografts were recorded daily in each treatment group. Upon termination of the experiment, wet tumor weight was measured. Significant inhibition in tumor xenograft growth and tumor weight was recorded in the periplocin-treated mice. * $P < 0.05$ compared to the control group. (B) Micrographs of hematoxylin/eosin-stained sections of tumor tissues. (C) Comparison of the apoptosis levels in the four treatment groups by TUNEL staining. The high dose group and positive group had significantly increased cellular apoptotic levels in the tumor tissues compared to controls. The apoptotic level of the low dose group also increased compared to controls.

demonstrated that the viability of cells knocked down for EGR1 expression was increased compared to cells transfected with control siRNA and treated with periplocin (Fig. 5D). Together these data suggest that periplocin may inhibit cell viability of gastric cancer cells via the ERK1/2-EGR1 pathway.

Effects of periplocin on gastric tumor formation in nude mice

To further explore the effects of periplocin in gastric cancer cells, we examined the inhibition of periplocin on tumor formation using SGC-7901 cells in BALB/c nude mice *in vivo*. Mice treated with periplocin did not exhibit impaired movement or any other visible sign of

Fig. 7. Immunohistochemistry of apoptosis-related proteins *in vivo*. Immunohistochemical staining for Mcl-1, Bid, caspase-3, Ki-67, EGR1 and p-ERK1/2 in tumor tissues. Expression levels of Mcl-1, pro-Bid, pro-caspase-3 and Ki-67 were decreased and expression levels of EGR1 and p-ERK1/2 were increased in periplocin-treated groups compared to controls.

physical toxicity. After 14 days, tumors were harvested and weighed (Fig. 6A, B). The tumor volumes of all drug-treated groups (Groups II, III, and IV) were significantly lower than the control group (Group I). The tumor volume in the group treated with a high concentration of periplocin (Group III) was similar to the cisplatin positive control (Group II).

To examine whether periplocin can lead to apoptosis of SGC-7901 cells *in vivo*, we performed TUNEL assays in tumor tissues (Fig. 6C). The results showed significantly higher numbers of apoptotic cells in the groups treated by cisplatin and periplocin compared to the control group.

To investigate the potential mechanism by which periplocin induces apoptosis *in vivo*, we performed immunohistochemistry on apoptosis-related molecules in tumor tissues. As shown in Fig. 7, the expression levels of Mcl-1, pro-caspase-3, pro-Bid and Ki-67 were decreased in all treatment groups compared with the control group. Notably, the expression levels of EGR1 and p-ERK1/2 were also increased in all treatment groups compared with the control group.

Discussion

Gastric cancer is associated with malignant tumors and with high incidence and mortality [13]. Identification of novel treatments for gastric cancer is required given the low sensitivity and high toxicity of chemotherapeutic drugs. Periplocin is a monomer compound obtained from cortex periplocae by butanol extraction. Previous studies have demonstrated that periplocin exhibits a potent inhibitory effect on the growth of several tumor cell lines [8, 14]. However, its effects on human gastric carcinoma cells have not yet been examined. In this study, we verified the effect of periplocin on gastric cancer cells using both *in vitro* and *in vivo* models.

Cancer is associated not only with abnormal proliferation and differentiation of cells but also with abnormal apoptosis [15, 16]. Apoptosis or programmed cell death is a process that results in the controlled elimination of unhealthy or damaged cells [17]. Many studies have reported that apoptosis contributes to the inhibition of tumor cell proliferation in malignant

Fig. 8. The results of performing the pathway analysis with differentially expressed genes.

tumors [18-20]. We examined the effect of periplocin on gastric cancer cells by MTS assays and found that after treatment with periplocin for 24 h and 48 h, the viability of several gastric cancer cell lines was significantly reduced. In addition, flow cytometry and TUNEL assays confirmed a dose-dependent increase in apoptosis in SGC-7901 and MGC-803 gastric cancer cells upon treatment with periplocin. Apoptosis is a complex cellular mechanism governed by several signaling molecules [21, 22]. The Bcl-2 family mediates a major apoptotic signal transduction cascade and includes anti-apoptotic and pro-apoptotic proteins [23, 24]. We found that induction of apoptosis in SGC-7901 and MGC-803 cells by periplocin was associated with a decrease in the levels of the anti-apoptotic protein Mcl-1 and non-activated Bid, a pro-apoptotic protein. In addition, the expression level of cleaved caspase-3 was increased by periplocin. Caspases are a group of cysteine-containing proteolytic enzymes that play a critical role in mediating apoptosis [25, 26]. Caspase-3 is a key enzyme that is activated by cleavage at the start of apoptosis and is one of the crucial downstream factors in executing the apoptotic program [27, 28]. Our *in vivo* immunohistochemistry and TUNEL assay results were consistent with these findings. Together, these data indicate that the inhibitory effects of periplocin on gastric cancer cells are mediated by induction of apoptosis.

The microarray results showed that several genes and pathways were modified upon treatment of gastric cancer cells with periplocin. In particular, the expression level of EGR1 was increased significantly. EGR1 is activated by periplocin in gastric cancer cells *in vitro* and *in vivo*, and knockdown of EGR1 can rescue gastric cancer cells treated with periplocin. The EGR1 gene is a member of the immediate early gene family encoding a zinc finger transcriptional factor [29] and is an important regulator of cell growth, differentiation and survival that is known to play a role in carcinogenesis and cancer progression [30]. Given that EGR1 is known to be upregulated via the activation of ERK1/2 [31], we also analyzed blots for increases in ERK1/2 phosphorylation. Our results showed that increased ERK1/2 phosphorylation appeared to correlate with the increases seen in EGR1 levels. Our *in vivo* immunohistochemistry results were consistent with these findings. Together these data indicate that periplocin might inhibit the cell viability of gastric cancer cells via the ERK1/2-EGR1 pathway.

In conclusion, our results show that periplocin can inhibit the proliferation of gastric cancer cells by inducing apoptosis *in vitro* and *in vivo* and that the ERK1/2-EGR1 pathway may mediate the cellular effects of periplocin. These findings provide an experimental basis for using periplocin as a chemotherapeutic drug against gastric cancer cells, thereby facilitating the development of new anticancer agents.

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Disclosure Statement

The authors have declared no conflicts of interest.

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Erratum

In the original article by Li et al. entitled “Periplocin Extracted from Cortex Periplocae Induced Apoptosis of Gastric Cancer Cells via the ERK1/2-EGR1 Pathway3” [Cell Physiol Biochem 2016;38:1939-1951 (DOI: 10.1159/000445555)] there is an error in Figures 3 and 4. The correct figures and the legends are reproduced correctly here.

The authors terribly apologized for this carelessness.

Fig. 3. TUNEL assay of gastric cancer cells treated with periplocin. TUNEL staining of (A) SGC-7901 cells and (B) MGC-803 cells treated with different concentrations of periplocin for 24 h. The number of apoptotic cells was increased in the periplocin treated group. * $P < 0.05$ compared to the untreated cell group.

Fig. 4. Periplocin induces changes in the expression of apoptosis-related proteins. Western blot analysis of pro-bid, Mcl-1, cleaved caspase-3 and p-ERK1/2 protein levels in (A) SGC-7901 cells and (B) MGC-803 cells treated with periplocin for 24 h. * $P < 0.05$ compared to the untreated cell group.