Original Article
EMMPRIN, SP1 and microRNA-27a mediate physcion 8-O-β-glucopyranoside-induced apoptosis in osteosarcoma cells

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Abstract: Physcion 8-O-β-glucopyranoside (PG), the main active ingredient of Rumex japonicus, induces apoptosis and causes cell cycle arrest in human lung cancer cells. However, its anti-tumor effects are not fully understood. In this study, we explored the mechanisms underlying PG induced apoptosis in the osteosarcoma cell line MG-63. Our results showed that PG exerted anti-proliferative effects and induced apoptosis in MG-63 cells via the intrinsic mitochondrial pathway, accompanied by loss of mitochondrial membrane potential (MMP) and cytochrome C release from the mitochondria. In addition, physcion treatment significantly inhibited extracellular matrix metalloproteinase inducer (EMMPRIN) expression in MG-63 cells, in a dose-dependent manner; meanwhile, EMMPRIN protein overexpression markedly reduced PG-induced apoptosis. Moreover, our findings suggested that the modulatory effects of PG on EMMPRIN were due, at least in part, to regulation of an ROS-miR-27a/ZBTB10-Sp1 transcription factor pathway.

Keywords: Physcion 8-O-β-glucopyranoside, osteosarcoma, EMMPRIN, Sp1, miR-27a, ROS

Introduction
Osteosarcoma (osteogenic sarcoma, OS), the most common malignant bone tumor in children and young adults, accounts for 6% of all childhood cancers [1]. The etiology of this neoplasm is complex, and multiple risk factors for OS occurrence have been identified, including genetic and environmental factors [2]. However, early diagnosis is rare due to lack of specific early symptoms and signs. Average diagnostic delay for OS is 9 weeks, and tendonitis has been found as the most common misdiagnosis [3]. Modern treatments, such as surgery and chemotherapy used as mono- or combination therapies, have been improved, but prognosis remains poor for patients with osteosarcoma [4, 5]. Furthermore, surgery cannot stop tumor metastasis, and chemotherapy is limited by cancer cell resistance and various side effects. Currently, traditional Chinese medicine (TCM) is commonly administered to cancer patients as an adjunct to conventional therapies, to improve the quality of life by alleviating symptoms and side effects [6]. Indeed, multiple active gradients of TCM inhibit tumor cell proliferation and induce tumor cell apoptosis [7, 8]. Therefore, developing novel agents for OS treatment can help improve the clinical outcome.

Extracellular matrix metalloproteinase inducer (EMMPRIN), a transmembrane glycosylated member of the immunoglobulin superfamily molecules expressed on the cell surface of most tumor cells, was firstly found to increase tumor invasion by inducing matrix metalloproteinase (MMP) synthesis by the surrounding stromal cells [9]. Other studies showed that EMMPRIN promotes survival, invasion and metastasis in tumor cells through multiple pathways and mechanisms, including loss of function of the tumor suppressor p53 [10], upregulated vascular endothelial growth factor (VEGF) [11, 12], disruption of the growth-modulating factor transforming growth factor-β1 (TGF-β1) [13], and regulation of the urokinase-type plasminogen activation (uPA) system of serine proteases [14]. Interestingly, multiple studies suggested EMMPRIN mediates cancer cell apoptosis in a hyaluronan-dependent manner via the PI3K and Erk1/2 pathways [15, 16]. Therefore, targeting EMMPRIN may provide a
mechanistic basis for apoptosis-inducing therapies. In the case of osteosarcoma, high levels of EMMPRIN are positively correlated with the clinicopathological degree, and negatively correlated with patient survival [17, 18]. However, whether EMMPRIN plays a role in OS cell apoptosis remains to be elucidated.

*Rumex japonicus* Houtt, a perennial herbal plant belonging to the family Polygonaceae widely distributed in China (known as Yang-Ti, in Chinese), has been used as antimicrobial, purgative, anti-inflammatory and anti-tumor agent in the folk medicine for many years [19-21]. Interestingly, a recent research showed that one of its main active ingredients, physcion 8-O-β-glucopyranoside (PG), induces apoptosis and causes cell cycle arrest in the human lung cancer cell line A549 [22]. However, the underlying mechanisms of PG induced apoptosis remain ununderstood. In this study, the anti-tumor properties of PG were assessed, examining its effects on OS cell proliferation and apoptosis. Furthermore, this study identified EMMPRIN as a target of PG action, through a pathway involving downregulation of EMMPRIN by modulating SP1 through the ROS/miR-27/ZBTB10 axis.

**Materials and methods**

**Cell culture**

Human osteosarcoma MG-63 cells were obtained from the Beijing Institute for Cancer Research (Beijing, China), and cultured in DMEM (Invitrogen) supplemented with heat-inactivated 10% fetal bovine serum (Invitrogen) at 37°C in a humidified environment containing 5% CO₂.

**Cell proliferation assay**

Cell proliferation was assessed using a WST-8 Cell Counting Kit-8 (CCK-8) (Beyotime, Nantong, China). Briefly, 3×10⁵ cells resuspended in 100 μl DMEM containing 10% fetal bovine serum were seeded in 96-well plates, and incubated at various times. Then, 10 μl CCK-8 solution was added to each well for 1 hour at 37°C. Absorbance at 450 nm was measured on an ELX-800 spectrometer reader (BioTek Instruments, Winooski, USA).

**Cell apoptosis assessment**

The proapoptotic effect of PG was determined by flow cytometry (FITC Annexin V apoptosis kit, BD Pharmingen, NJ, USA). Briefly, cells were rinsed with ice-cold PBS buffer and resuspended in binding buffer at a final density of 1×10⁶ cells/ml. Then, cells were stained with Annexin V-FITC and propidium iodide (PI) for 15 minutes in the dark and analyzed on a flow-cytometer (Beckman Coulter Inc., FL, USA). Annexin V-FITC positive cells were considered to be apoptotic, while those negative for FITC were regarded as living cells.

**Apoptosis detection by morphological changes using Hoechst staining**

Apoptotic cells were confirmed by Hoechst 33258 staining. Apoptosis was indicated by the presence of condensed or fragmented nuclei which bind Hoechst 33258 with high affinity. MG-63 cells were treated with various PG concentrations for 48 h, washed with PBS, and fixed with pre-cooled methanol at 500 μl/well for 10 min. Afterwards, cells were stained with 1 μM Hoechst 33258 (Sigma-Aldrich, MO, USA) for 10 min and analyzed on a Leica fluorescence microscope. Two hundred cells in three randomly selected fields were counted and scored for the incidence of apoptotic chromatin.

**Caspase-3 and caspase-9 activity quantitation**

To assess the activities of caspase-3 and caspase-9, cytosolic proteins were extracted from cells using a hypotonic cell lysis buffer. Then, cytosolic extracts containing 30 μg of protein were analyzed using a colorimetric assay kit specific for caspase-3 and caspase-9 (Ray Biotech, Guangzhou, China).

**Mitochondrial membrane potential (MMP) assessment**

Changes in MMP were examined using the fluorochrome dye JC-1 following a standard protocol. Briefly, MG-63 cells were challenged with PG for 48 hours before incubation with JC-1. The cells were then rinsed with PBS to remove excess dye before quantitation of fluorescence signals by flow cytometry.

**Determination of miRNA and mRNA expression levels**

Gene expression was assessed by quantitative real time PCR (qPCR) using gene-specific primers as described previously [23]. In brief, total RNA was extracted using a commercial kit.
(RNeasy Mini kit, Qiagen, Dusseldorf, Germany). For miRNA expression analysis, 40 ng of cDNA, obtained by reverse-transcription, was used as a template for PCR [23]. For mRNA quantitation, primers for EMMPRIN and Sp1 were synthesized based on published sequences [24]. First-strand cDNA was synthesized from 1 μg RNA using the Reverse Transcription System (Takara, Dalian, China). The resulting cDNA (2 μg) was subjected to PCR amplification. The PCR reactions contained SYBR GREEN Master Mix (Solarbio Co., Beijing, China), forward and reverse primers, and 10 ng of template cDNA. PCR was carried out for 5 minutes at 95°C, followed by 40 cycles of 95°C for 30 seconds, 60°C for 30 s, and 72°C for 30 seconds. Gene expression was analyzed with U6 or GAPDH as internal controls.

**Plasmid construction and cell transfection**

To assess the role of EMMPRIN/Sp1 in PG-induced apoptosis in MG-63 cells, EMMPRIN/Sp1 was overexpressed as previously described [25]. Briefly, full-length cDNA was obtained by reverse transcription, amplified with specific EMMPRIN/Sp1 primers, and inserted into the pEGFP-N1 vector (Takara Biomedical Technology Co., Ltd., Beijing, China). The resulting plasmid was named pEGFP-N1-EMMPRIN/Sp1, and cloned into MG-63 cells to induce EMMPRIN/Sp1 expression. MG-63 cells were transfected with empty pEGFP-N1 were used as controls. 48 hours after transfection, G418 was used to select stable clones.

**EMMPRIN/Sp1 gene silencing**

shRNA oligos for EMMPRIN/Sp1 gene knockdown were designed as previously described [26]. Two different shRNA sequences and a scramble control sequence were subcloned into the plasmid vector pGCsi-H1 following the manufacturer’s instructions, and designated p-shRNA1, p-shRNA2 and p-shRNA-control, respectively. MG-63 cells in logarithmic growth phase were seeded in 6-well plates at a density of 3×10⁵ cells per well, incubated overnight, and transfected with p-shRNA1, p-shRNA2 and p-shRNA-control, respectively, using Lipofectamine-2000 (Millipore, MA, USA). Proteins were probed with specific primary antibodies following standard protocols. After washing with TBST, secondary antibodies were added for 2 hours. The blots were washed with TBST before signal detection with chemiluminescence substrate (KPL., Guildford, UK). The BandScan software (Glyko, Novato, CA) was used to quantify protein band intensities, with β-actin included as a loading control.

**In vivo anti-tumor activity assay**

Four-week-old male nude mice (BALB/c, nu/nu; SPF laboratory animal center of Soochow University, Suzhou, China) were housed under...
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Figure 1. PG reduces cell viability and induces apoptosis in MG-63 cells. *P<0.05 vs. PG at 0 μg/ml, **P<0.01 vs. PG at 0 μg/ml, ^P<0.05 vs. PG, ^^P<0.01 vs. PG. MG-63 cells were incubated with PG at indicated dosages for 48 hours unless otherwise stated.
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pathogen-free conditions. All animal experiments were approved by the Animal Ethics Committee of Soochow University. A total of 1.5 \( \times 10^6 \) MG-63 cells resuspended in 50% Matrigel were subcutaneously injected into the animals. Tumor volumes were calculated as \( \frac{1}{2} a b \) (a and b are the short and long tumor axes, respectively). After euthanasia, the tumors were harvested for RNA preparation and EMMPRIN, Sp1 and miR-27a gene expression assessment by qRT-PCR.

Statistical analysis

All experiments were performed three times in triplicate unless otherwise stated. Data are mean ± SD (standard deviation), and were compared by one-way ANOVA using the SPSS 13.0 software. P<0.05 was considered statistically significant.

Results

PG decreases MG-63 cell viability in a time- and concentration-dependent manner

To assess the effect of PG on cell viability, MG-63 cells were exposed to various concentrations (0, 20, 50, 100 \( \mu \)g/ml) of PG for 24, 48 or 72 hours. As shown in Figure 1A, PG reduced MG-63 cell viability in a time- and concentration-dependent manner. PG at 50 and 100 \( \mu \)g/ml could significantly suppress MG-63 cell proliferation after 24 h of treatment. When treatment was prolonged to 48 hours, all PG dosages caused a significant loss of cell viability. As expected, cell proliferation was further decreased after treatment with PG for 72 hours. To exclude the time-caused loss of cell viability, 48 hours was selected for subsequent experiments.

PG induces apoptosis via the intrinsic mitochondrial pathway

Next, we investigated the proapoptotic effect of PG at different concentrations. As shown in Figure 1B, PG induced apoptosis in MG-63 cells in a dose-dependent manner. PG treatment at all three tested dosages induced a significant increase in MG-63 cell apoptosis rate compared with the vehicle control. The proapoptotic effect of PG was also confirmed by Hoechst staining (Figure 1C). Since apoptotic cell death is featured by cleavage of caspase-3 and PARP, activation of caspase-3 and PARP was examined to confirm apoptosis. Our results showed that PG increased caspase-3 activation in a dose-dependent manner (Figure 1D). In addition, Western blot results showed that PG treatment was associated with activation of both caspase-3 and PARP (Figure 1E). Since apoptosis in tumor cells can occur via caspase-independent or caspase-dependent pathway [27], a caspase inhibitor (Z-VAD-FMK) was utilized to fully explore the underlying mechanism of the proapoptotic effect of PG. As shown in Figure 1F, Z-VAD-FMK treatment significantly abolished the proapoptotic effect of PG, suggesting that PG treatment induced apoptosis in MG-63 cells by activating the caspase cascade.

Apoptosis may occur through either intrinsic or extrinsic pathway, depending on mitochondrial involvement [28]. Mitochondria-mediated (or intrinsic) apoptosis is evidenced by caspase-9 activation, decreased MMP levels, and cytochrome C translocation from mitochondria to the cytosol. Therefore, to further explore the role of mitochondria in PG-induced apoptosis, changes in caspase-9 activity, MMP levels and cytosolic cytochrome C amounts were examined. Our results showed that PG treatment led to dose-dependent loss of MMP, caspase-9 activation, and increased cytosolic cytochrome C amounts compared with cells treated with the vehicle (Figure 2A-C). To confirm these findings, activation of caspase-8, an indicator of extrinsic apoptosis, was also examined. As shown in Figure 2D, PG caused no significant change in activated caspase-8 levels, further demonstrating that PG induced apoptosis in MG-63 cells via the mitochondrial pathway.

PG induces apoptosis in MG-63 cells by modulating EMMPRIN

The involvement of EMMPRIN in tumor cell apoptosis was previously reported [15, 29]. In addition, a recent study found that PG prevents hypoxia-induced epithelial-mesenchymal transition in colorectal cancer HCT116 cells by modulating EMMPRIN [30]. Therefore, we assessed whether PG induced apoptosis by modulating EMMPRIN. As shown in Figure 3A and 3B, PG treatment reduced EMMPRIN expression at both mRNA and protein levels. To further explore the role of EMMPRIN in
Figure 2. PG induces apoptosis via the intrinsic mitochondrial pathway. *P<0.05 vs. PG at 0 μg/ml, **P<0.01 vs. PG at 0 μg/ml. MG-63 cells were incubated with PG at indicated dosages for 48 hours unless otherwise started.
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PG-induced apoptosis, MG-63 cells were transfected with an EMMPRIN overexpression plasmid. Interestingly, PG-induced apoptosis was significantly abolished by EMMPRIN overexpression (Figure 3C). In contrast, EMMPRIN knockdown by shRNA led to a significantly increased apoptotic population (Figure 3C), supporting the role of EMMPRIN in apoptosis modulation. Meanwhile, EMMPRIN overexpression in MG-63 cells significantly blocked PG-induced activation of caspase-3 (Figure 3D). Together, our results suggested that PG induced apoptosis in MG-63 cells, at least partly, by modulating EMMPRIN.

**PG modulates EMMPRIN expression by down-regulating Sp1**

The transcription factor Sp1 has been shown to regulate EMMPRIN expression in human malignancies [31]. Here, we examined the role of Sp1 in the regulatory effect of PG on EMMPRIN. As shown in Figure 4A and 4B, a dose-dependent decrease in Sp1 expression was observed in MG-63 cells treated with PG. Next, Sp1 expression was artificially manipulated to assess its role in EMMPRIN regulation. Our results showed that Sp1 knockdown led to significantly reduced EMMPRIN levels (Figure 5C and 5D). In addition, the suppressing effect of PG on EMMPRIN expression was significantly attenuated by Sp1 overexpression in MG-63 cells (Figure 4C and 4D). Moreover, Sp1 overexpression in Mg-63 cells significantly attenuated the proapoptotic effect of PG in MG-63 cells (Figure 4E). Taken together, the above findings confirmed the hypothesis that PG induced apoptosis by modulating EMMPRIN via Sp1.

**PG modulates Sp1 expression by inducing the Sp repressor ZBTB10 via ROS/miR-27a signaling**

A recent study highlighted that natural products could mediate Sp1 downregulation in tumor cells by generating ROS [32]. In addition, a PG related chemical, PG, induces ROS in tumor cells [33]. Therefore, we explored whether ROS induction was involved in PG-mediated downregulation of Sp1. As shown in Figure 5A, PG caused a significant accumulation of intracellular ROS. Then, the ROS activator CoCl2 and inhibitor NAC were used to further explore the role of ROS in reducing PG effect on Sp1 and EMMPRIN expression. As shown in Figure 5B and 5C, increasing ROS levels by CoCl2 treatment resulted in significantly increased Sp1 and EMMPRIN levels both at the mRNA and protein levels, similar to PG. Meanwhile, treatment with both PG and NAC almost completely abrogated the PG-induced repression on Sp1 and EMMPRIN expression. The above results
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indicated that PG downregulated SP1 and EMMPRIN in a ROS-dependent manner. Moreover, our results also demonstrated that ROS generation was involved in PG-induced MG-63 cell apoptosis, since NAC significantly abolished the proapoptotic effect of PG.

Previous studies showed that ROS-dependent downregulation of Sp1, Sp3, and Sp4 in human malignancies is due to reduced miR-27a and/or miR-20a/miR-17 levels, resulting in the induction of miR-regulated “Sp repressors” ZBTB10 and ZBTB4, respectively [32], which competitively bind GC-rich Sp binding sites in the promoters of Sp1, Sp3, Sp4, and Sp-regulated genes, causing decreased transactivation [34, 35]. Therefore, the effects of PG on miR-27a/ZBTB10 and miR-20a or miR-17/ZBTB4 axis were assessed to explore the mechanism by which PG modulated Sp1 expression. As shown in Figure 6A-C, PG treatment did not significantly alter ZBTB4 levels and miR-20a or miR-17 expression. In contrast, PG treatment induced a remarkable increase in ZBTB10 expression as well as significantly decreased miR-27a cell levels (Figure 6A-C). Regulation of SP1 was confirmed by using miR-27a mimics that decreased ZBTB10 expression, abrogating the modulating effect of PG on SP1 (Figure 6D and 6E). In agreement, the suppressive effect of PG on EMMPRIN levels was significantly reduced by transfection with miR-27a mimics (Figure 6D and 6E). Collectively, our results indicated that PG disrupted the miR-27a/ZBTB10 axis, resulting in repressed SP1 and subsequent downregulation of EMMPRIN. As expected, increasing levels of miR-27a using miR-27a mimics could alter PG-induced apoptosis, indicating miR-27a involvement in the proapoptotic effect of PG (Figure 6F).

In vivo anticancer effect of PG

Next, we sought to assess the in vivo effect of PG. Nude mice were injected with KB cells and tumors were allowed to grow to 100 mm³. Then,
the animals were treated with different doses of PG, including 100 mg, 50 mg, and 10 mg/kg/day, respectively. Treatment efficacy was evaluated by measuring tumor volumes. As shown in Figure 7A, PG inhibited tumor growth dose-dependently, and this anti-tumor effect was significant at 50 and 100 mg/kg/day. Moreover, PCR data showed that PG caused a marked decrease in EMMPRIN mRNA levels, suggesting in vivo PG effectiveness in inhibiting tumor growth was mediated by EMMPRIN downregulation (Figure 7B). Moreover, the inhibitory effect of PG on tumor growth was associated with significantly decreased Sp1 and miR-27a expression levels (Figure 7C), further supporting our in vitro findings that apoptosis induced by PG was associated with EMMPRIN downregulation via miR-27a modulation.

**Discussions**

Apoptosis, also called type I programmed cell death, is associated with the activation of catabolic enzymes, eventually resulting in nuclear chromatin condensation, nuclear fragmentation, and formation of distinct apoptotic bodies [36]. Mounting evidence suggests that apoptosis induction in tumor cells is a major protective mechanism against the development and progression of cancer [37]. Our results revealed that PG exhibited dose- and time-dependent anti-proliferation effects on OS cells by promoting apoptosis, suggesting the potential of PG as a chemotherapeutic agent in the treatment of OS. Apoptosis is mainly mediated by the death receptor-triggered extrinsic pathway and mitochondrial-initiated intrinsic pathway, featured by activation of caspase-8 and caspase-9, respectively [38, 39]. In the present study, the observed PG-mediated caspase-9 and caspase-3 activation as well as cytochrome C release into cytosol and loss of MMP suggest that PG induces apoptosis mainly via the mitochondrial-mediated caspase pathway.

EMMPRIN is highly expressed on the surface of various malignant tumor cells, and mainly functions as a cellular adhesion molecule that induces secretion of matrix metalloproteinases (MMP; mainly MMP-1, MMP-2 and MMP-9), thus promoting invasion and metastasis [40, 41]. Besides its function in invasion and metastasis [41], the role of EMMPRIN in apoptosis...
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was demonstrated by RNA interference showing that EMMPRIN knockdown induces apoptosis in both solid tumors and hematological malignancies [26, 42, 43]. In addition, recent studies proposed that natural products exert apoptosis-inducing effects by downregulating EMMPRIN [29, 44]. In this study, the proapoptotic effect of PG in MG-63 cells was mediated, at least partly, by EMMPRIN regulation, corroborating recent studies and confirming the role of EMMPRIN in OS cell apoptosis.

Gene regulation by transcription factors is critical to many biological processes and carcinogenesis [45]. One of the first transcription factors identified in mammalian cells is Sp1 [45], a member of the zinc-finger Sp family of proteins that includes the Kruppel-like factor (KLF) family [46]. Sp1 is expressed ubiquitously in various mammalian cells and implicated in the transcription of many genes that contain GC boxes in their promoters [47], particularly housekeeping genes and those involved in cell growth and development. Sp1 is overexpressed in a number of human malignancies, and regulates many aspects of cancer biology by regulating pro-oncogenic genes important for cell growth (cyclin D1, EGFR, and c-Met) [48], survival (bcl-2 and survivin) [48], angiogenesis (VEGF and VEGF receptors) [49], and metastasis [50, 51]. In a previous study, Kong et al reported that differential Sp1 expression and activity directly regulate EMMPRIN levels in human lung cancer [31]. Ke et al also found that Sp1, combined with HIF-1α, induces EMMPRIN expression in hypoxia [52]. As shown above, PG regulated EMMPRIN expression by Sp1 repression; in addition, similar effects were

Figure 6. PG exerts ROS-dependent regulatory effect on Sp1 by modulating miR-27a/ZBTB10. *P<0.05 vs. PG at 0 μg/ml, **P<0.01 vs. PG at 0 μg/ml, ^^P<0.01 vs. PG. MG-63 cells were incubated with PG at 100 μg/ml for 48 hours unless otherwise stated.
obtained by artificially manipulating Sp1 amounts in MG-63 cells, corroborating previous findings demonstrating the regulatory role of Sp1 in EMMPRIN expression. Furthermore, our findings highlight the potential of PG to interfere with various neoplastic activities through Sp1 repression, thus regulating Sp1-targeting genes.

In normal cells, reactive oxygen species (ROS), including radicals (superoxide, nitric oxide, and hydroxyl radicals) and non-radical species (hydrogen peroxide, ozone, and peroxynitrates) are generated as by-products of cellular metabolism, and in cellular redox balance with biochemical antioxidants [53]. In cancer cells, a modest increase in ROS can enhance cell proliferation, survival, and drug resistance; however, further increase of ROS that cannot be attenuated by intracellular redox systems can induce apoptosis and cell cycle arrest [53]. Given that ROS levels are higher in cancer than in non-cancer cells, ROS-inducing drugs can selectively kill cancer cells without causing overt toxicity to normal cells [53]. Therefore, increasing ROS production is considered an important strategy for cancer therapies. In the context of OS, a number of natural products have been found to induce apoptosis by generating excessive cellular ROS and triggering various signaling pathways, including JNK [54], p38 MAPK [55], and JAK2/STAT3 [56] pathways. In this study, PG induced ROS and decreased miR-27a levels in MG-63 cells, which resulted in the upregulation of the Sp-repressor ZBTB10; this resulted in SP1 downregulation and the subsequent repression of EMMPRIN. On the other hand, it has been found that ROS-

Figure 7. In vivo anti-tumor effect of PG is associated with downregulation of EMMPRIN, Sp1 and miR-27a as well as elevated expression of ZBTB10. *P<0.05 vs. Vehicle, **P<0.01 vs. Vehicle.
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inducing agents could exert pro-autophagic effects on OS cells [54, 57, 58]. Therefore, by generating ROS, PG might also kill OS cells by inducing autophagy.

In conclusion, our results showed that PG induces apoptosis in OS cells, at least in part, through a pathway involving EMMPRIN down-regulation, by modulating SP1 via the ROS/miR-27/ZBTB10 axis, highlighting the potential of PG as an anticancer agent. However, further studies, including clinical trials, are needed to fully evaluate PG as a novel therapeutic in cancer prevention and treatment.

Disclosure of conflict of interest

None.

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