# Arctigenin hinders the invasion and metastasis of cervical cancer cells via the FAK/paxillin pathway

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## ARTICLE INFO

## ABSTRACT

*Context:* Cervical cancer is the most common gynecological pernicious tumor with high morbidity and mortality worldwide, especially in developing countries. Arctigenin (ARG), a nature-derived component, has exhibited anti-tumor activity in various tumors.
*Objective:* To explore the effect of ARG on cervical cancer.
*Materials and methods:* The effect and mechanism of ARG on cervical cancer cells were explored by cell counting kit-8 (CCK-8), flow cytometry, transwell and Western blot assays. Additionally, *in vivo* experiment was conducted in xenografted mice by immunohistochemistry (IHC), terminal deoxynucleotidyl transferase deoxyuridine triphosphate (dUTP) nick end labeling (TUNEL) and Western blot assays.
*Results:* ARG treatment induced both concentration-dependent and time-dependent reductions in the cell viability of SiHa and HeLa cells with a IC50 value of 9.34 μM and 14.45 μM, respectively. ARG increased the apoptosis rate and the protein levels of cleaved-caspase 3 and E-cadherin, but decreased the invaded cell numbers and the protein levels of Vimentin and N-cadherin *in vitro*. Mechanically, ARG inhibited the expression of focal adhesion kinase (FAK)/paxillin pathway, which was confirmed by the overexpression of FAK in SiHa cells. The inhibitory role of over-expression of FAK in proliferation and invasion, as well as its promoted role in apoptosis were reversed with ARG treatment. Meanwhile, ARG suppressed growth and metastasis, and enhanced apoptosis *in vivo*. Consistently, ARG administration reduced the relative protein level of *p*-FAK/FAK and *p*-paxillin/paxillin in tumor tissues of xenografted mice.

*Conclusion:* ARG inhibited proliferation, invasion and metastasis, but enhanced apoptosis of cervical cancer via the FAK/paxillin axis.

## 1. Introduction

Cervical cancer is one of the most common pernicious tumors in female. 604,000 new cases and 342,000 deaths in cervical cancer have been reported around the world, which makes it rank to the fourth most common diagnosed cancer and dominating cause of cancer death in women in 2020 [1]. Surgery is still the mainstay for the treatment of cervical cancer, and chemotherapy and radiotherapy are also extremely significant therapies for cervical cancer [2]. Besides, drugs, such as carboplatin and cisplatin are verified to be effective for the treatment of cervical cancer [3]. Nevertheless, facts of chemotherapy and radiotherapy with resistance and drugs can cause systemic toxicity, which severely impede the progress of therapy for cervical cancer in clinical practice [4,5]. Therefore, it is still a substantial demand for the discovery of potential therapy for cervical cancer with effectivity and controllable toxicities.

Nature-derived products are low toxicity, which have exhibited anti-tumor effects on a wide spectrum of tumors, including cervical cancer [6,7]. For instance, lycorine, an alkaloid isolated from Lycoris bulbs induces apoptosis and suppresses proliferation and migration in cervical cancer cells [8]. Astragaloside IV, a saponin from Astragalus polysaccharides restrains growth and invasion of cervical cancer cells [9]. *Arctium lappa* L. a biennial plant, is a traditional Chinese herbal medicine [10], which has exhibited inhibitory effect on various cancers. For instance, Taleb et al. [11] report that the extracts of aerial parts of *Arctium lappa* L. exerts a proapoptotic and antiangiogenic activities on breast cancer. Arctiin, a lignan glycoside, isolated from *Arctium lappa* L. suppresses migration and invasion in cervical cancer [12]. Arctigenin (ARG) is a phenylpropane dibenzylbutyrolactone lignan that is mainly extracted from *Arctium lappa* L. [13]. As a vital bioactive ingredient from Fructus Arctii, ARG has a series of pharmacological properties, such as anti-leukemia activity, anti-inflammatory effect, anti-colitis effect, anti-viral effect, vascular protective effect, protection against brain damage and memory problems, hepatoprotective effect, and anti-insect activity [14]. More importantly, ARG has been identified as an anti-tumor agent [15]. ARG restrains proliferation, invasion and stemness of breast cancer cells [16]. An inhibitory effect of ARG is reported on cell growth, mobility and epithelial-mesenchymal transition (EMT) with the enhanced apoptosis in cholangiocarcinoma [17]. ARG attenuates cisplatin resistant and proliferation and elicited apoptosis in colorectal cancer [18]. However, the effect of ARG is still not clearly identified on cervical cancer.

This study aimed to explore the effect and potential mechanism of ARG on cervical cancer by the both cell and animal models. The effect of ARG on proliferation, apoptosis, invasion and EMT was first investigated both *in vitro* and *in vivo*. In addition, the potential mechanism was also addressed in both *in* cell and animal models. We wish our data can establish a foundational understanding for the treatment of cervical cancer.

## 2. Materials and methods

### 2.1. Cell culture

Endocervical End1/E6E7 (CRL-2615) cells were prepared from American Type Culture Collection (ATCC, Manassas, VA, USA) and cultured in keratinocyte serum-free media (KSFM, 17005042, Thermo Fisher Scientific, Waltham, MA, USA) added with bovine pituitary extract (0.05 mg/mL, 02–104, Merek, Whitehouse Station, NJ, USA) and human recombinant epidermal growth factor (0.5 ng/mL, PHG0311, Thermo Fisher Scientific) at 37 °C with 5% carbon dioxide ($CO_2$). SiHa cells (CL-0210) and HeLa cells (CL-0101) were bought from Procell (Wuhan, China) and maintained in Minimum Essential Medium (MEM, PM150410, Procell) with 10% fetal bovine serum (FBS, 10099141C, Thermo Fisher Scientific) and 1% streptomycin-penicillin (ST488, Beyotime, Shanghai, China) with 5% $CO_2$ at 37 °C.

### 2.2. Cell treatment and transfection

End1/E6E7, SiHa and HeLa were incubated with 0, 1.25, 2.5, 5, 10, 20, 40 μM ARG for 24 h [16] to determine the toxic effect of ARG on cells. Among them, 0 μM ARG designated that cells were treated with phosphate buffer saline (PBS, C0221A, Beyotime). Besides, SiHa and HeLa cells were administrated with 20 μM ARG for 24, 48 and 72 h to screen the optical time for the further assays. To confirm the role of focal adhesion kinase (FAK)/paxillin in cervical cancer, FAK sequences were sub-cloned into pcDNA3 plasmid to upregulate the level of FAK. The overexpression plasmids were transfected into SiHa cells by using Lipofectamine 3000 (L3000008, Invitrogen, Carlsbad, CA, USA). After the transfection for 48 h, cells were yielded and treated with 20 μM ARG for 24 h. ARG was purchased from MedChemExpress (HY–N0035, Monmouth Junction, NJ, USA) with a purity of 99.69% and mixed in dimethylsulfoxide (DMSO, ST038, Beyotime).

### 2.3. Cell counting kit-8 (CCK-8) analysis

End1/E6E7, SiHa or HeLa cells with a density of $1 \times 10^4$ per well were plated into 96-well plates and cultured with 5% $CO_2$ at 37 °C. Following the different treatments, the cell viability was determined with a cell counting kit-8 kit (C0037, Beyotime) based on the

operating manual. The absorbance was measured at 450 nm using a microplate reader (Thermo Fisher Scientific).

### 2.4. Cell apoptosis detection

HeLa or SiHa cells with a density of $2.5 \times 10^5$ cells/well were distributed into 24-well plates and the cell apoptosis was examined by the flow cytometry. Following the various treatments, cells were resuspended with 500 μL of binding buffer and incubated with 5 μL of FITC-conjugated *anti*-annexin V antibody (Thermo Fisher Scientific) and 5 μL of propidium iodide (PI) (Thermo Fisher Scientific) at room temperature without light for 15 min. The results were analyzed on a FACScan flow cytometry with the CellQuest software (BD Biosciences, NJ, USA).

### 2.5. Transwell assay

SiHa or HeLa cells ($5 \times 10^4$ cells/well) with serum-free MEM were plated into the upper compartment of transwell plates (3422, Corning Company, New York, NY, USA) coated with Matrigel (356234, Solarbio, Beijing, China). MEM with 20% FBS and ARG (0, 5, 10, or 20 μM) were filled into the lower chamber of transwell plates. After being cultured for 24 h, the Matrigel was erased with a cotton swab and the cells were immobilized with 4% paraformaldehyde (P0099, Beyotime), stained with 0.1% crystal violet (C0121, Beyotime), and imaged by a microscope (Olympus, Tokyo, Japan). Five random and different fields were chosen for the count of the number of invasion cells to determine the cell invasion ability.

### 2.6. Animal experiment

The animal experiment was approved by Ethical Review Committee of SSL Central Hospital of Dongguan and was conducted following ARRIVE guidelines. BALB/c nude mice with four weeks old were obtained from Cyagen (Jiangsu, China), and raised in a specified pathogen free (SPF) animal environment with a 12-h cycle of light-dark at 22–24 °C. Mice were received with $1 \times 10^6$ SiHa cells via the subcutaneous administration [19], and then randomly assigned into sham group and 50 mg/kg ARG group (n = 5). Mice in 50 mg/kg ARG group were intraperitoneally injected with 50 mg/kg ARG [16], while mice in sham group were received with the equal amount of PBS. Mice body weight and tumor volume were monitored every seven days for continuous five weeks. Tumor volume was determined according to the following formula: volume $= 1/2 \times$ length $\times$ width$^2$. Mice were intraperitoneally injected with sodium pentobarbital (100 mg/kg) for sacrifice, and then tumors samples were isolated, weighted and preserved in 4% formaldehyde for immunohistochemistry (IHC) and terminal deoxynucleotidyl transferase deoxyuridine triphosphate (dUTP) nick end labeling (TUNEL) examinations or in liquid nitrogen for Western blot assays.

### 2.7. IHC detection

Tumor samples were fixed in 4% formaldehyde (P0099, Beyotime) overnight and dehydrated with gradient concentrations ethanol. After being embedded into parrafin (YA0011, Solarbio), tissues were cut into sections with the 5 μm thick on an Automatic Microtome (E0972, Beyotime). Slices were then dried, deparaffinized and subjected to antigen retrieval with Citrate Antigen Retrieval Solution (pH 6.0, P0081, Beyotime) at 94 °C for 15 min. Subsequently, sections were blocked with 1% BSA (ST2249, Beyotime) for half an hour before the incubation with primary antibodies targeting Vimentin (ab92547, 1:200, Abcam, Cambridge, UK) and secondary antibody labeled with HRP (ab6721, 1:1000, Abcam). The sections were re-stained hematoxylin (C0107, Beyotime) and photographed with a light microscope (Olympus).

### 2.8. TUNEL assay

The apoptosis was detected by a Colorimetric TUNEL Apoptosis Assay Kit (C1091, Beyotime) in tumor tissues in line with the instruction for use. Paraffin-embedded sections were dewaxed in xylene and dehydrated in graded ethanol. Slices were treated with proteinase K (ST533, Beyotime) at room temperature for 15 min, immobilized with 10% formalin (G2161, Solarbio) for 10 min at room temperature and administrated with 2% $H_2O_2$ for 5 min at room temperature. Slices were incubated with TUNEL reaction mixtures for 60 min at 37 °C before the incubation of the 50 μL Streptavidin-HRP (A0305, Beyotime) for half an hour at room temperature and 500 μL DAB (P0202, Beyotime) for 15 min at room temperature. The sections were re-stained hematoxylin (C0107, Beyotime) and photographed under a light microscope (Olympus). The apoptosis was determined by the ration of TUNEL positive cells and the total cells number.

### 2.9. Western blot

Total protein was collected from SiHa or HeLa cells and tumor samples with the RIPA Lysis Buffer (P0013K, Beyotime) and measured with the BCA Protein Assay Kit (P0012S, Beyotime) for the quantification of the protein concentrations. 20 μg protein samples were separated with 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and electrically transferred onto PVDF membranes (FFP70, Beyotime). Then, the membranes were blocked with 3% BSA (ST2249, Beyotime) for 1 h at room temperature and treated with primary antibodies against cleaved-caspase 3 (ab2302, 1:200, Abcam), E-cadherin (ab40772, 1:20000, Abcam), Vimentin (ab45939, 1:1000, Abcam), N-cadherin (ab18203, 1:1000, Abcam), FAK (ab40794, 1:2000, Abcam), phospho FAK

(*p*-FAK, ab81298, 1:1000, Abcam), paxillin (ab32084, 1:5000, Abcam), *p*-paxillin (ab24402, 1:1000, Abcam) and GAPDH (1:10000, ab181602, Abcam). Bounds were developed with goat anti-rabbit IgG H&L (HRP) (ab6721, 1:10000, Abcam) and BeyoECL Plus (P0018S, Beyotime). The band intensity was measured by QUANTITY ONE software (Bio-Rad, Hercules, CA, USA).

*2.10. Statistical analysis*

Experiment in cells were repeated three times and experiment in animal were repeated five times. All results were shown as the form of mean ± standard deviation (SD). All data were analyzed with the Student's *t*-test (only two groups) or one-way analysis of variance (ANOVA) (≥2 groups) followed by *Post Hoc* Bonferroni test using SPSS 20.0 software (IBM, Armonk, New York, USA). Statistical difference was significant when $P < 0.05$.
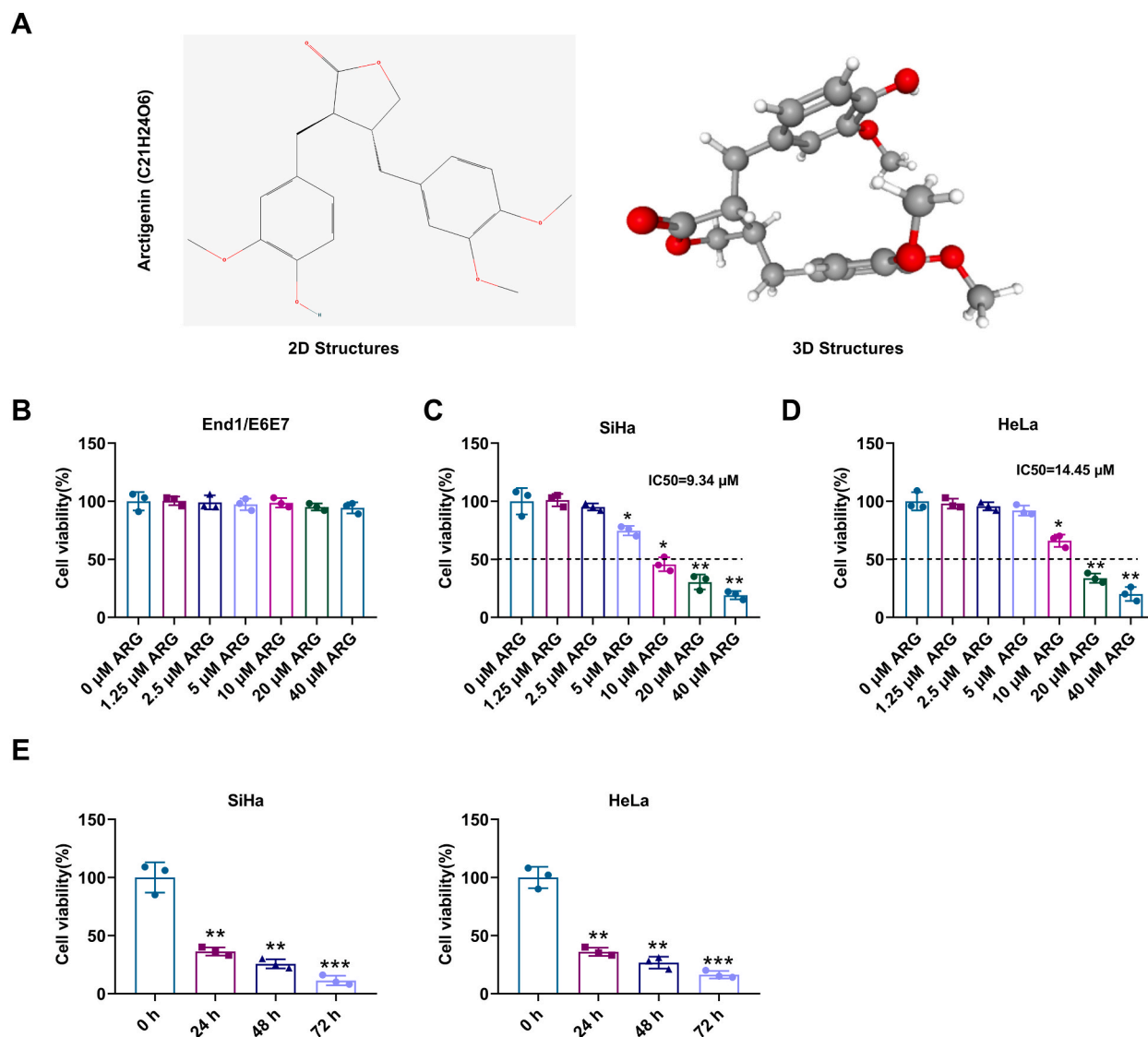


**Fig. 1.** ARG inhibited the proliferation of SiHa and HeLa cells. (A) The 2D and 3D structure of ARG downloaded from https://pubchem.ncbi.nlm.nih.gov/compound/64981. (B–D) The cell viability of End1/E6E7, SiHa or HeLa cells was examined by CCK-8 assays after cells were incubated with 0, 1.25, 2.5, 5, 10, 20, 40 μM ARG for 24 h *P<0.05 and **P<0.01 *vs.* 0 μM ARG. (E) The cell viability of SiHa or HeLa cells was detected by CCK-8 assays after cells were hatched with 20 μM ARG for 24, 48 and 72 h, respectively. **P<0.01 and ***P<0.001 *vs.* 0 h.

## 3. Results

### 3.1. ARG inhibited the proliferation of cervical cancer cells

To investigate the effect of ARG (Fig. 1A) on cervical cancer, diverse concentrations of ARG, including 0, 1.25, 2.5, 5, 10, 20, 40 μM were incubated with End1/E6E7, SiHa or HeLa cells for 24 h. ARG treatment induced a concentration-dependent diminishment in the cell viability of both cells with a IC50 value of 9.34 μM in SiHa cells and 14.45 μM in HeLa cells, but not in the cell viability of End1/E6E7 cells (Fig. 1B–D). Among them, ARG ranged from 5 to 40 μM significantly decreased the cell viability of SiHa cells and ARG ranged from 10 to 40 μM prominently diminished the cell viability of HeLa cells. Thus, three concentrations of ARG, including 5, 10 and 20 μM were selected for the following assays and designated as the low, middle and high concentration, respectively. In addition, ARG incubation also triggered a time-dependent decrease in the cell viability of both cells after cells were hatched with 20 μM ARG for 24, 48 and 72 h, severally (Fig. 1E). Since 20 μM ARG treatment for all three time points observably declined the cell viability of both cells, the time point of 24 h was chosen for subsequent examination. Altogether, ARG suppressed the proliferation of cervical cancer *in vitro*.

### 3.2. ARG induced apoptosis of cervical cancer cells

Then, the effect of ARG on cell apoptosis was addressed in both cells. Results from Fig. 2A and B showed that the three concentrations of ARG (5, 10 and 20 μM) all markedly elevated the SiHa cells apoptosis rate, and only 10 and 20 μM ARG observably enhanced the SiHa cells apoptosis rate. Besides, all the three concentrations of ARG memorably increased the relative protein expression of cleaved-caspase 3, while just two high concentrations of ARG significantly promoted the protein expression of cleaved-caspase 3
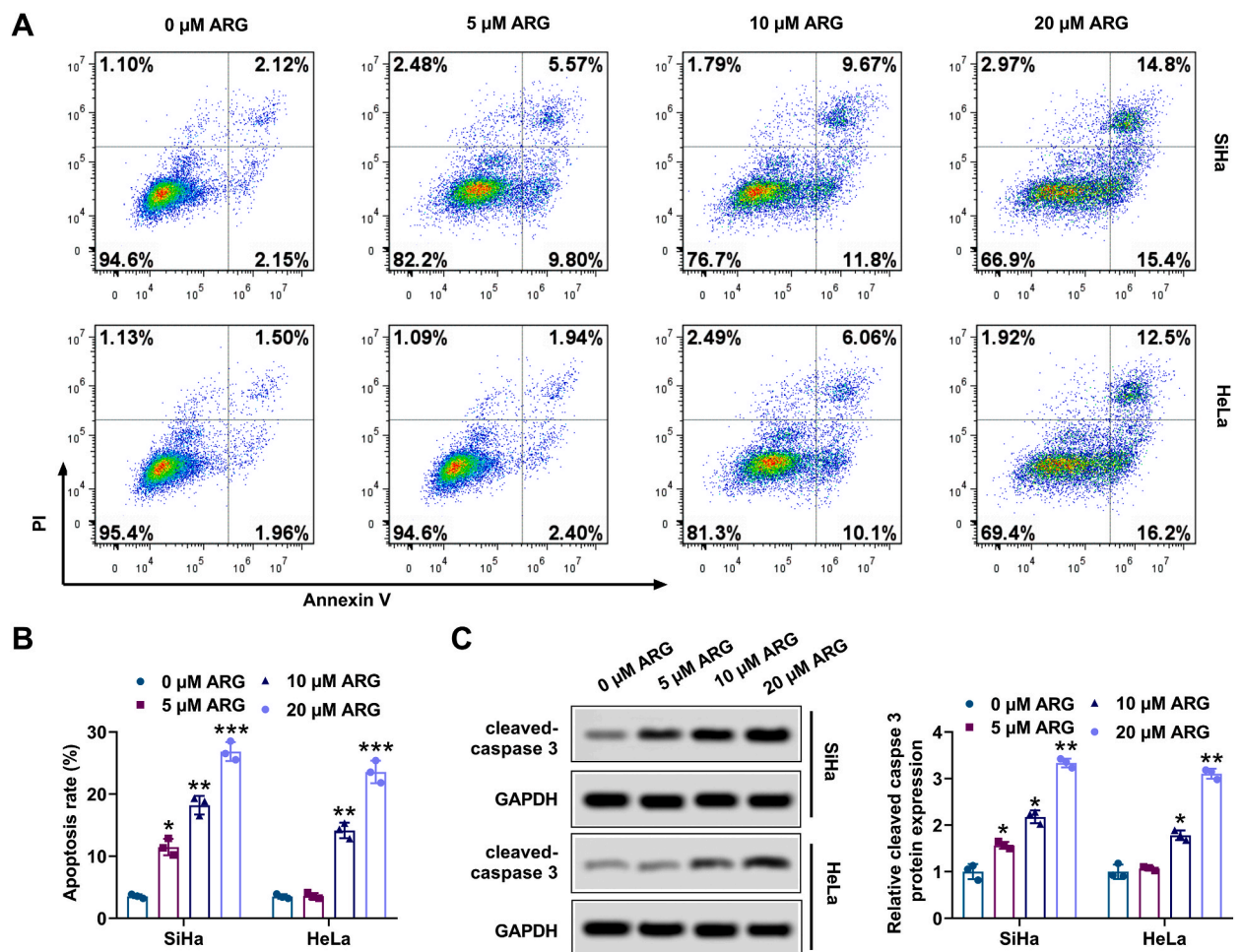


**Fig. 2.** ARG accelerated apoptosis of SiHa and HeLa cells. (A and B) The apoptosis rate of SiHa and HeLa cells was assessed by flow cytometry after both cells were treated with 5, 10 and 20 μM ARG for 24 h. (C) The relative protein expression of cleaved-caspase 3 was detected by Western blot. Data were present after normalized with GAPDH. *$P<0.05$, **$P<0.01$ and ***$P<0.001$ *vs.* 0 μM ARG.
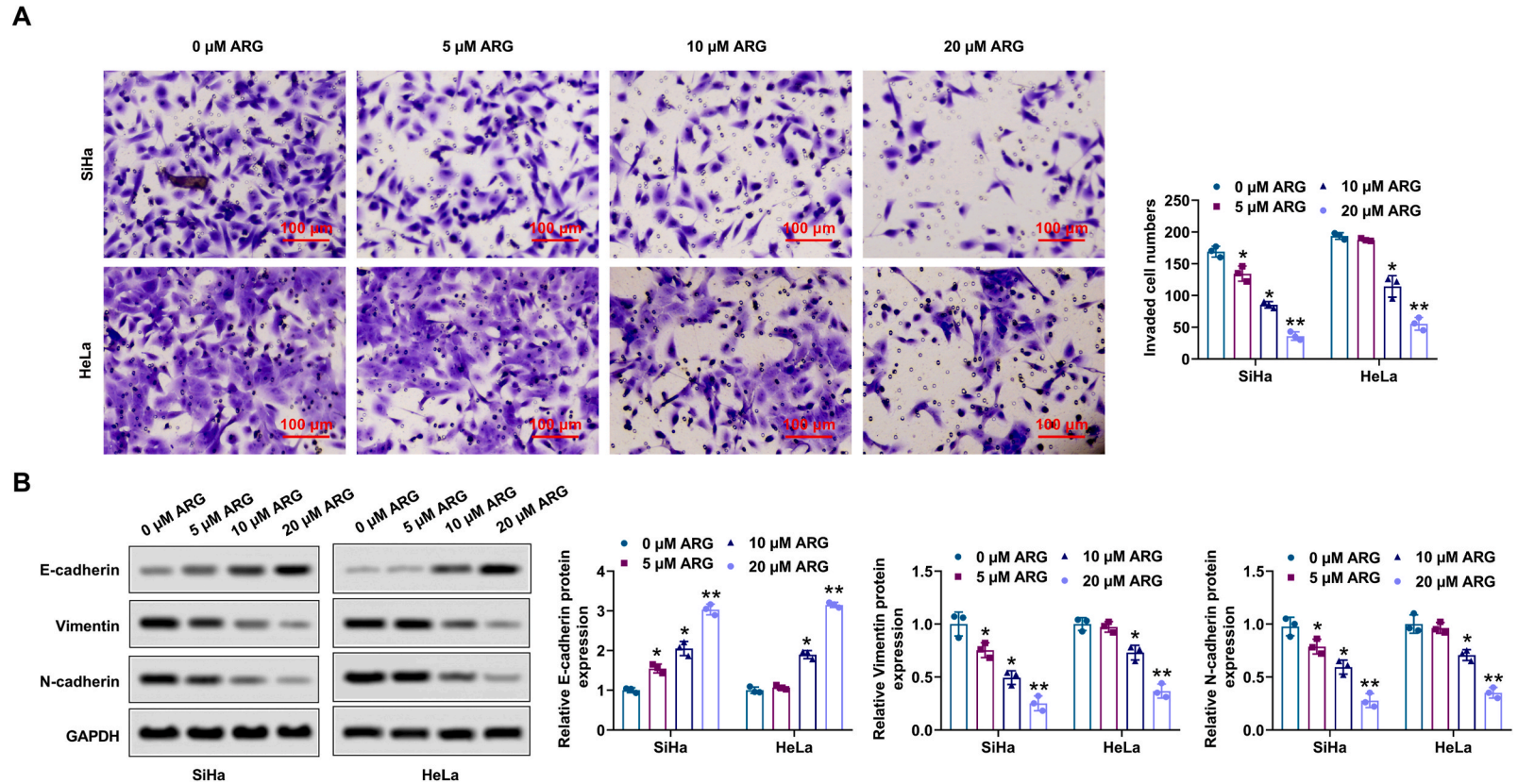
**A**



**B**



**Fig. 3.** ARG inhibited the invasion and EMT of SiHa and HeLa cells. (A) The invaded cell numbers of SiHa and HeLa cells were counted by transwell assays after cell were administrated with 5, 10 and 20 μM ARG for 24 h. (B) The relative protein expressions of E-cadherin, Vimentin and N-cadherin in SiHa and HeLa cells were determined by Western blot after cell were incubated with 5, 10 and 20 μM ARG for 24 h. Data were exhibited after normalized with GAPDH. *$P$<0.05 and **$P$<0.01 *vs.* 0 μM ARG.

**A**



**B**



**C**



**D**



**E**
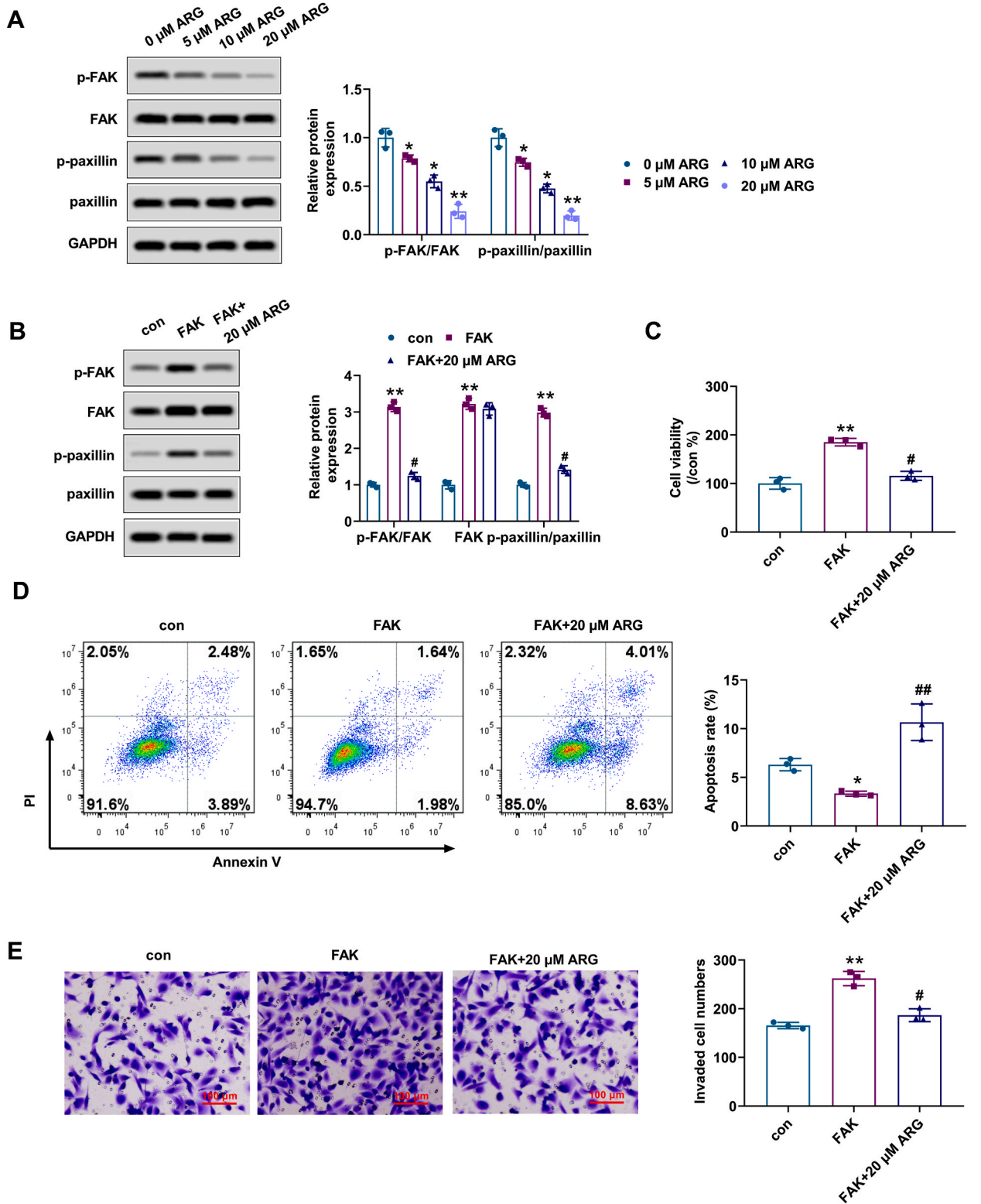


*(caption on next page)*

**Fig. 4.** ARG restrained cell proliferation and invasion with enhanced apoptosis of SiHa cells through suppressing the FAK/paxillin axis. (A) The relative protein expression of FAK, *p*-FAK, paxillin and *p*-paxillin was examined by Western blot after SiHa and HeLa cells were treated with ARG (5, 10 and 20 μM) for 24 h. Data were displayed after normalized with GAPDH. *$P<0.05$ and **$P<0.01$ *vs.* 0 μM ARG. (B) The relative protein expression of FAK, *p*-FAK, paxillin and *p*-paxillin was detected by Western blot after SiHa cells transfected with FAK overexpression plasmid before the treatment of 20 μM ARG for 24 h. Data were displayed after normalized with GAPDH. **$P<0.01$ *vs.* con; #$P<0.05$ *vs.* FAK. (C) The SiHa cell viability was measured by CCK-8 assays. *$P<0.05$ *vs.* con; ##$P<0.01$ *vs.* FAK. (D) The apoptosis of SiHa cells was analyzed by flow cytometry. (E) The invaded cell numbers of SiHa and HeLa cells were determined by transwell assays. **$P<0.01$ *vs.* con; #$P<0.05$ *vs.* FAK.

(Fig. 2C). Thus, ARG enhanced apoptosis of SiHa and HeLa cells.

### 3.3. ARG suppressed the invasion and EMT of cervical cancer cells

Also, the effect of ARG on invasion and EMT was explored. ARG (5, 10 and 20 μM) prominently reduced the invaded cell numbers of SiHa cells, while 10 and 20 μM ARG notably decreased the invaded cell numbers of HeLa cells (Fig. 3A). Additionally, the relative protein level of E-cadherin in SiHa cells was significantly increased with treatment of 5, 10 and 20 μM ARG, while that in HeLa cells was prominently enhanced with administration of 10 and 20 μM ARG. On the other hand, the three concentrations of ARG (5, 10 and 20 μM) all observably attenuated the relative protein levels of Vimentin and N-cadherin in SiHa cells, while only 10 and 20 μM ARG markedly declined the relative protein expression of Vimentin and N-cadherin in HeLa cells (Fig. 3B). Therefore, ARG hindered the invasion and EMT of SiHa and HeLa cells.

### 3.4. ARG mediated the malignant phenotype of cervical cancer cells by inhibiting the FAK/paxillin axis

Mechanically, ARG (5, 10 and 20 μM) observably downregulated the relative level of *p*-FAK/FAK and *p*-paxillin/paxillin in both SiHa and HeLa cells (Fig. 4A), which indicated that ARG inhibited the expression of FAK/paxillin pathway in cervical cancer. To confirm the direct regulative role of ARG in FAK/paxillin pathway, SiHa cells were overexpressed with FAK and subsequently administrated with 20 μM ARG for 24 h. The relative protein expression of FAK was significantly increased after SiHa cells were transfected with FAK overexpressed plasmids, suggesting a successful transfection (Fig. 4B). Overexpression of FAK in SiHa cells markedly increased the relative protein level of both *p*-FAK/FAK and *p*-paxillin/paxillin, which was both observably neutralized with ARG treatment (Fig. 4B). However, ARG incubation did not affect the enhanced relative protein expression of FAK caused by overexpression of FAK in SiHa cells, which indicated that ARG administration just influenced the phosphorylated expression of FAK. Also, ARG incubation notably offset the enhanced cell viability caused by overexpression of FAK in SiHa cells (Fig. 4C), while inverse results were observed in the SiHa cells apoptosis rate (Fig. 4D). Besides, overexpression of FAK significantly enhanced the invaded cell numbers of SiHa cells, which was markedly counteracted with ARG introduction (Fig. 4E). Totally, ARG suppressed cell growth and invasion with increased apoptosis of SiHa cells via downregulating the FAK/paxillin pathway.

### 3.5. ARG repressed the growth of cervical cancer cells in vivo

To assess the effect of ARG on cervical cancer *in vivo*, BALB/c nude mice were subcutaneously inoculated with SiHa cells and intraperitoneally administrated with ARG. After the monitor for sequential five weeks, results from Fig. 5A–C showed that ARG significantly declined the tumor volume and weight, but did not affect the mice body weight. ARG injection prominently reduced the relative protein expression of Vimentin but enhanced the apoptosis in tumor tissues from mice (Fig. 5D and E). Additionally, ARG administration notably diminished the relative protein level of *p*-FAK/FAK and *p*-paxillin/paxillin in tumor tissues from mice (Fig. 5F). The full and non-adjusted Western blot images were provided in supplementary material 1. Collectively, ARG impeded cervical tumor cell growth and promoted cell apoptosis *in vivo*.

## 4. Discussion

The effect of ARG on cervical cancer was addressed both in the cells model and animal model in the present study. The results showed that ARG inhibited cell growth, invasion and metastasis, but induced apoptosis of cervical cancer *in vitro* and *in vivo*. Mechanically, ARG downregulated the level of FAK/paxillin pathway in cervical cancer cells and in tumor tissues. Overexpression of FAK enhanced cell viability and the invaded cell numbers but reduced apoptosis rate in SiHa cells, which were all reversed with ARG treatment. Taken together, ARG restrained growth, invasion and metastasis with the enhanced apoptosis of cervical cancer via the FAK/paxillin pathway.

According to the review recapitulated by Hanahan D [20], proliferation and apoptosis serve a pivotal role in the progression of cancer. Thus, agents targeting to cancer cell proliferation and apoptosis are expected to be a potential drug for the cancer therapy. Plant derivatives or extractives have shown therapeutic potential in the treatment of cervical cancer through working on cell proliferation and apoptosis. For instance, the methanolic extract of Azadirachta indica stem bark restricts proliferation and enhances apoptosis of HeLa, SiHa, and ME-180 cells, which makes it being as a promising anti-cervical cancer drug [21]. Secundiflorol G extracted from the Aeschynomene fascicularis root bark is an isoflavan, which elicits apoptosis of HeLa cells, thereby exhibiting the therapeutic potential of cervical cancer [22]. Triterpenes a terpenoid of plant secondary metabolites, presents a prominent anti-proliferative effect on DoTc2 cells, holding an underlying role on the treatment of cervical cancer [23]. Here, ARG a
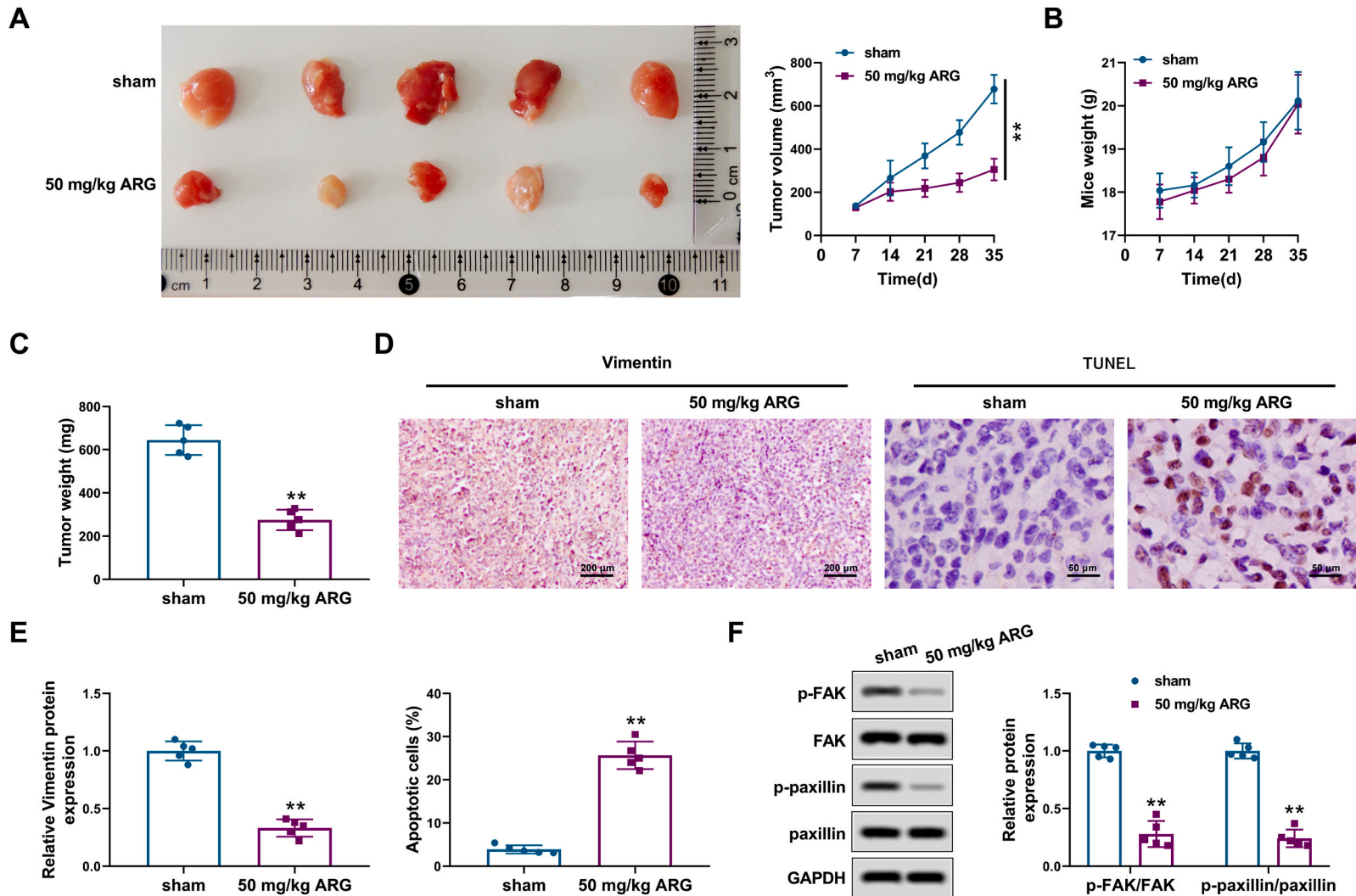
**Fig. 5.** ARG restrained cervical tumor cell growth and enhanced cell apoptosis *in vivo*. (A) Representative images of tumors from nude mice in two groups, and the supervision of tumor volumes for successive seven weeks. **$P < 0.01$ (B) The monitor of mice body weight for sequential thirty-five days. (C) Tumor weight after nude mice were administrated with PBS or 50 mg/kg ARG for five weeks. **$P < 0.01$ *vs.* sham. (D) Representative images of IHC staining for Vimentin (Scale bar = 200 μm) and TUNEL staining (Scale bar = 50 μm). (E) The quantitative analysis of IHC staining for Vimentin and TUNEL staining with the histograms. **$P < 0.01$ *vs.* sham. (F) The relative protein expressions of *p*-FAK, FAK, *p*-paxillin and paxillin were detected by the Western blot. The results were normalized to GAPDH. **$P < 0.01$ *vs.* sham.

phenylpropane dibenzylbutyrolactone lignan isolated from *Arctium lappa* L. [13] reduced the cell viability and enhanced the apoptosis rate and the protein expression of cleaved-caspase 3 of SiHa and HeLa cells. Caspase 3 is a critical effector of apoptosis, whose active form cleaved-caspase 3 can regulate the various phases in the apoptotic pathway [24]. Moreover, lignans isolated from other plants has displayed the inhibitory effect on the progression of cervical cancer, such as sesamin a sesame lignan on the suppression of growth and induction of apoptosis in HeLa and SiHa cells [25], lignan rich portion of Phyllanthus amarus on the enhancement of apoptosis in HeLa and C33 cells [26], and lignans from the creosote bush on the promotion of apoptosis in C33-A and HeLa cells [27]. Moreover, ARG injection also reduced tumor volume and weight, as well as enhanced apoptotic cells in tumor samples from mice xenografted with SiHa cells. Totally, ARG suppressed growth and elicited apoptosis of cervical cancer both *in vivo* and *in vitro*.

Invasion and metastasis are also considerable hallmarks of cancers [20]. ARG reduced the invaded cell numbers and the relative protein levels of Vimentin and N-cadherin, but increased the protein expression of E-cadherin in both SiHa and HeLa cells. Besides, ARG decreased the relative level of Vimentin protein in the tumor tissues from mice inoculated with SiHa cells. E-cadherin as the typical epithelial cell marker, and Vimentin and N-cadherin as representative mesenchymal cell markers are strongly complicated with EMT, in which the level of E-cadherin is diminished and that of Vimentin and N-cadherin is enhanced [28]. It has been demonstrated that EMT is tightly intertwined with the invasion and metastasis of a variety of cancers [29]. Thus, our results suggested that ARG attenuated invasion and metastasis of cervical cancer. Similar findings are also reported in other diverse tumors. For instance, ARG diminishes invasion and the N-cadherin expression with the elevated E-cadherin level in lung cancer [30]. Also, in colorectal cancer, ARG mediates EMT via elevating the level of E-cadherin and declining the expression of Vimentin and N-cadherin, thereby repressing invasion and metastasis [31]. Collectively, ARG inhibited invasion and metastasis of cervical cancer both in cells and animal models.

FA is one of significant structure associated with cell migration via the determination of the cell-matrix interactions dynamic [32]. FAK, as a nonreceptor tyrosine kinase joins in the formation of FA complex, whose dysregulation is closely related to metastasis in a wide variety of tumors [33]. Besides, paxillin a structural protein of the FA complex likewise promotes metastasis [34]. Therefore, FAK/paxillin signaling axis has been revealed to exert a crucial role in regulating the progression of different tumors, such as ovarian cancer [35], melanoma [36], lung cancer [37], and gastric cancer [38]. It has been natural plant-derived component can mediate the development of cervical cancer by inactivating the FAK/paxillin axis. For instance, genistein a natural phytoestrogen suppresses cell mobility and invasion via inhibiting FAK-paxillin pathway in HeLa cells [39]. Apigenin a natural flavonoid represses cell growth, EMT and migration, and enhances apoptosis and G2/M-phase cell cycle arrest in cervical cancer through blocking FAK-paxillin pathway [40]. More importantly, enterolactone, an active polyphenol metabolite of lignan restrains the development of breast cancer by attenuating FAK/paxillin pathway [41]. Consistent with these findings, the results from the current study showed that ARG down-regulated the expression of FAK/paxillin axis in cervical cancer both *in vivo* and *in vitro*. Furthermore, overexpression of FAK promoted the growth and invasion but repressed apoptosis in cervical cancer cells, which were reversed with the ARG incubation. Altogether, ARG restrained cell growth and invasion with enhanced apoptosis in cervical cancer via downregulating the FAK/paxillin pathway.

In summary, ARG impeded the growth, invasion and EMT, but promoted apoptosis of cervical cancer in both cells and animal models. Mechanically, the inhibitory effect of ARG on the development of cervical cancer was strongly associated with the FAK/paxillin axis. However, the direct repressive effect of ARG on mice with cervical cancer via the FAK/paxillin pathway should be studied in the future. Besides, to detect whether there is an effect of the other forms of cell death, the cell viability should be examined after both SiHa and HeLa cells were incubated with ferroptosis inhibitor ferrostatin-1, necrosis inhibitor, necrosulfonamide, and pyroptosis inhibitor, ZVAD-FMK. Briefly, combined with the further preclinical and clinical trials, the results can illustrate that ARG works as an alternative agent for the treatment of cervical cancer.

## Author contribution statement

Dan Liao: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.
Yanyan Liu and Cuifen Li: Analyzed and interpreted the data.
Bin He and Yejia Cui: Performed the experiments.
Guanghui Zhou: Interpreted the data.
Haohai Huang: Conceived and designed the experiments; Analyzed and interpreted the data: Revised and submited the paper.

## Funding statement

## Data availability statement

Data will be made available on request.

## Declaration of competing interest

The authors declare that there is no conflict of interests.

# Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e16683.

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