Vitexin exerts anti-tumor and anti-angiogenesis effects on cervical cancer through VEGFA/VEGFR2 pathway

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Abstract
Vitexin is widely known as a bioactive flavonoid compound, and exerts analgesic and anti-oxidative properties. Antitumor effect of vitexin was identified in various tumors. Role and related mechanism of vitexin in cervical cancer were investigated. Cervical cancer cells were treated with vitexin, and treatment with vitexin reduced cell viability, and decreased number of colonies in cervical cancer. Moreover, cell migration and invasion were repressed by vitexin, and vitexin inhibited angiogenesis of cervical cancer. Vitexin reduced protein expression of Vascular Endothelial Growth Factor A (VEGFA) and Vascular endothelial growth factor receptor 2 (VEGFR2) of cervical cancer in a dosage dependent way. In conclusion, vitexin reduced cell proliferation, migration, invasion and angiogenesis of cervical cancer through inhibition of VEGFA/VEGFR2 signaling.

Keywords
Vitexin; Cervical cancer; Proliferation; Migration; Invasion; Angiogenesis; VEGFA/VEGFR2

1. Introduction
Cervical cancer is a common malignancy in women, and ranks fourth in total cancer-related mortality in China [1, 2]. Therapeutic strategies, including surgery, chemotherapy, radiation, and immunotherapy, are used for cervical cancer patients, while the 5-year survival rate still remains unsatisfactory due to the tumor metastasis and recurrence [3]. Therefore, there is an urgent need to determine effective therapeutic target for cervical cancer.

Vitexin is widely known as apigenin flavone glycoside, and isolated from distinct medicinal plants, such as bamboo, Zappi, Mimosa L., Passiflora cristalina Vanderpl., and Crataegus L., Vigna Savi [4]. Vitexin exerts anti-spasmodic, cardioprotective, anti-metastatic potential, and hypotensive properties [5, 6]. The anti-tumor effect of vitexin has also been reported through suppression of cell proliferation and metastasis [7]. For example, vitexin induced G2/M phase arrest of glioblastoma cells [8], triggered cell apoptosis of leukemia cells [9], and esophageal cancer [10]. Vitexin also reduced cell proliferation of breast cancer [11] and suppressed carcinogenesis of melanoma [12]. Vitexin exerted anti-neoplastic potential against ovarian cancer [13], and suppressed tumor growth of cervical cancer [14]. However, the underlying mechanism involved in suppressive effect of vitexin against cervical cancer has not been reported yet.

Blood vessels drive growth and metastasis of cancer cells, and angiogenesis contributes to development of tumors [15]. Angiogenesis also plays a significant role in cervical cancer [16]. Vascular Endothelial Growth Factor (VEGF) binds to VEGFR to induce endothelial cell proliferation, and is closely related to tumor angiogenesis through promotion new blood vessel formation [17]. VEGFA/VEGFR2 signaling has been shown to contribute to carcinogenesis of cervical cancer [18], and inhibition of VEGFA/VEGFR2 signaling inhibited tumor cell proliferation, metastasis, epithelial-mesenchymal transition and angiogenesis [19, 20]. Increasing evidence confirmed the antiangiogenic potential of vitexin [21]. Therefore, vitexin might also inhibit angiogenesis of cervical cancer through inhibition of VEGFA/VEGFR2 signaling.

This study investigated the effects of vitexin on cell proliferation, migration, invasion and angiogenesis of cervical cancer, as well as the related mechanism.

2. Materials and methods

2.1 Cell culture and treatment
Cervical cancer cells (Hela and Siha) were cultured in DMEM (Gibco, Grand Island, NY, USA) containing 10% fetal bovine serum (Invitrogen, Carlsbad, CA, USA). Cells with at least 80% confluence were seeded in plates, and incubated with 5, 10, or 20 μM vitexin (Sigma-Aldrich, San Francisco, CA, USA), dissolved in normal saline, for 24 hours. Cells in control group were treated with normal saline.

2.2 Cell viability and proliferation assays
Hela and Siha post vitexin treatment were cultured for 24, 48, 72, or 96 hours. Cell Counting Kit-8 (CCK-8) reagent (10 μL; Beyotime, Beijing, China) were applied to incubate...
FIGURE 1. Vitexin reduced cell proliferation of cervical cancer. (a) Treatment with vitexin decreased cell viability of Hela and Siha. (b) Treatment with vitexin reduced number of colonies in Hela and Siha. *** $p < 0.001$.

Hela and Siha for 2 hours. Microplate reader was used to detect absorbance at 450 nm. For cell proliferation assay, Hela and Siha post vitexin treatment were seeded in plates, and cultured for 10 days. Cells were fixed in methanol, and stained with crystal violet before observe under microscope (Olympus, Tokyo, Japan).

2.3 Cell migration and invasion assays

Hela and Siha post vitexin treatment were suspended in serum-free medium, and plated into upper chambers of Transwell chambers (Corning Incorporated, Corning, NY, USA). Medium with 15% fetal bovine serum were planted into lower chambers. Cells in lower chamber were stained, and then observed under the microscope. For cell migration assay, Hela and Siha post vitexin treatment were seeded in plates, and scratched by pipette tip. Cells were observed under the microscope 24 hours later.

2.4 Tube formation assay

Plates were precoated with basement membrane matrix containing reduced growth factor (Invitrogen), and the human umbilical vein endothelial cells (HUVECs) were seeded in the plates. The culture medium of Hela and Siha post vitexin treatment were added into each well, and cultured for 6 hours. Cells were fixed, and then observed under the microscope.
2.5 Western blot

Hela and Siha post vitexin treatment were lysed in Radio-Immunoprecipitation Assay (RIPA) buffer (Beyotime). Samples were separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and transferred onto nitrocellulose membranes. The membranes were blocked in 5% Bovine Serum Albumin (BSA) and probed with specific antibodies: anti-VEGFA (1:2000, Abcam, Cambridge, MA, USA), anti-VEGFR2 (1:3000, Abcam), anti-glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (1:4000, Abcam). The membranes were then washed and incubated with horseradish peroxidase-conjugated secondary antibody (1:5000, Abcam) and tetramethylbenzidine, the immunoreactivities were visualized using enhanced chemiluminescence (Sigma-Aldrich).

2.6 Statistical analysis

All the data with at least triple replicates were expressed as mean ± standard error of the mean (SEM), and analyzed by student’s t test or one-way analysis of variance under SPSS 22.0 software (IBM, Armonk, NY, USA). A p value of < 0.05 was considered as statistically significant.

3. Results

3.1 Vitexin reduced cell proliferation of cervical cancer

Treatment with vitexin decreased cell viability of Hela and Siha in a dosage dependent way (Fig. 1a). The number of colonies in Hela and Siha were also reduced by vitexin (Fig. 1b), suggesting the anti-proliferative effect of vitexin against cervical cancer.

3.2 Vitexin reduced cell migration and invasion of cervical cancer

Number of invasive cells in Hela and Siha were reduced by vitexin (Fig. 2a). Treatment with vitexin suppressed the cell migration of Hela and Siha (Fig. 2b), demonstrating anti-invasive effect of vitexin against cervical cancer.

3.3 Vitexin reduced angiogenesis of cervical cancer

Vitexin reduced number of tubes in Hela and Siha (Fig. 3), indicating the anti-angiogenic effect of vitexin against cervical cancer.
3.4 Vitexin suppressed activation of VEGFA/VEGFR

Protein expression of VEGFA and VEGFR were down-regulated in Hela and Siha by vitexin treatment (Fig. 4), revealing that vitexin inactivated VEGFA/VEGFR signaling to suppress cell proliferation, migration, invasion and angiogenesis in cervical cancer.

4. Discussion

The naturally flavonoids have been shown to mediate cell cycle, apoptosis, autophagy, cell growth, redox metabolism, immunity, and inflammation in various tumors, exerts anti-neoplastic potential and regarded as adjuvants in cancer therapy [22]. The pharmacological effects, including anti-tumor, of vitexin were investigated in previous study [7]. Vitexin functioned as a chemo preventive compound, and protected against progression of various cancers through autophagy and proapoptotic processes [7]. This study confirmed the anti-neoplastic potential of vitexin on cervical cancer through inhibition of cell proliferation, migration, invasion and angiogenesis.

Previous study has shown that vitexin suppressed cell proliferation and metastasis of leukaemia, brain tumors, esophageal cancer breast and oral carcinoma [6]. Vitexin reduced activation of Phosphoinositide 3-kinase (PI3K)/protein kinase B (Akt)/mammalian target of rapamycin (mTOR) signaling to repress proliferation of hepatocellular carcinoma, renal cell carcinoma, non-small-cell lung cancer, and glioblastoma [23]. Tumor growth of cervical tumor xenograft was also suppressed by vitexin [14]. Results in this study also indicated that cell proliferation, invasion and migration of cervical cancer were suppressed by vitexin. Epithelial-mesenchymal transition contributes to cervical cancer metastasis through promotion of chemo-resistance, evasion of immune surveillance, anti-apoptosis and stem cell traits [24]. Effects of vitexin on cell apoptosis and epithelial-mesenchymal transition in cervi-
VEGFA/VEGFR signaling.

Increasing evidence has shown that angiogenesis attributes to tumor growth, invasion and metastasis of cervical cancer [25]. Anti-angiogenic therapies showed promising prevention for the metastasis and recurrence of cervical cancer [25]. Vitexin exerted cardioprotective role in ischaemia-reperfusion injury through suppression of angiogenesis [26]. Moreover, vitexin promoted the inactivation of Akt signaling and activation of forkhead box protein O3α to repress the tumor growth and angiogenesis in hepatocellular carcinoma [27]. Data from tube formation assay in this study showed that vitexin reduced number of tubes in Hela and Siha in a dosage dependent way. Therefore, vitexin exerted anti-angiogenic effect against cervical cancer.

VEGFA/VEGFR2 signaling network is implicated in the pathogenesis of angiogenesis in tumors [28]. VEGFA contributed to activation of PI3K/Akt/mTOR signaling in cervical cancer [29]. Inhibitors targeting VEGFR2 retarded progression and angiogenesis in cervical cancer [30], and apatinib, a VEGFR2 inhibitor, was clinically used for patients with recurrent or refractory cervical cancer [30]. Vitexin has been shown to reduce VEGF in sevoflurane-induced newborn rats [31], and decreased VEGFA and VEGFR2 to suppress carcinogenesis of epithelial ovarian cancer [32]. Here, protein expression of VEGFA and VEGFR in cervical cancer cells were down-regulated by vitexin. Therefore, vitexin might exert anti-angiogenic effect on cervical cancer through inactivation of VEGFA/VEGFR signaling.

5. Conclusions

In sum, vitexin exhibited anti-neoplastic potential against cervical cancer through inhibition of cell proliferation migration, and invasion. Angiogenesis of cervical cancer was also repressed by vitexin through mediation of VEGFA/VEGFR signaling.

AUTHOR CONTRIBUTIONS

QW, JZ and JY designed the research study. QW, JZ, JY and JG performed the research. QW, JZ and JY analyzed the data. QW, JZ and JY wrote the manuscript. All authors read and approved the final manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES


