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# Effects of polydatin on the proliferation, migration, and invasion of ovarian cancer

Xiuchun ZHANG

Gynecology, Liaocheng University Hospital, Liaocheng, 252000, China

**Key words:** Polydatin, Ovarian cancer, Proliferation, Migration, Invasion

**Abstract:** To investigate the effects of polydatin on the proliferation, migration, and invasion of ovarian cancer, the change of proliferative ability, migration ability, and invasive ability of human ovarian cancer cell OVCAR-3, A2780, and HO-8910 was detected by using polydatin and up-regulating PI3K. The anticancer activity and mechanism of polydatin in ovarian cancer were analyzed. Polydatin could effectively inhibit the proliferation, migration, and invasion of OVCAR-3, A2780, and HO-8910, and inhibit the expression of PI3K protein. After the expression level of PI3K protein was up-regulated, the inhibitory effect of polydatin on the proliferative ability, migration ability, and invasive ability of OVCAR-3, A2780, and HO-8910 significantly decreased, suggesting that PI3K was the target of polydatin. Therefore, we concluded that polydatin could inhibit the proliferation, migration, and invasion of ovarian cancer cells by inhibiting the expression of PI3K protein, which provides an experimental basis for polydatin in the treatment of ovarian cancer.

## Introduction

Ovarian cancer is one of the main reasons of death in patients with gynecological cancers worldwide (Howlader *et al.*, 2013; Howlader *et al.*, 2016). Although the morbidity of ovarian cancer has decreased in recent decades and the morbidity of ovarian cancer patients has decreased by about 30% since the 1970s. When platinum-based therapy is used to treat patients with platinum sensitivity, the efficacy will decrease and toxicity will cumulate and increase due to the superiority of invasive high-grade serous cancer and the lack of specific early symptoms and effective early detection methods as well as the decrease of efficacy over time, thus less than half of women can survive for more than 5 years after they are diagnosed (Hanker *et al.*, 2013; Torre *et al.*, 2018).

In recent years, many scholars have found that Chinese medicine has good efficacy in anti-tumor, with few side effects low costs (Xie *et al.*, 2016; Lu *et al.*, 2016). Polydatin is a natural precursor and glycoside form of resveratrol and exists in grapes, peanuts, hops, cocoa, and other products. It has a variety of biological functions, including anti-platelet aggregation, anti-oxidation, cardioprotection, anti-inflammation, anti-tumor, and immune regulation (Zhang *et al.*, 2014; Du *et al.*, 2013). In a study by Chen *et al.* (2019), there was reported that polydatin could improve the sensitivity of colorectal cancer cells to radiotherapy, inhibit cell proliferation, and facilitate apoptosis. In a study by Chen *et al.* (2017), there was also found that polydatin could facilitate the apoptosis of breast cancer cells. Moreover, other studies reported that polydatin had an anticancer effect in oral cancer (Martano *et al.*, 2018), laryngeal cancer (Li *et al.*, 2017), and non-small-cell lung cancer (Zou *et al.*, 2018). These studies indicate that polydatin had a broad-spectrum and anti-tumor effect. However, there are few reports about the effects of polydatin on ovarian cancer. Only Hogg *et al.* (2015) reported that resveratrol and its derivatives could inhibit the growth of three-dimensional cell aggregates of ovarian cancer cell lines and reported the mechanism of the dose dependence of resveratrol. However, the mechanism of polydatin in ovarian cancer needs to be researched deeply.

Therefore, in this study, the impacts of polydatin on the proliferation, migration, and invasion of ovarian cancer cell lines were researched, and its specific mechanism was analyzed to investigate its effects in ovarian cancer deeply.

## Materials and Methods

*Cell culture*

Human ovarian cancer cell OVCAR-3, A2780, and HO-8910 were purchased from Beina Biotechnology. Their serial numbers were BNCC338624, BNCC341157, and BNCC100717. The medium was: RPMI-1640 medium (Thermo Fisher Scientific, article number: 61870044) + 10% of fetal bovine serum (Thermo Fisher Scientific, article number: 16250086) + penicillin (100 U/mL) and streptomycin (100 μg/mL) (Thermo Fisher Scientific, article number: 15140163). The medium was incubated in a constant temperature incubator at 37°C, with 5% of $CO_2$, then a

*Address correspondence to: Xiuchun Zhang, xiuchunzhang@aliyun.com

passage was carried out for 2 or 3 times.

*The construction of PI3K expression vectors*
sh-PI3K (PI3K overexpression vectors) and blank vectors were constructed by Thermo Fisher Scientific (China). sh-PI3K, blank group, and not transfection group were constructed and exposed to trypsin before they were transfected. When the cells reached 80% confluence, the expression vectors were transfected. Specific procedures were carried out according to the instructions of the kits. Then the expression vectors were cultured in an incubator at 37°C with 5% of $CO_2$ for 48 h. The medium was replaced every 6 h. Transfection results were detected by Western blot. Lipofectamine TM 2000 transfection kits were purchased from Shanghai Yanjin Biotechnology Co., Ltd.

*Cell intervention and grouping*
Each ovarian cancer cell line was divided into a control group, a polydatin group, and a polydatin + sh-PI3K group (combination group). The dose of polydatin (Sigma, article number: 15721) was 50 μM, and it was then added into the medium.

*Cell proliferation experiment in vitro*
The cells were prepared into a single array cell suspension with a concentration of $4 \times 10^6$ cells/mL. 200 μL of cell suspension was routinely inoculated in 96-well cell culture plates. Five time points were constructed, they were 6, 12, 24, 48, and 96 h. 20 μL of MTT (5 mg/mL) solution was added into the plates at each time point. The supernatant containing impurities was removed. Then dimethyl sulfoxide preparation was added into the plates, and the plates were placed in a horizontal vibration table for 10 min. The optical density value at the wavelength of 570 nm was calculated by a microplate reader (Shanghai Flash Spectrum Biological Technology Co., Ltd.). MTT test kits were purchased from Beijing Fangcheng Jiahong Technology Co., Ltd.

*Wound healing assay*
The cells were routinely inoculated in 6-well plates. 3 sets of duplicate wells were constructed. When the cell fusion reached about 90%, the tip of a pipette with a volume of 20 μL was used to draw according to prepared horizontal lines; the pipette was perpendicular to the 6-well plates. PBS was used to wash the 6-well plates for 3 times. Then the cells were cultured in a DMEM culture solution with 10% of FBS for 48 h (Thermo Fisher Scientific, article number: 10569044).

*Transwell invasion experiment in vitro*
100 μL of the prepared cell suspension ($5 \times 10^5$/mL) was inoculated in Transwell chambers; then it was cultured for 24 h. PBS buffer solution was used to rinse the cells that did not pass the membrane; then, paraformaldehyde was used to fix the matrix membrane for 10 min, and double-distilled water was used to rinse the redundant paraformaldehyde. After the cells were dried, they were stained with crystal violet with a concentration of 0.5%. Then the number of cells that passed the membrane was counted. Three parallel experiments were performed simultaneously. Transwell chambers and the reagents were purchased from Shanghai SunBio Biomedical technology Co., Ltd.

*Western blot*
The total protein was extracted by the RIPA lysis method. The concentration of the protein was detected by the BCA method. The concentration of the protein was adjusted to 4 μg/μL, then the protein was separated by a polyacrylamide gel electrophoresis with a concentration of 12%. The initial voltage was 90 V, then the voltage was increased to 120 V to move the sample to a proper position in the separation gel. After the electrophoresis was finished, the membrane was transferred with a constant voltage of 100 V for 100 min, then it was closed at 37°C for 60 min, next the transferred membrane was closed in skim milk with a concentration of 5%, lastly, an immune reaction was carried out. The membrane was incubated with a primary antibody (1:1000) overnight at 4°C, on the next day, it was washed by PBS for three times, with 5 min each time. Then, it was incubated with a secondary antibody (1:1000) for 1 h at room temperature. After this, an ECL luminescence reagent was used to develop and fix the cells. Quantity One was used to statistically analyze the strips that were scanned by films. The relative expression level of protein = the gray value of strips/the gray value of internal references. RIPA kits, BCA protein kits, ECL luminescence kits, and trypsin were purchased from Thermo ScientificTM (article numbers: 89901, 23250, 35055, 90058). Rabbit anti-PI3K monoclonal antibody and goat anti-rabbit IgG secondary antibody, were both purchased from Abcam in the United States (article numbers: ab32089 and ab6721).

*Statistical methods*
SPSS19.0 (AsiaAnalyticsFormerlySPSSChina) was used. Measurement data were expressed as mean value ± standard deviation (mean ± SD). The comparison between the two groups was performed by independent sample *t*-test. The comparison between groups was performed by one-way analysis of variance. Back testing was performed by the LSD test. When $p < 0.05$, differences were statistically significant.

## Results

*The effect of polydatin on cell proliferation*
The proliferative ability of OVCAR-3, A2780, and HO-8910 in the polydatin group at each time point was lower than that of OVCAR-3, A2780, and HO-8910 in the control group ($p < 0.05$; Fig. 1).

*The effect of polydatin on migration ability*
The migration distance of OVCAR-3, A2780, and HO-8910 in the polydatin group was shorter than that of OVCAR-3, A2780, and HO-8910 in the control group ($p < 0.05$; Fig. 2).

*The effect of polydatin on invasive ability*
The number of transmembrane cells of OVCAR-3, A2780, and HO-8910 in the polydatin group was less than that of OVCAR-3, A2780, and HO-8910 in the control group ($p < 0.05$; Fig. 3).

*The effect of polydatin on the expression of PI3K protein*
Expression levels of PI3K protein in OVCAR-3, A2780, and HO-8910 in the polydatin group were lower than those in OVCAR-3, A2780, and HO-8910 in the control group ($p < 0.05$; Fig. 4).

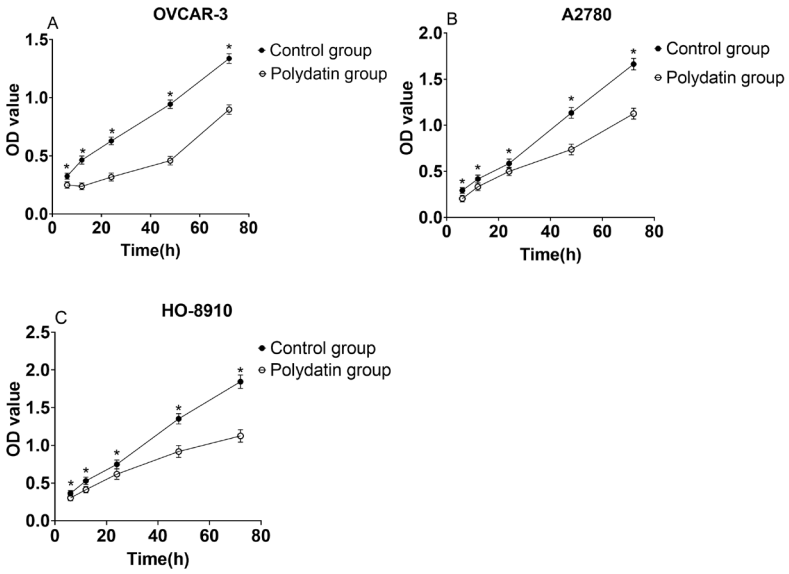


**FIGURE 1. The effect of polydatin on cell proliferation.** The proliferative ability of OVCAR-3, A2780, and HO-8910 in the polydatin group at each time point was lower than that of OVCAR-3, A2780, and HO-8910 in the control group. *means compared to the polydatin group, $p < 0.05$.

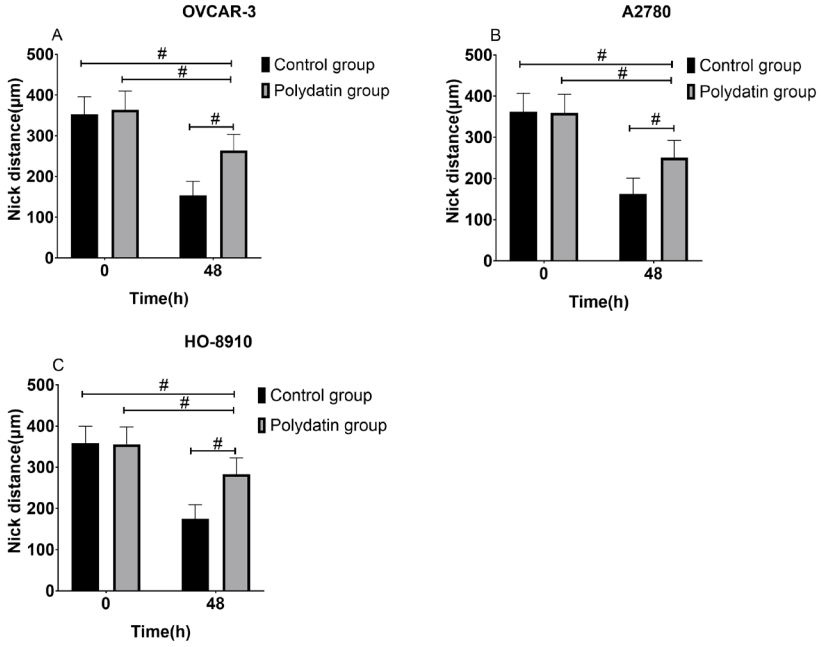


**FIGURE 2. The effect of polydatin on migration ability.** The migration distance of OVCAR-3, A2780, and HO-8910 in the polydatin group was shorter than that of OVCAR-3, A2780, and HO-8910 in the control group. # $p < 0.05$.

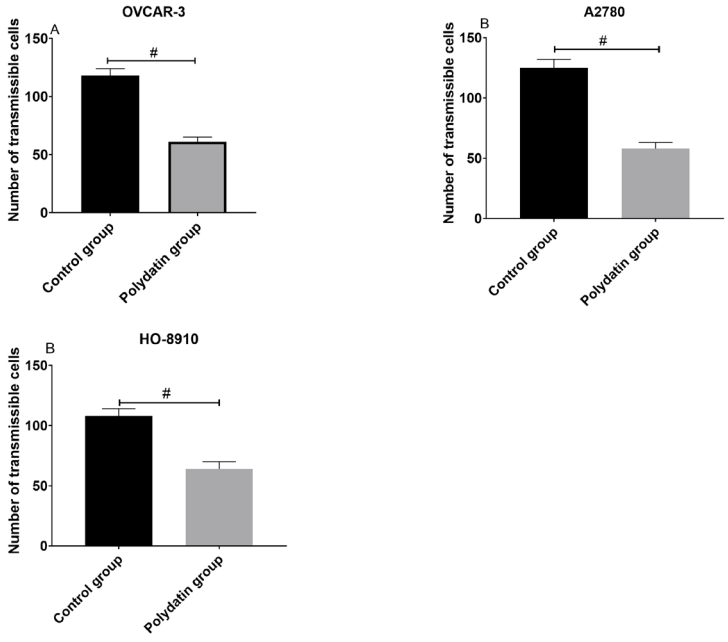


**FIGURE 3. The effect of polydatin on invasive ability.** The number of transmembrane cells of OVCAR-3, A2780, and HO-8910 in the polydatin group was less than that of OVCAR-3, A2780, and HO-8910 in the control group. # $p < 0.05$.

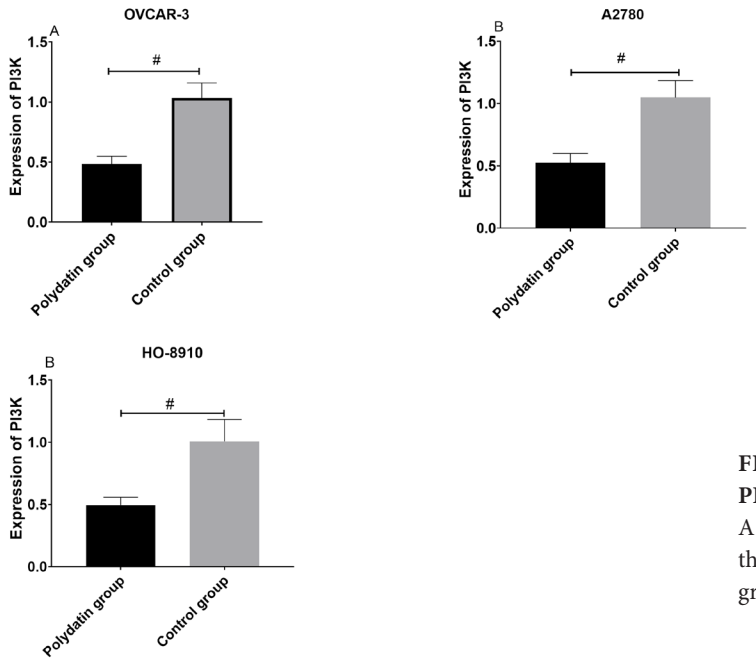


**FIGURE 4. The effect of polydatin on the expression of PI3K protein.** Expression levels of PI3K protein in OVCAR-3, A2780, and HO-8910 in the polydatin group were lower than those in OVCAR-3, A2780, and HO-8910 in the control group. # $p < 0.05$.

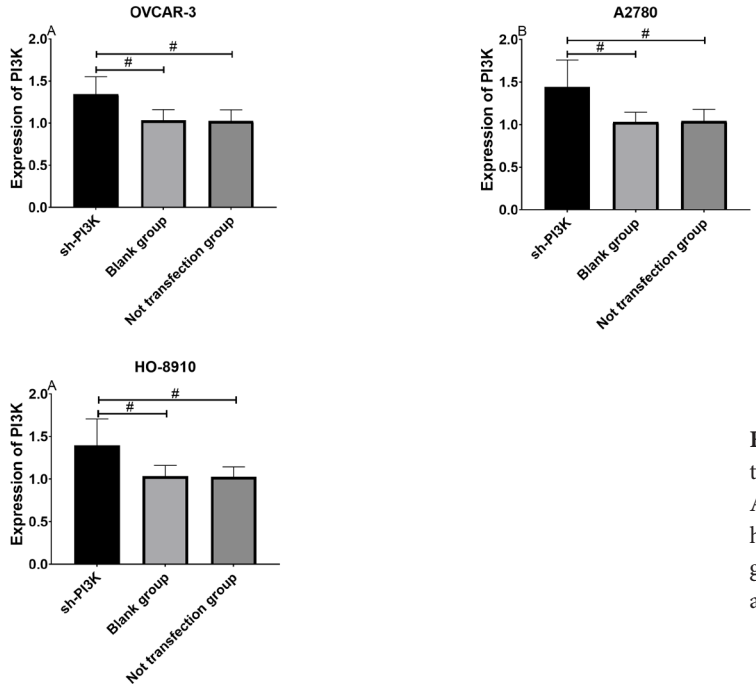


**FIGURE 5. Transfection results of PI3K.** After PI3K was transfected, expression levels of PI3K protein in OVCAR-3, A2780, and HO-8910 in the sh-PI3K group were significantly higher than those in the blank group and the not transfection group. There were no differences between the blank group and the not transfection group. # $p < 0.05$.

*The mechanism of polydatin in biological behaviors of cells*

After PI3K was transfected, expression levels of PI3K protein in OVCAR-3, A2780, and HO-8910 in the sh-PI3K group were significantly higher than those in the blank group and the not transfection group ($p < 0.05$). There were no differences between the blank group and the not transfection group ($p > 0.05$; Fig. 5).

The proliferative ability, migration ability, and invasive ability of OVCAR-3, A2780, and HO-8910 in the combination group were significantly higher than those of OVCAR-3, A2780, and HO-8910 in the polydatin group ($p < 0.05$; Fig. 6).

## Discussion

The PI3K pathway is a classical signaling pathway, it is involved in the regulation of proliferation, survival, and death of cells (Roy *et al.*, 2002; Xu *et al.*, 2004). In a study by Pan *et al.* (2017), it was reported that polydatin could induce the apoptosis of cervical cancer cells through PI3K/AKT/mTOR signaling pathway and that polydatin played a protective role in ischemia-reperfusion injury (Liu *et al.*, 2015) and inflammatory injury through PI3K/AKT signaling pathway (Ye *et al.*, 2017). This result indicates that the PI3K/AKT signaling pathway is a very important target of polydatin. It is assumed that polydatin takes effects in anti-ovarian cancer by regulating the PI3K/AKT signaling pathway, which provides experimental evidence for verifying the anticancer effect of Polydatin in ovarian cancer.

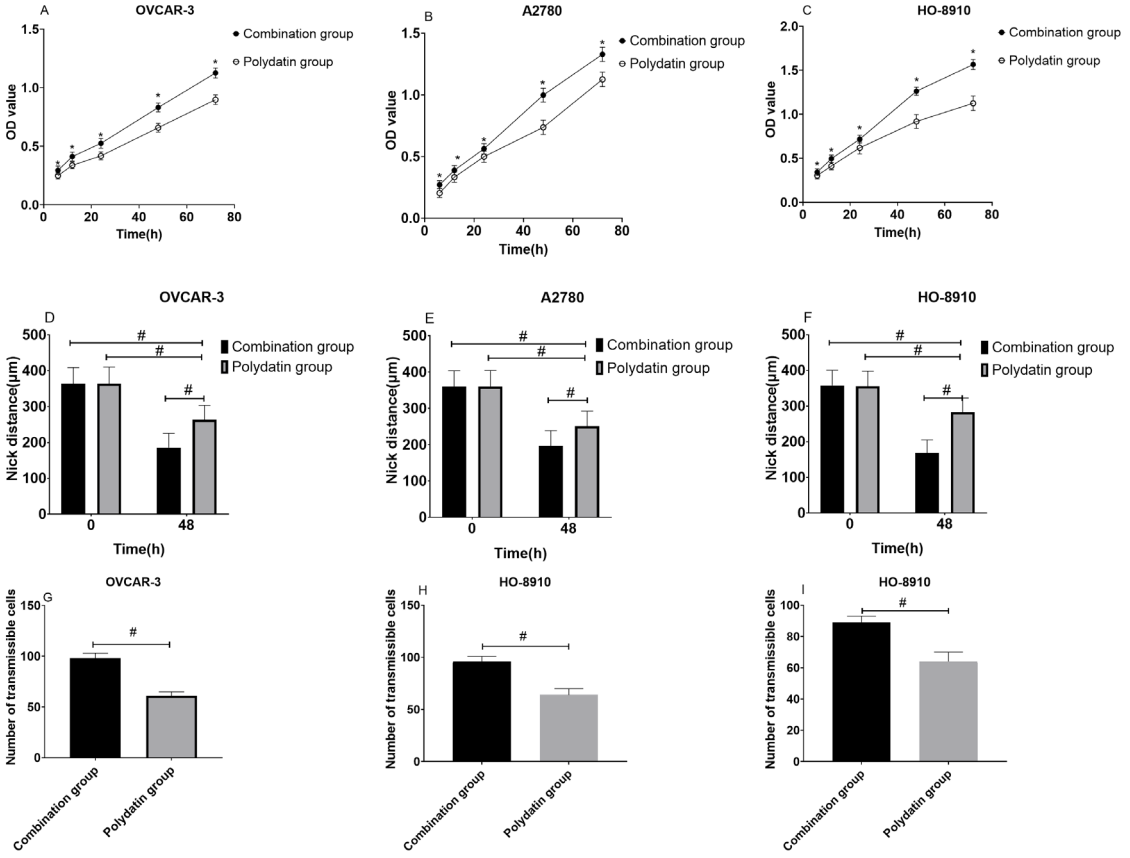


**FIGURE 6. The effect of up-regulating the level of PI3K protein on the anti-ovarian cancer effect of polydatin.** The proliferative ability, migration ability, and invasive ability of OVCAR-3, A2780, and HO-8910 in the combination group were significantly higher than those of OVCAR-3, A2780, and HO-8910 in the polydatin group ($p < 0.05$). *means compared to the polydatin group, $p < 0.05$. # $p < 0.05$.

In this study, three ovarian cancer cell lines, OVCAR-3, A2780, and HO-8910 were used. The results showed that polydatin could inhibit the three cell lines effectively. After OVCAR-3, A2780, and HO-8910 were interposed by polydatin, their proliferative ability, migration ability, and invasive ability weakened obviously. In addition, it was found that polydatin down-regulated the expression level of PI3K protein. According to this result, it is speculated that PI3K may be a target of polydatin in ovarian cancer. A rescue experiment was carried out, the result showed that after the expression level of PI3K protein was up-regulated, the inhibitory effect of polydatin on the proliferative ability, migration ability, and invasive ability of OVCAR-3, A2780, and HO-8910 reduced obviously. This result confirmed the hypothesis in this study. Polydatin is a natural polyphenolic compound. In a rat experiment, resveratrol was found in the small intestine and feces of the rats after they took polydatin. This result indicates that polydatin can convert to activated resveratrol and then take effects *in vivo* (Zhang *et al.*, 2008). Therefore, the target of resveratrol may also be the target of polydatin. In the study by Vergara *et al.* (2012), it was reported that polydatin increased the extracellular signaling and regulated the phosphorylation level of kinase (ERK) by inhibiting PI3K, thereby inhibiting the proliferation of breast cancer cells and reversing drug resistance. In studies about other cancers, it was shown that resveratrol could also inhibit the PI3K/Akt signaling pathway by up-regulating BMP7, thereby reducing the phosphorylation level of tumor suppressor genes and taking an anti-colon cancer effect (Zeng *et al.*, 2017). Resveratrol can also inhibit the transformation of prostate cancer epithelial cells into mesenchymal cells (Wang *et al.*, 2016). Some studies reported that resveratrol affected the proliferation and apoptosis of leukemia cells by regulating the PTEN/PI3K/AKT pathway (Meng *et al.*, 2019). These studies verified the credibility of the results of this study to some extent, but there are some new questions. PI3K may take an anti-tumor effect through direct targets and indirect targets of polydatin. This result needs to be confirmed by later studies. In addition, in a study about rat colitis, it was reported that the anti-inflammatory effect of polydatin was better than that of resveratrol (Yao *et al.*, 2011). Whether polydatin has a stronger anti-tumor activity needs to be confirmed by studies. However, from the above studies, it is believed that polydatin has a good anticancer activity for ovarian cancer and PI3K is one of the targets of polydatin.

There are some shortcomings in this study. Although several ovarian cancer cell lines were used to verify effects of polydatin in this study, the microenvironment in the human body is complex, the results and conclusions of this study need to be confirmed through experiments *in vivo*. Adverse reactions also need to be found through experiments in vivo. In addition, the effect of polydatin on the apoptosis of ovarian cancer cells needs to be supplemented in the next experiment. In this study, just a mechanism of polydatin was

investigated, thus more studies are required to investigate polydatin deeply.

In summary, polydatin can inhibit the proliferation, migration, and invasion of ovarian cancer cells by inhibiting the expression of PI3K protein, which provides an experimental basis for the treatment of ovarian cancer.

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**Conflicts of Interests**

None.

**References**

Chen Q, Zeng YN, Zhang K, Zhao Y, Wu YY, Li G, Cheng HY, Zhang M, Lai F, Wang JB, Cui FM (2019). Polydatin increases radiosensitivity by inducing apoptosis of stem cells in colorectal cancer. *International Journal of Biological Sciences* **15**: 430.

Chen S, Tao J, Zhong F, Jiao Y, Xu J, Shen Q, Wang H, Fan S, Zhang Y (2017). Polydatin down-regulates the phosphorylation level of Creb and induces apoptosis in human breast cancer cell. *PLoS One* **12**: e0176501.

Du QH, Peng C, Zhang H (2013). Polydatin: a review of pharmacology and pharmacokinetics. *Pharmaceutical Biology* **51**: 1347-1354.

Hanker LC, Loibl S, Burchardi N, Pfisterer J, Meier W, Pujade-Lauraine E, Ray-Coquard I, Sehouli J, Harter P, du Bois A (2012). The impact of second to sixth line therapy on survival of relapsed ovarian cancer after primary taxane/platinum-based therapy. *Annals of Oncology* **23**: 2605-2612.

Hogg SJ, Kenny C, Wafaa H, Sykes PH, Ashley G (2015). Resveratrol, acetyl-resveratrol, and Polydatin exhibit antigrowth activity against 3D cell aggregates of the skov-3 and ovcar-8 ovarian cancer cell lines. *Obstetrics and Gynecology International* **2015**: 1-14.

Howlader N, Noone AM, Krapcho M, Garshell J, Neyman N, Altekruse SF, Cho H (2013). SEER cancer statistics review, 1975-2010. *Bethesda, MD: National Cancer Institute* **12**.

Howlader N, Noone AM, Krapcho M, Miller D, Bishop K, Altekruse SF, Mariotto A (2016). SEER cancer statistics review, 1975-2013. *Bethesda, MD: National Cancer Institute* **19**.

Li H, Shi B, Li Y, Yin F (2017). Polydatin inhibits cell proliferation and induces apoptosis in laryngeal cancer and HeLa cells via suppression of the PDGF/AKT signaling pathway. *Journal of Biochemical and Molecular Toxicology* **31**: e21900.

Liu HB, Meng QH, Huang C, Wang JB, Liu XW(2015). Nephroprotective effects of polydatin against ischemia/reperfusion injury: a role for the PI3K/Akt signal pathway. *Oxidative Medicine and Cellular Longevity* **2015**: 1-13.

Lu DY, Lu TR, Lu Y, Sastry N, Wu HY (2016). Discover natural chemical drugs in modern medicines. *Metabolomics* **6**: 2153-0769.

Martano M, Stiuso P, Facchiano A, De Maria S, Vanacore D, Restucci B, Rubini C, Caraglia M, Ravagnan G, Lo Muzio L (2018). Aryl hydrocarbon receptor, a tumor grade-associated marker of oral cancer, is directly downregulated by polydatin: a pilot study. *Oncology Reports* **40**: 1435-1442.

Meng J, Liu GJ, Song JY, Chen L, Wang AH, Gao XX, Wang ZJ (2019). Preliminary results indicate resveratrol affects proliferation and apoptosis of leukemia cells by regulating PTEN/PI3K/AKT pathway. *European Review for Medical and Pharmacological Sciences* **23**: 4285-4292.

Pan JH, Wang HB, Du XF, Liu JY, Zhang DJ (2017). Polydatin induces human cervical cancer cell apoptosis via PI3K/AKT/mTOR signaling pathway. *Zhongguo Zhong yao za zhi=Zhongguo Zhongyao Zazhi=China Journal of Chinese Materia Medica* **42**: 2345-2349.

Roy HK, Olusola BF, Clemens DL, Karolski WJ, Ratashak A, Lynch HT, Smyrk TC (2002). AKT proto-oncogene overexpression is an early event during sporadic colon carcinogenesis. *Carcinogenesis* **23**: 201-205.

Torre LA, Trabert B, DeSantis CE, Miller KD, Samimi G, Runowicz CD, Gaudet MM, Jemal A, Siegel RL (2018). Ovarian cancer statistics, 2018. *CA: A Cancer Journal for Clinicians* **68**: 284-296.

Vergara D, Simeone P, Toraldo D, Del Boccio P, Vergaro V, Leporatti S, Pieragostino D, Tinelli A, De Domenico S, Alberti S, Urbani A, Salzet M, Santino A, Maffia M (2012). Resveratrol downregulates Akt/GSK and ERK signalling pathways in OVCAR-3 ovarian cancer cells. *Molecular BioSystems* **8**: 1078-1087.

Wang Z, Wu L, Tong S, Hu X, Zu X, Li Y, He W, Liu L, Chen M, Qi L (2016). Resveratrol suppresses the epithelial-to-mesenchymal transition in PC-3 cells by down-regulating the PI3K/AKT signaling pathway. *Animal Cells and Systems* **20**: 77-85.

Xie T, Li Y, Li SL, Luo HF (2016). Astragaloside IV enhances cisplatin chemosensitivity in human colorectal cancer via regulating NOTCH3. *Oncology Research Featuring Preclinical and Clinical Cancer Therapeutics* **24:** 447-453.

Xu X, Sakon M, Nagano H, Hiraoka N, Yamamoto H, Hayashi N, Dono K, Nakamori S, Umeshita K, Ito Y, Matsuura N, Monden M (2004). Akt2 expression correlates with prognosis of human hepatocellular carcinoma. *Oncology Reports* **11**: 25-32.

Yao J, Wang JY, Liu L, Zeng W-S, Li YX, Xun AY, Zhao L, Jia CH, Feng JL, Wei XX, Wang LS (2011). Polydatin ameliorates DSS-induced colitis in mice through inhibition of nuclear factor-kappaB activation. *Planta Medica* **77**: 421-427.

Ye J, Piao H, Jiang J, Jin G, Zheng M, Yang J, Jin X, Sun T, Choi YH, Li L, Yan G (2017). Polydatin inhibits mast cell-mediated allergic inflammation by targeting PI3K/Akt, MAPK, NF-κB and Nrf2/HO-1 pathways. *Scientific Reports* **7**: 11895.

Zeng YH, Zhou LY, Chen QZ, Li Y, Shao Y, Ren WY, Liao YP, Wang H, Zhu J-H, Huang M, He F, Wang J, Wu K, He BC (2017). Resveratrol inactivates PI3K/Akt signaling through upregulating BMP7 in human colon cancer cells. *Oncology Reports* **38**: 456-464.

Zhang W, Li Q, Zhu M, Huang Q, Jia Y, Bi K (2008). Direct determination of polydatin and its metabolite in rat excrement samples by high-performance liquid chromatography. *Chemical and Pharmaceutical Bulletin* **56**: 1592-1595.

Zhang Y, Zhuang Z, Meng Q, Jiao Y, Xu J, Fan S (2014). Polydatin inhibits growth of lung cancer cells by inducing apoptosis and causing cell cycle arrest. *Oncology Letters* **7**: 295-301.

Zou J, Yang Y, Yang Y, Liu X (2018). Polydatin suppresses proliferation and metastasis of non-small cell lung cancer cells by inhibiting NLRP3 inflammasome activation via NF-κB pathway. *Biomedicine & Pharmacotherapy* **108**: 130-136.