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Effects of estradiol and progesterone on the growth of HeLa cervical cancer cells

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Abstract. – OBJECTIVE: The purpose of this project was to examine the effects of estradiol (E_2) and progesterone (P) on the proliferation of HeLa cells. E_2 promoted and P inhibited the proliferation of HeLa cells.

MATERIALS AND METHODS: Then, we tested the consequence of combining the different activities of E_2 + P.

RESULTS: We found that P inhibited the proliferative effect of E_2 on HeLa cells. Analysis of fotocitometry (FCM) cell cycle and apoptosis rate showed that E_2 decreased significantly G0/G1 phase, P and E_2 + P significantly increased G0/G, and decreased significantly S phase. P inhibits tumor growth and induces apoptosis mainly by blocking the progression from G1 to S phase. Thus, the E_2 + P combination can effectively inhibit the effect of E_2 on HeLa cell proliferation. Meanwhile, the E_2 + P combination can block the progression from G1 to S phase to induce apoptosis.

CONCLUSIONS: Overall, these results suggest that the combined application of E_2 and P can be a new effective strategy for hormone replacement therapy after treatment of cervical adenocarcinoma.

Key Words:
Estradiol (E_2), Progesterone (P), Human HeLa cervical adenocarcinoma cell.

Introduction

Cervical cancer is the most common malignant tumor of the female reproductive system. Cervical squamous cell carcinoma accounts for 80% of all the tumors and adenocarcinoma for another 15%. In recent years, epidemiologic studies have shown that the prevalence of cervical cancer has increased and tended to affect younger females. Specifically, the prevalence of cervical adenocarcinoma among young patients has significantly risen, accounting for 20~30% of all cervical cancers[1,2]. The rate of ovary metastasis for cervical adenocarcinoma is around 4%, significantly higher than for cervical squamous cell carcinoma (0.2%). Therefore, resection of bilateral ovaries is often conducted together with radical hysterectomy in patients with cervical adenocarcinoma. Radical radiotherapy on advanced cervical cancer may lead to loss of ovarian function. In short, patients may suffer partial or complete loss of ovarian function after cervical carcinoma therapy, leading to estradiol deficiency, hectic fever, perspiration, insomnia, coleostenosis, dyspareunia, and other symptoms that seriously affect the life quality of patients. In particular, loss of ovarian function in young patients alters several aspects of their life and even result in family breakdown. Hormone replacement therapy (HRT) can effectively relieve female climacteric symptom, urogenital atrophy, osteoporosis, and other symptoms associated with estrogen deficiency and improve their life quality[3]. However, the application of HRT in cervical cancer patients is still controversial. Most scholars believe that HRT does not increase the incidence of cervical squamous cell carcinoma. Whereas Xenoestrogen does not increase the occurrence of cervical squamous cell carcinoma, the occurrence of cervical adenocarcinoma may be associated with estradiol. Thus, HRT may affect the prognosis of these tumors[4]. However, other studies show that the using progesterone (P) to counter the effect of estradiol (E_2) may significantly decrease the occurrence of cervical adenocarcinoma. This combination provides an opportunity to apply HRT after comprehensive therapy of cervical adenocarcinoma. Most research on the application of HRT in patients with cervical adenocarcinoma focus on clinical observations, but few studies

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have focused on the mechanisms of this therapy. By determining the effect of different E_2 and P treatments (concentrations and time), we studied whether E_2 and P can induce proliferation and apoptosis of cervical adenocarcinoma HeLa cells. Additionally, we detected the changes in cell cycle and apoptosis rate in HeLa cells after the E_2 and P treatments. Our ultimate objective is to provide the mechanistic foundation for the application of HRT after comprehensive therapy of cervical adenocarcinoma.

Material and Methods

Materials

The Yunnan Provincial Tumor Institution provided the cryopreserved cervical adenocarcinoma HeLa cell line, which is positive for estradiol and progesterone receptors. The cell line is ready for experiment use after cell recovery and continuous cell culture.

Experimental Groups

We established the following treatment groups for our studies: E_2, P, E_2 + P, and control. The E_2 and P groups were set with 5 concentration gradients in the MTT test: 0.01, 0.1, 1, 10, and 100 μmol/l. Each treatment was observed at 24, 48, and 72 h. The optimal drug concentration and time for E_2 and P as well as the optimal compatibility concentration for E_2 and P in the E_2 + P group were selected based on the MTT results.

HeLa Cell Cycle and Apoptosis Rates

E_2, P, and E_2 + P (1 μmol/l) were applied on cervical adenocarcinoma HeLa cells for 72 h. Beckman-Coulter FCM was used to detect results, and then Count WinCycle software was used for DNA analysis to identify cells in G_0/G_1, G_2/M, and S phases. The TUNEL method was applied to detect apoptosis in HeLa cells. Cell proliferation inhibition rate (%) = value of the experimental group- value of the control group)/ value of the control group × 100%.

Statistical Analysis

The SPSS17.0 software package was used for statistical analysis of the data. Data were expressed as $\bar{x} \pm sd$. Analysis of variance was used for inter-group comparison and q test for pairwise comparison. Spearman test was use for correlation analysis. When $p < 0.05$, it was considered statistically significant.

Results

Effects of E_2 and P on HeLa Cell Proliferation

We first examined the effect of different E_2 and P concentrations and incubation times on the proliferation of HeLa cells. E_2 treatments from 0.01, 0.1, 1 μmol/l promoted the growth of HeLa cells, with the longer treatment showing the strongest growth (Figure 1 and Table I). In contrast, P inhibited the growth of HeLa cells, showing time and concentration dependence (Figure 2 and Table II). E_2 and P treatments for 96 h led to massive cell floating and death, which was an indication that long-term exposure to E_2 led to limited nutrition and growth space for the cells, while P was too toxic. Therefore, 72 h was used as the maximum test time for E_2 and P in subsequent experiments.

The best effect of E_2 in HeLa cell growth was observed at 1 μmol/l for 72 h. The IC_{50} for P group after 72 h was 1.2576 μmol/l. Therefore, for the E_2 + P group we selected the 0.01, 0.1, 10, and 100 μmol/l concentrations for E_2 for the MTT assay and 1 μmol/l for morphological observation and FCM detection.

E_2 Effect on Proliferation of Cervical Adenocarcinoma HeLa Cells

The E_2 treatments for 24 h showed no statistical differences in the proliferation of HeLa cells with respect to control, except for the 10 μmol/l treatment (Table I). In 48 h treatment, 1 and 10 μmol/l E_2 significantly promoted the growth of cervical adenocarcinoma HeLa cell (Table I). In the 72 h E_2 treatment, 0.01, 0.1, 1, and 10 μmol/l significantly promoted the growth of HeLa cell

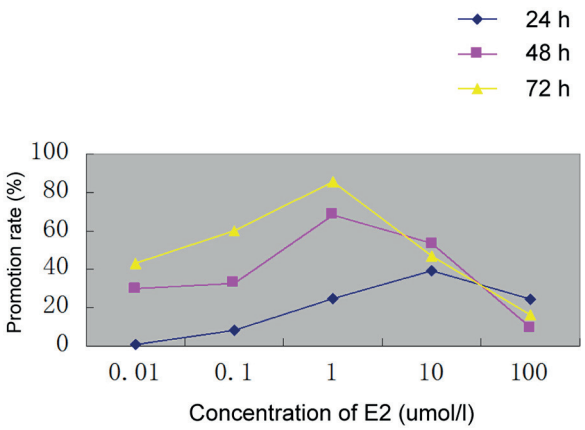

Figure 1. E_2 promotes the proliferation of HeLa cells.

Table I. Value of HeLa cells in the E_2 and control groups.

Groups	Cases (n)	Time		
		24h	48h	72h
Control	9	0.849 ± 0.229	0.987 ± 0.263	1.227 ± 0.237
E_2 (μmol/l)				
0.01	9	0.856 ± 0.175	1.282 ± 0.303	1.756 ± 0.156*▲
0.10	9	0.919 ± 0.192	1.309 ± 0.428	1.963 ± 0.401*▲
1.00	9	1.059 ± 0.214	1.660 ± 0.389*	2.277 ± 0.172*
10.0	9	1.182 ± 0.273*	1.515 ± 0.232*	1.803 ± 0.156*
100	9	1.056 ± 0.309	1.083 ± 0.287	1.426 ± 0.196▲

*: compared with control group, $p < 0.05$; ▲: compared with 1 μmol/ L after 72h, $p < 0.05$. Proliferation promotion rate of E_2 of different concentrations after 24h, 48h and 72h was calculated to draw the proliferation promotion rate figure as follows. (See Figure 1)[6].

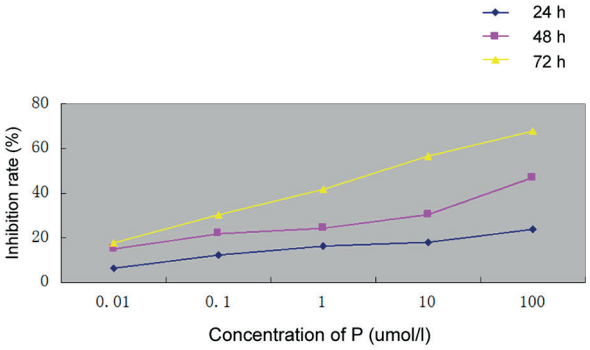

Figure 2. P inhibits the proliferation of HeLa cells.

(Table I). The correlation analysis showed that there was no significant correlation between E_2 effect on HeLa cell proliferation and concentration ($p > 0.05$).

We conducted pairwise comparison of the 10 and 100 μmol/l concentrations after 24, 48, and 72 h incubations and found no statistical differences ($p > 0.05$). Pairwise comparisons of 0.01, 0.1, and 1 μmol/l after 24, 48, and 72 h showed a statistical difference ($p < 0.05$). This result suggests that the effect on proliferation of HeLa cells of low E_2 concentrations (0.01, 0.1 and 1 μmol/l) was time-dependent[6].

Effect of P on Proliferation of Cervical Adenocarcinoma HeLa Cells

Different concentrations of P (0.01, 0.1, 1, 10, and 100 μmol/l) after 24, 48, and 72 h significantly inhibited the proliferation of HeLa cells, showing time and concentration dependence (Table II)[6]]. Cell proliferation inhibition rate of P of different concentrations after 24, 48, and 72 h was calculated to draw the proliferation inhibition rate (Figure 2)[6].

Effect of E_2 + P on Proliferation of Cervical Adenocarcinoma HeLa Cells

The E_2+ P treatments with different concentrations (0.01, 0.1, 1, 10, and 100 μmol/l)

Table II. Value of HeLa cells in P group and the control group.

Groups	Cases (n)	Time		
		24h	48h	72h
Control	9	0.577 ± 0.008	0.802 ± 0.051	1.038 ± 0.100
P (μmol/l)				
0.01	9	0.540 ± 0.011*	0.682 ± 0.037*	0.854 ± 0.023*
0.1	9	0.506 ± 0.014*	0.627 ± 0.066*	0.724 ± 0.047*
1	9	0.483 ± 0.015*	0.607 ± 0.078*	0.610 ± 0.060*
10	9	0.473 ± 0.024*	0.558 ± 0.025*	0.450 ± 0.007*
100	9	0.376 ± 0.014*	0.338 ± 0.065*	0.203 ± 0.890*

*: compared with the control group, $p < 0.05$.

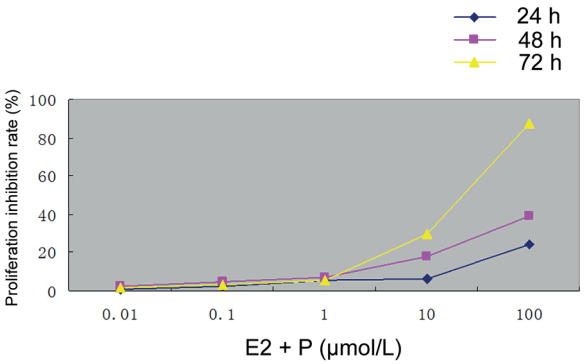

Figure 3. Effect of E_2 + P on the proliferation of HeLa promotion rate.

inhibited the proliferation of cervical adenocarcinoma HeLa cells after 24, 48, and 72 h. The 24 and 48 h treatments with 0.01, 0.1, 1, and 10 μmol/l of E_2 +P had the same effect on HeLa cell proliferation as the control group (Figure 3 and Table III). Only the E_2 + P 100 μmol/l treatment significantly inhibited HeLa cell proliferation (Figure 3 and Table III). The proliferation inhibition rates of cervical adenocarcinoma HeLa cell of E_2 + P with different concentrations after 24, 48, and 72 h were drawn (Figure 3).

Change of Cell Cycle and Apoptosis in HeLa Cells After E_2 + P Treatment

By morphological observation, E_2 had no effect on HeLa cells. Similarly, treating HeLa cells with P and E_2 + P showed no morphological differences. Using FCM, the proportion of HeLa cell at G_0/G_1 phase dropped significantly under the effect of E_2 (Table IV). The proportion of cells in S and G_2/M phases increased, but with no statistical significance (Table IV). In the treatments with P and E_2 + P, the proportion of HeLa cell in G0/G1 phase increased significantly, while cells in S phase dropped significantly (Table IV). The proportion of HeLa cells in G2/M phase showed no differences of statistical significance compared with the control group. A characteristic hypodiploid peak appeared at pre-peak of G0/G1 phase. Compared with the P group, the proportion of HeLa cells in G0/G1 phase in the E_2 + P group increased significantly (Table IV and Figure 4)[6].

HeLa Cell Apoptosis After E_2 and P Treatments detected by TUNEL

The E_2 treatment decreased the apoptosis of HeLa cells, but the difference was not significant (Table V). The P and E_2 + P treatment significantly increased apoptosis of HeLa cells (Table V). Compared with the P group, the apoptosis rate in

Table III. Value of HeLa cells in E_2 + P group and the control group ($\bar{x} \pm s$).

Groups	Cases (n)	Time		
		24h	48h	72h
Control	9	0.577 ± 0.008	0.802 ± 0.051	1.038 ± 0.100
E_2 + P (μmol/l)				
0.01	9	0.565 ± 0.006	0.785 ± 0.070	1.025 ± 0.081
0.1	9	0.563 ± 0.010	0.759 ± 0.066	1.006 ± 0.025
1	9	0.546 ± 0.012	0.745 ± 0.121	0.984 ± 0.035
10	9	0.542 ± 0.003	0.660 ± 0.055	0.732 ± 0.059*
100	9	0.435 ± 0.012*	0.489 ± 0.036*	0.128 ± 0.011*▲

*: compared with the control group, $p < 0.05$; ▲: compared with 10 μmol/L group, $p < 0.05$.

Table IV. FCM analysis of E_2 and P effects on HeLa cycle (%, $\bar{x} \pm s$).

Groups	n	G_0/G_1 phase	S phase	G_2/M phase
Control	6	63.73 ± 0.74	17.70 ± 1.61	18.57 ± 1.24
E_2	6	56.83 ± 1.72*	19.73 ± 3.56	23.40 ± 3.92
P	6	71.50 ± 1.37*	6.87 ± 0.86*	20.13 ± 1.70
E_2+ P	6	74.37 ± 0.67*▲	4.47 ± 1.2*	22.67 ± 1.03

*: compared with the control group, $p < 0.05$; ▲: compared with P group, $p < 0.05$.

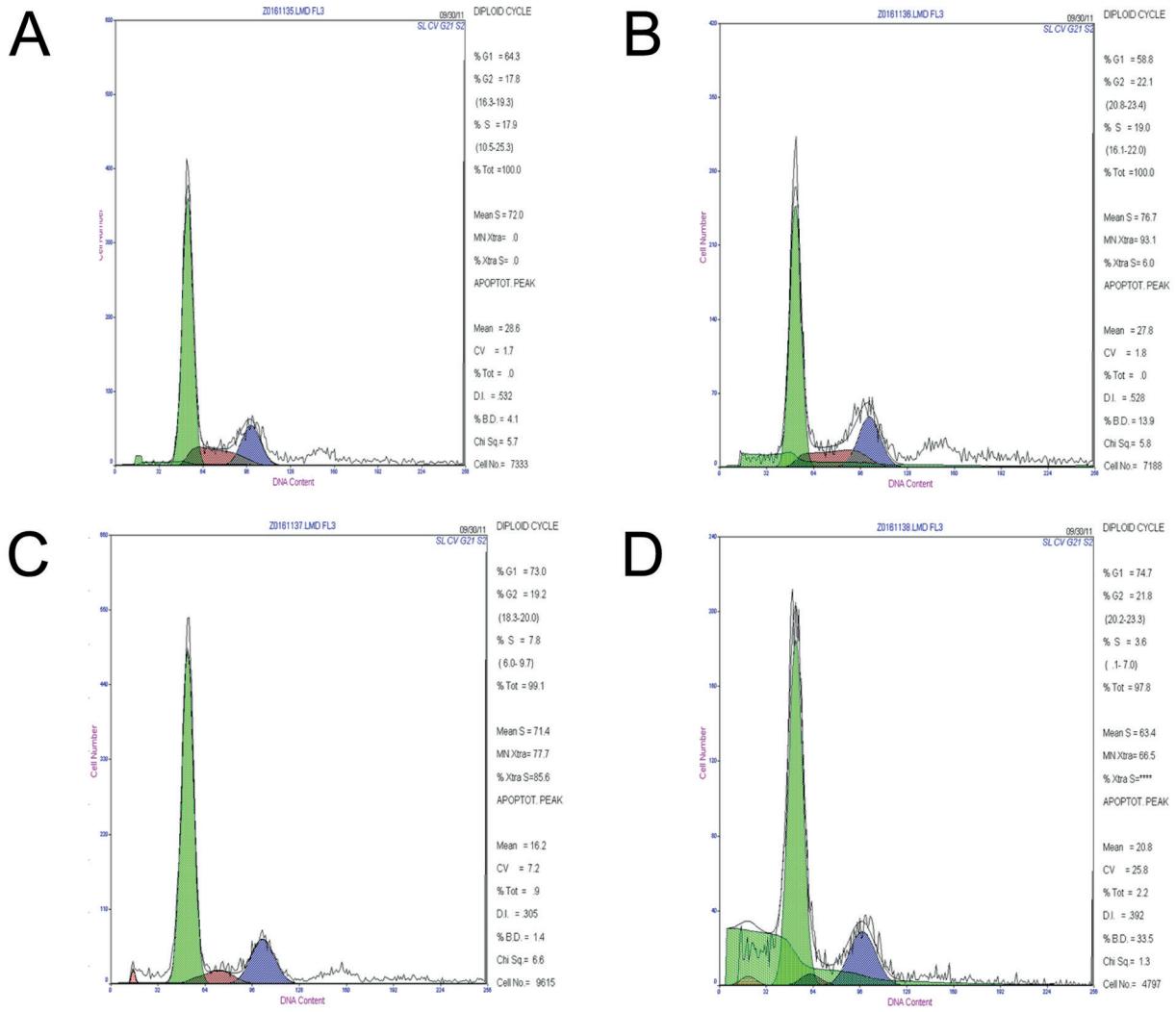

Figure 4. HeLa cycle detected by DNA content analysis. ***A,*** Control group: the first peak (G0/G1) represents the cells with 2N DNA content, the second peak (G2/M) represents the cells with 4N DNA content, and in between are cells in active DNA synthesis (S phase) with 2N-4N DNA content. ***B,*** E_2 group: the proportion of HeLa cells in G0/G1 phase is reduced significantly. ***C,*** P group: the proportion of HeLa cells at S phase is decreased, and the proportion of HeLa cells at G0/G1 phase is increased, characteristic hypodiploid peak appeared at pre-peak of G0/G1 phase. ***D,*** E_2 + P group: the proportion of HeLa cells at S phase is reduced, G0/G1 is increased, and characteristic hypodiploid peak appeared at pre-peak of G0/G1 phase.

the E_2 +P group showed a more robust increase (Table V). No necrotic cells were observed in each group (Table V and Figure 5)[6].

Discussion

E_2 and P are the most important steroid hormones in the female endocrine system. They regulate the proliferation and differentiation of target organ cells, promote the maturity of reproductive organs and the appearance of secondary sex characters of female, and maintain sexual desire and

Table V. FCM analysis of E_2 and P effects on HeLa apoptosis rate

Group	n	Apoptosis rate
Control	6	8.47 ± 1.03
E_2	6	7.13 ± 0.80
P	6	23.87 ± 0.93*
E_2 + P	6	26.17 ± 9.36*▲

*: compared with the control group, $p < 0.05$; ▲: compared with P group, $p < 0.05$.

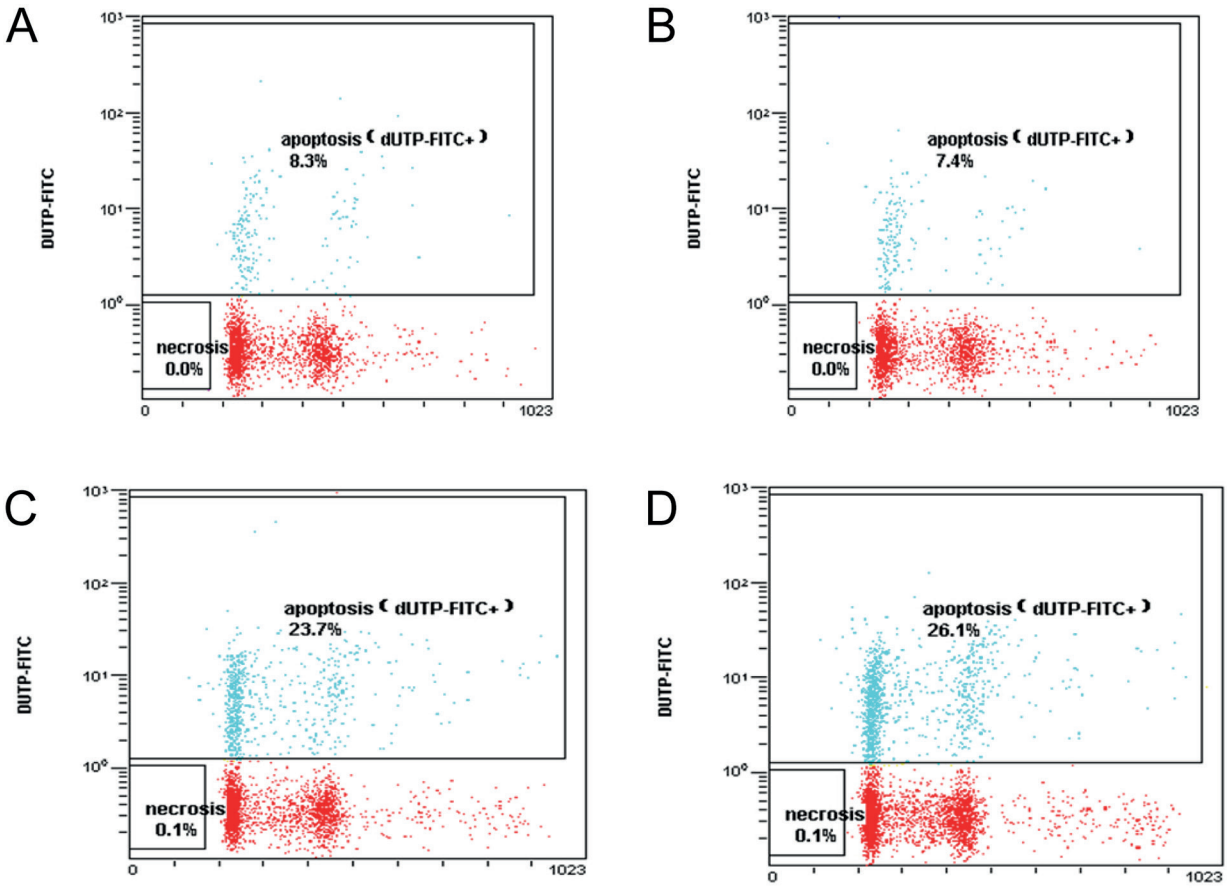

Figure 5. Detection of apoptosis in HeLa cells by TUNEL. ***A,*** Control group: apoptotic cells are scattered through the green zone, percentage indicated the apoptosis rate. The red zone indicated the necrotic cells, percentage indicated the necrosis rate. ***B,*** E_2 group: no significant difference in apoptotic cells. ***C,*** P group: apoptotic cells are significantly increased. ***D,*** E_2 + P group: apoptotic cells are significantly increased.

reproductive function. In recent years, the relationship between E_2 and P and female malignant tumors has been revealed. For example, E_2 can promote the growth of an endometrial and breast cancer cell lines *in vitro*[6]. P can inhibit proliferation of normal breast epithelial cells *in vivo* and inhibit the proliferation of a mammary carcinoma[7] and an endometrial cancer cell line *in vitro*[8]. P also inhibits the proliferation of ovarian carcinoma cells, showing dosage-effect relationship[9]. Zheng et al[10] have showed that progesterone inhibits the growth of breast cancer cells resulted from E_2 mainly by progesterone receptor subtype B (PR-B).

Our work showed that E_2 promoted proliferation of HeLa cells and P significantly inhibited proliferation of HeLa cells. The E_2 + P combination at high concentrations significantly inhibited HeLa cell proliferation, indicating that P can effectively antagonize the cell proliferative effect of E_2. This result is consistent with the work of Chen Zeng-yan et al[11], and is also similar to other results in endometrial cancer. Therefore, E_2 may correlate with adenocarcinoma of endometrial tissue[12].

Although low E_2 concentration stimulated proliferation, higher concentrations had a weaker effect on proliferation of HeLa cells. This is consistent with the statement from Rees et al[13]: "estradiol may stimulate the proliferation of breast cancer cell and also inhibit its growth". However, this is inconsistent with the conclusion of Chen Zeng-yan et al[11] that "E_2 effect on proliferation of HeLa cell is positively related to drug concentration". The above result may be associated with the relationship between cell amount and drug concentration, where too high drug concentration may produce drug toxicity and further cause HeLa cell damage and death. The purpose of HRT is to find the minimum effective concentration. Therefore, safe and effective treating time and dosage should be continuously explored in follow-up test and clinical application[14,15].

FCM showed that E_2 significantly decreased proportion of cells at G0/G1 phase. This suggested that E_2 promotes cell proliferation through changing HeLa cell cycle phase. Additionally, P and E_2 + P, significantly increased G0/G1 phase and significantly decreased S-phase. Thus, P inhibits HeLa cell proliferation and induces apoptosis by blocking the progression of cell cycle from G1 phase to S phase. It appears that the combination of E_2 and P promotes the expression of progesterone receptor, and thus enhances the apoptosis-promoting effect of P.

To sum up, E_2 promotes the proliferation of HeLa cells at low concentrations, but high concentrations. E_2 promotes cell proliferation mainly through changing the cell cycle of HeLa cells. P inhibits the proliferation of HeLa cells and induces apoptosis mainly by blocking the progression of cell cycle from G1 to S phase. The E_2 and P combination effectively antagonizes the proliferation effect of E_2 on HeLa cell, and induces cell apoptosis through blocking the progression of cell cycle from G1 to S phase while inhibiting proliferation. Therefore, treating patients with cervical adenocarcinoma with E_2 alone is highly risky, but adding P may effectively antagonize the proliferation effect of E_2 and induce apoptosis. Therefore, the combined application of E_2 and P may be a new estrategy for HRT in cervical adenocarcinoma.

Conclusions

Overall, we found that the combined application of E_2 and P can be a new effective strategy for hormone replacement therapy after treatment of cervical adenocarcinoma.

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Conflict of Interest

The Authors declare that they have no conflict of interests.

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