

Estradiol agonists inhibit human LoVo colorectal-cancer cell proliferation and migration through p53

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estrogen receptors (ER) or direct administration of ER agonists on human colorectal cancer.

METHODS: LoVo cells were established from the Bio-resource Collection and Research Center and cultured in phenol red-free DMEM (Sigma, United States). To investigate the effects of E2 and/or ER selective agonists on cellular proliferation, LoVo colorectal cells were treated with E2 or ER-selective agonists for 24 h and 48 h and subjected to the MTT (Sigma) assay to find the concentration. And investigate the effects of E2 and/or ER selective agonists on cell used western immunoblotting to find out the diversification of signaling pathways. In order to observe motility and migration the wound healing assay and a transwell chamber (Neuro Probe) plate were used. For a quantitative measure, we counted the number of migrating cells to the wound area post-wounding for 24 h. We further examined the cellular migration-regulating factors urokinase-type plasminogen activator (u-PA), tissue-type plasminogen activator (t-PA) and matrix metalloproteinase (MMP)-9 in human LoVo cells so gelatin zymography that we used and gelatinolytic activity was visualized by Coomassie blue staining. And these results are presented as means \pm SE, and statistical comparisons were made using Student's *t*-test.

RESULTS: The structure was first compared with E2 and ER agonists. We then treated the LoVo cells with E2 and ER agonists (10^{-8} mol/L) for 24 h and 48 h and subsequently measured the cell viability using MTT assay. Our results showed that treatment with 17β -estradiol and/or ER agonists in human LoVo colorectal cancer cells activated p53 and then up-regulated p21 and p27 protein levels, subsequently inhibiting the downstream target gene, cyclin D1, which regulates cell proliferation. Taken together, our findings demonstrate the anti-tumorigenesis effects of 17β -estradiol and/or ER agonists and suggest that these compounds may prove to be a potential alternative therapy in the treatment

Abstract

AIM: To investigate the effects of 17β -estradiol *via*

of human colorectal cancer. These results demonstrate that 17 β -estradiol and/or ER agonists downregulate migration-related proteins through the p53 signaling pathway in human LoVo colorectal cancer cells. These findings suggest that p53 plays a critical role in the 17 β -estradiol and/or ER agonist-mediated protective activity against colorectal cancer progression. In addition, 17 β -estradiol and/or ER agonists dramatically inhibited cell migration and reduced the expression of u-PA, t-PA and MMP-9 as well as MMP-2/9 activity in LoVo cells, which regulate cell metastasis. Moreover, we observed that pretreatment with a p53 inhibitor significantly blocked the anti-migration effects of E2 and/or ER agonists on LoVo cells. That E2 and/or ER agonists may impair LoVo cell migration by modulating migration-related factors *via* the *p53* tumor suppressor gene.

CONCLUSION: Direct ER treatment may prove to be an attractive alternative therapy in the treatment of human colorectal tumors in the future.

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Key words: Estrogen; Estrogen agonist; Estrogen receptors; Human colon cancer cell; p53

Core tip: The present study is to investigate the effects of 17 β -estradiol *via* estrogen receptors or directly administration of ERs agonist on the development of human colorectal cancer, and to elucidate whether the effect was regulated by tumor suppressor gene *p53*. Here, our results showed that 17 β -estradiol and/or ERs agonist treatment in human LoVo colorectal cancer cells could activate p53, then up-regulated p21 and p27 protein levels, subsequently inhibited downstream target gene, cyclin D1, which regulated the cell proliferation.

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INTRODUCTION

Epidemiological studies suggest that cancers of the lung/bronchus, prostate and colon/rectum in men and cancers of the lung/bronchus, breast and colon/rectum in women continue to be the most common cancers in the United States. Colorectal cancer is the third most common cause of cancer death^[1]. Colorectal cancers include nonhereditary and hereditary types. Hereditary colon cancers include familial adenomatous polyposis and hereditary non-polyposis colon cancer (HNPCC). HNPCC is the most common form of colorectal cancer, accounting

for 5%-10% of total hereditary colorectal cancers, and it occurs as early as age 25 with an average age of 45 years at diagnosis^[2].

The role of the female sex hormone, 17 β -estradiol, in tumorigenesis has been studied for many years. It has been proposed that the lower incidence of colorectal cancer (CRC) in women might be due to the influence of female sex steroid hormones^[3]. Many studies have confirmed that hormone replacement therapy (HRT) in postmenopausal women reduces the incidence of colorectal cancer^[4], whereas only one study has reported an adverse effect of HRT^[5].

One might ask what is the mechanism behind this protective effect of female sex hormones against cancer cell proliferation and carcinogenesis? The biological activity of 17 β -estradiol is mediated mainly by its binding to two specific receptors: estrogen receptor alpha (ER α), the prevalent form in the breast, cardiovascular system and liver, and estrogen receptor beta (ER β), the prevalent form in the gastrointestinal tract^[6]. Both ER α and ER β exist in colorectal cancer cells^[7].

Various proteases are expressed in cancer progression and metastasis^[8]. The systems primarily responsible for extracellular matrix (ECM) degradation *in vivo* are matrix metalloproteinase (MMP) and plasminogen activator (PA) systems^[9]. MMPs are a family of functionally related zinc-containing enzymes that include interstitial collagenases, gelatinases, metalloelastase and membrane-type MMPs^[10,11]. The gelatinases MMP-2 and MMP-9 have been implicated in colorectal cancer progression and metastasis in animal models and patients^[12]. In the proteolytic plasminogen system, the up-regulation of urokinase-type plasminogen activators (u-PAs) and tissue-type plasminogen activators (t-PAs) has been shown to activate MMPs and is involved in colon cancer progression^[13,14]. In addition, a mutation in the adenomatous polyposis coli (APC) tumor suppressor gene occurs in most colorectal tumors, resulting in the accumulation of β -catenin due to reduced ubiquitin-mediated proteolysis, which may play a causal role in promoting carcinogenesis^[15,16]. The current results indicate that the accumulation of nuclear β -catenin can be used as a prognostic marker in patients with stage IIA colon cancer^[17].

The *p53* tumor suppressor gene mediates many cellular processes, including cell cycle regulation, DNA repair, differentiation and apoptosis, in response to various extracellular and intracellular signals^[18,19]. In contrast, it is well known that *p53* mutations contribute to the malignant progression of colorectal cancer and resistance to anticancer therapy^[20-22]. Interestingly, the precise anti-metastasis mechanisms underlying the protective effects of 17 β -estradiol/ERs on colorectal cancer *via* the *p53* tumor suppressor protein remain unclear. This study examines the effects of 17 β -estradiol and/or ER agonists on the regulation of cell proliferation and migration in human LoVo colorectal cancer cells. The roles of *p53* and the precise molecular mechanisms behind this protective property are identified.

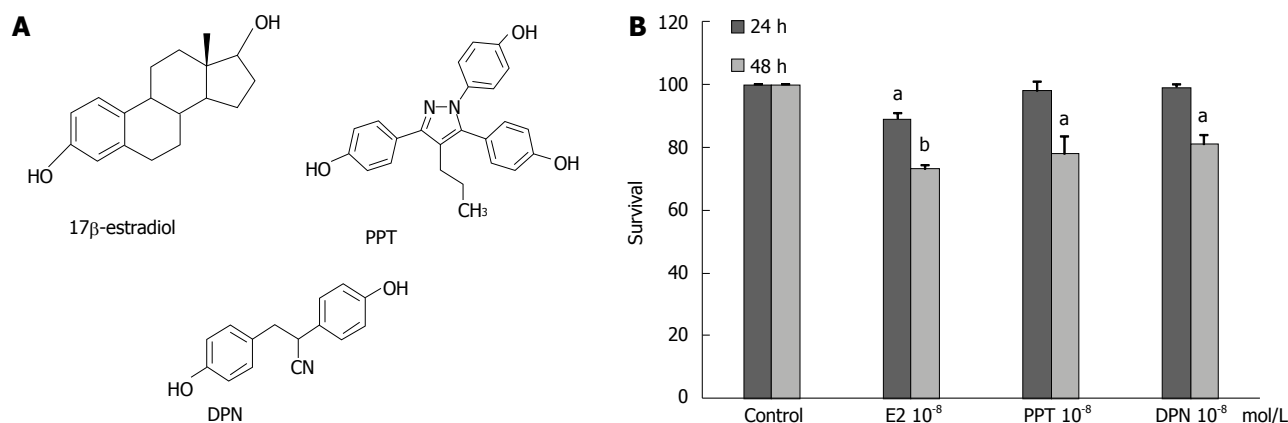


Figure 1 Estradiol and ER selective agonists impair cell proliferation in human LoVo cells. A: Structures of 17 β -estradiol, propylpyrazole-triol (PPT), a selective agonist of ER α , and diarylpropionitrile (DPN), a selective agonist of ER β ; B: LoVo cells cultured in DMEM were treated with 17 β -estradiol, PPT and DPN for 24 h and 48 h and were then analyzed for cell viability by the MTT assay. ^a $P < 0.05$ vs control; ^b $P < 0.001$ vs control (means \pm SE, $n = 3$).

MATERIALS AND METHODS

Cell, chemicals and materials

The human colon cancer cell line LoVo was obtained from the Bioresource Collection and Research Center (BCRC). LoVo cells were established from a metastatic nodule resected from a 56-year-old Caucasian male colon adenocarcinoma patient.

The following reagents were used for experiment: 17 β -estradiol (E2) (Sigma, Louis), an ER α -selective agonist [propylpyrazole-triol (PPT)], an ER β -selective agonist [diarylpropionitrile (DPN)] (Figure 1A), an ER α -selective antagonist [methyl-piperidinopyrazoledihydrochloride (MPP)], an ER β -selective antagonist 4-[2-Phenyl-5,7-bis(trifluoromethyl) pyrazolo [1,5-a] pyrimidin-3-yl] phenol (PHTPP), the ER antagonist ICI 182780 (ICI) (all from TOCRIS), and the p53 inhibitor Pifithrin- α , *p*-Nitro, Cyclic (Merck).

Cell culture

LoVo colon cancer cells were cultured in phenol red-free DMEM (Sigma, United States) supplemented with 1.5 g/L sodium bicarbonate, 3.5 g/L glucose, 1% penicillin-streptomycin and 10% cosmic calf serum (Hyclone, United States) in a humidified atmosphere at 37 °C with 5% CO₂. The medium was changed to phenol red-free DMEM with 0% serum 4 h before the experiment was started.

Cell proliferation assay

To investigate the effects of E2 and/or ER selective agonists on cellular proliferation, LoVo colorectal cells were treated with E2 or ER-selective agonists for 24 h and 48 h and subjected to the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) (Sigma) assay. The blue formazan crystal absorbance was measured at 570 nm using an enzyme-linked immunosorbent assay plate reader.

Western immunoblotting and antibodies

Total proteins were extracted using lysis buffer [50 mmol/L Tris-base, pH = 7.5, 0.5 mol/L NaCl, 1.0

mmol/L EDTA, pH = 7.5, 10% glycerol, 1 mmol/L β -Mercaptoethanol and a proteinase inhibitor cocktail (Roche Molecular Biochemicals)]. The cell lysate proteins were analyzed using SDS-PAGE. The following primary antibodies were used for incubations: MMP-9 (Chemicon), Cyclin D1 sc-246, β -Catenin sc-7963, GSK-3 β sc-9166, p53 sc-1311, u-PA sc-14019, t-PA sc-5239, α -Tubulin sc-5286, and β -actin sc-47778 (all from Santa Cruz Biotechnology). Following primary antibody incubations, membranes were incubated with horseradish peroxidase-linked secondary antibodies (anti-rabbit, anti-mouse, or anti-goat IgG) (all from Santa Cruz Biotechnology).

Wound healing assay

LoVo cells were seeded into six-well plates at 1×10^5 cells/well in culture medium. Confluent monolayers were scratched with a sterile micro-pipette tip and then washed with PBS to remove floating cells in serum-free medium. Then, the cells were serum starved for 4 h. Wound healing was performed by treatment with E2, ER-selective agonists, a p53 inhibitor (10 μ mol/L), ER-selective antagonists or ICI182780 (1 μ mol/L), respectively. For a quantitative measure, we counted the number of migrating cells to the wound area post-wounding for 24 h.

Cell migration and invasion assays

The cell migration assay was carried out using a modified Boyden chamber consisting of a trans well chamber (Neuro Probe) plate with 8- μ m pore size polycarbonate membrane filters^[23]. Serum-deprived LoVo cells were added to the upper part of the Boyden chamber, and the bottom chamber was filled with DMEM containing 10% serum. After incubation for 48 h, the cells were allowed to migrate to the underside of the membrane. The cells on the membrane filter were then fixed with methanol and stained with 0.05% Giemsa (Sigma). The number of migrated cells was quantified by cell counting in at least three random fields (magnification, $\times 200$) per filter.

Gelatin zymography

LoVo cells cultured in DMEM were treated with E2 (10⁻⁸

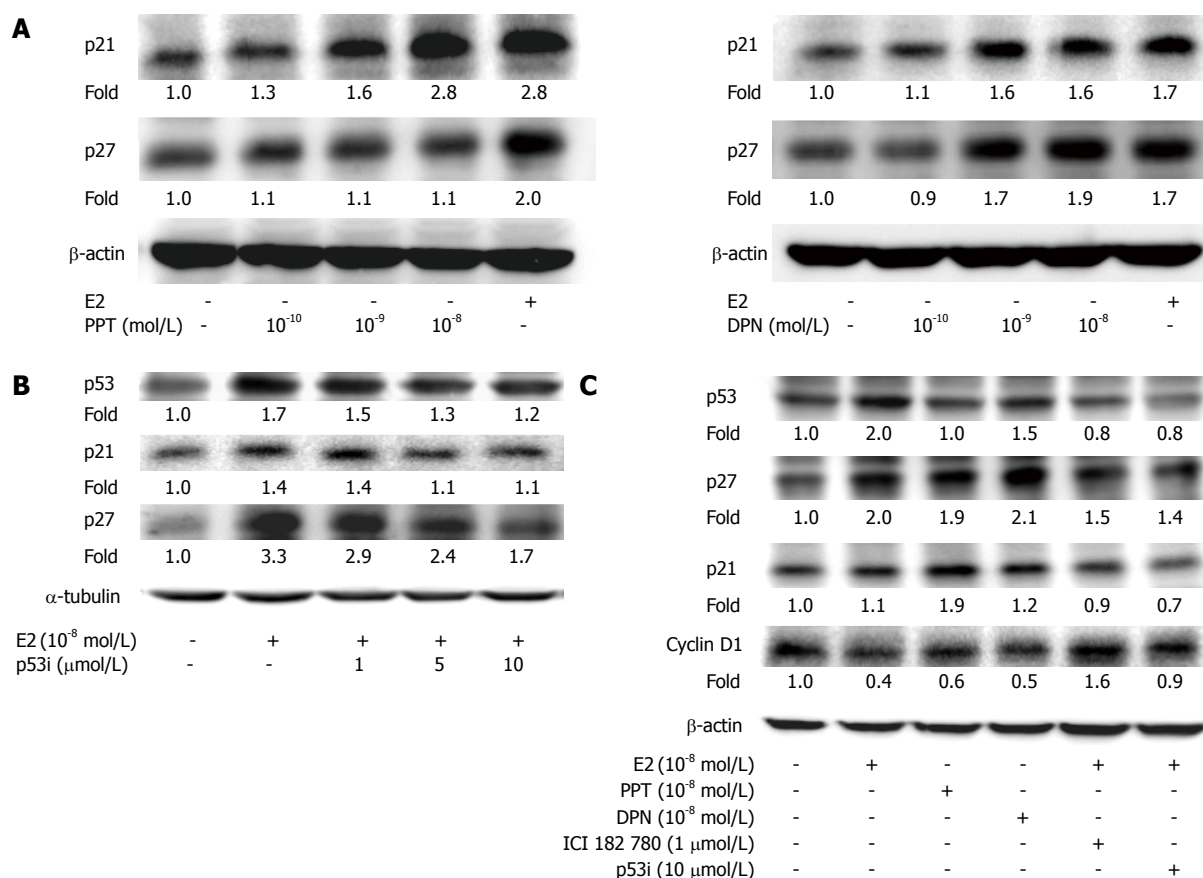


Figure 2 17β-estradiol and/or ER selective agonists upregulate p53 signaling proteins to change the cell cycle progression in human LoVo colorectal cancer cells. A: LoVo cells were treated with E2 and various concentrations (10⁻¹⁰ mol/L, 10⁻⁹ mol/L and 10⁻⁸ mol/L) of PPT or DPN for 48 h; B: LoVo cells were pretreated with various concentrations (1 μmol/L, 5 μmol/L and 10 μmol/L) of a p53 inhibitor for 1 h followed by E2 (10⁻⁸ mol/L) treatment for 48 h; C: LoVo cells were pretreated with vehicle, the ER antagonist ICI 182780 (ICI), or a p53 inhibitor for 1h, followed by E2 administration for 48 h. Subsequently, cells were harvested and measured by Western blotting. A β-actin standard was used as a loading control for all proteins. All experiments were repeated twice with identical results.

mol/L) for 24h and subsequently collected in conditional medium. Samples were electrophoresed without reduction (no DTT) on 8% SDS polyacrylamide gels copolymerized with 0.1% gelatin. When the tracking dye at the front reached the bottom of the gel, the gel was removed and shaken gently for 30 min in 2.5% Triton X-100 to remove SDS. The gels were then transferred to a bath (without Triton X-100) and washed for 30 min to remove Triton X-100. The gels were then incubated overnight (37 °C) in reaction buffer containing 40 mmol/L Tris-HCl (pH = 8.0), 0.01% NaCl and 10 mol/L CaCl₂. Finally, gelatinolytic activity was visualized by Coomassie blue staining.

Statistical analysis

Each experiment was repeated at least twice with identical results. The results are presented as means ± SEs, and statistical comparisons were made using Student's *t*-test. Significance was presented as a *P* < 0.05 or *P* < 0.01.

RESULTS

Effects of 17β-estradiol and ER selective agonist on cell proliferation and viability in human LoVo colorectal cancer cells

To determine the effects of E2 and ER-selective agonists

on the proliferation of human LoVo colorectal cancer cells, the structure was first compared with E2 and ER agonists. We then treated the LoVo cells with E2 and ER agonists (10⁻⁸ mol/L) for 24 h and 48 h and subsequently measured the cell viability using MTT assay. The results showed a significant reduction in LoVo colorectal cancer cell viability, with a reduction of approximately 28.0% following E2 treatment for 48 h, 21.0% following PPT treatment for 48 h and 15.8% following DPN treatment for 48 h (Figure 1B). We further examined the level of p53 signaling and downstream proteins through Western blotting. After LoVo cells were treated with E2 (10⁻⁸ mol/L) or various concentrations (10⁻¹⁰ mol/L, 10⁻⁹ mol/L and 10⁻⁸ mol/L) of PPT or DPN for 24 h, we observed a significant dose-dependent reduction in the expression of p21 and p27 (Figure 2A). In LoVo cells, administration of a p53 inhibitor (1 μmol/L, 5 μmol/L, 10 μmol/L) significantly inhibited the E2-induced activation of p53, p27 and p21 in a dose-dependent manner (Figure 2B). The serum-starved human LoVo colorectal cancer cells were pretreated with ICI or p53 inhibitor (1 μmol/L), which significantly inhibited the E2-induced increases in p53, p27 and p21 protein levels and blocked the E2-dependent reduction in the protein levels of cell cycle-regulating proteins such as cyclin D1. At the same time,

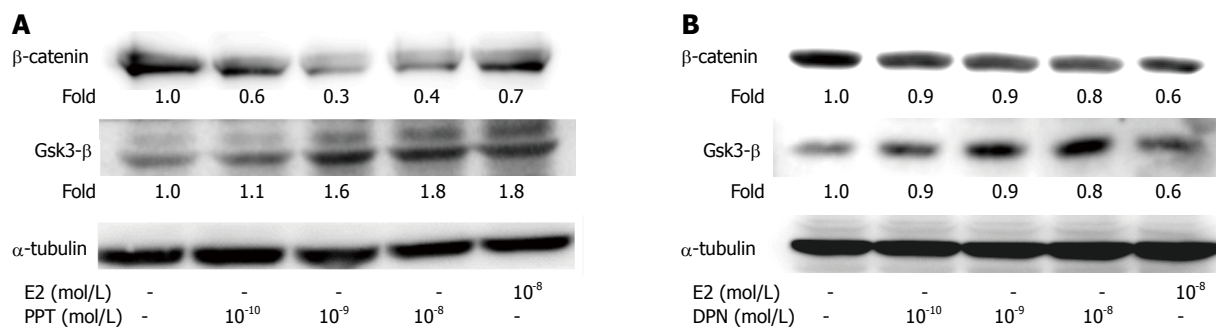


Figure 3 17β-estradiol and ER selective agonists impair the Wnt-β-catenin signaling pathway in human LoVo colorectal cancer cells. LoVo cells were treated with E2 (10⁻⁸ mol/L) or various concentrations (10⁻¹⁰ mol/L, 10⁻⁹ mol/L and 10⁻⁸ mol/L) of PPT (A) or DPN for 24 h (B). Cells were harvested and measured by Western blotting. An α-tubulin standard was used as a loading control for all proteins. All experiments were repeated twice with identical results.

we observed that treatment with the ER agonists PPT or DPN alone also had the same effect as E2 treatment on human LoVo cells (Figure 2C). These results suggest that the administration of 17β-estradiol or ER agonist can inhibit cell proliferation in human LoVo cells by modulating p53 signaling and its downstream cell cycle target gene, cyclin D1.

Suppression of the Wnt-β-catenin signaling pathway by E2 and ER-selective agonists in human LoVo colorectal cancer cells

After LoVo cells were treated with various concentrations (10⁻¹⁰ mol/L, 10⁻⁹ mol/L and 10⁻⁸ mol/L) of PPT or DPN for 24 h, the level of the Wnt-β-catenin complex protein was investigated. The GSK-3β protein content was dramatically increased, and the β-catenin level was decreased in a dose-dependent manner (Figure 3). These results might indicate that E2 or ER agonist treatment can inhibit colorectal cancer cells growth *via* Wnt/wingless signaling suppression.

17β-estradiol and/or ER selective agonists down-regulate the u-PA, t-PA and MMP-9 protein levels in LoVo cells

It is known that proteolytic plasminogen system activation by u-PA and t-PA is involved in the up-regulation of downstream MMPs in cancer cells^[24]. We therefore further examined the cellular migration-regulating factors u-PA, t-PA and MMP-9 in human LoVo cells. In our studies, we observed that a significant reduction in u-PA, t-PA, MMP-2 and MMP-9 protein expression was induced by E2 (10⁻⁸ mol/L) treatment within 24 h. Subsequently, the quantitative results showed that PPT (10⁻¹⁰ mol/L, 10⁻⁹ mol/L and 10⁻⁸ mol/L) treatment alone also significantly reduced the u-PA, t-PA and MMP-9 levels. DPN (10⁻¹⁰ mol/L, 10⁻⁹ mol/L and 10⁻⁸ mol/L) treatment alone reduced the u-PA and MMP-9 levels in a dose-dependent manner (Figure 4A). Administration of a p53 inhibitor (1 μmol/L, 5 μmol/L, 10 μmol/L) to LoVo cells significantly blocked the E2-induced inhibition of u-PA and t-PA in a dose-dependent manner (Figure 4B). The serum-starved human LoVo colorectal cancer cells that were pretreated with ICI or p53 inhibitor (1 μmol/L)

significantly blocked the E2-dependent reduction in t-PA protein levels (Figure 4C). Therefore, these results suggest that 17β-estradiol or ER agonist administration can inhibit cellular migration-regulating factors, including u-PA, t-PA and MMP-9, in a p53-dependent manner.

17β-estradiol inhibits MMP-2/9 activities in LoVo colorectal colon cells

It is known that MMPs function as proteases in extracellular matrix protein degradation^[25]. Thus, MMP activities play important roles in cell cycle regulation. We therefore examined whether E2 can suppress MMP-2/9 expression and activity in LoVo cells. In Figure 5, gel images from gelatin zymography show that MMP-2 (72 kDa) and MMP-9 (92 kDa) activities were significantly suppressed in LoVo cells treated with E2.

17β-estradiol and/or ER agonists inhibit human LoVo colorectal cancer cell motility through the tumor suppressor gene p53

We observed that the migration ability of LoVo cells could be inhibited after E2 (10⁻⁸ mol/L) treatment. We further determined that E2 and/or ER agonists played a role in LoVo cell migration ability. We then cultured LoVo cells with E2 (10⁻⁸ mol/L) in the presence or absence of ICI 182780, a p53 inhibitor, ERα antagonists (MPP) or ERβ antagonists (PHTPP). We also treated LoVo cells with PPT or DPN (10⁻⁸ mol/L), which are ER agonists, alone for 48 h. We observed the migration ability in LoVo cells using scratch motility and migration assays. In the scratch motility (Figure 6A) and migration assays (Figure 6B), we observed that ICI 182780 and the p53 inhibitor dramatically blocked the inhibitory effects of E2 on cellular migration in LoVo cells. In addition, treatment with either of the ER agonists, *i.e.*, PPT or DPN, alone also significantly inhibited LoVo colorectal cancer cell migration by approximately 24% and 39%, respectively. Both ERα and ERβ are involved in down regulating cellular mobility in human LoVo colorectal cancer cells. These findings showed that p53 mediates the E2-ER-modulated migration ability of human LoVo cells and that ER agonist treatment alone has the same ability to inhibit LoVo cell migration.

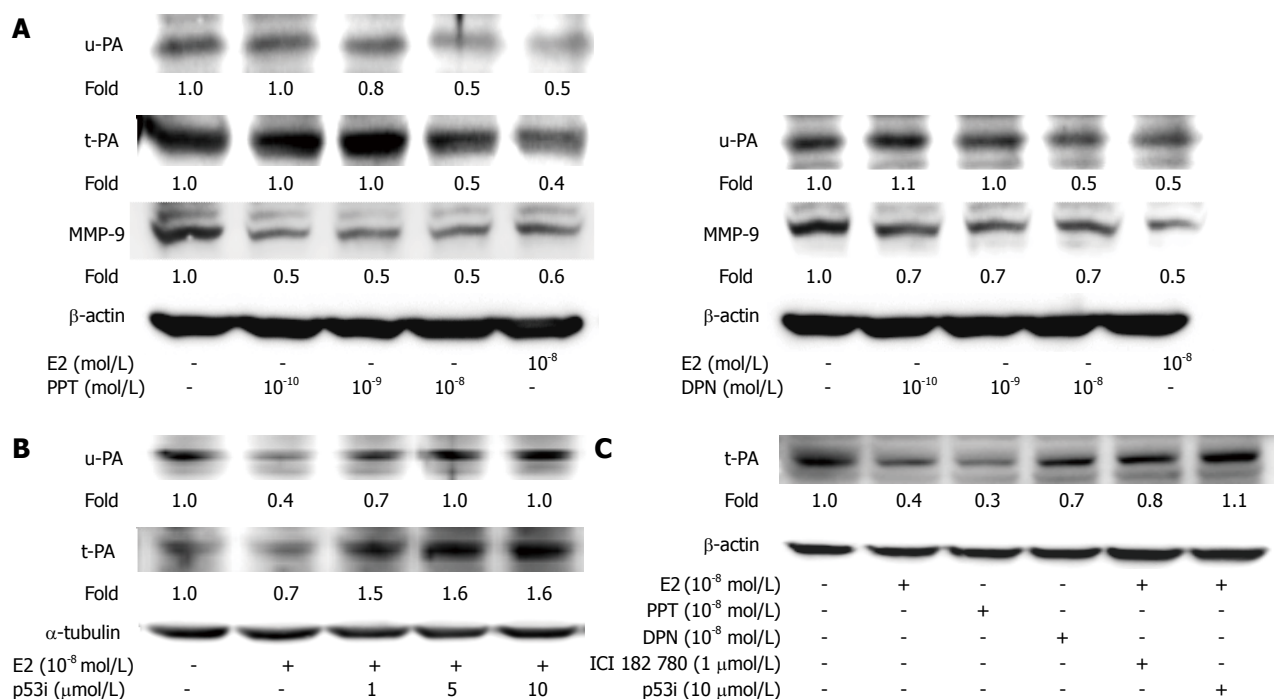


Figure 4 17β-estradiol and/or ER selective agonists down-regulate the protein levels of u-PA, t-PA and MMP-9 in LoVo cells *via* p53. A: LoVo cells were treated with E2 or various concentrations (10⁻¹⁰ mol/L, 10⁻⁹ mol/L and 10⁻⁸ mol/L) of PPT or DPN for 24 h; B: LoVo cells were pretreated with various concentrations (10⁻¹⁰ mol/L, 10⁻⁹ mol/L and 10⁻⁸ mol/L) of a p53 inhibitor for 1 h, followed by E2 administration for 24 h; C: LoVo cells were pretreated with vehicle, the ER antagonist ICI 182780, or a p53 inhibitor for 1 h, followed by E2 administration for 24 h. Cells were harvested and examined by Western blotting. A β-actin or α-tubulin standard was used as a loading control for all proteins. All experiments were repeated twice with identical results.

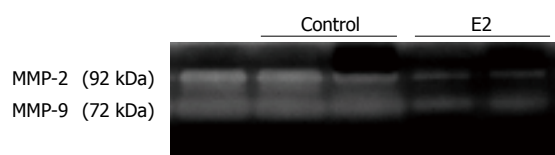


Figure 5 17β-estradiol inhibits MMP-2/9 activity in LoVo colorectal colon cells. LoVo cells cultured in DMEM were treated with E2 (10⁻⁸ mol/L) for 24 h, and then, the culture medium was collected. Samples were electrophoresed without reduction on 8% SDS polyacrylamide gels copolymerized with 0.1% gelatin.

DISCUSSION

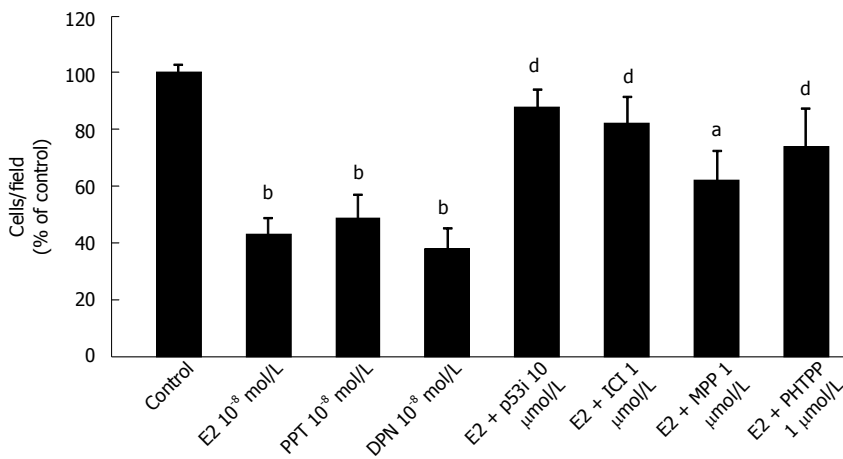
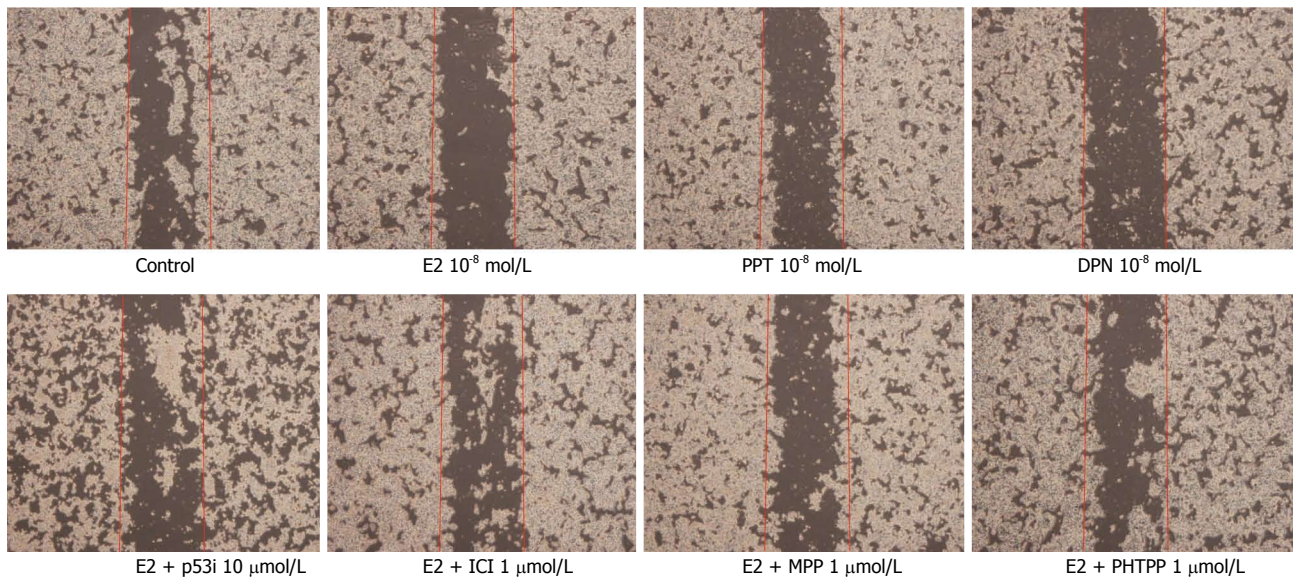
Previous studies have shown that E2 binding to ERs can regulate tissue and cellular responses *via* multiple signaling pathways^[26]. The ERs act at estrogen-responsive target genes to either trans-activate or trans-repress gene expression^[27]. In our previous studies, we found that over-expressed ERα induces apoptosis and inhibits the proliferation of human LoVo colorectal cancer cells^[28]. The apoptotic effects of over-expressed ERβ acted in a ligand-dependent manner^[29]. These published results provide evidence that E2 and/or the over-expression of ERs plays a critical role in inducing the apoptosis of human LoVo colorectal cancer cells. Here, we were interested in further determining the anti-motility effects of the ERα- and ERβ-selective agonists PPT and DPN on LoVo colorectal cancer cells.

The major findings of this study can be summarized as follows: (1) treatment with E2 and/or ER agonists (10⁻¹⁰ mol/L, 10⁻⁹ mol/L and 10⁻⁸ mol/L) significantly

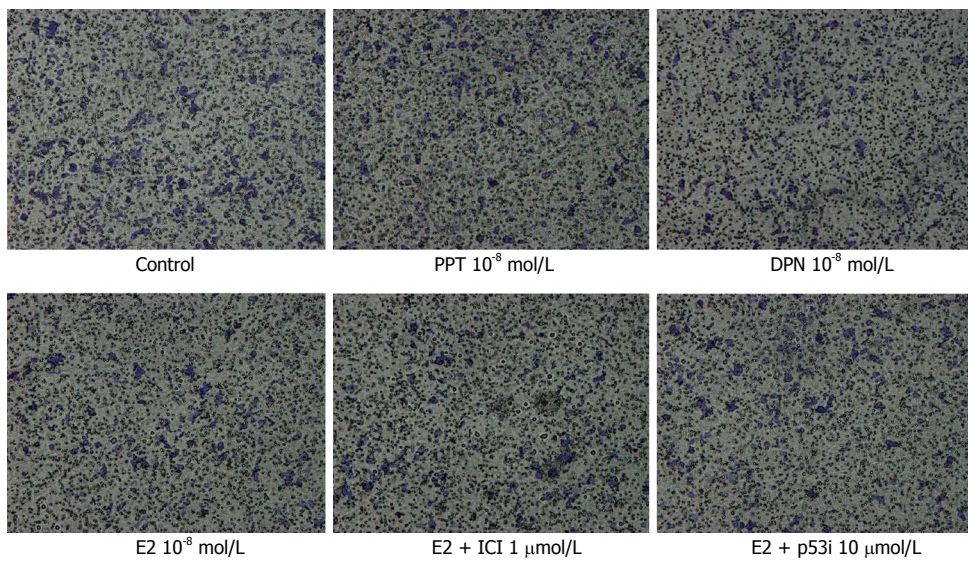
inhibits human LoVo colon cancer cell proliferation by increasing p53, p21 and p27 protein levels and decreasing the expression of the downstream target gene cyclin D1. These results suggest that E2 and/or ER agonists (10⁻⁸ mol/L) greatly suppress cell proliferation by activating the p53 signaling pathway and modulating cell cycle-regulating factors in human LoVo cells; (2) the motility of LoVo colorectal cancer cells is significantly suppressed by E2 and/or ER agonist treatment. We simultaneously observed that the decrease in the migratory ability of LoVo cells due to treatment with E2 (10⁻⁸ mol/L) and/or ER agonists (10⁻¹⁰ mol/L, 10⁻⁹ mol/L and 10⁻⁸ mol/L) was accompanied by the down regulation of migration-related proteins, including u-PA, t-PA and MMP-9, as well as the suppression of MMP-2/9 activities (Figure 5); and (3) the inhibitory effects of u-PA, t-PA and MMP-9 in human LoVo cells that had been treated with E2 and/or ER agonists were completely inhibited by a p53 inhibitor. These results demonstrate that E2 and/or ER agonists down regulate migration-related proteins through the p53 signaling pathway in human LoVo colorectal cancer cells. These findings suggest that p53 plays a critical role in the E2- and/or ER agonist-mediated protective activity against colorectal cancer progression.

Changes in the expression of cell cycle regulators, such as cyclin D1, are a critical step in tumor development and progression, which are the most critical events in colorectal cancer^[30]. The cell cycle regulator Cyclin D1 is a key intracellular regulator that is involved in cell cycle progression through the G1 phase and is over-expressed in colorectal carcinoma, leading to a worse prognosis^[31].

A



B



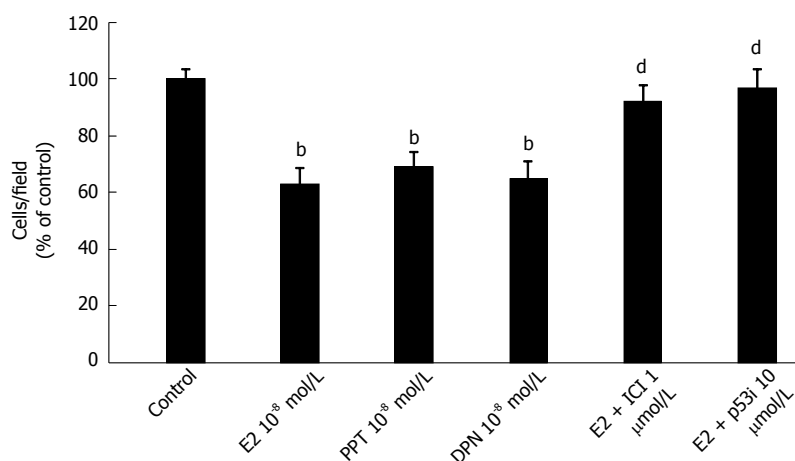


Figure 6 17β-estradiol and/or ER selective agonists inhibit human LoVo colorectal cancer cell motility through p53 signaling. LoVo cells cultured in DMEM were pretreated with vehicle, ICI 182780 (ER antagonist 1 μmol/L), p53 inhibitor (10 μmol/L), MPP (ERα antagonist 1 μmol/L), or PHTPP (ERβ antagonist 1 μmol/L) for 1 h, followed by treatment with E2 (10⁻⁸ mol/L), PPT (10⁻⁸ mol/L) or DPN (10⁻⁸ mol/L) administration for 48h. The migration ability of LoVo cells was analyzed by a scratch motility assay (A) and a migration assay (B). The results were observed and analyzed with a fluorescence microscope. All experiments were repeated twice with identical results. ^aP < 0.05 vs control; ^bP < 0.01 vs control; ^dP < 0.01 vs E2.

In our study, we observed that cell proliferation and the expression of cyclin D1 in LoVo cells was significantly inhibited by E2 and/or ER agonist treatment. These findings suggest that E2 and/or ER agonists may protect against colorectal cancer proliferation by modulating cell cycle regulators.

Many studies have reported that increased MMP levels contribute to ECM remodeling and tumor cell motility, thus leading to the progression of malignant tumors^[8,12]. Activation of the plasminogen activator system, which includes u-PA and t-PA, is reported to be involved in MMP activation and colorectal cancer development. Expression of u-PA and t-PA is considered a marker of malignant colon cancer^[13,14,32,33]. Here, we found that the administration of E2 and/or ER agonists dramatically inhibited cell migration and reduced the expression of u-PA, t-PA and MMP-9 as well as MMP-2/9 activity in LoVo cells. Moreover, we observed that pretreatment with a p53 inhibitor significantly blocked the anti-migration effects of E2 and/or ER agonists on LoVo cells. These findings suggest that E2 and/or ER agonists may impair LoVo cell migration by modulating migration-related factors *via* the p53 tumor suppressor gene.

Taken together, our results suggest that the tumor suppression protein p53 may mediate downstream signaling when E2 binds to ERs and, further, that it modulates E2-mediated anti-tumorigenic properties by inhibiting the expression of u-PA, t-PA and MMP-9. In addition, ER agonists directly activate ERα or ERβ. Anti-migration effects were observed after E2 treatment of human LoVo colorectal cancer cells. These results show that direct ER treatment may prove to be an attractive alternative therapy in the treatment of human colorectal tumors in the future.

COMMENTS

Background

Epidemiological studies suggest that cancers of the lung/bronchus, prostate

and colon/rectum in men and cancers of the lung/bronchus, breast and colon/rectum in women continue to be the most common cancers in the United States. Colorectal cancer is the third most common cause of cancer death.

Innovations and breakthroughs

This study examines the effects of 17β-estradiol and/or ER agonists on the regulation of cell proliferation and migration in human LoVo colorectal cancer cells. The roles of p53 and the precise molecular mechanisms behind this protective property are identified.

Applications

ER agonists directly activate ERα or ERβ. Anti-migration effects were observed after E2 treatment of human LoVo colorectal cancer cells. These results show that direct ER treatment may prove to be an attractive alternative therapy in the treatment of human colorectal tumors in the future.

Peer review

The authors mainly focus on to explore whether estrogen or estradiol agonists inhibit human LoVo colorectal-cancer cell proliferation and migration through p53. Their findings showed that treatment with 17β-estradiol and/or ER agonists in human LoVo colorectal cancer cells activated p53 and then up-regulated p21 and p27 protein levels, subsequently inhibiting the downstream target gene, cyclin D1, which regulates cell proliferation.

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