Mol Cell Biochem (2010) 342:63–70
DOI 10.1007/s11010-010-0469-7

17β-Estradiol inhibits prostaglandin E2-induced COX-2 expressions and cell migration by suppressing Akt and ERK1/2 signaling pathways in human LoVo colon cancer cells

Tung-Yuan Lai · Li-Mien Chen · Jing-Ying Lin · Bor-Show Tzang · James A. Lin · Chang-Hai Tsai · Yueh-Min Lin · Chih-Yang Huang · Chung-Jung Liu · Hsi-Hsien Hsu

Received: 10 January 2010 / Accepted: 12 April 2010 / Published online: 6 May 2010
© Springer Science+Business Media, LLC. 2010

Abstract Epidemiological studies demonstrate that the incidence and mortality rates of colorectal cancer in women are lower than in men. However, it is unknown if 17β-estradiol treatment is sufficient to inhibit prostaglandin E2 (PGE2)-induced cellular motility in human colon cancer cells. Upregulation of cyclooxygenase-2 (COX-2) is reported to associate with the development of cancer cell mobility, metastasis, and subsequent malignant tumor. After administration of inhibitors including LY294002 (Akt activation inhibitor), U0126 (ERK1/2 inhibitor), SB203580 (p38 MAPK inhibitor), SP600125 (JNK1/2 inhibitor), or QNZ (NFκB inhibitor), we found that PGE2 treatment increases COX-2 via Akt and ERK1/2 pathways, thus promoting cellular motility in human LoVo cancer cells. We further observed that 17β-estradiol treatment inhibits PGE2-induced COX-2 expression and cellular motility via suppressing activation of Akt and ERK1/2 in human LoVo cancer cells. Collectively, these results suggest that 17β-estradiol treatment dramatically inhibits PGE2-induced progression of human LoVo colon cancer cells.

Chih-Yang Huang, Chung-Jung Liu, and Hsi-Hsien Hsu contributed equally to this article.

T.-Y. Lai
College of Chinese Medicine, School of Post-Baccalaureate Chinese Medicine, China Medical University, Taichung 404, Taiwan

T.-Y. Lai
Department of Chinese Medicine, China Medical University Hospital, Taichung, Taiwan

L.-M. Chen
Division of Medical Technology, Department of Internal Medicine, Armed-Force, Taichung General Hospital, Taichung, Taiwan

J.-Y. Lin
Department of Medical Imaging and Radiological Science, Central Taiwan University of Science and Technology, Taichung, Taiwan

B.-S. Tzang
Institute of Biochemistry and Biotechnology, Chung Shan Medical University, Taichung, Taiwan

J. A. Lin · C.-Y. Huang
Institute of Basic Medical Science, China Medical University, Taichung, Taiwan

C.-H. Tsai
Department of Healthcare Administration, Asia University, Taichung, Taiwan

Y.-M. Lin
Department of Pathology, Changhua Christian Hospital, Changhua, Taiwan

C.-Y. Huang · C.-J. Liu
Graduate Institute of Chinese Medical Science, China Medical University, Taichung, Taiwan

C.-Y. Huang
Department of Health and Nutrition Biotechnology, Asia University, Taichung, Taiwan

H.-H. Hsu
Division of Colorectal Surgery, Mackay Memorial Hospital, Taipei, Taiwan

H.-H. Hsu (✉)
Mackay Medicine, Nursing and Management College, Taipei, Taiwan
e-mail: cyhuang@mail.cmu.edu.tw

Keywords Estrogen · Prostaglandin E2 · Human colon cancer cell · COX-2 · Cell motility

Abbreviations

E2	17β-Estradiol
PGE2	Prostaglandin E2
ERK	Extracellular signal regulated kinase
p38 MAPK	p38 Mitogen-activated protein kinase
JNK	c-Jun N-terminal kinase
NF-κB	Nuclear factor κ B
PI3-K	Phosphatidylinositol 3-kinase
PKB	Protein kinase B
DMEM	Dulbecco's modified Eagle's medium
QNZ	6-Amino-4-(4-phenoxyphenylethylamino) quinazoline
ECM	Extracellular matrix
PBS	Phosphate-buffered saline

Introduction

Colorectal carcinoma (CRC) is one of the most prevalent cancers world-wide [1], and is the secondary leading cause of cancer-related mortality in the developed countries [2]. Colon cancer accounts for more than 130,000 new cases per year [3] and causes more than 56,000 deaths per year in United States [4] despite the advanced chemotherapeutic treatments.

Cyclooxygenase 2 (COX-2) is overexpressed in several human cancers. COX-2 enzymes converted arachidonic acid into various structurally related prostaglandins, including prostaglandin E2 (PGE2), PGD2, PGF2a, PGI2, and thromboxane A2 by specific prostaglandin synthases [5]. COX-2 expression is normally low or absent in most cells and tissues but greatly upregulated by tumor promoters [6], which indicate that COX-2 is a pharmacological target for anticancer therapy. Among the COX-2-derived prostaglandins, PGE2 has emerged as one of the most studied because that COX-2 mediated PGE2 synthesis is showed to further induce expression of COX-2 positive feedback, thus leading to the development of malignant tumor [5, 7].

Epidemiological studies demonstrate that the incidence and mortality rates of colorectal cancer in women are lower than in men [8]. Estrogen (E_2) performing the profound effect on target tissue is mediated by two estrogen receptor (ER) subtypes ERα and ERβ [9]. ERα and ERβ have been identified in colon tissue in both the sexes [10]. In observational studies, estrogen exerts a protective role against the development of fatal colon cancer with a substantially decreased risk in women receiving hormone replacement therapy (HRT) [11–13], and a reduced mortality from this disease [14]. However, the precise mechanism behind protective effects of 17β-estradiol against PGE2-induced progression in colon cancer remains unclear. In this study, we examined the effects of 17β-estradiol on PGE2-induced cellular motility in human LoVo colon cancer cells, and further identified the precise molecular and cellular mechanisms behind this protective property. The results demonstrated that 17β-estradiol treatment inhibits PGE2-induced cellular motility and expression of COX-2 by suppressing the activation of Akt and ERK1/2 in LoVo cells. This study suggests that 17β-estradiol presents the properties of anti-cancer by inhibiting PGE2-induced progression in human LoVo cancer cells.

Methods

Cells, antibodies, reagents, and enzymes

Human colon cancer cell lines, LoVo, were obtained from the American Tissue Culture Collection (ATCC) (Rockville, MD, USA). LoVo cells were established from the metastatic nodule resected from a 56-year-old colon adenocarcinoma patient. 17β-Estradiol (E_2) purchased from Sigma (Sigma Chemical Co., St. Louis, Missouri, USA). Prostaglandins E2 (PGE2) was purchased from CALBIOCHEM (Darmstadt, Germany). The LY294002 (PI3K inhibitor), U0126 (MEK1/2 inhibitor), SB203680 (p38 MAPK inhibitor), SP600125 (JNK inhibitor), and ER antagonist ICI 182,780 (ICI) were purchased from TOCRIS (Ellisville, Missouri, USA). 6-Amino-4-(4-phenoxyphenylethylamino) quinazoline (QNZ), NFκB activation inhibitor was purchased from Calbiochem, Darmstadt, Germany. We utilized the following antibodies against Akt and phospho-Akt, (Cell Signaling Technology, Inc. Beverly, MA, USA); ERK1/2, phospho-ERK1/2, and COX-2 (Santa Cruz Biotechnology, Inc. Santa Cruz, California, USA); α-tubulin (Lab Vision Corporation, Fremont, California, USA) as loading control. Goat anti-mouse IgG antibody conjugated to horseradish peroxidase and goat anti-rabbit IgG antibody conjugated to horseradish peroxidase and rabbit anti-goat IgG horseradish peroxidase conjugate were purchased from Santa Cruz Biotechnology, Inc., California, USA.

Cell culture

LoVo colon cancer cell line from the American Type Culture Collection (ATCC) (Rockville, MD) were cultured on 100- or 60-mm culture dishes in Dulbecco's modified

Eagle's medium (DMEM) supplemented with 100-μg/ml penicillin, 100-μg/ml streptomycin, 2-mM glutamine, 1-mM HEPS buffer, and 10% Clontech fetal bovine serum in humidified air (5% CO_2) at 37°C

Immunoblotting

To isolate total proteins, cultured LoVo cells were washed with cold PBS and resuspended in lysis buffer (50-mM Tris, pH 7.5, 0.5-M NaCl, 1.0-mM EDTA, pH 7.5, 10% glycerol, 1-mM BME, 1% IGEPAL-630 and a proteinase inhibitor cocktail; Roche Molecular Biochemicals). After incubation for 30 min on ice, the supernatant was collected by centrifugation at 12,000×*g* for 15 min at 4°C, and the protein concentration was determined by the Bradford method. Sample containing equal proteins (60 μg) were loaded and analyzed by Western blot analysis. Briefly, proteins were separated by 12% SDS-PAGE and transferred onto PVDF membrane (Millipore, Belford, Massachusetts, USA). Membrane was blocked with blocking buffer (5% non-fat dry milk, 20-mM Tris-HCl, pH 7.6, 150 mM NaCl, and 0.1% Tween 20) for at least 1 h at room temperature. Membranes were incubated with primary antibodies (1:1000) in the above solution on an orbit shaker at 4°C overnight. Following primary antibody incubations, membranes were incubated with horseradish peroxidase-linked secondary antibodies (1:2000) (anti-rabbit, anti-mouse, or anti-goat IgG).

Migration assay

Migration assay was performed using the 48-well Boyden chamber (Neuro Probe) plate with the 8-μm pore size polycarbonate membrane filters [15]. The lower compartment was filled with DMEM containing 20% FCS. LoVo cells were placed in the upper part of the Boyden chamber containing serum-free medium and incubated for 48 h. After incubation, the cells on membrane filter were fixed with methanol and stained with 0.05% Giemsa for 1 h. The cells on upper surface of the filter were removed with a cotton swab. The filters were then rinsed in double distilled water until additional stain was leached. The cells then were air-dried for 20 min. The migratory phenotypes were determined by counting the cells that migrated to the lower side of the filter with microscopy at ×200 and ×400 magnifications, respectively. The fourth fields were counted for each filter, and each sample was assayed in triplicate.

Statistical analysis

Each experiment was duplicated at least three times. Results were presented as the mean ± SE, and statistical comparisons were made using the Student's *t* test. Significance was defined at the $P < 0.05$ or 0.01 levels.

Results

The effect of prostaglandin E2 on expression of COX-2 in human LoVo colon cancer cells

We detected the expression of COX-2 in LoVo cells treated with PGE2. In this study, we observed that the significant increase in expression level of COX-2 (Fig. 1) is induced following PGE2 (10^{-6} M) treatment within 3 h, and is maintained up for 24 h. The quantitative results showed that COX-2 is significantly increased by approximately 2.77-fold within 3 h, 2.86-fold within 6 h, 2.58-fold within 12 h, and 2.49-fold within 24 h.

Both Akt and ERK1/2 co-mediate PGE2-upregulated COX-2 in human LoVo colon cancer cells

To further identify which signal transduction pathway(s) is involved in the mechanism behind PGE2-upregulated expression of COX-2 in human colon cancer cells, we applied the following inhibitors such as Akt (LY294002), U0126 (ERK1/2 activation inhibitor), SB203580 (p38

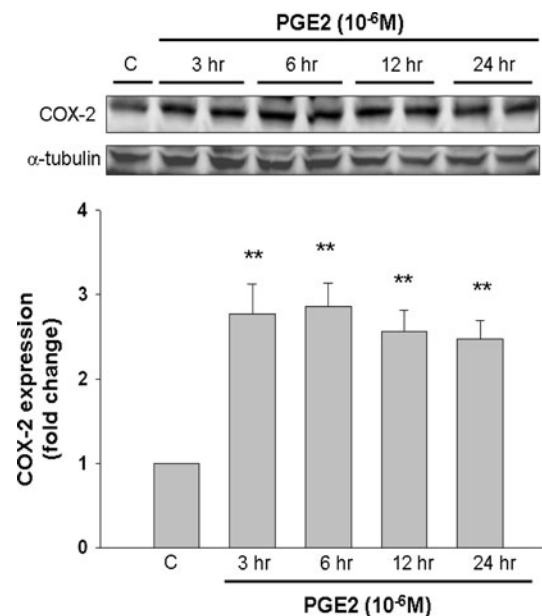

Fig. 1 PGE2 induces expression of COX-2 in human LoVo colon cancer cells. LoVo cells cultured in DMEM were treated with PGE2 (10^{-6} M) for 3, 6, 12, and 24 h, and subsequently observed protein level of COX-2 in LoVo cells by immunoblotting assay. The responses to different time periods of PGE2 treatment were measured by the immunoblotting assay. * $P < 0.05$ versus control, ** $P < 0.01$ versus control (mean ± SE, $n = 3$)

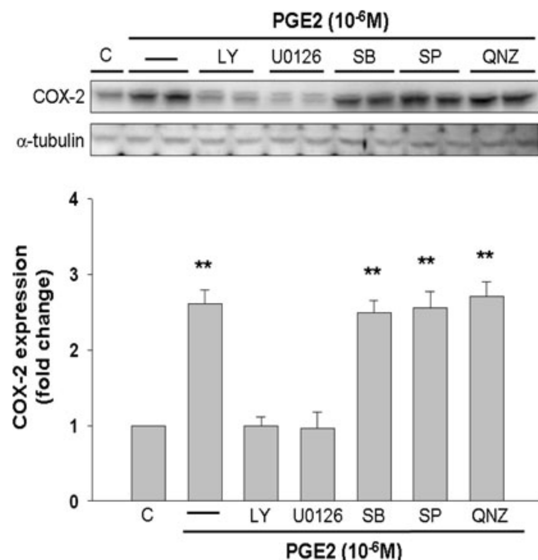

Fig. 2 PGE2 upregulates COX-2 via Akt and ERK1/2 pathways in human LoVo colon cancer cells. LoVo cells were pretreated with vehicle, LY294002 (Akt activation inhibitor), U0126 (ERK1/2 activation inhibitor, 1 μM), SB203580 (p38 MAPK inhibitor, 1 μM), SP600125 (JNK1/2 inhibitor, 1 μM), or QNZ (NFκB inhibitor, 1 μM) for 1 h and followed by PGE2 (10^{-6} M) administration for 24 h, and then were harvested for immunoblotting assays. Total protein of cell extracts was separated by 12% SDS-PAGE, transferred to PVDF membranes, and immunoblotted with antibodies against COX-2 protein. Equal loading was assessed with an anti-α-tubulin antibody. * $P < 0.05$ versus control, ** $P < 0.01$ versus control (mean ± SE, $n = 3$)

MAPK inhibitor), SP600125 (JNK1/2 inhibitor), and QNZ (NFκB activation inhibitor) to, respectively, block these pathways following the administration of PGE2. LoVo cells were preincubated with LY294002 (1 μM), U0126 (1 μM), SB203580 (1 μM), SP600125 (1 μM), or QNZ (1 μM) for 1 h and followed by the administration of PGE2 (1 μM) for 24 h, and subsequently were subjected to immunoblotting assay to assess the effect of these inhibitors on PGE2-induced expression of COX-2. The finding showed that Akt inhibitor (LY294002) and ERK1/2 inhibitor (U0126) significantly suppress PGE2-induced protein levels of COX-2 (Fig. 2). It suggested that cooperation of Akt and ERK1/2 mediates PGE2-induced COX-2 expression in human LoVo colon cancer cells.

The effect of prostaglandin E2 on activation of Akt and ERK1/2 in human LoVo colon cancer cells

To further explore the effects of prostaglandin E2 (PGE2) on activation of Akt, and ERK1/2 in human LoVo colon cancer cells, we first treated LoVo cells with PGE2 (10^{-6} M) for various time periods (15 min, 30 min, 1 h, 3 h, 6 h, 12 h, and 24 h), and subsequently measured the phosphorylation/activation of proteins by immunoblotting

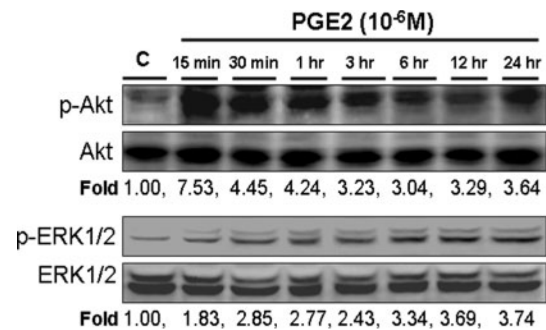

Fig. 3 PGE2 induces activation of Akt and ERK1/2 in human LoVo colon cancer cells. LoVo cells cultured in DMEM were treated with PGE2 (10^{-6} M) for various periods (15 min, 30 min, 1 h, 3 h, 6 h, 12 h, and 24 h), and subsequently measured the phosphorylation/activation of proteins by immunoblotting assay. The responses to different time periods of PGE2 treatment were measured by the immunoblotting assay

assay. Phosphorylation of Akt and ERK1/2 is significantly induced within 15 min in response to PGE2 stimulation, and was maintained up for 24 h (Fig. 3). The findings suggested that administration of PGE2 may induce the progression of human colon cancer by inducing activation of Akt and ERK1/2.

17β-Estradiol inhibits PGE2-induced COX-2 expression by suppressing activation of Akt and ERK1/2

In this study, we treated LoVo cells with 17β-estradiol (10^{-8} M) for various time periods (5 min, 15 min, 30 min, 1 h, 3 h, 6 h, 12 h, and 24 h), and subsequently measured the phosphorylation/activation of proteins by immunoblotting assay. The results showed that phosphorylation of Akt and ERK1/2 are significantly reduced within 5 min in response to 17β-estradiol stimulation, and are maintained up for 24 h (Fig. 4a). Serum-starved human LoVo colon cancer cells were preincubated with 17β-estradiol (10^{-8} M) for 30 min, followed by PGE2 (10^{-6} M) treatment for 3 or 24 h, and then were subjected to immunoblotting assay for protein detection of phospho-Akt, phospho-ERK1/2, and COX-2, respectively. We observed that PGE2-induced phosphorylation of Akt and ERK1/2 are dramatically inhibited by 17β-estradiol, which further led to the down-regulation of COX-2 expression within 24 h in human LoVo colon cancer cells (Fig. 4b).

17β-Estradiol inhibits PGE2-induced cell migration in human LoVo colon cancer cells

In this study, we examined the effects of PGE2 on the migration ability in human LoVo colon cancer cells by culturing LoVo cells with PGE2 (10^{-6} M) in the presence

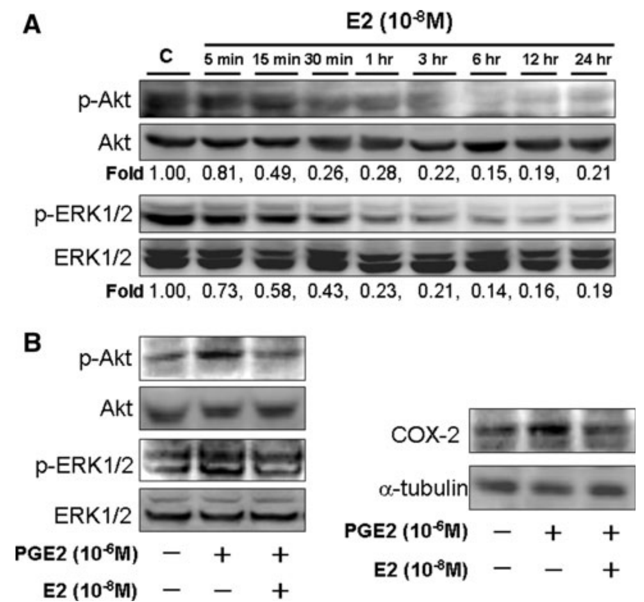

Fig. 4 17β-Estradiol down-regulates PGE2-induced COX-2 expression by suppressing activation of Akt and ERK1/2 in human LoVo cells. **a** LoVo cells cultured in DMEM were treated with 17β-estradiol (10^{-8} M) for various periods (5 min, 15 min, 30 min, 1 h, 3 h, 6 h, 12 h, and 24 h), and subsequently measured the phosphorylation/activation of proteins by immunoblotting assay. The responses to different time periods of 17β-estradiol treatment were measured by the immunoblotting assay. **b** LoVo cells were pretreated with 17β-estradiol (10^{-8} M) for 30 min, followed by PGE2 (10^{-6} M) treatment for 3 or 24 h, and then were subjected to immunoblotting assay for protein detection of phospho-Akt and phospho-ERK1/2 (PGE2 stimulation within 3 h); COX-2 (PGE2 stimulation within 24 h). * $P < 0.05$ versus control, ** $P < 0.01$ versus control (mean ± SE, $n = 3$)

or in the absence of Akt inhibitor (LY294002), ERK1/2 inhibitor (U0126) for 48 h. Subsequently, we observed the ability of migration in LoVo cells by migration assay. In migration assay (Fig. 5), we observed that PGE2 induces a dramatic increase in cellar migration in LoVo cells. A significant increase of cell migration about 85.67% following PGE2 treatment (10^{-6} M) for 48 h was observed in human LoVo cancer cells. However, inhibitors such as LY294002 and U0126 significantly blocked PGE2-induced cell migration about 47.13 and 43.15%, respectively. In addition, pretreatment of 17β-estradiol (10^{-8} M) notably inhibited PGE2-promoted LoVo cancer cell migration. Alone 17β-estradiol treatment showed a significant reduction in LoVo cell migration in the absence of PGE2. ICI 182780 treatment further confirmed the inhibitory property of 17β-estradiol/estrogen receptor (ER) complex on LoVo cellular motility by suppressing function of ERs. These findings suggested that 17β-estradiol may inhibit PGE2-promoted cellular motility by suppressing activation Akt and ERK1/2 in human LoVo colon cancer cells.

Discussion

The major findings of this study can be summarized as followings: (1) PGE2 treatment dramatically induces phosphorylation of Akt and ERK1/2 in human LoVo colon cancer cells. (2) Migration of LoVo colon cancer cells is significantly promoted by PGE2 (10^{-6} M) treatment. We simultaneously observed that an increase in cell migration is accompanied with the upregulation of COX-2, following PGE2 (10^{-6} M) treatment. In addition, both Akt and ERK1/2 signaling pathways contribute to COX-2 expression in response to PGE2 treatment. (3) PGE2-induced expression of COX-2 in human LoVo cells is significantly inhibited by 17β-estradiol (10^{-8} M) pretreatment. 17β-Estradiol dramatically inhibits PGE2-induced COX-2 expression by suppressing activation of both Akt and ERK1/2. These results demonstrate that 17β-estradiol may efficiently inhibit PGE2-induced progression in human LoVo colon cancer cells (Fig. 6).

An increase in COX-2 expression has been detected in 50% of colorectal adenomas and in up to 85% of colorectal cancer [16], which further indicates that great expression of COX-2 is a marker for poor survival [17]. Evidences have shown that deficiency or inhibition of COX-2 function is associated with a reduced risk of malignant tumor formation and growth in colorectal cancer in clinical and animal studies [18–21]. Stimuli such as growth factors, cytokines, and tumor promoters have been shown to up-regulate level of COX-2 by various signaling pathways such as Akt, protein kinase C (PKC), and mitogen-activated protein kinases (MAPKs) [5, 22–26]. In this study, we observed that 17β-estradiol dramatically inhibits PGE2-induced COX-2 expression by suppressing the activation of Akt and ERK1/2 signaling pathways.

A large number of studies have been dedicated to exploring the molecular mechanisms involved in the downregulation of cancer development. Mitogen-activated protein kinases (MAPKs) include tree major subfamilies such as the extracellularly responsive kinases (ERKs), the c-Jun N-terminal kinases (JNKs), also known as stress-activated protein kinases (SAPKs), and the p38 MAPKs [27]. Studies have showed that ERK1/2 is involved in hepatoma-derived growth factor-induced promotion of carcinogenesis of gastric epithelial cells [28] and in the proliferation of pancreatic stellate cells [29]. p38 MAPK mediates TNFa-induced MMP-9 expression, thus leading to the progression of human urinary bladder cancer cells [30]. JNK/AP-1 signaling pathway may contribute to cellular migration and invasion of prostate cancer cells [31]. Hepatocyte growth factor activated both Akt and JNK which enhance the proteolysis and invasiveness of human nasopharyngeal cancer cells [32]. Abnormal response of

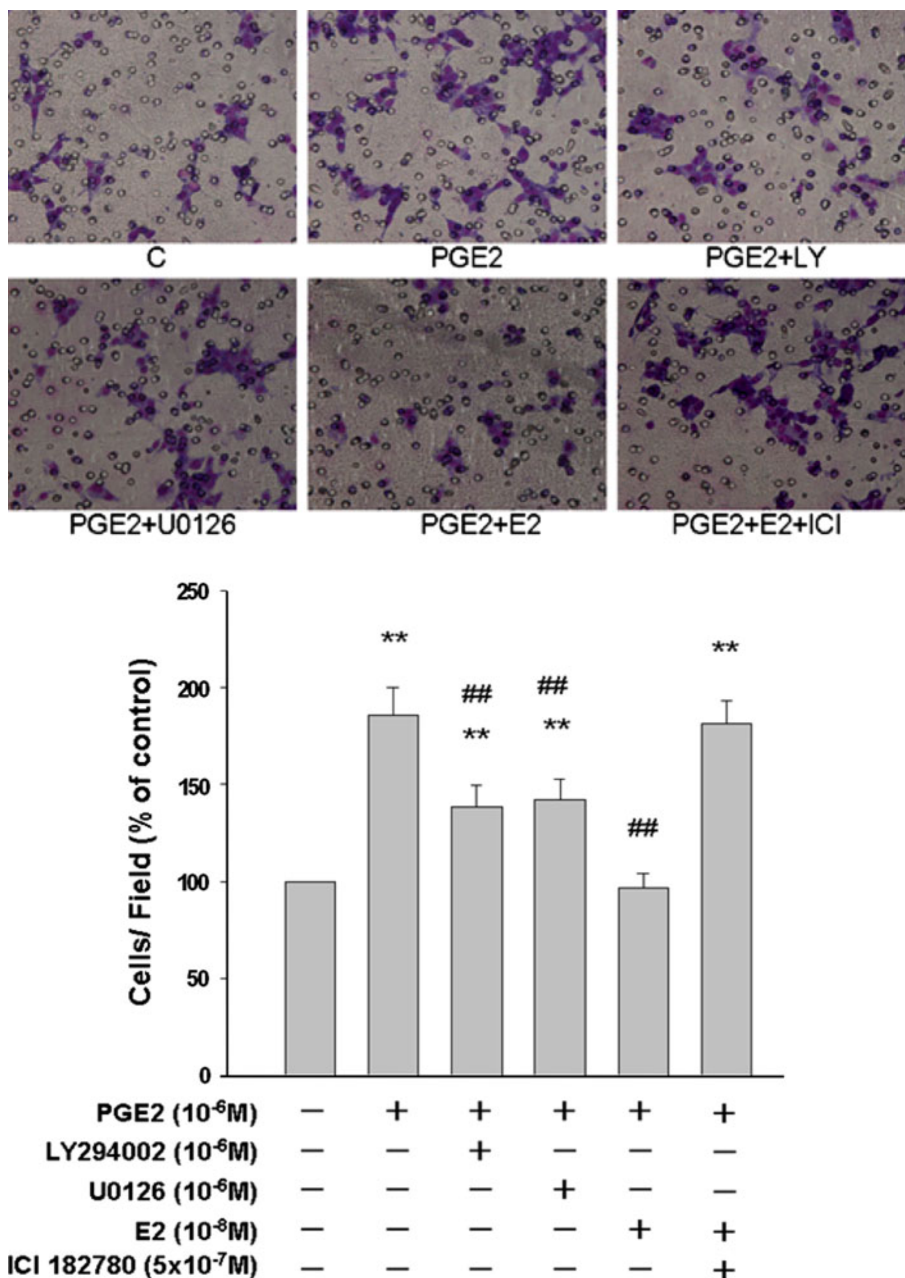

Fig. 5 17β-Estradiol inhibits PGE2-promoted cellular motility in human LoVo cells. LoVo cells cultured in DMEM were pretreated with vehicle, LY294002 (Akt activation inhibitor), U0126 (ERK1/2 activation inhibitor, 1 μM), or 17β-estradiol (10^{-8} M) for 1 h prior to PGE2 (10^{-6} M) treatment for another 48 h, and subsequently observed the ability of migration in LoVo cells by migration assay. At the same time, LoVo cells were treated 17β-estradiol (10^{-8} M) in the presence or in the absence of ERs inhibitor, ICI 182780 (5×10^{-6} M). The responses to different treatments were observed and analysis with a fluorescence microscope. * $P < 0.05$ versus control, ** $P < 0.01$ versus control (mean ± SE, $n = 4$)

NFkB signaling pathway may contribute to the chemoresistance in acute lymphoblastic leukemia [33].

Therefore, we further attempted to identify the role of these factors including Akt, ERK1/2, p38 MAPK and JNK1/2 and NFkB in PGE2-induced expression of COX-2 and PGE2-promoted cellular mobility in human LoVo colon cancer cells. Using Akt activation inhibitor LY294002, ERK1/2 activation inhibitor U0126, p38 MAPK inhibitor SB203580, JNK1/2 inhibitor SP600125, and NFkB activation inhibitor QNZ, we observed that both Akt and ERK1/2 signaling pathways co-mediate PGE2-induced COX-2 expression, which further contributes to cellular motility of human LoVo cancer cells. Previous studies have shown that 17β-estradiol (E_2) binding to estrogen receptors (ERs) can regulate tissue/cellular responses through multiple signaling pathways [27]. In this study, we further observed that 17β-estradiol pretreatment inhibits PGE2-induced expression of COX-2 and cellular motility via suppressing activation of Akt and ERK1/2 in LoVo cells. It suggested that 17β-estradiol presents the anti-cancer properties by inhibiting PGE2-promoted progression in human LoVo cancer cells. Estrogen receptors α and β (ERα and β) have been identified in colon tissue. Therefore, further more studies are also required to identify

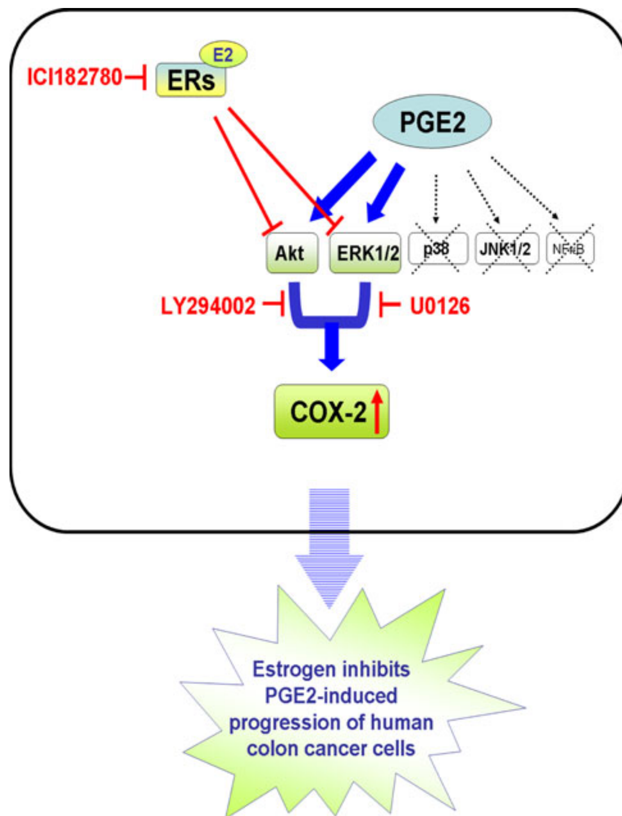

Fig. 6 A schematic representation showing 17β-estradiol inhibition of COX-2 expression and cell migration via suppression of Akt and ERK1/2 pathways in human LoVo colon cancer cells. Administration of prostaglandin E2 (PGE2) rapidly activates kinases such as Akt and ERK1/2, thus leading to the expression of downstream targets including COX-2, which further promotes cellular motility in human LoVo cancer cells. Estrogen receptor is activated by 17β-estradiol binding to form 17β-estradiol-ER complex. 17β-Estradiol-ER complex presents the properties of anti-cancer by down-regulating expression of COX-2 via deactivation of Akt and ERK1/2 in LoVo cells. It suggests that 17β-estradiol dramatically inhibits PGE2-induced progression of human LoVo colon cancer cells

which ER subtype (ERα and/or ERβ) is involved in downregulation of cellular mobility in human LoVo colon cancer cells.

Acknowledgments This study is supported by Taiwan Department of Health Clinical Trial and Research Center of Excellence (DOH99-TD-B-111-004) and in part by Taiwan Department of Health Cancer Research Center of Excellence (DOH99-TD-C-111-005).

References

1. Jemal A, Murray T, Ward E, Samuels A, Tiwari RC, Ghafoor A et al (2005) Cancer statistics. CA Cancer J Clin 55:10–30
2. Greenwald P (1992) Colon cancer overview. Cancer 70(5):1206–1215
3. Jemal A, Thomas A, Murray T, Thun M (2002) Cancer statistics. CA Cancer J Clin 52:23–47
4. Jemal A, Tiwari RC, Murray T, Ghafoor A, Samuels A, Ward E et al (2004) Cancer statistics. CA Cancer J Clin 54:8–29
5. Subbaramaiah K, Dannenberg AJ (2003) Cyclooxygenase 2: a molecular target for cancer prevention and treatment. Trends Pharmacol Sci 24:96–102
6. Vane JR, Botting RM (1998) Mechanism of action of nonsteroidal anti-inflammatory drugs. Am J Med 104:2S–8S discussion 21S–22S
7. Hull MA, Ko SC, Hawcroft G (2004) Prostaglandin EP receptors: targets for treatment and prevention of colorectal cancer? Mol Cancer Ther 3:1031–1039
8. Jacobs ET, Thompson PA, Martinez ME (2007) Diet, gender, and colorectal neoplasia. J Clin Gastroenterol 41:731–746
9. Katzenellenbogen JA, Katzenellenbogen BS (1996) Nuclear hormone receptors: ligand-activated regulators of transcription and diverse cell responses. Chem Biol 3:529–536
10. Campbell-Thompson M, Lynch IJ, Bhardwaj B (2001) Expression of estrogen receptor (ER) subtypes and ERbeta isoforms in colon cancer. Cancer Res 61:632–640
11. Gambacciani M, Monteleone P, Sacco A, Genazzani AR (2003) Hormone replacement therapy and endometrial, ovarian and colorectal cancer. Best Pract Res Clin Endocrinol Metab 17: 139–147
12. Grodstein F, Martinez ME, Platz EA, Giovannucci E, Colditz GA, Kautzky M et al (1998) Postmenopausal hormone use and risk for colorectal cancer and adenoma. Ann Intern Med 128:705–712
13. Chlebowski RT, Wactawski-Wende J, Ritenbaugh C, Hubbell FA, Ascensao J, Rodabough RJ et al (2004) Estrogen plus progestin and colorectal cancer in postmenopausal women. N Engl J Med 350:991–1004
14. Calle EE, Miracle-McMahill HL, Thun MJ, Heath CW Jr (1995) Estrogen replacement therapy and risk of fatal colon cancer in a prospective cohort of postmenopausal women. J Natl Cancer Inst 87:517–523
15. Hsieh YH, Wu TT, Huang CY, Hsieh YS, Hwang JM, Liu JY (2007) p38 mitogen-activated protein kinase pathway is involved in protein kinase Calpha-regulated invasion in human hepatocellular carcinoma cells. Cancer Res 67:4320–4327
16. Eberhart CE, Coffey RJ, Radhika A, Giardiello FM, Ferrenbach S, DuBois RN (1994) Up-regulation of cyclooxygenase 2 gene expression in human colorectal adenomas and adenocarcinomas. Gastroenterology 107:1183–1188
17. Sheng H, Shao J, Kirkland SC, Isakson P, Coffey RJ, Morrow J et al (1997) Inhibition of human colon cancer cell growth by selective inhibition of cyclooxygenase-2. J Clin Invest 99:2254–2259
18. Thun MJ, Henley SJ, Patrono C (2002) Nonsteroidal anti-inflammatory drugs as anticancer agents: mechanistic, pharmacologic, and clinical issues. J Natl Cancer Inst 94:252–266
19. Oshima M, Dinchuk JE, Kargman SL, Oshima H, Hancock B, Kwong E et al (1996) Suppression of intestinal polyposis in Apc delta716 knockout mice by inhibition of cyclooxygenase 2 (COX-2). Cell 87:803–809
20. Oshima M, Murai N, Kargman S, Arguello M, Luk P, Kwong E et al (2001) Chemoprevention of intestinal polyposis in the Apcdelta716 mouse by rofecoxib, a specific cyclooxygenase-2 inhibitor. Cancer Res 61:1733–1740
21. Steinbach G, Lynch PM, Phillips RK, Wallace MH, Hawk E, Gordon GB et al (2000) The effect of celecoxib, a cyclooxygenase-2 inhibitor, in familial adenomatous polyposis. N Engl J Med 342:1946–1952
22. Smith WL, DeWitt DL, Garavito RM (2000) Cyclooxygenases: structural, cellular, and molecular biology. Annu Rev Biochem 69:145–182

23. Zhang F, Subbaramaiah K, Altorki N, Dannenberg AJ (1998) Dihydroxy bile acids activate the transcription of cyclooxygenase-2. J Biol Chem 273:2424–2428
24. Subbaramaiah K, Hart JC, Norton L, Dannenberg AJ (2000) Microtubule-interfering agents stimulate the transcription of cyclooxygenase-2. Evidence for involvement of ERK1/2 AND p38 mitogen-activated protein kinase pathways. J Biol Chem 275:14838–14845
25. Subbaramaiah K, Cole PA, Dannenberg AJ (2002) Retinoids and carnosol suppress cyclooxygenase-2 transcription by CREB-binding protein/p300-dependent and -independent mechanisms. Cancer Res 62:2522–2530
26. Zhang Z, Sheng H, Shao J, Beauchamp RD, DuBois RN (2000) Posttranscriptional regulation of cyclooxygenase-2 in rat intestinal epithelial cells. Neoplasia 2:523–530
27. Marino M, Galluzzo P, Ascenzi P (2006) Estrogen signaling multiple pathways to impact gene transcription. Curr Genomics 7:497–508
28. Mao J, Xu Z, Fang Y, Wang H, Xu J, Ye J et al (2008) Hepatoma-derived growth factor involved in the carcinogenesis of gastric epithelial cells through promotion of cell proliferation by Erk1/2 activation. Cancer Sci 99:2120–2127
29. Schwer CI, Guerrero AM, Humar M, Roesslein M, Goebel U, Stoll P et al (2008) Heme oxygenase-1 inhibits the proliferation of pancreatic stellate cells by repression of the ERK1/2 pathway. J Pharmacol Exp Ther 327:863–871
30. Lee SJ, Park SS, Lee US, Kim WJ, Moon SK (2008) Signaling pathway for TNF-alpha-induced MMP-9 expression: Mediation through p38 MAP kinase, and inhibition by anti-cancer molecule magnolol in human urinary bladder cancer 5637 cells. Int Immunopharmacol 8:1821–1826
31. Kwon GT, Cho HJ, Chung WY, Park KK, Moon A, Park JH (2009) Isoliquiritigenin inhibits migration and invasion of prostate cancer cells: possible mediation by decreased JNK/AP-1 signaling. J Nutr Biochem 20:663–676
32. Zhou HY, Wan KF, Ip CK, Wong CK, Mak NK, Lo KW et al (2008) Hepatocyte growth factor enhances proteolysis and invasiveness of human nasopharyngeal cancer cells through activation of PI3 K and JNK. FEBS Lett 582:3415–3422
33. Stankovic T, Marston E (2008) Molecular mechanisms involved in chemoresistance in paediatric acute lymphoblastic leukaemia. Srp Arh Celok Lek 136:187–192