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# Suppression of Prostaglandin $E_2$-Mediated Cell Proliferation and Signal Transduction by Resveratrol in Human Colon Cancer Cells

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**Abstract** − Although the overproduction of prostaglandin $E_2$ ($PGE_2$) in intestinal epithelial cells has been considered to be highly correlated with the colorectal carcinogenesis, the precise mechanism of action remains poorly elucidated. Accumulating evidence suggests that the PGE receptor (EP)-mediated signal transduction pathway might play an important role in this process. In the present study, we investigated the mechanism of action underlying $PGE_2$-mediated cell proliferation and the effect of resveratrol on the proliferation of human colon cancer cells in terms of the modulating $PGE_2$-mediated signaling pathway. $PGE_2$ stimulated the proliferation of several human colon cancer cells and activated growth-stimulatory signal transduction, including Akt and ERK. $PGE_2$ also increased the phosphorylation of GSK-3β, the translocation of β-catenin into the nucleus, and the expressions of c-myc and cyclin D1. Resveratrol, a cancer chemopreventive phytochemical, however, inhibited $PGE_2$-induced growth stimulation and also suppressed $PGE_2$-mediated signal transduction, as well as β-catenin/T cell factor-mediated transcription in human colon cancer cells. These findings present an additional mechanism through which resveratrol affects the regulation of human colon cancer cell growth.

**Keywords:** β-catenin, Colon cancer cells, EP receptors, Prostaglandin $E_2$, Resveratrol

## INTRODUCTION

Elevated cyclooxygenase-2 (COX-2) and prostaglandin $E_2$ ($PGE_2$) levels have been associated with inflammation and carcinogenic processes. Increased expression of COX-2 was predominant in colorectal cancer tissues and chemically-induced tumors in animals (Eberhart *et al.*, 1994; DuBois *et al.*, 1996). $PGE_2$ levels are also elevated in colorectal tumor tissues when compared to normal mucosa (Rigas *et al.*, 1993; Pugh and Thomas, 1994). Indeed, $PGE_2$ treatment was also shown to enhance the incidence of colorectal tumors in azoxymethane (AOM)-treated rats and to alleviate the effect of non-steroidal anti-inflammatory drug (NSAID)-mediated tumor regression in ApcMin/+ mice, through increasing intestinal epithelial cell proliferation and reducing apoptosis (Hansen-Petrik *et al.*, 2002; Kawamori *et al.*, 2003; Wang *et al.*, 2005). Additionally, the reduction of tissue $PGE_2$ levels by NSAID treatment led to more effective regression of human adenoma (Giardiello *et al.*, 2002; Ulrich *et al.*, 2006). $PGE_2$ also increased cell survival, invasion, and migration of human colon cancer cells, suggesting the involvement of $PGE_2$ in colorectal carcinogenesis (Sheng *et al.*, 2001; Buchanan *et al.*, 2003; Pai *et al.*, 2003). Therefore, $PGE_2$-mediated signal transduction might be an important target for the development of cancer chemopreventive agents.

It is known that the $PGE_2$-mediated signal is transduced through four G-protein coupled receptors, designated as EP1, EP2, EP3, and EP4 within the family of prostaglandin E receptors (Hull *et al.*, 2004; Sugimoto and Narumiya, 2007). EP1 is linked to Gq protein and thus activates PLC/$IP_3$, leading to the increase of intracellular [$Ca^{2+}$]; EP2 and EP4 bind to Gs protein and elevate cAMP concentration; (Editor Note: Do you mean “activate” or “elevates?” EP3 is linked to Gi protein and thus decreases intracellular cAMP levels (Hull *et al.*, 2004). Therefore, the effects of $PGE_2$ on cancer cell growth seem to be associated with overall secondary responses in the cells mediated through EP receptors. EP receptors are distributed throughout several

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organs, and mediate inflammation response and maintenance of tissue homeostasis (Sugimoto and Narumiya, 2007). EP receptors are also associated with the carcinogenic processes. In particular, EP receptors are over-expressed in colorectal and lung cancer cells and tissues when compared to normal cells (Hull *et al.*, 2004; Sugimoto and Narumiya, 2007). In addition, activation of EP receptors is known to up-regulate downstream signaling involved in cell proliferation. For example, EP receptor induces the activation of CREB and thus increases the expression of a growth factor - amphiregulin, resulting in an increase of cell proliferation through the phosphorylation of EGFR and its downstream signaling pathways such as ERK/MAPK and PI3K/Akt (Pai *et al.*, 2002; Shao *et al.*, 2003; Hull *et al.*, 2004). EP receptors are also associated with the activation of Wnt signaling by stimulating PI3K/Akt signaling and thus inhibiting GSK-3β (Fujino *et al.*, 2002; Castellone *et al.*, 2005; Shao *et al.*, 2005).

Resveratrol, a naturally occurring stilbenoid, has been shown to possess antioxidant, anti-inflammatory, anti-cancer and cancer chemopreventive activities (Bhat and Pezzuto, 2002). Previous studies showed that resveratrol suppressed growth and induced apoptosis in colon cancer cells that either expressed COX-2 or showed no detectable COX-2, suggesting COX-2-dependent and independent modes of action of resveratrol on the regulation of cancer cell growth (Mutoh *et al.*, 2000; Wolter *et al.*, 2001; Bhat and Pezzuto, 2002; Joe *et al.*, 2002; Liang *et al.*, 2003). Additionally, few studies have been performed to investigate the effect of resveratrol on $PGE_2$-induced proliferation of human cancer cells. Herein, we report that resveratrol inhibits $PGE_2$-stimulated colon cancer cell growth with the suppression of $PGE_2$-mediated signal transduction, including Akt and ERK, and through the regulation of β-catenin/T cell factor-mediated transcription.

## MATERIALS AND METHODS

### Cell culture and reagents

Human colorectal cancer cells (HCT-15, HCT-116, and LS 174T) were maintained in RPMI 1640 medium supplemented with 10% heat-inactivated FBS and antibiotics - antimycotics (PSF; 100 units/ml penicillin G sodium, 100 μg/ml streptomycin, and 250 ng/ml amphothericin B) at 37°C with 5% $CO_2$. SW 480 colorectal carcinoma cells were grown in RPMI 1640 medium containing 25 mM HEPES, 10% FBS, and PSF at 37°C. Mouse monoclonal anti-cyclin D1, anti-PARP, and anti-GSK-3β antibodies were purchased from BD Biosciences (San Diego, CA, USA). Mouse monoclonal anti-c-myc, ant-ERK1/2, and anti-phospho-ERK (Tyr204) were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Rabbit polyclonal antibodies against anti-phospho-β-catenin (Ser45), anti-phospho-β-catenin (Ser33/37, Thr41), anti-phospho-Akt (Ser473), anti-phopsho-Akt (Thr308), and anti-Akt were from Cell Signaling (Danvers, MA, USA). Rabbit polyclonal anti-EP1, EP2, EP3, and EP4 antibodies and $PGE_2$ were purchased from Cayman Chemical (Ann Arbor, MI, USA). Dual luciferase assay system was purchased from Promega (Madison, WI, USA). $PGE_2$ was dissolved in ethanol and stored at −20°C until use. Resveratrol (3,5,4'-trihydroxy-trans-stilbene) was obtained from Sigma (St. Louis, MO, USA), and dissolved in dimethyl sulfoxide (DMSO) (Fig. 1A).

### Cell proliferation assay (SRB assay)

Cell proliferation was evaluated using the sulforhodamine B (SRB) assay as previously described (Nam *et al.*, 2003). Cells were seeded into 96 well plates at a density of $5\times10^3$ cells/well and incubated for 24 h. The cells were serum starved for 24 h, and then treated with test sample for an additional 48 h. Cells were fixed with 10% trichloroacetic acid solution for 30 min at 4°C, washed 5 times with tap water, and dried in the air. Cells were stained with 0.4% SRB in 1% acetic acid solution for 30 min at room

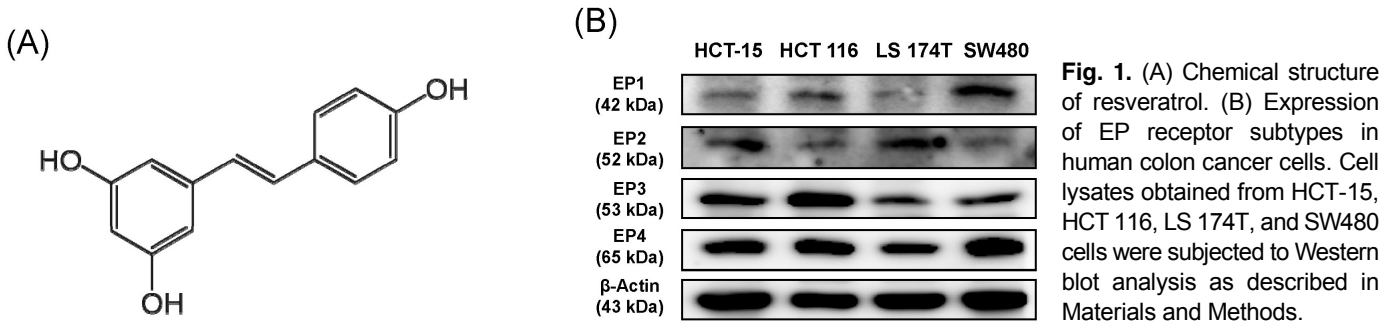


**Fig. 1.** (A) Chemical structure of resveratrol. (B) Expression of EP receptor subtypes in human colon cancer cells. Cell lysates obtained from HCT-15, HCT 116, LS 174T, and SW480 cells were subjected to Western blot analysis as described in Materials and Methods.

temperature. After washing unbound dye and drying, stained cells were dissolved in 10 mM Tris (pH 10.0), and absorbance was measured at 515 nm. Cell proliferation was calculated by comparison with absorbance of a vehicle-treated control group.

**Western blot analysis**

Cultured cells were seeded into 100 mm dishes at a density of $8\times10^5$ cells/dish and incubated for 24 h. Cells were washed twice with PBS, and then incubated for 24 h without serum. The cells were washed with PBS, treated with test sample 30 min prior to the addition of $PGE_2$, and then incubated for the indicated time periods. After harvesting, cells were washed twice with PBS, suspended with boiling 2xsample loading buffer (250 mM Tris-HCl (pH 6.8), 4% SDS, 10% glycerol, 0.006% bromophenol blue, 2% β-mercaptoethanol, 50 mM sodium fluoride, and 5 mM sodium orthovanadate) and further incubated for 5-20 min at 100°C for complete lysis. After cooling at room temperature, samples were stored at −20°C until the experiment. Protein concentration of cell lysates was determined using the BCA method.

Equal amounts (30-50 μg) of protein samples were subjected to 8-11% SDS-PAGE. Separated proteins were electrically transferred onto PVDF membranes (Millipore, MA, USA). Membranes were blocked with blocking buffer (5% non-fat dry milk in PBS containing 0.1% Tween-20 (PBST)) for 1 h at room temperature. After washing 3 times with PBST, membranes were incubated with primary antibodies diluted in 3% non-fat dry milk in PBS (1:1,000-1:2,000) overnight at 4°C. Membranes were washed 3 times with PBST and incubated with corresponding secondary antibodies diluted in 3% non-fat dry milk in PBS (1:2,000-1:5,000) for 2-3 h at room temperature. Membranes were washed 3 times with PBST, and then exposed to enhanced an chemiluminescence (ECL) detection kit (LabFrontier, Suwon, Korea). Blots were detected by LAS 3000 (Fuji Film Corp., Japan).

**Isolation of cytosolic and nuclear extracts**

LS 174T cells were seeded into 100 mm dishes at a density of $8\times10^5$ cells/dish and incubated for 24 h. Cells were treated with test sample diluted in the serum-free medium for 24 h. Harvested cells were washed with PBS, suspended with ice-cold lysis buffer (10 mM HEPES (pH 7.4), 250 mM sucrose, 1.5 mM $MgCl_2$, 10 mM KCl, 0.1 mM EDTA, 1 mM DTT, 2% NP-40, 10 mM PMSF) on ice for 5 min. Following centrifugation at 2,500×rpm for 4 min at 4°C, supernatant was collected as the cytosolic fraction, and pellets were washed twice with ice-cold lysis buffer without NP-40. Pellets were resuspended in hypertonic nuclear extract buffer (20 mM Tris-HCl (pH 8.0), 420 mM NaCl, 1.5 mM $MgCl_2$, 0.2 mM EDTA, 25% glycerol, 50 mM sodium fluoride, and protease inhibitor cocktail) on ice for 10 min, and then centrifuged at 14,000×rpm for 15 min at 4°C. The supernatant containing nuclear extract was collected and stored in aliquots at −70°C. The protein content of cell lysates was determined using the Bradford assay.

**Reporter gene assay**

Transient transfection was performed using lipofectamine 2000 (Invitrogen, CA, USA). Briefly, LS 174T cells ($8\times10^4$ cells/ml) were seeded in a 48-well plate. After 24 h, cells were co-transfected for 24 h with the luciferase reporter constructs [500 ng of TOPflash (β-catenin/Tcf reporter plasmids) or FOPflash (mutated β-catenin/Tcf reporter plasmids) (Millipore, MA, USA) and 25 ng of pRL-SV40 reporter plasmids (Promega, Madison, WI, USA)]. After transfection, cells were incubated with $PGE_2$ and/or test sample for 24 h. Luciferase activity was measured using the dual-luciferase reporter gene assay system (Promega). Transfection efficiency was normalized by the values of *Renilla* luciferase activity. The activity was expressed as a relative value compared to control.

**Statistics**

Data were presented as the means ± S.D. for the indicated number of independently performed experiments. Fig. data are shown as one representative of at least three independent experiments. Non-linear regression analysis for calculation of $EC_{50}$ values was performed by Tablecurve program (ver. 1.0, AISN software). Statistical significance was analyzed by Student's t-test (SigmaStat 3.1, Systat software Inc.). A difference was considered to be statistically significant when $p < 0.05$.

## RESULTS

**Expression of EP receptor subtypes in human colon cancer cells**

It is well known that $PGE_2$-mediated signal transduction is associated with the G protein-coupled receptors designated subtypes of EP1, EP2, EP3 and EP4 [13]. Therefore, we primarily determined the protein expression profiles of EP receptor subtypes in several human colon cancer cells. As illustrated in Fig. 1B, Western blot showed that all EP receptors were expressed to a certain level in human colon cancer cells, and especially, EP4 expression was the most prominent in all cell lines tested. In LS 174T

cells, the expressions of EP2 and EP4 were more abundant compared to EP3, which is consistent with previous findings by Sheng *et al.* (2001).

### Effects of resveratrol on $PGE_2$-mediated cell proliferation in human colon cancer cells

To determine whether $PGE_2$ enhances cell proliferation, we evaluated the growth of colon cancer cells treated with 0.5 μM of $PGE_2$ in a serum-free condition for 48 h. $PGE_2$ increased the growth of colon cancer cells >1.2-fold compared to control group (Fig. 2A). Based on this result, the effect of resveratrol on $PGE_2$-enhanced cell proliferation was evaluated in cultured human colon cancer cells. To determine the non-cytotoxic concentration ranges of resveratrol in cancer cells, the growth inhibitory activity of resveratrol on LS 174T cells was measured using the SRB assay. The resultant $IC_{50}$ value was 51.2 μM, and the survival rate was over 90% at 25 μM (Fig. 2B). Therefore, the subsequent experiments were performed with less than 25 μM resveratrol to exclude the possibility of unexpected effects due to its cytotoxicity. As shown in Fig. 2C, we found that resveratrol effectively inhibited the $PGE_2$ stimulated growth of cancer cells in all tested human colon cancer cells.

### Suppression of $PGE_2$-stimulated Akt and ERK activation by resveratrol

Based on the $PGE_2$-mediated enhancement of cell proliferation, the signaling molecules which are related to the activation of cell proliferation were monitored in colon cancer cells. Especially, Akt and ERK activation by treatment with $PGE_2$, in the presence or absence of resveratrol, was

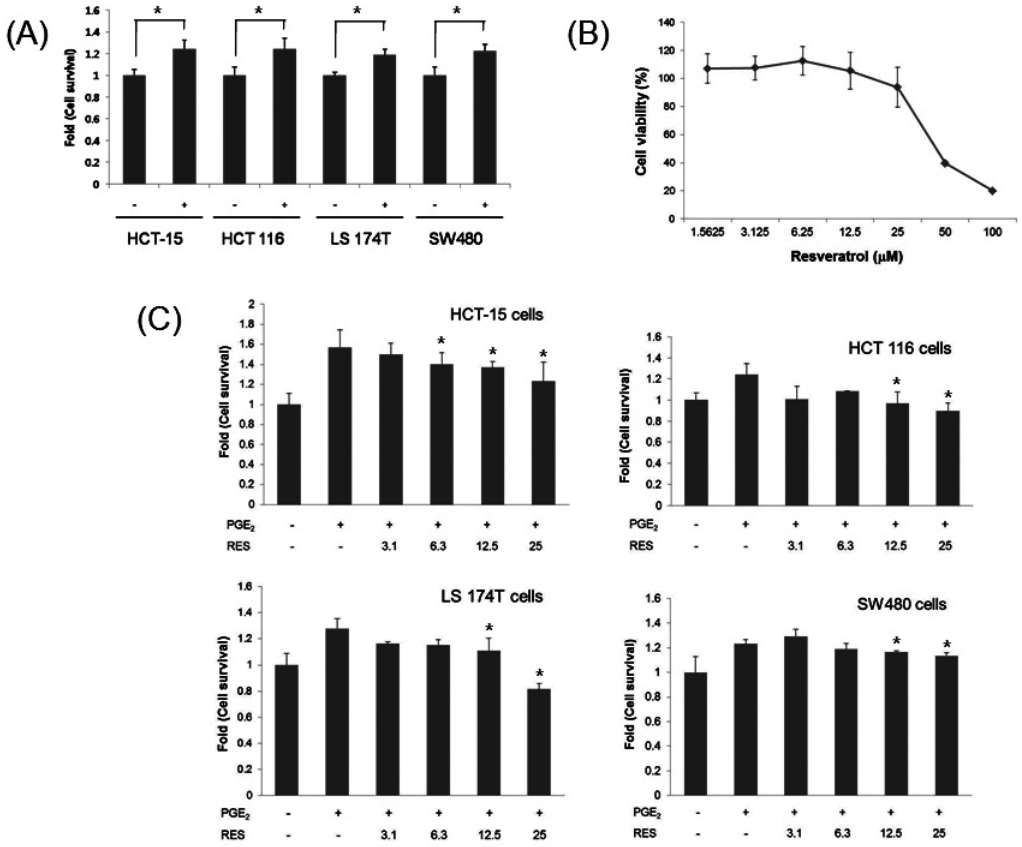


**Fig. 2.** (A) Stimulation of cell proliferation in various colon cancer cell lines by treatment with 0.5 μM $PGE_2$. Serum-starved cells were treated with 0.5 μM $PGE_2$ for 48 h. Cell proliferation was determined by SRB assay. Fold-changes of cell proliferation in $PGE_2$-treated cells were calculated by comparison of the absorbance of $PGE_2$-treated cells with that of vehicle-treated control cells. (*$p<0.05$). (B) Effect of resveratrol on the proliferation of LS 174T cells. Cells were exposed to the increasing concentrations of resveratrol for 48 h. Cell viability was determined by SRB assay. (C) Effect of resveratrol on $PGE_2$-stimulated cell proliferation in various human colon cancer cells. Serum-starved cells were treated with $PGE_2$ alone or in combination with various concentrations of resveratrol for 48 h. Cell proliferation was evaluated by SRB assay. Fold-changes were determined by comparing the absorbance of treated cells to that of vehicle-treated control cells. RES: resveratrol. *$p<0.05$ compared to $PGE_2$-treated control group.

determined. When monitored during a short-term course of 2 h in LS 174T cells, $PGE_2$ (0.5 μM) increased Akt phosphorylation at an early time point, reached peak levels at 30 and 60 min, and then decreased. The pre-treatment with resveratrol (30 min prior to $PGE_2$ treatment) alleviated the increased phosphorylation of Akt during the incubation as shown Fig. 3A. We next examined the activation of Akt and ERK with $PGE_2$ for up to 24 h in LS 174T cells. As shown in Fig. 3B, the Akt activation by $PGE_2$ was suppressed in cells treated with resveratrol during the incubation. However, the activation of ERK phosphorylation was induced at a relatively late time point after 2 h incubation and was predominant at 8 h. This activation was also alleviated by pre-treatment with resveratrol.

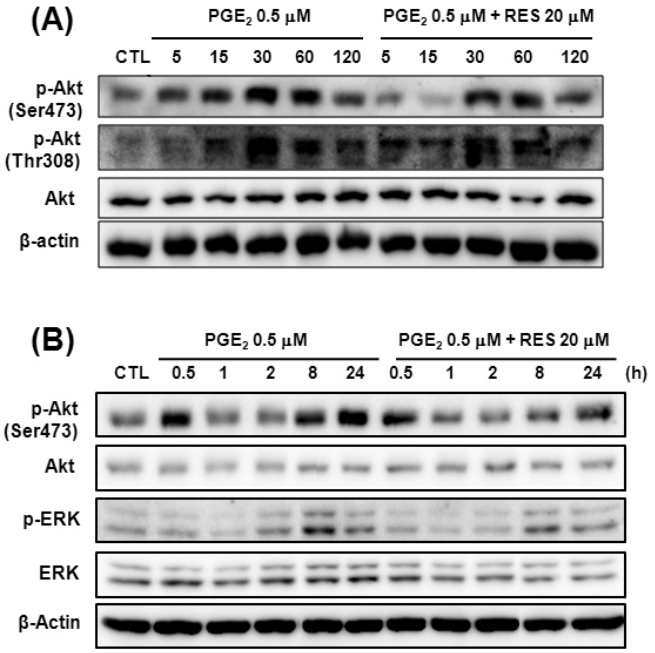


**Fig. 3.** Suppression of PGE2-mediated phosphorylation of Akt and ERK by treatment with resveratrol in LS 174T cells. Cells were starved of serum for 24 h, and then stimulated with $PGE_2$ (0.5 μM) in the presence or absence of resveratrol (20 μM) at various time points for 120 min (A) or for 24 h (B). Resveratrol was added to the cells 30 min prior to $PGE_2$ stimulation. After harvesting cells, the levels of total and phosphorylated proteins were analyzed by Western blot analysis, as described in Materials and Methods. RES: resveratrol.

### Suppression of $PGE_2$-stimulated GSK-3 phosphorylation and β-catenin phosphorylation by resveratrol

It is reported that $PGE_2$ increases the phosphorylation of GSK-3 in human embryonic kidney and neural cells that express prostanoid receptors (Fujino *et al.*, 2002). The phosphorylation of GSK-3 inhibits its kinase activity, which is required for phosphorylation and degradation of β-catenin. To determine whether $PGE_2$ increased the phosphorylation of GSK-3 in colon cancer cells, LS 174T cells were treated with $PGE_2$ (0.5 μM). Treatment with $PGE_2$ rapidly induced the increase of phosphorylation of GSK-3α/β at the early time point of 0.5 h. An increased level of β-catenin was also detected. Consequently, the phosphorylation of β-catenin was decreased after 2 h. When LS 174T cells were treated with resveratrol, the phosphorylation of GSK-3α/β was decreased at 0.5 and 1 h, and the total β-catenin was also decreased throughout a time period of 0.5 to 24 h incubation (Fig. 4).

### Resveratrol suppression of $PGE_2$-stimulated c-myc and cyclin D1 expression

Based on the influence of $PGE_2$ treatment on β-catenin expression, the protein expressions of β-catenin/Tcf-target

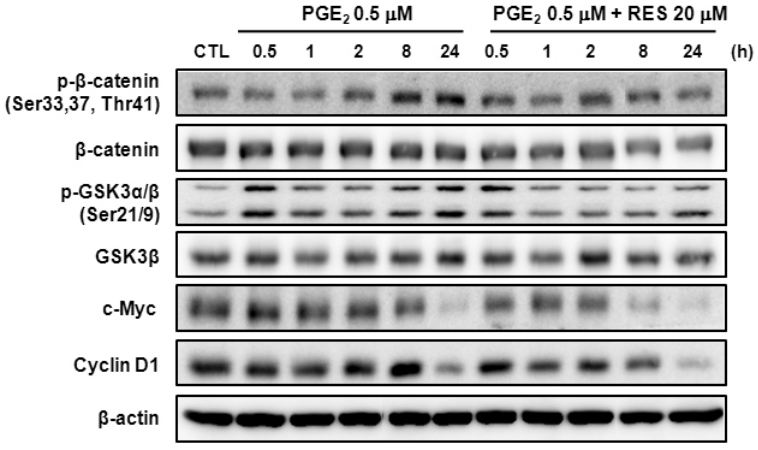


**Fig. 4.** Modulation of $PGE_2$-mediated protein expression and activation associated with Wnt signaling by resveratrol treatment in LS 174T cells. Cells were starved of serum for 24 h, and then stimulated with $PGE_2$ (0.5 μM) in the presence or absence of resveratrol (20 μM) at different time points up to 24 h. Resveratrol was added to the cells 30 min prior to $PGE_2$ stimulation. After harvesting cells, the levels of total and phosphorylated proteins were determined by Western blot analysis. RES: resveratrol.

genes, such as c-myc and cyclin D1, were determined in LS 174T cells. As shown in Fig. 4, a considerable suppression of c-myc and cyclin D1 was detected at 8 h incubation with $PGE_2$ and resveratrol.

### Regulation of β-catenin translocation by resveratrol in $PGE_2$-stimulated colon cancer cells

To further elucidate the underlying mechanism of action by which resveratrol suppresses c-myc and cyclin D1 expression, (which are known as β-catenin/Tcf-target genes), in LS 174T cells, the effect of resveratrol on the translocation of β-catenin into the nucleus as mediated by $PGE_2$ was determined. As shown in Fig. 4, $PGE_2$ treatment increased the phosphorylation of GSK-3α/β at 0.5 h and decreased the phosphorylated β-catenin level. This event might lead to the decrease of β-catenin in cytosol as well as the increase of β-catenin translocation into the nucleus. When treated with $PGE_2$ (0.5 μM) for 24 h, the level of β-catenin in the nucleus was increased (Fig. 5A). However, nuclear translocation of β-catenin by $PGE_2$ was definitely alleviated by treatment with resveratrol, as illustrated in Fig. 5B.

### Suppression of $PGE_2$-induced TCF-dependent transcription in reporter gene assay

Accumulated β-catenin binds and activates the transcriptional factor of the Tcf/Lef family, which in turn, up-regulates the transcription of target genes. To determine whether $PGE_2$ was able to stimulate β-catenin/Tcf-mediated transcription, LS 174T cells were transfected with a reporter vector harboring the Tcf-binding site - TOPflash. Exposure to $PGE_2$ increased the transcriptional activity of TOPflash. In contrast, in the reporter construct containing mutated TCF sites, FOPflash showed minimal transcriptional activity in $PGE_2$-treated LS 174T cells, suggesting the specificity of the transcriptional activation by

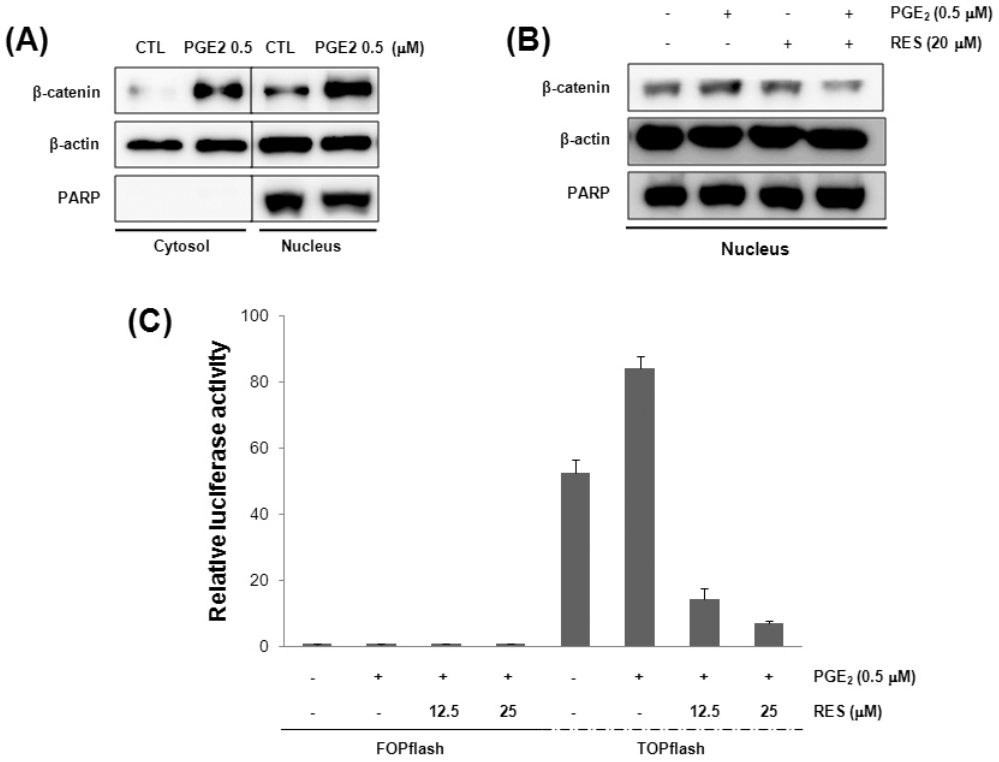


**Fig. 5.** Inhibition of $PGE_2$-mediated nuclear translocation of β-catenin in cells treated with resveratrol. (A) The expression level of β-catenin in cytosol and nucleus after treatment with $PGE_2$ in LS 174 T cells. β-catenin expression in both cytosol and nucleus was analyzed by Western blot analysis. (B) LS 174T cells were stimulated with $PGE_2$ alone, or in combination with resveratrol for 24 h. Nuclear extracts were prepared as described in Materials and Methods, and the expression levels was determined by Western blot analysis. (C) Suppression of β-catenin/Tcf-mediated transcriptional activity by resveratrol treatment. LS 174T cells were co-transfected with TOPflash or FOPflash, and pRL-SV40 reporter plasmids. Cells were then incubated with $PGE_2$ and/or resveratrol for an additional 24 h. β-Catenin/Tcf transcriptional activity was evaluated using the dual luciferase reporter gene assay system, and normalized by the values of *Renilla* luciferase activity. The activity was expressed as a relative value compared to control. RES: resveratrol.

$PGE_2$ stimulation. In this experimental condition, resveratrol markedly inhibited the TOPflash activity without affecting FOPflash activity (Fig. 5C).

## DISCUSSION

Accumulating evidence suggests that chronic inflammation is highly associated with the carcinogenic process. Indeed, inflammatory mediators such as $PGE_2$ and NO enhance the proliferation and metastasis of cancer cells, and also suppress the apoptotic death of cancer cells (Wiseman and Halliwell, 1996; Sheng *et al.*, 2001; Torok *et al.*, 2002). $PGE_2$-mediated signal transduction is mediated by triggering the activation of EP receptors which are distributed in several tissues and play important roles in inflammation and maintenance of homeostasis in the body. Therefore, signal transduction activated by $PGE_2$/EP receptors might be a candidate target for cancer chemoprevention. In the present study, we investigated the effects of resveratrol on $PGE_2$-stimulated cell proliferation and the mechanisms of action regarding $PGE_2$/EP receptors/Wnt signaling in colon cancer cells.

Primarily, we determined whether EP receptors are expressed in human colon cancer cells (LS 174T, HCT-15, HCT 116 and SW480). All these colon cancer cells express subtypes of EP receptors (EP1-4) to various extents. Especially, LS 174T cells which do not express endogenous COX-2, also show the expression of EP2 and EP4 receptors, which are major receptors of $PGE_2$-mediated signal transduction. These results suggest that $PGE_2$-mediated signal transduction might be linked with the relay of EP receptors in these colon cancer cells. Although $PGE_2$ was known to enhance the proliferation of cells, we confirmed whether $PGE_2$ stimulates the growth of colon cancer cells. $PGE_2$ stimulated the proliferation of all the tested colon cancer cells, with ~1.2-fold induction at 0.5 $\mu$M $PGE_2$ after a 3 day incubation (Fig. 2).

To further determine whether the enhancement of cell proliferation might be associated with the expression of cell proliferation-related biomarker proteins, Western blot analysis was performed in LS 174T cells. $PGE_2$ effectively stimulated the expression of phosphorylated Akt and ERK in LS 174T cells, indicating the activation of growth-stimulatory signaling by $PGE_2$ treatment (Fig. 3). Next, we investigated the effect of $PGE_2$ on the Wnt signaling pathway linked with a downstream signaling of EP receptors. $PGE_2$ also affected GSK-3$\alpha/\beta$, which is associated with the Wnt signaling, and subsequently activates downstream oncogenic protein expression, including c-myc and cyclin D1 (Fig. 4).

Based on the information of $PGE_2$-mediated signaling and activation of proliferation biomarkers, we examined, for the first time, whether a cancer chemopreventive agent (resveratrol) could modulate the $PGE_2$-stimulated growth-promoting signaling pathway in colon cancer cells. Resveratrol, a natural stilbenoid, exhibits potential cancer chemopreventive activity, and is known to inhibit the COX-2 activity and induce apoptosis of cancer cells (Mutoh *et al.*, 2000; Wolter *et al.*, 2001; Bhat and Pezzuto, 2002; Joe *et al.*, 2002; Liang *et al.*, 2003). However, the cancer cells which do not express COX-2 were also growth inhibited by resveratrol, suggesting that resveratrol-mediated growth inhibition might involve both COX-2-dependent and -independent mechanisms. Because there is no report showing the regulatory effects of resveratrol in the $PGE_2$-mediated signaling pathway, in the present study, we determined the effect of resveratrol on the stimulation of cell proliferation as mediated by $PGE_2$ and its action mechanism in colon cancer cells. Resveratrol inhibited the $PGE_2$ stimulated cell proliferation of colon cancer cells in a dose-dependent manner over a range of non-cytotoxic concentrations (Fig. 2). The extent of the decrease of cell proliferation by treatment with resveratrol appears to vary in each cell line, which might be due to different expression levels of each EP receptor subtype. Resveratrol exhibited the most profound suppression of cell proliferation in LS 174T cells, which express relatively higher levels of EP2 receptor and relatively lower levels of EP3 and 4 receptors among the tested cell lines. At the concentration of 25 $\mu$M, resveratrol induced decreased viability of LS 174T cells at a level lower than that of vehicle-treated control cells (the fold-change of cell viability was $<1.0$). It is reported that activation of EP2 receptor by $PGE_2$ results in promotion of colon cancer cell growth through the modulation of $\beta$-catenin-mediated signaling, a component of the Wnt signaling pathway (Castellone *et al.*, 2005). Moreover, in previous studies, resveratrol triggered cell cycle arrest and apoptosis in human prostate and uterine cancer cells (Hsieh and Wu, 1999; Sexton *et al.*, 2006). Since resveratrol modulated growth-promoting signal transduction, including $\beta$-catenin/Tcf-mediated transcription as discussed below, it is suggested that modulation of EP2-mediated signaling might partially explain the effectiveness of resveratrol on the proliferation of LS 174T cells. In addition, the down-regulation of cell proliferation at the highest concentration of resveratrol might be mediated by induction of cell cycle arrest and/or apoptosis. Additional studies should be conducted to prove these suggestions.

Next, we examined the effect of resveratrol on the signal transduction activated by $PGE_2$ in LS 174T cells, which do

not express endogenous COX-2. Again, resveratrol suppressed the phosphorylation of Akt and ERK as stimulated by $PGE_2$ (Fig. 3). In addition, resveratrol remarkably inhibited the expressions of oncoproteins c-myc and cyclin D1, which were known to be induced by Wnt signaling activation in $PGE_2$-stimulated colon cancer cells (Fig. 4). To further examine the inhibitory mechanism of resveratrol on the Wnt signaling activation by $PGE_2$ treatment, we next determined the translocation of β-catenin into the nucleus. Resveratrol suppressed the translocation of β-catenin into the nucleus (Fig. 5B), and this event might lead to the inhibition of Tcf/β-catenin transcriptional activity in the reporter gene assay (Fig. 5C). These results again suggest that resveratrol might inhibit $PGE_2$-mediated cell proliferation through modulating growth-stimulatory signal transduction in LS 174T colon cancer cells.

In summary, $PGE_2$, which is known to be highly correlated with the carcinogenic process in colon, stimulates cell proliferation of colon cancer cells through activation of growth-promoting signal transduction, and a cancer chemopreventive agent (resveratrol) modulates the $PGE_2$-mediated signal transduction and thereby inhibits the cell proliferation stimulated by $PGE_2$ treatment in colon cancer cells. Therefore, these results suggest an additional mechanism of action for resveratrol in its cancer chemopreventive effect.

## ACKNOWLEDGMENTS

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## REFERENCES

Bhat, K. P. L. and Pezzuto, J. M. (2002). Cancer chemopreventive activity of resveratrol. *Ann. N.Y. Acad. Sci.* **957**, 210-229.

Buchanan, F. G., Wang, D., Bargiacchi, F. and DuBois, R. N. (2003). Prostaglandin $E_2$ regulates cell migration via the intracellular activation of the epidermal growth factor receptor. *J. Biol. Chem.* **278**, 35451-35457.

Castellone, M. D., Teramoto, H., Williams, B. O., Druey, K. M. and Gutkind, J. S. (2005). Prostaglandin $E_2$ promotes colon cancer cell growth through a Gs-axin-β-catenin signaling axis. *Science* **310**, 1504-1510.

DuBois, R. N., Radhika, A., Reddy, B. S. and Entingh, A. J. (1996). Increased cyclooxygenase-2 levels in carcinogen-induced rat colonic tumors. *Gastroenterology* **110**, 1259-1262.

Eberhart, G. E., Coffey, R. J., Radhika, A., Giardiello, F. M., Ferrenbach, S. and DuBois, R. N. (1994). Up-regulation of cyclooxygenase-2 gene expression in human colorectal adenoma and adnocarcinomas. *Gastroenterology* **107**, 1183-1188.

Fujino, H., West, K. A. and Regan, J. W. (2002). Phosphorylation of glycogen synthase-3 and stimulation of T-cell factor signaling following activation of EP2 and EP4 prostanoid receptors by prostaglandin $E_2$. *J. Biol. Chem.* **277**, 2614-2619.

Giardiello, F. M., Yang, V. W., Hylind, L. M., Krush, A. J., Petersen, G. M., Trimbath, J. D., Piantadosi, S., Garrett, E., Geiman, D. E., Hubbard, W., Offerhaus, G. J. and Hamilton, S. R. (2002). Primary chemoprevention of familial adenomatous polyposis with sulindac. *New Engl. J. Med.* **346**, 1054-1059.

Hansen-Petrik, M. B., McEntee, M. F., Jull, B., Shi, H., Zemel, M. B. and Whelan J. (2002). Prostaglandin $E_2$ protects intestinal tumors from nonsteroidal anti-inflammatory drug-induced regression in Apc(Min/+) mice. *Cancer Res.* **62**, 403-408.

Hsieh, T. C. and Wu, J. M. (1999). Differential effects on growth, cell cycle arrest, and induction of apoptosis by resveratrol in human prostate cancer cell lines. *Exp. Cell Res.* **249**, 109-115.

Hull, M. A., Ko, S. C. W. and Hawcroft, G. (2004). Prostaglandin EP receptors: targets for treatment and prevention of colorectal cancer? *Mol. Cancer Ther.* **3**, 1031-1039.

Joe, A. K., Liu, H., Suzui, M., Vural, M. E., Xiao, D. and Weinstein, I. B. (2002). Resveratrol induces growth inhibition, S-phase arrest, apoptosis, and changes in biomarker expression in several human cancer cell lines. *Clin. Cancer Res.* **8**, 893-903.

Kawamori, T., Uchiya, N., Sugimura, T. and Wakabayashi, K. (2003). Enhancement of colon carcinogenesis by prostaglandin $E_2$ administration. *Carcinogenesis* **24**, 985-990.

Liang, Y. C., Tsai, S. H., Chen, L., Lin-Shiau, S. Y. and Lin, J. K. (2003). Resveratrol-induced G2 arrest through the inhibition of CDK7 and $p34^{CDC2}$ kinases in colon carcinoma HT29 cells. *Biochem. Pharmacol.* **65**, 1053-1060.

Mutoh, M., Takahashi, M., Fukuda, K., Matsushima-Hibiya, Y., Mutoh, H., Sugimura, T. and Wakabayashi, K. (2000). Suppression of cyclooxygenase-2 promoter-dependent transcriptional activity in colon cancer cells by chemopreventive agents with a resorcin-type structure. *Carcinogenesis* **21**, 959-963.

Nam, K. A., Heo, Y. H. and Lee, S. K. (2003). Cytotoxic activity and G2/M cell cycle arrest mediated by antofine, a phenanthroindolizidine alkaloid isolated from Cynanchum paniculatum. *Planta Med.* **69**, 21-25.

Pai, R., Nakamura, T., Moon, W. S. and Tarnawski, A. S. (2003). Prostaglandins promote colon cancer cell invasion: signaling by cross-talk between two distinct growth factor receptors. *FASEB J.* **17**, 1640-1647.

Pai, R., Soreghan, B., Szabo, I. L., Pavelka, M., Baatar, D. and Tarnawski, A. S. (2002). Prostaglandin $E_2$ transactivates EGF receptor: a novel mechanism for promoting colon cancer growth and gastrointestinal hypertrophy. *Nat. Med.* **8**, 289-293.

Pugh, S. and Thomas, G. A. (1994). Patients with adenomatous polyps and carcinomas have increased colonic mucosal prostaglandin $E_2$. *Gut* **35**, 675-678.

Rigas, B., Goldman, I. S. and Levine, L. (1993). Altered eicosanoid levels in human colon cancer. *J. Lab. Clin. Med.* **122**,

518-523.

Sexton, E., van Themsche, C., Leblanc, K., Parent, S., Lemoine, P. and Asselin, E. (2006). Resveratrol interferes with AKT activity and triggers apoptosis in human uterine cancer cells. *Mol. Cancer* **5**, 45.

Shao, J., Jung, C., Liu, C. and Sheng, H. (2005). Prostaglandin $E_2$ stimulates the β-catenin/T cell factor-dependent transcription in colon cancer. *J. Biol. Chem.* **280**, 26565-26572.

Shao, J., Lee, S. B., Guo, H., Evers, B. M. and Sheng, H. (2003). Prostaglandin $E_2$ stimulates the growth of colon cancer cells via induction of amphiregulin. *Cancer Res.* **63**, 5218-5223.

Sheng, H., Shao, J., Washington, M. K. and DuBois, R. N. (2001). Prostaglandin $E_2$ increases growth and motility of colorectal carcinoma cells. *J. Biol. Chem.* **276**, 18075-18081.

Sugimoto, Y. and Narumiya, S. (2007). Prostaglandin E receptors. *J. Biol. Chem.* **282**, 11613-11617.

Torok, N. J., Higuchi, H., Bronk, S. and Gores, G. J. (2002). Nitric oxide inhibits apoptosis downstream of cytochrome C release by nitosylating caspase-9. *Cancer Res.* **62**, 1648-1653.

Ulrich, C. M., Bigler, J. and Potter, J. D. (2006). Non-steroidal anti-inflammatory drugs for cancer prevention: promise, perils, and pharmacogenetics. *Nat. Rev. Cancer* **6**, 130-140.

Wang, D., Buchanan, F. G., Wang, H., Dey, S. K. and DuBois, R. N. (2005). Prostaglandin $E_2$ enhances intestinal adenoma growth via activation of the Ras-mitogen-activated protein kinase cascade. *Cancer Res.* **65**, 1822-1829.

Wiseman, H. and Halliwell, B. (1996). Damage to DNA by reactive oxygen and nitrogen species: role in inflammatory disease and progression to cancer. *J. Biol. Chem.* **313**, 17-29.

Wolter, F., Akoglu, B., Clausnitzer, A. and Stein, J. (2001). Down-regulation of the cyclin D1/cdk4 complex occurs during resveratrol-induced cell cycle arrest in colon cancer cell lines. *J. Nutr.* **131**, 2197-2203.