17-Beta Estradiol and Hydroxyestradiols Interact via the NF-Kappa B Pathway to Elevate Cyclooxygenase 2 Expression and Prostaglandin E2 Secretion in Human Bronchial Epithelial Cells

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Some epidemiological studies suggest women may be at greater risk for lung cancer than men. Hydroxyestradiols (OHE2) are genotoxic and considered as carcinogenic metabolites of estrogens. In this study, we demonstrate that treatment with 0.1 or 1 nM E2 significantly increased intracellular oxidative stress, nuclear factor kappa B (NF-κB) activity, and cyclooxygenase-2 (COX-2) expression within 24 h in human bronchial epithelial cells BEAS-2B. Cotreatment with the NF-κB inhibitor, Bay 117085, prevented OHE2-induced COX-2 mRNA accumulation, suggesting that E2 induced COX-2 expression via the NF-κB dependent pathway. Furthermore, cotreatment with 10 nM 17-beta estradiol (E2) significantly enhanced OHE2-increased intracellular oxidative stress and significantly increased not only NF-κB activity but also COX-2 levels. As COX-2 participates in biosynthesis of prostaglandin E2 (PGE2), PGE2 secretion was enhanced by the cotreatment of 1 nM OHE2 and 10 nM E2. To understand the enhancement mechanism between OHE2 and E2, cells were cotreated with an antioxidant, N-acetylcysteine (NAC), or NF-κB inhibitor, Bay 117085. Both NAC and Bay 117085 prevented the enhancement in COX-2 expression and PGE2 secretion by the cotreatment of E2 and OHE2 in BEAS-2B cells. Similarly, Bay 117085 prevented PGE2 secretion induced by the cotreatment of E2 and OHE2 in rat lung slice cultures. These results suggest that E2 enhanced OHE2-increased intracellular oxidative stress which increased NF-κB activity, COX-2 expression, and PGE2 secretion. Elevated COX-2 expression and PGE2 secretion have been shown to increase the risk of cancer development. Our present data suggest a pathway that contributes an epigenetic mechanism to the overall mechanism of carcinogenesis.

Key Words: nongenotoxic; endocrine; estrogens.

Estrogens have been considered as human carcinogens (Gasperino and Rom, 2004). Many studies suggest that estrogens might induce tumors via metabolic activation of estrogens (Van Aswegen et al., 1989). 17-Beta estradiol (E2) is the most potent and major estrogen in humans. E2 is readily metabolized to 2-hydroxy (2OHE2) and 4-hydroxy estradiols (4OHE2) by cytochrome P450 enzymes (Dawling et al., 2003). 4OHE2 has been demonstrated to induce renal tumors in hamsters (Cavaliere et al., 2000). More recently, Lareef et al. (2005) demonstrated that 4OHE2 induced cell transformation in human breast epithelial cells.

OHE2s are believed to undergo two-electron oxidation to quinone via semiquinone (Cavaliere et al., 2000). During this process, reactive oxygen species (ROS) are generated which causes oxidative DNA damage (Cavaliere et al., 2000). OHE2-induced DNA damage eventually causes mutations in human cells (Fernandez et al., 2006; Lareef et al., 2005). Therefore, OHE2 are thought to increase carcinogenesis by generating DNA damage. In addition to inducing DNA damage, OHE2 also have epigenetic effects, including increasing cell invasiveness (Paquette et al., 2005), enhancing cell proliferation, and activating metalloproteinases (Paquette et al., 2005) nuclear factor kappa B (NF-κB) (Chen et al., 2005), and extracellular signal-regulated protein kinases in human mammary epithelial cells. These effects were implied to contribute to OHE2-induced carcinogenesis.

It is well established that aryl hydrocarbon receptor (AhR) mediates the expression of cytochrome P450 1A1 (CYP1A1) and 1B1 (CYP1B1), which catalyzes 2- and 4-hydroxylation of E2, respectively, to form 2OHE2 and 4OHE2 (Hayes et al., 1996; Spink et al., 1992). Recently, we demonstrated that AhR and CYP1B1 expression was elevated in human lung adenocarcinomas (Chang et al., 2007a). Cigarette smoking, which has AhR agonist activity (Kasai et al., 2006), positively associated with CYP1A1 expression in human lung cancers (Chang et al., 2007a). In cultivated lung cells, CYP1A1 and CYP1B1 are highly inducible by AhR agonists, such as benzo[a]pyrene (BaP) and dioxin (Chang et al., 2007b; Lin et al., 2004). Furthermore, exposure to BaP significantly caused the accumulation of OHE2 in the media of cultivated human lung cells (Chang et al., 2007b). These results suggested that...
OHE2 might be accumulated in CYP1A1 and/or CYP1B1 overexpressed lung cancer tissues. Therefore, it is worthwhile to explore the biological function of OHE2 in lung carcinogenesis. (Tang et al., 2006) reported that nuclear NF-xB was frequently expressed in lung cancer and preneoplastic lesions and suggested that NF-xB activation played a role in lung cancer pathogenesis (Tang et al., 2006). Recently, we reported that OHE2 increased COX-2 mRNA levels in human lung cells (Chang et al., 2007b). Prostaglandins, the primary metabolites of COX-2-catalyzed oxygenation of arachidonic acid, are important immune modulators in inflammatory lung diseases (Martey et al., 2004). Increased COX-2 expression and prostaglandin E2 (PGE2) secretion were common in lung cancer (Funahashi et al., 1994; Hasturk et al., 2002; Ochiai et al., 1999) and seem to facilitate survival of tumor cells (Chen et al., 2003, 2005; Fujita et al., 2002; Prosperi et al., 2004). Thus, it is likely that COX-2 induction may contribute to OHE2-induced carcinogenesis.

Previously we demonstrated that BaP increased CYP1A1 and CYP1B1 expression to result in the accumulation of 2OH E2 and 4OH E2 in lung cells (Chang et al., 2007b). Furthermore, both 2OH E2 and 4OH E2 activated NF-xB and increased COX-2 expression in lung cells (Chang et al., 2007b). In the biological system, E2 always coexists with OHE2. However, the interaction between E2 and OHE2 has never been studied. Many studies suggest that estrogens activate estrogen receptor (ER) to induce carcinogenesis. Although E2 and OHE2 have similar structures, OHE2 has not been reported to activate or antagonize ER (Gupta et al., 1998). In human BEAS-2B cells, we observed that E2 interacted with BaP to increase COX-2 expression and PGE2 accumulation. This raises the question of whether E2 interacts with OHE2 to increase COX-2 expression and PGE2 secretion.

**MATERIALS AND METHODS**

**Materials.** E2 (more than 98% purity), liquid chromatograph-grade ethanol, methanol, N-acetylcycteine (NAC), BaP, and dimethyl sulfoxide (DMSO) were purchased from Sigma Chemical Co. (St Louis, MO). BaP was dissolved in DMSO, stored in aliquots, and kept at -20°C until used. 2OH E2 and 4OH E2 were purchased from Steraloids Inc. (Newport, RI). Bay 117085 was purchased from Biomol International, L.P. (Plymouth Meeting, PA).

**Cell culture.** The human bronchial epithelial cell line BEAS-2B cells immortalized with SV40 (American Type Culture Collection, Manassas, VA) were maintained in serum-free LHC-9 medium (BioSource International Inc., Nivelles, Belgium) in a 37°C incubator with a humidified mixture of 5% CO2 and 95% air. The medium was changed twice a week, and cells were passaged by trypsinization every week.

**Quantitative real-time reverse transcription PCR assay.** Total RNA was prepared using TriReagent (Life Technologies, Rockville, MD) and the chloroform extraction method. Synthesis of cDNA was performed using moloney marine leukemia virus reverse transcriptase, deoxy-nucleotide triphosphate, and RNase inhibitor (Promega, Madison, WI) with 1.5 µg total RNA mixed with 0.5 µg oligo-dT (Mission Biotech, Taipei, ROC). Quantitative PCR was carried out using the TaqMan Universal PCR Master Mix (Perkin-Elmer Applied Biosystems, Foster City, CA) and analyzed on an ABI PRISM 7700 Sequence Detector System (Perkin-Elmer Applied Biosystems). Primers were chosen with the assistance of the computer program Primer Express (Perkin-Elmer Applied Biosystems). The primer sequences and their optimal primer concentrations of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) have been previously described (Chang et al., 2006). The primers for COX-2 were 5'-GCTGGGAACTGGAAATCCTCC-3' and 5'-ATCTGGCTGTGTTGCTAT-3'. The probe for COX-2 was 5'-ACCAGCAACCTGGCGCA-CAA-3'. The PCR reactions consisted of an initial step of 2 min at 50°C, then a polymerase activation step for 1 min at 95°C, followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. Quantitative values were obtained from the threshold cycle (Ct) number. The expression level of the target gene for each sample was normalized to its GAPDH mRNA content. The relative mRNA levels of the target gene = 2-(Ct target gene – Ct GAPDH). Each experiment was repeated twice with three replicates each time.

**Western immunoblot.** Cytosolic homogenates were prepared for COX-2 immunoblotting as described previously (Lin et al., 2003). The samples were separated by 15% sodium dodecyl sulfate polyacrylamide gel electrophoresis. Proteins were transferred to a polyvinylidene difluoride membrane. Membranes were blotted with either a rabbit polyclonal anti-COX-2 (Cayman Chemical Co., Ann Arbor, MI), or anti-GAPDH (Santa Cruz Biotechnology, Santa Cruz, CA). Bands were visualized using an enhanced chemiluminescence kit according to the manufacturer’s instructions (Amersham, Buckinghamshire, UK). Each experiment was repeated four times with duplicates. The data was presented as the mean of four repeated experiments.

**Enzyme-linked immunosorbent assay.** BEAS-2B cells (1 × 10^5 cells per well) were seeded into 24-well dishes and then treated with 2/4OH E2 and/or E2 for 3 days, and in some cases with inhibitors (Bay 117085). The culture medium was replaced with 0.5 ml of LHC-9 medium per well, and conditioned medium was collected 72 h later. PGE2 concentration was determined using the human PGE2 ELISA (enzyme-linked immunosorbent assay) kit and human PGI2 ELISA kit (Cayman Chemical Co., Ann Arbor, MI) according to the manufacturer’s instructions. Each experiment was repeated twice with four replicates each time.

**Animals.** Female Sprague-Dawley rats (150–250 g) 6 weeks old were purchased from BioLASCO Biotechnology Inc. (Taiwan). The animals were housed in standard cages under a 12-h light/dark cycle and received food and water ad libitum. All procedures and experiments with animals in this study were approved by the Animal Care and Use Committee of the National Health Research Institutes, Taiwan.

**Rat lung slice preparation and incubation.** The procedures for preparing rat lung slices has been previously described (Chang et al., 2006). Rats were anesthetized with pentobarbital sodium (100 mg/kg) via intraperitoneal injection. Cylindrical tissue cores (8 mm diameter) were prepared from the lung tissues, which were cut to form 450-µm-thick lung slices using a Vitrin tissue slicer (Vitrin Inc., Tucson, AZ). A total of two lung slices were floated onto the titanium mesh of a single Teflon roller insert (Vitron Inc.). Each insert was placed in a 20-ml glass scintillation culture vial containing 1.8 ml of RPMI1640 medium. Culture vials were incubated at 37°C, 5% CO2 and 95% O2. After 2 h the culture medium was changed and the lung slices were treated as described in the figure legend for 72 h. The culture medium was collected at the end of the 72-h incubation, and the concentration of secreted PGE2 was determined using human a PGE2 ELISA kit (Cayman Chemical Co.). The slices were collected for quantifying protein concentrations with Bio-Rad method (Bio-Rad Laboratories, Hercules, CA). Secreted PGE2 levels were normalized with protein contents of slices.

**Reporter gene assay.** To assess ER activity on a canonical E2 response element, the pG2.2ERE reporter containing three consensus estrogen responsive elements (EREs) upstream of a PRL TATA box and the firefly luciferase gene was obtained from Clontech (San Diego, CA) (Boerner et al., 2005). The nNF-xB-Luc (Stratagene, La Jolla, CA) vector contained the Photinus pyralis (firefly) luciferase reporter gene driven by a basic promoter element (TATA box) plus five repeats of the kappa B cis-enhancer element.
To detect 2\textsuperscript{-}dichlorofluorescein diacetate (DCFDA) as previously described (Frenkel and Gleichauf, 1991). Briefly, after treatment with OHE2 for 10 min, cells were incubated with 50\textmu M DCFDA for 30 min at 37°C. A flow cytometer (Becton, Dickson and Co., San Jose, CA) was used to detect 2\textsuperscript{-}7\textsuperscript{-}dichlorofluorescein (DCF) formed by the reaction of DCFH with intracellular peroxides. Relative levels of intracellular ROS were determined by measuring the mean value of fluorescence per cell. The data is presented as the percentage of DMSO-treated control cells. Each experiment was repeated at least three times.

**Statistical analysis.** Treated and control groups were compared using the one-way ANOVA followed by Tukey’s Honestly Significant Difference test ($p < 0.05$ significance level).

### RESULTS

#### 2/4OHE\textsubscript{2} Increased COX-2 Expression and NF-\kappaB Activity

When AhR agonist, BaP, induced CYP1A1 and CYP1B1 expression in BEAS-2B cells, the amounts of both 2OHE\textsubscript{2} and 4OHE\textsubscript{2} were simultaneously accumulated in the media (Chang et al., 2007b). To mimic simultaneous accumulation of 2/4OHE\textsubscript{2} in lung cells, cells were treated with equal amounts of 2OHE\textsubscript{2} and 4OHE\textsubscript{2}. Similar to our previous study (Chang et al., 2007b), combined treatment with equal amounts of 0.1 to 10nM 2OHE\textsubscript{2} and 4OHE\textsubscript{2} dose-dependently increased the mRNA levels of COX-2 by 1.8- and 2.5-fold of controls in BEAS-2B cells (Fig. 1A). NF-\kappaB has been reported to regulate transcription of COX-2 (Allport et al., 2000; Chen et al., 2003; Martey et al., 2004; Singer et al., 2003). Previously, we reported that 10nM 2OHE\textsubscript{2} and 4OHE\textsubscript{2} increased NF-\kappaB reporter activity in BEAS-2B cells (Chang et al., 2007b). At the doses of 0.1 and 1nM, 2/4OHE\textsubscript{2} significantly increased NF-\kappaB reporter activity to 1.5 and 1.7-fold of controls, respectively (Fig. 1B). These results suggested that 2/4OHE\textsubscript{2}, at doses as low as 0.1nM, activated NF-\kappaB and increased COX-2 expression in BEAS-2B cells.

**E\textsubscript{2} Enhanced 2/4OHE\textsubscript{2}-Induced COX-2 Expression and PGE\textsubscript{2} Secretion**

E\textsubscript{2} always coexists with OHE\textsubscript{2} in the biological system. In the absence of AhR agonists, E\textsubscript{2} was barely converted into OHE\textsubscript{2} in BEAS-2B cells (Chang et al., 2007b). However, cotreatment with 10nM E\textsubscript{2} significantly enhanced 0.1 and 1nM 2/4OHE\textsubscript{2}-induced COX-2 mRNA accumulation from 1.5- and 1.7-fold to 1.8- and 2.1-fold of controls, respectively (Fig. 2A). The enhancement was also significant in the cotreatment of 1nM E\textsubscript{2} and 1nM 2/4OHE\textsubscript{2} (Fig. 2B). The enhancement was further confirmed at protein levels (Fig. 2C). COX-2 participates in biosynthesis of prostaglandins, especially PGE\textsubscript{2} (Dubois et al., 1998; Herschman, 1996; Smith et al., 2000). Cotreatment with 1nM 2/4OHE\textsubscript{2} enhanced 10nM E\textsubscript{2} induced PGE\textsubscript{2} secretion from 2.1-fold to 4.0-fold of controls (Fig. 2D). In contrast, the cotreatment of E\textsubscript{2} and 2/4OHE\textsubscript{2} reduced prostaglandin I2 (PGI\textsubscript{2}) secretion (Fig. 2D).

**FIG. 1.** Effect of 2/4OHE\textsubscript{2} on COX-2 genes expression and NF-\kappaB activity in BEAS-2B cells. BEAS-2B cells were treated with 0.1% methanol or 0.1–10nM 2/4OHE\textsubscript{2}. (A) COX-2 mRNA levels were determined with the realtime reverse transcription PCR method 24 h later. The data were the means of three replicates. (B) NF-\kappaB activity was determined with reporter assay. BEAS-2B cells were transfected with the pNF-\kappaB-Luc reporter. Transfected cells were treated with indicated conditions for 72 h before collected for measuring luciferase activity. The data are the means of four replicates. *Represents $p < 0.05$ as compared with methanol-treated cells.
NF-κB and Oxidative Stress Involved in the Interaction between E2 and 2/4OHE2

Because 2/4OHE2 increased NF-κB reporter activity (Fig. 1B), we further examined if E2 enhanced 2/4OHE2 increased NF-κB reporter activity. Indeed, cotreatment with 10nM E2 enhanced 2/4OHE2 induced NF-κB reporter activity from 1.7-fold to 2.4-fold of controls (Fig. 3A). The data suggested that E2 enhanced NF-κB activation by 2/4OHE2 to potentiate 2/4OHE2-induced COX-2 mRNA accumulation. Previously, we reported that E2 increased PGE2 secretion via an ER signaling pathway in BEAS-2B cells (Chang et al., 2007b). Here, we showed that 1nM 2/4OHE2 failed to increase ER reporter activity (Fig. 3A), suggesting that 2/4OHE2 did not increase ER activity to enhance E2-induced PGE2 secretion. It is well known that oxidative stress may activate NF-κB (Janssen-Heininger et al., 1999; Ndengele et al., 2005; Rahman et al., 2001). Furthermore, 4OHE2 has been reported to induce oxidative stress (Chen et al., 2005). We found that 1nM 2/4OHE2 increased oxidative stress to 1.3-fold of controls (Fig. 3B). Although E2 alone failed to increase oxidative stress, cotreatment with 10nM E2 significantly enhanced 1nM 2/4OHE2 increased oxidative stress to 1.8-fold of controls (Fig. 3B). These results implied that E2 might enhance 2/4OHE2-induced ROS to further increase NF-κB activity.

To understand the role of oxidative stress and NF-κB activation in the interaction between 2/4OHE2 and E2, cells were cotreated with either an antioxidant, NAC, or NF-κB antagonist, Bay 117085. NAC significantly prevented the induction of COX-2 expression by 2/4OHE2 and by the

![Fig. 2](image_url)
A combination of E₂/OHE₂ (Fig. 4A), as well as the increase in PGE₂ secretion by the combination of E₂/OHE₂ (Fig. 4B). Furthermore, NAC also prevented NF-κB activation by 2/4OHE₂ and by the combination of E₂/OHE₂ (Fig. 4C). Similarly, Bay 117085 significantly prevented the induction of COX-2 expression by 2/4OHE₂ and by the combination of E₂/OHE₂ (Fig. 4A). As shown in Figure 5C, cotreatment with E₂ significantly enhanced 2/4OHE₂-induced PGE₂ secretion from 2.7-fold to 4.5-fold of controls, which was inhibited by Bay 117085. A summary of the findings is presented in Figure 5. *Represents p < 0.05 as compared with methanol-treated cells. †Represents p < 0.05 as compared with 10nM E₂-treated cells. §Represents p < 0.05 as compared with 1nM 2/4OHE₂-treated cells.

FIG. 3. Effect of E₂ and 2/4OHE₂ on NF-κB activity and oxidative stress in BEAS-2B cells. Cells were treated with 0.1% methanol, 10nM E₂, 1nM 2/4OHE₂ plus 10nM E₂ and 1nM 2/4OHE₂. (A) ER and NF-κB activities were detected with reporter assay. Cells were transfected with the pGL2-3ERE luciferase reporter or the pNF-κB-Luc reporter. Transfected cells were treated with indicated conditions for 72 h before being collected for measuring luciferase activity. The data are the means of four replicates. (B) Intracellular ROS was measured with the DCFDA method. Cells were treated with 10nM E₂ and/or 1nM 2/4OHE₂ for 10 min. The data are the means of four replicates. *Represents p < 0.05 as compared with methanol-treated cells. †Represents p < 0.05 as compared with 10nM E₂-treated cells.

FIG. 4. Effects of antioxidant (NAC) on 2/4OHE₂ and E₂ interaction in BEAS-2B cells. Cells were pretreated with 1mM NAC for 30 min and then treated with indicated conditions, including 1mM NAC. (A) COX-2 mRNA level was quantified with the real-time reverse transcription PCR assay 24 h later. (B) Amounts of PGE₂ released into culture media were quantified with ELISA assay at 72 h. (C) NF-κB activity was measured with reporter assay. BEAS-2B cells were transfected with the pNF-κB-Luc reporter. Transfected cells were treated under indicated conditions for 72 h before being collected for measuring luciferase activity. The data are the means of four replicates. *Represents p < 0.05 as compared with methanol-treated cells. †Represents p < 0.05 as compared with 10nM E₂-treated cells.
These results suggest that oxidative stress-triggered NF-κB pathway was involved in the interaction between OHE2 and E2 in lung cells and tissues.

DISCUSSION

Conversion of estrogens into carcinogenic metabolites is one of the potential mechanisms to increase cancer risk (Fernandez et al., 2006; Liehr et al., 1986; Mueck et al., 2002). Previously we demonstrated that BaP increased CYP1A1 and CYP1B1 expression to result in the accumulation of 2OHE2 and 4OHE2 in lung cells (Chang et al., 2007b). Furthermore, both 2OHE2 and 4OHE2 activated NF-κB and increased COX-2 expression in lung cells. In the biological system, E2 always coexists with OHE2. In our present study, we demonstrated that cotreatment with E2 and OHE2 enhanced COX-2 expression and PGE2 secretion in human lung BEAS-2B cells. Because it has been proposed that COX-2 and PGE2 promote carcinogenesis, E2 levels may modulate OHE2 associated carcinogenesis.

Our present study suggested that NF-κB activation played a critical role in the effect between OHE2 and E2. The NF-κB pathway is known to regulate COX-2 expression (Allport et al., 2000; Singer et al., 2003). Cotreatment with 10nM E2 significantly increased 1nM OHE2-induced NF-κB activity in BEAS-2B cells. Furthermore, the IκB kinase inhibitor, Bay 117085, prevented COX-2 induction by OHE2 and combined OHE2/E2 (Fig. 6). Previously we demonstrated that E2 induced PGE2 secretion via an ER-dependent pathway (Chang et al., 2007b). Our present study shows that 10nM E2 significantly increased ER reporter activity in BEAS-2B cells. However, cotreatment with OHE2 slightly reduced ER reporter activity, suggesting that the ER pathway might not be involved in the effect on PGE2 secretion.

FIG. 6. A model for the interaction of E2 and OHE2 on COX-2 expression and PGE2 secretion.
COXs mediate the oxidative metabolism of arachidonic acid into prostaglandin H2 (Smith et al., 2000) (Fig. 6). Prostaglandin H2 is subsequently metabolized by PGI2 synthase or microsomal PGE synthase-1 and converted into PGI2 or PGE2, respectively (Smith et al., 2000) (Fig. 6). PGE2 and PGI2 are suggested to have opposite effects on lung carcinogenesis, with PGE2 increasing lung cancer growth but PGI2 having an antineoplastic function (Keith et al., 2004, 2006; Kreutzer et al., 2007; Yamaki et al., 2004). PGE2 increased growth of lung tumor cells in vitro (Yamaki et al., 2004). Nevertheless, increasing pulmonary production of PGI2 by lung-specific overexpression of prostacyclin synthase decreased lung tumorigenicity in mice (Keith et al., 2004). Furthermore, increased PGI2 levels were coupled with reduced PGE2 levels in the lung. The balance between PGE2 and PGI2 production seems to play a role in promoting or preventing tumor progression in the lung. In our present study, cotreatment with OHE2 not only increased PGE2 secretion, but also reduced PGI2 secretion. This implies that the combination of OHE2 and E2 will favor the promotion or progression of lung carcinogenesis.

Oxidative stress is known to activate the NF-κB signaling pathway (Janssen-Heininger et al., 1999; Rahman et al., 2001). Although E2 alone failed to increase intracellular oxidative stress or NF-κB activity, cotreatment with E2 enhanced OHE2-induced oxidative stress and NF-κB activity (Fig. 6). It has been proposed that OHE2s are oxidized to quinones accompanying the generation of free radicals. In addition to superoxide, semiquinones of OHE2 are formed during the conversion of OHE2 into quinones (Cavalleri et al., 1997, 2000). It is possible that semiquinones, which are neutral free radicals, can abstract hydrogen atoms from E2 to generate E2 free radicals, which enhance OHE2-induced oxidative stress (Fig. 7). This hypothesis shall be tested in the future.

It is well known that OHE2 is readily converted into OHE2 by CYP1 enzymes. However, the expression of CYP1 enzyme is low in BEAS-2B cells. When we quantified OHE2 in the presence of 1 µM E2 in BEAS-2B cells, OHE2 was not detectable (data not shown). Furthermore, our present study showed that E2 enhanced OHE2-induced oxidative stress within 10 min. Therefore, it is likely that this interaction was not a result of increased OHE2 accumulation from E2 in BEAS-2B cells.

In humans, expression of CYP1 enzymes in the lung is inducible by cigarette smoking and diseases, such as cancer. For example, CYP1A1 enzymes are highly expressed in the lung epithelia of smokers (Chang et al., 2007a; Hukkanen et al., 2002; Smith et al., 2001), and CYP1B1 protein levels are elevated in lung adenocarcinomas (Chang et al., 2007a). Because elevation of CYP1 enzyme activities in the lung are expected to increase the formation of OHE2 in females, OHE2 may play a role in female lung carcinogenesis. OHE2 is known to initiate carcinogenesis by altering DNA structure and inducing mutation (Cavalleri et al., 2000; Fernandez et al., 2006). Here, we demonstrate that OHE2 enhanced PGE2 secretion, which is associated with chronic inflammation. Chronic inflammation is considered as one of the most important epigenetic factors contributing to cancer development (Wistuba, 2007). Therefore, our present study offers an epigenetic mechanism for OHE2-associated lung carcinogenesis. More experimental evidence will be investigated in the future.

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