Effect of 17β-estradiol on the expression of cytochrome P450 1A1 gene via an estrogen receptor dependent pathway in cellular and xenografted ovarian cancer models

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Introduction
Cytochrome P450 (CYP) 1A1 plays a major role in the metabolic activation of procarcinogens to carcinogens via aryl hydrocarbon receptor (AhR) pathway. Estrogen responsive cancers, 17β-estradiol (E2) may influence on AhR dependent expression of CYP1A1 via the interaction between estrogen receptor (ER) and AhR. In the present study, the effect of E2/ER on the expression of AhR and CYP1A1 genes was investigated for BG-1 ovarian cancer expressing ER.

Materials and Methods
BG-1 cell was cultured with 0.1% DMSO (control), TCDD (10-8 M) and E2 (10-8 M). And to observe the effects of TCDD and E2 on the RNA or protein expression of AhR or CYP1A1, RT-PCR or western blot were performed. Also, the xenografted mice transplanted with BG-1 ovarian cancer cells were ovariectomized for removal of endogenous estrogen and then were injected (i.p. with TCDD, E2 or 0.1% DMSO in PBS during the experiment (80 days). To evaluate the ability of TCDD or E2 to induce in vivo expression of AhR and CYP1A1, we performed an immunohistochemistry for AhR and CYP1A1 proteins on the tumors tissues obtained from the mice.

Results
In reverse transcription (RT)-PCR and western blot analysis, the expression levels of CYP1A1 appeared to be increased by TCDD or E2. The increased expression of AhR and CYP1A1 induced by E2 was restored to the control level by the co-treatment of ICI 182,780 (10-8 M). These results indicate that E2 induced the expression of AhR and CYP1A1 like TCDD via an ER dependent pathway. In an in vivo xenograft mouse model transplanted with BG-1 cells, the protein levels of AhR and CYP1A1 of tumor masses were also increased by TCDD or E2.

Conclusion
These results indicate that E2 may promote AhR dependent expression of CYP1A1 via ER dependent pathway in BG-1 ovarian cancer expressing ER in the absence of DMSO, an agonist of AhR. The relevance of E2 and ER in CYP1A1 activation of estrogen responsive cancers may be targeted for developing more effective cancer treatments.

Abstract
The effects of TCDD and E2 on the protein expression of AhR and CYP1A1 were investigated for the proteins extracted from BG-1 ovarian cancer cells treated with TCDD or E2. The expression of AhR protein was significantly increased by the treatment of TCDD or E2 compared to a control (0.1% DMSO) as shown in Figure 1. Because the expression of CYP1A1 was also induced by DMSO at 3 and 9 h, TCDD or E2-induced alteration in the expression of CYP1A1 mRNA was reversed to the control level at each time point. In the co-treatment of ICI 182,780, E2-induced increase in the expression of CYP1A1 mRNA was reversed to the control level via ER dependent pathway.

1. Effects of TCDD and E2 on the expression of AhR and CYP1A1 mRNAs.
To examine the effects of TCDD (a positive control) or E2 on the expression of AhR and CYP1A1 mRNAs, RT-PCR was performed for total RNAs extracted from TCDD or E2 treated BG-1 ovarian cancer cells. The mRNA expression of AhR was not changed by TCDD or E2, while the expression of CYP1A1 mRNA was significantly increased by E2 at 24 h and by TCDD at 3, 9, and 24 h compared to a control (0.1% DMSO) as shown in Figure 2. A time-dependent increase in the expression of CYP1A1 mRNA in response to TCDD or E2 was reversed to the control level at each time point. In the co-treatment of ICI 182,780, E2-induced increase in the expression of CYP1A1 mRNA was reversed to the control level via ER dependent pathway.

2. Effects of TCDD and E2 on the expression of AhR protein
To investigate the effects of TCDD and E2 on the protein expression of AhR, western blot assay was performed for the proteins extracted from BG-1 ovarian cancer cells treated with TCDD or E2. The expression of AhR protein was significantly increased by the treatment of TCDD or E2 compared to a control (0.1% DMSO) as demonstrated in Figure 3. However, the increased expression of AhR protein was reversed to the control level by the co-treatment of ICI 182,780 at 24 and 72 h (Fig. 2). These results indicate that E2 induced the expression of AhR protein like TCDD via an ER dependent pathway.

3. Effects of TCDD and E2 on the expression of CYP1A1 protein
To investigate the effects of TCDD and E2 on the protein expression of CYP1A1, western blot assays were performed for the proteins extracted from TCDD or E2 treated BG-1 ovarian cancer cells treated with TCDD or E2. The expression of CYP1A1 protein was significantly increased by the treatment of TCDD or E2 for 24 and 72 h compared to a control (0.1% DMSO) as shown in Figure 3. However, the increased expression of CYP1A1 protein was significantly reduced by the co-treatment of ICI 182,780 for 48 or 72 h (Fig. 3). These results indicate that the E2 induced the expression of CYP1A1 protein as did TCDD via an ER dependent pathway.

4. Immunohistochemistry for analysis of in vivo expression of CYP1A1
The xenografted mice transplanted with BG-1 ovarian cancer cells were ovariectomized for removal of endogenous estrogen and then were injected (i.p. with TCDD, E2 or 0.1% DMSO in PBS during the experiment period (80 days) according to a time schedule shown in Figure 4A. To evaluate the ability of TCDD or E2 to induce in vivo expression of AhR in the xenografted ovarian cancer cells, performed immunohistochemistry for AhR and CYP1A1 proteins on the tumor tissues obtained from the mice by using antibodies against these proteins. As shown in Figure 4B & C, the expression of AhR and CYP1A1 genes in the tumors of BG-1 ovarian cancer cells were significantly increased by the treatment of TCDD or E2 compared to the control, respectively (Fig. 4B & C), which is similar to the in vitro results of western blot assay shown in Figure 2 and 3.

Conclusion
These results indicate that E2 may promote AhR dependent expression of CYP1A1 via ER dependent pathway in BG-1 ovarian cancer expressing ER in the absence of DMSO, an agonist of AhR. The relevance of E2 and ER in CYP1A1 activation of estrogen responsive cancers may be targeted for developing more effective cancer treatments.

Figure 1. Altered mRNA expressions of AhR and CYP1A1 following the treatment with E2 or TCDD. BG-1 cells were seeded in 60-mm dish and treated with 0.1% DMSO (control), TCDD (10-8 M), or E2 (10-8 M) in the presence or absence of ICI 182,780 (10-8 M) for 0, 24, and 72 h. (A) The expression levels of AhR mRNA were increased by TCDD or E2. The increased expression of AhR mRNA and CYP1A1 mRNA at 24 h was reversed to the control level by the co-treatment of ICI 182,780 (10-8 M) for 24 and 72 h as shown in Figure 1. (B) In the co-treatment of ICI 182,780, E2-induced increase in the expression of CYP1A1 mRNA at 24 h was reversed to the control level via ER dependent pathway.

Figure 2. Altered protein expressions of CYP1A1 following the treatment with E2 or TCDD. BG-1 cells were seeded in 60-mm dish and treated with 0.1% DMSO (control), TCDD (10-8 M), or E2 (10-8 M) in the presence or absence of ICI 182,780 (10-8 M) for 0, 24, and 72 h.

Figure 3. Altered protein expressions of CYP1A1 following the treatment with E2 or TCDD. BG-1 cells were seeded in 60-mm dish and treated with 0.1% DMSO (control), TCDD (10-8 M), or E2 (10-8 M) in the presence or absence of ICI 182,780 (10-8 M) for 0, 24, and 72 h. These results indicate that E2 induced the protein expression of CYP1A1 like TCDD via an ER dependent pathway.

Figure 4. Representative immunohistochemical images of AhR, and CYP1A1 proteins in the isolated tumors. (A) The mice were injected i.p. with DMSO, E2, and TCDD every 3 days during experiment period (80 days). Paraffin blocks were cut into 3 to 5 thick sections and each section was treated with primary antibody by IHC staining protocol for measuring the immunohistochemical images of AhR (a) and CYP1A1 (b) (magnification, 40 x and 200 x). (c) The expression levels of AhR and CYP1A1 proteins were quantified by cell sers dimension software.

Figure 5. The cooperation of E2 and ER with AhR signaling in the induction of CYP1A1 expression in estrogen responsive BG-1 ovarian cancer cells. According to a well-known signaling pathway, AhR is phosphorylated by PKC. This complex binds to XREs and modulates the expression of CYP1A1. In addition, the present study suggests that E2 may have the potential to induce the CYP1A1 expression via ER dependent-AhR signaling in estrogen responsive BG-1 ovarian cancer cells even though AhR is not changed with its agonist.


