An estrogen receptor-dependent pathway is involved in fludioxonil-induced cancer growth and metastasis linked with epithelial mesenchymal transition in cellular and xenografted ovarian cancer models

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Abstract

Fludioxonil is an antifungal agent used in agricultural applications that is present at measurable amounts in fruits and vegetables. In this study, the effects of fludioxonil on cancer cell viability, epithelial-mesenchymal transition (EMT) and metastasis were examined in BG-1 ovarian cancer cells with estrogen receptors (ERs). BG-1 cells were cultured with 0.1% DMSO (control), 17β-estradiol (E2; 1 x 10^{-9} M), or fludioxonil (10^{-5}-10^{-8} M). MTT assay revealed that fludioxonil increased BG-1 cell viability 1.2 to 1.5 times compared to the control, while E2 markedly increased BG-1 cell viability by about 3.5 times. When the samples were co-treated with ICI 182,780 (10^{-8} M), an ER antagonist, fludioxonil-induced BG-1 cell viability was reversed to the level of the control. Protein levels of cyclin E, cyclin D1, snail and N-cadherin increased in response to fludioxonil as E2 did, but these increases were not observed when fludioxonil was administered with ICI 182,780. Moreover, the protein level of p21 and E-cadherin decreased in response to treatment with fludioxonil, but remained at the control level when co-treated with ICI 182,780. In xenografted mouse models transplanted with BG-1 cells, fludioxonil significantly increased the tumor mass formation by about 2.5 times as E2 did when compared to vehicle (0.1% DMSO) during the experimental period (80 days). Immunohistochemistry revealed that the protein level of proliferating cell nuclear antigen (PCNA), snail and cathepsin D increased in response to fludioxonil as E2 did. These results imply that fludioxonil may have disruptive effects on ER expressing cancers by inducing alterations in the expression of cell cycle- and EMT-related genes via the ER dependent pathway.

Results

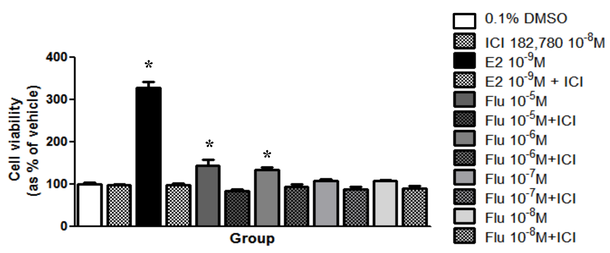

Figure 1. Increased cell viability of BG-1 cells following the treatment with E2 or Fludioxonil. To evaluate the effect of E2 and Fludioxonil on cell viability, BG-1 cells were plated at 3,000 cells/well in 200 μl of phenol red-free DMEM with 5% CD–FBS medium. Cells were cultured in phenol red-free DMEM with vehicle (0.1% DMSO, control), E2 (10^{-8} M), Fludioxonil (10^{-5}-10^{-8} M) in the present or absence ICI 182,780 (10^{-8} M) for 6 days. The cell viability was measured using an MTT assay. Values are the means ± SD. *: Mean values were significantly different from 0.1% DMSO (vehicle), P<0.05. (Dunnett's multiple comparison test).

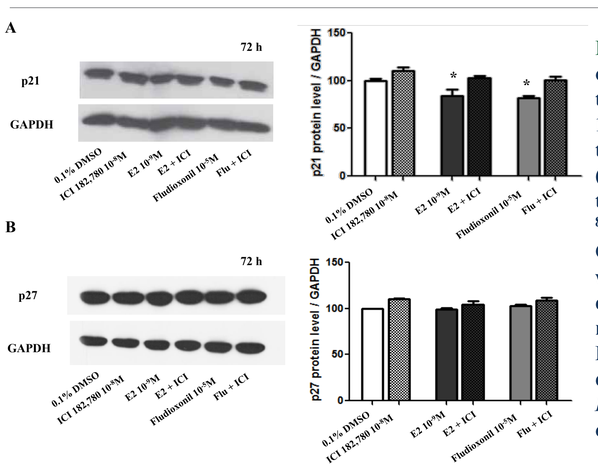

Figure 2. Altered protein expressions of cell cycle related gene following the treatment with E2 or Fludioxonil. BG-1 cells were seeded in 60-mm dish and treated with 0.1% DMSO (control), E2 (10^{-8} M), and Fludioxonil (10^{-5} M) in the present or absence ICI 182,780 (10^{-8} M) at p21(A) and p27 (B) at 72h. Quantification of proteins of these were conducted by scanning the densities of bands on a transfer membrane using Gel Doc 2000. *: Mean values were significantly different from 0.1% DMSO (vehicle), *P*<0.05. (Dunnett's multiple comparison test).

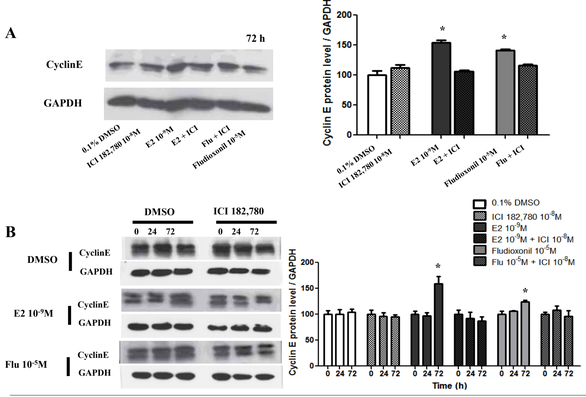

Figure 3. Altered protein expressions of CyclinE gene following the treatment with E2 or Fludioxonil. BG-1 cells were seeded in 60-mm dish and treated with 0.1% DMSO (control), E2 (10^{-8} M), and Fludioxonil (10^{-5} M) in the present or absence ICI 182,780 (10^{-8} M) at 72 h (A) or in time-dependent manner (B).

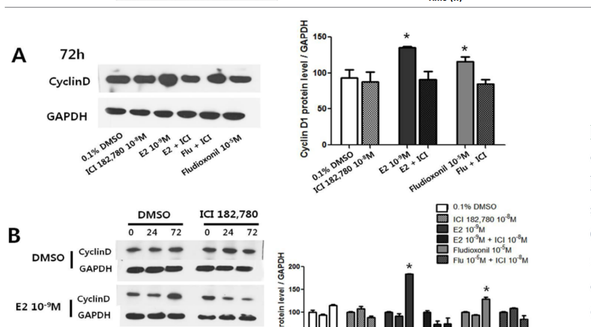

Figure 4. Altered protein expressions of CyclinD following the treatment with E2 or Fludioxonil. BG-1 cells were seeded in 60-mm dish and treated with 0.1% DMSO (control), E2 (10^{-8} M), and Fludioxonil (10^{-5} M) in the present or absence ICI 182,780 (10^{-8} M) at 72h (A) and time-dependent manner (B).

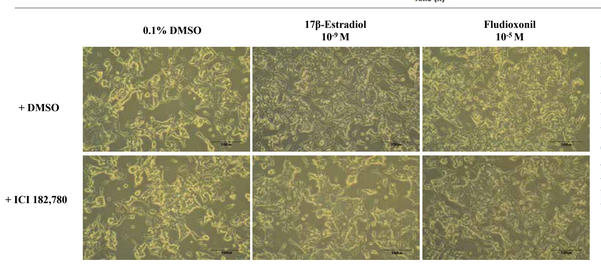

Figure 5. Change of the morphology following the treatment with E2 or Fludioxonil. BG-1 cells were seeded in 60-mm dish and treated with 0.1% DMSO (control), E2 (10^{-8} M), and Fludioxonil (10^{-5} M) in the present or absence ICI 182,780 (10^{-8} M) at 96 h.

Results

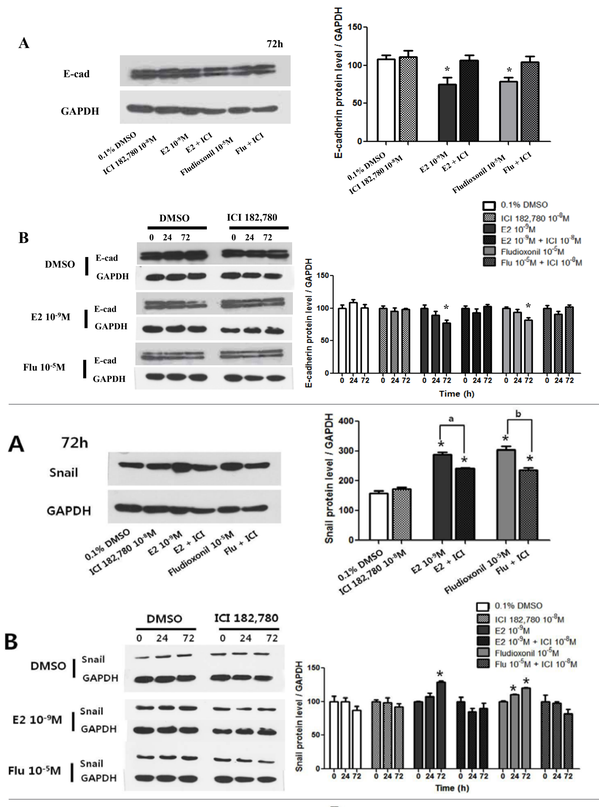

Figure 4. Altered protein expressions of Snail following the treatment with E2 or Fludioxonil. BG-1 cells were seeded in 60-mm dish and treated with 0.1% DMSO (control), E2 (10^{-8} M), Fludioxonil (10^{-5} M) in the present or absence ICI 182,780 (10^{-8} M) at 72h (A) and in a time-dependent manner (B).

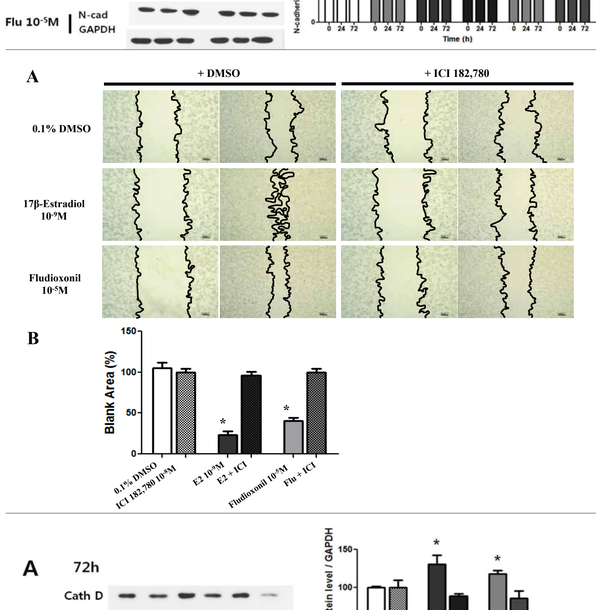

Figure 5. Altered protein expressions of Snail following the treatment with E2 or Fludioxonil at 72h (A) and in a time-dependent manner (B). * : Mean values were significantly different from 0.1% DMSO (vehicle), a: mean values were significantly different from the E2 10^{-9} M and b: mean values were significantly different from the Fludioxonil 10^{-5} M, P<0.05. (Dunnett's multiple comparison test).

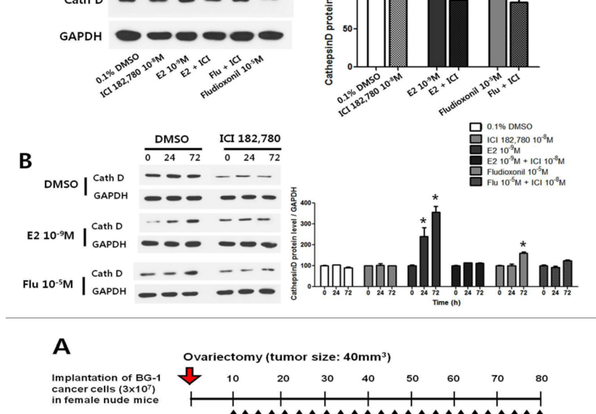

Figure 6. Altered protein expressions of N-cadherin following the treatment with E2 or Fludioxonil. BG-1 cells were seeded in 60-mm dish and treated with 0.1% DMSO (control), E2 (10^{-8} M), Fludioxonil (10^{-5} M) in the present or absence ICI 182,780 (10^{-8} M) at 72 h (A) and in a time-dependent manner (B).

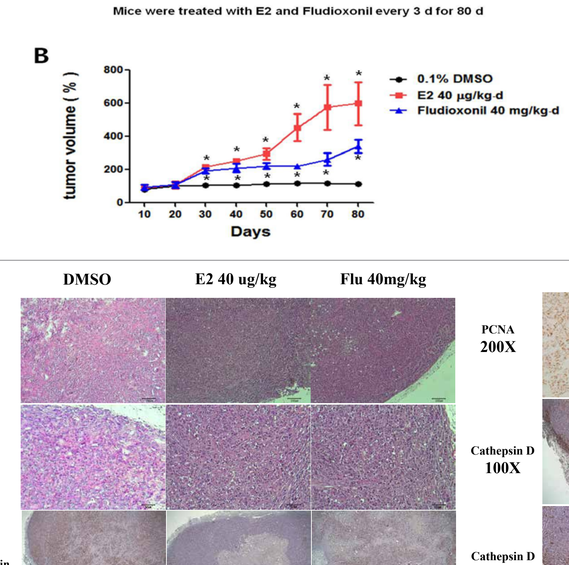

Figure 7. Wound healing assay for BG-1 cells following treatment with E2 and fludioxonil. To evaluate the effects of E2 and fludioxonil on cell mobility, BG-1 cells were plated at 1 x 10^6 cells/well in 6-well plates in phenol red-free DMEM with 5% CD–FBS. The cell were cultured with 0.1% DMSO (control), E2 (10^{-8} M), or fludioxonil (10^{-5} M) in the presence or absence of ICI 182,780 (10^{-8} M) for 96 h.

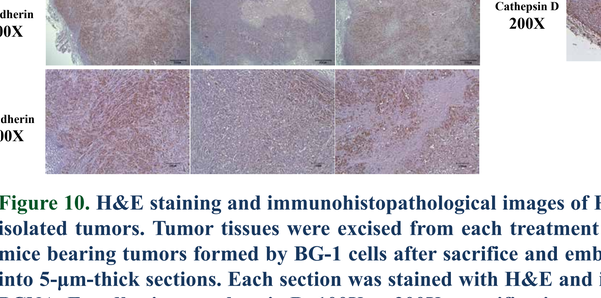

Figure 8. Altered protein expressions of CathepsinD (CathD) following the treatment with E2 or Fludioxonil. BG-1 cells were seeded in 60-mm dish and treated with 0.1% DMSO (control), E2 (10^{-8} M), fludioxonil (10^{-5} M) in the present or absence ICI 182,780 (10^{-8} M) at 72 h (A) and in a time-dependent manner (B).

Figure 9. Effect of E2 and Fludioxonil on the tumor growth in the absence of endogenous estrogen. The mice were injected i.p. with DMSO, E2, and fludioxonil every 3 days (A) and tumor volumes were measured by length x width x height x 0.5236 (mm^3) using a vernier calipers every 10 days during the experiment period of 80 days (B). *: Mean values were significantly different from DMSO (vehicle), P<0.05. (Student's t-test).

Figure 10. H&E staining and immunohistopathological images of PCNA, E-cadherin and cathepsin D proteins in isolated tumors. Tumor tissues were excised from each treatment group (0.1% DMSO, E2, and Fludioxonil) of mice bearing tumors formed by BG-1 cells after sacrifice and embedded in paraffin. Paraffin blocks were cut in into 5-μm-thick sections. Each section was stained with H&E and incubated with primary antibodies specific for PCNA, E-cadherin or cathepsin D. 100X or 200X magnification.

Conclusions

1. In a cell viability assay, the treatment of E2 or Fludioxonil induced a significant proliferation in BG-1 ovarian cancer cells. When ICI 182,780 was treated with E2 or Fludioxonil, E2 or Fludioxonil -induced cell proliferation was reversed as much as the control (0.1% DMSO). These results provide that Fludioxonil induced BG-1 ovarian cancer cell proliferation via ER dependent pathway, as did E2.

2. The exposure of E2 or Fludioxonil in BG-1 cancer cells induced the increased expression of both cell cycle induced protein as a Cyclin D, Cyclin E proteins at 72 h or in a time-dependent manner, which was also reduced completely by the co-treatment of ICI 182,780. And Protein of p21 and p27 decreased by E2 or fludioxonil in principle. These results suggest that Fludioxonil induced the increased gene expression of cell cycle induced protein level via ER dependent pathway in BG-1 ovarian cancer cells.

3. The morphology of BG-1 was changed a spindle and scatter shape by E2 or fludioxonil than DMSO or ICI 182,780 treatment group.

4. The exposure of E2 or Fludioxonil in BG-1 cancer cells induced the increased expression of both EMT related gene such as Snail and N-cadherin proteins and decreased expression of E-cadherin proteins at 72 h or in a time-dependent manner, which was also reduced completely or partially by the co-treatment of ICI 182,780. These results suggest that Fludioxonil induced the increased gene expression of EMT induced protein level via ER dependent pathway.

5. Also, in wound healing assay, blank area of BG-1 cell was closed by E2 or fludioxonil. And when ICI 182,780 treated with E2 or fludioxonil, blank area was not closed. This result indicated that invasion/metastasis of BG-1 by E2 or fludioxonil mediated in estrogen receptor pathway.

6. The exposure of E2 or Fludioxonil induced the increased expression of Cathepsin D proteins at 72 h or in a time-dependent manner, which was also reduced by the co-treatment of ICI 182,780. These results suggest that Fludioxonil induced the increased gene expression of metastasis induced protein level via ER dependent pathway.

7. These in vitro results were confirmed by in vivo animal models using the ovariectomized mice exposed to E2 or Fludioxonil. E2 and Fludioxonil stimulated the BG-1 ovarian tumor growth compared with the control (0.1% DMSO) *in vivo*.

8. Tumor tissue sections from mice exposed to E2 or fludioxonil displayed hyperproliferative cellular formations with higher density compared to the control in H&E staining. Also, expression of PCNA significantly was increased by E2 or fludioxonil compared to the control.

9. Expression of E-cadherin of EMT repress gene was decreased by E2 or fludioxonil compared to the control. On the other hand, expression of cathepsin D of metastasis marker was increased by E2 or fludioxonil compared to the control. This result indicated that fludioxonil may induced EMT and metastasis as did E2 in vivo.

10. In conclusion, the present study shows that both E2 and Fludioxonil may have the capability of proliferating BG-1 ovarian cancer and stimulating the protein expression of cell cycle, EMT and metastasis protein via ER dependent pathway.

11. Based on these results, further studies are needed to elucidate the detail cell cycle, EMT or metastasis relationship between ER pathways. Because its importance in estrogen responsive cancers for developing more effective cancer treatments targeting this process.

References

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CHUNGBUK NATIONAL UNIVERSITY

ECE 2016 180-GP

Reproduction & Endocrine Disruption

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Poster presented at: ECE2016 28 – 31 May 2016 MUNICH, GERMANY 18th EUROPEAN CONGRESS OF ENDOCRINOLOGY

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