An estrogen receptor-dependent pathway is involved in fludioxonil-induced cancer growth and metastasis linked with epithelial-mesenchymal transition in cellular and xenograft 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 co-treated with 0.1% DMSO (control), I7P estradiol (E2; 1 x 10^-8 M), or fludioxonil (10^-8-10^-5 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 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, but remained at the control level when co-treated with ICI 182,780 (10^-8 M) at 72h (A) and in a time-dependent manner (B).

Results

Figure 1. Increased cell viability of BG-1 cells following the treatment with E2 or Fludioxonil. To evaluate the effects of E2 and Fludioxonil on cell viability, BG-1 cells were seeded in 60-mm dish and treated with 0.1% DMSO (control), E2 (10^-9 M), Fludioxonil (10^-8 M) in the present or absence ICI 182,780 (10^-8 M) at 72h (A) and in a time-dependent manner (B).

Figure 2. Altered protein expressions of cyclin E 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^-9 M), Fludioxonil (10^-8 M) in the present or absence ICI 182,780 (10^-8 M) at 72h (A) and in a time-dependent manner (B).

Figure 3. Altered protein expression of Cyclin D1 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^-9 M), Fludioxonil (10^-8 M) in the present or absence ICI 182,780 (10^-8 M) at 72h (A) or in a time-dependent manner (B).

Figure 4. Altered protein expressions of Cyclin E1 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^-9 M), Fludioxonil (10^-8 M) in the present or absence ICI 182,780 (10^-8 M) at 72h (A) and in a 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^-9 M), Fludioxonil (10^-8 M) in the present or absence ICI 182,780 (10^-8 M) at 72h.

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^-9 M), Fludioxonil (10^-8 M) in the present or absence ICI 182,780 (10^-8 M) at 72h (A) and in a time-dependent manner (B).

Figure 7. Wound healing assay for BG-1 cells 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^-9 M), Fludioxonil (10^-8 M) in the present or absence ICI 182,780 (10^-8 M) at 96 h.

Figure 8. Altered protein expressions of Cathespin D 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^-9 M), Fludioxonil (10^-8 M) in the present or absence ICI 182,780 (10^-8 M) at 72h (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 DMEM, E2, and fludioxonil every 3 days (A) and tumor volumes were measured by length x width x 0.5236 (mm3) using a vernier caliper over 18 days during the experiment period of 80 days (B). *: Mean values were significantly different from DMEM (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 experiment period of 80 days. PCNA, E-cadherin or cathepsin D. 100X or 200X magnification.

Conclusions

1. 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 proteins as Cyclin E and Cyclin D proteins at 72 h in a time-dependent manner, which was also reduced completely partially by the co-treatment of ICI 182,780. These results suggest that Fludioxonil induced the increased gene expression of cell cycle proteins induced by 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 (vehicle).

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 in a time-dependent manner, which was also reduced completely partially by the co-treatment of ICI 182,780. These results suggest that Fludioxonil induced the increased gene expression of E-cadherin protein level via ER dependent pathway.

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

6. The exposure of E2 or Fludioxonil induced the increased expression of Cathespin D protein at 72 h in a time-dependent manner, which was also reduced completely partially 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 ovariochomized 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 features with higher density compared to the control in H&E staining. Also, expression of PCNA 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 Cathespin D of metastasis marker was increased by E2 or fludioxonil compared to the control. This result indicated that E2 or Fludioxonil-induced EMT and metastasis as did E2 in vivo.

10. In conclusion, the present study shows that E2 and Fludioxonil may have the capability of proliferating BG-1 ovarian cancer and stimulating the protein expression of cell cycle, EMT and metastasis proteins 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 response cancers for developing more effective cancer treatments targeting this process.

References


