# Dual face of sex-steroid hormones, estrogen and progesterone, on ovarian cancer metastasis *via* the regulation of epithelial-mesenchymal transition
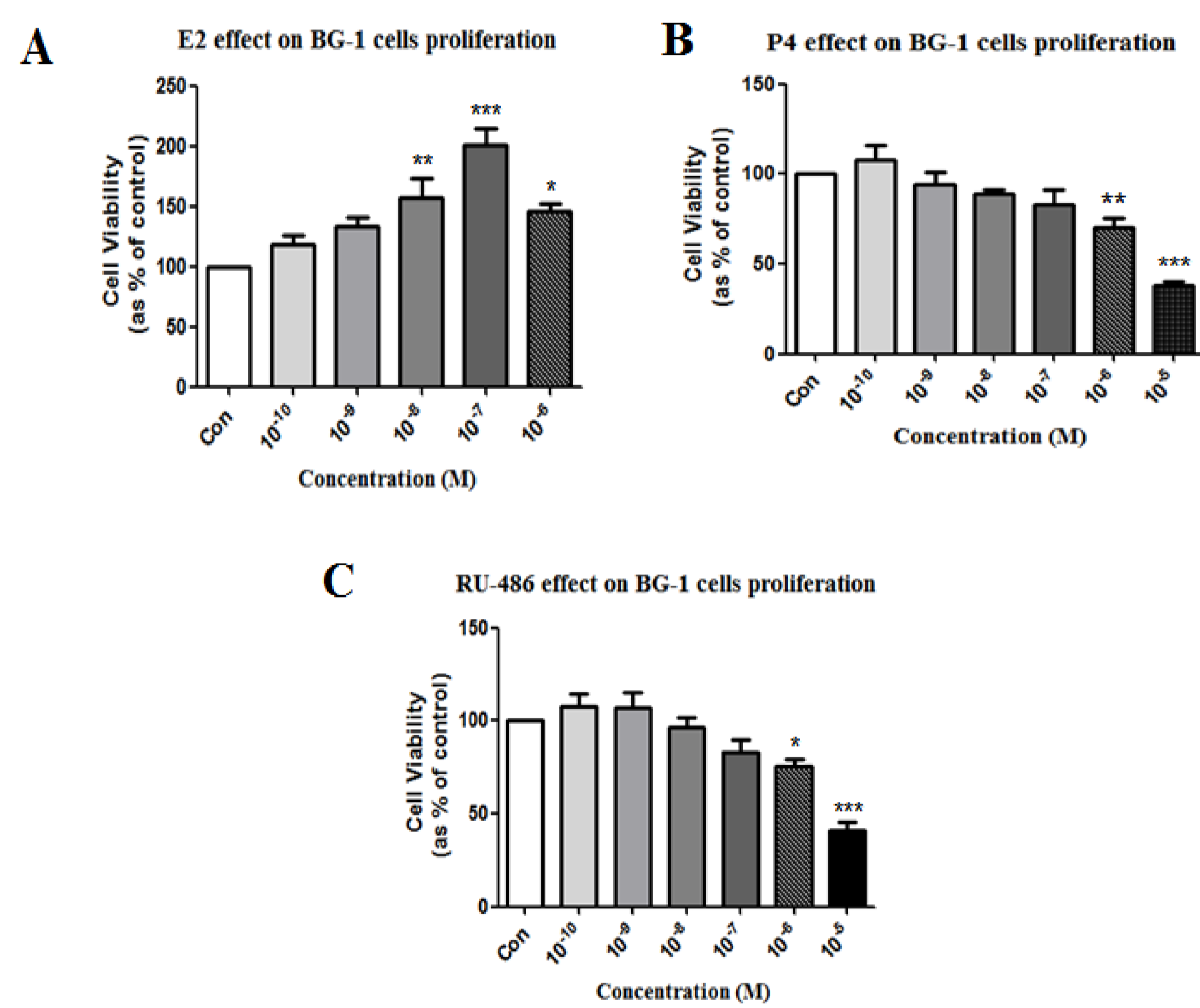
Jae-Rim Heo, So-Ye Jeon and Kyung-Chul Choi [Correspondence]

*Laboratory of Biochemistry and Immunology, College of Veterinary Medicine, Chungbuk National University, Cheongju, Chungbuk 361-763 Republic of Korea*

## ABSTRACT

Ovarian carcinoma is the most deadly and leading cause of cancer death occurring in the female reproductive tracts. 17β-estradiol (E2) has long been considered as one of the effective causes of ovarian cancer through its actions via estrogen receptors (ERs). In contrast, progesterone (P4) offers protective effect against ovarian carcinogenesis. We predicted that P4 would inhibit the metastasis of BG-1 human epithelial ovarian cancer cells, which was induced by E2. In the present study, we confirmed that E2 increased BG-1 cell viability in a dose-dependent manner, while the effect of E2 was inhibited by the co-treatment of P4. Also we showed that P4 decreased the metastatic potential of BG-1 cells. P4 treatment clearly led to functional changes in cancer cell migratory and invasive propensity. We performed scratch assay and invasion assay to evaluate these functional changes. The results showed that P4 inhibited the migration and invasion activity of BG-1 ovarian cancer cells, which was increased by E2 via its receptor interaction. These alterations were also related with changes in the epithelial-mesenchymal transition (EMT) markers such as E-cadherin, Vimentin, and N-cadherin and EMT-associated transcriptional factors, Snail and Slug, too. Upon P4 stimulation, the expression of the epithelial marker E-cadherin was strikingly increased, whereas the expression of mesenchymal makers like N-cadherin and Vimentin was decreased. EMT-associated transcriptional factors, Snail and Slug, were also significantly down-regulated. These results indicate that P4 can inhibit the migration of BG-1 ovarian cancer cells by reducing EMT. Consequently, the present results represent that P4 is a potent substance which may inhibit the growth of human ovarian cancer cells and metastasis via regulation of PR. Therefore, the hormone therapy using P4 may be a clinically effective tool for the treatment of human epithelial ovarian cancer.

## RESULTS



**Figure 1. The effects of E2, P4, RU-486 on cell proliferation of BG-1 cells.** BG-1 cells were seeded at 5000 cells/well in 96-well plates in the presence of phenol red-free DMEM (+5% CD-FBS). After 2 days, the medium was replaced by phenol red-free DMEM (+5% CD-FBS) containing (A) Estradiol (B) Progesterone and (C) RU-486 (mifepristone), a PR antagonist, at the indicated concentration for 4 days. The cell viability was determined by MTT assay. * P<0.05 compared to a vehicle.
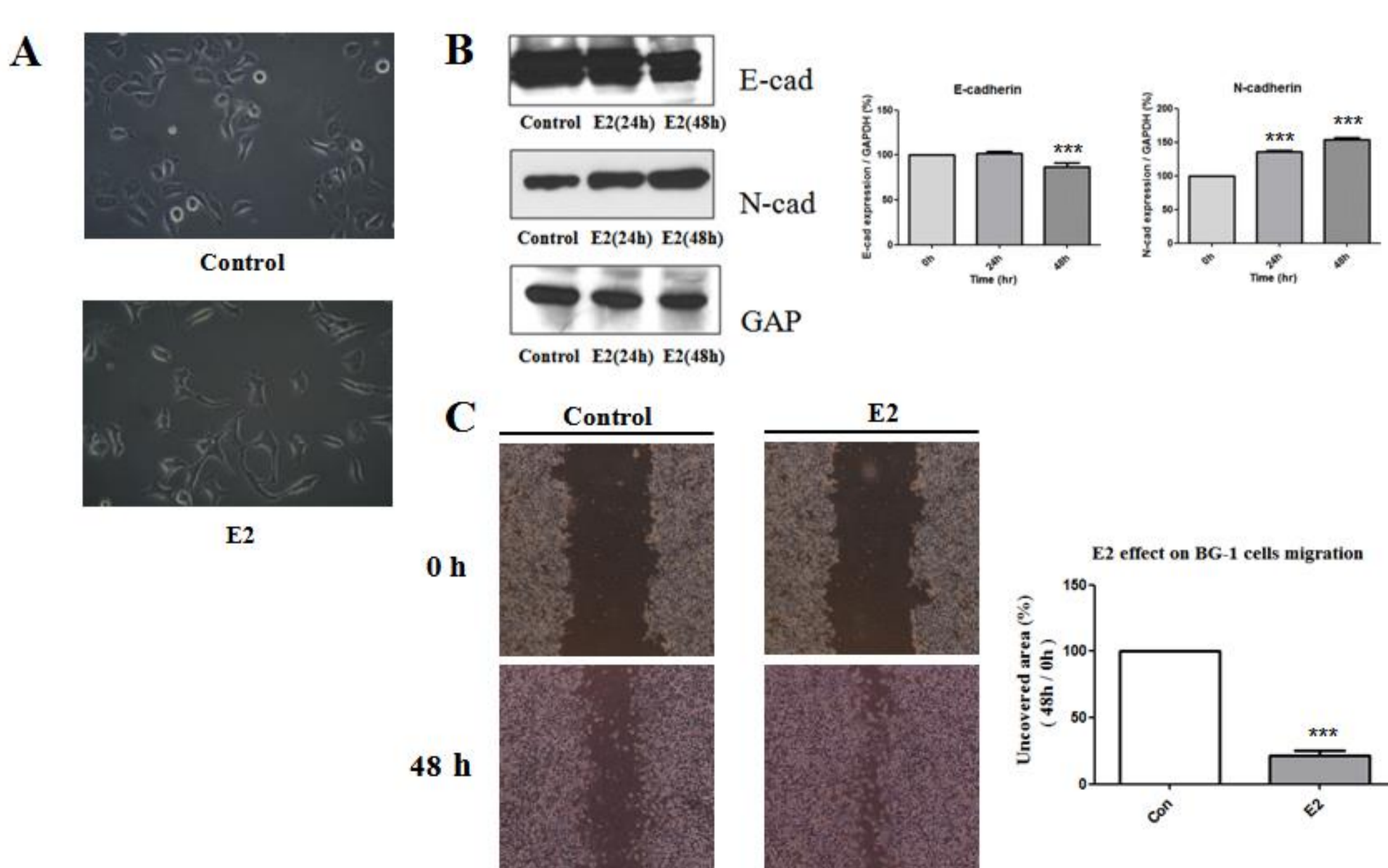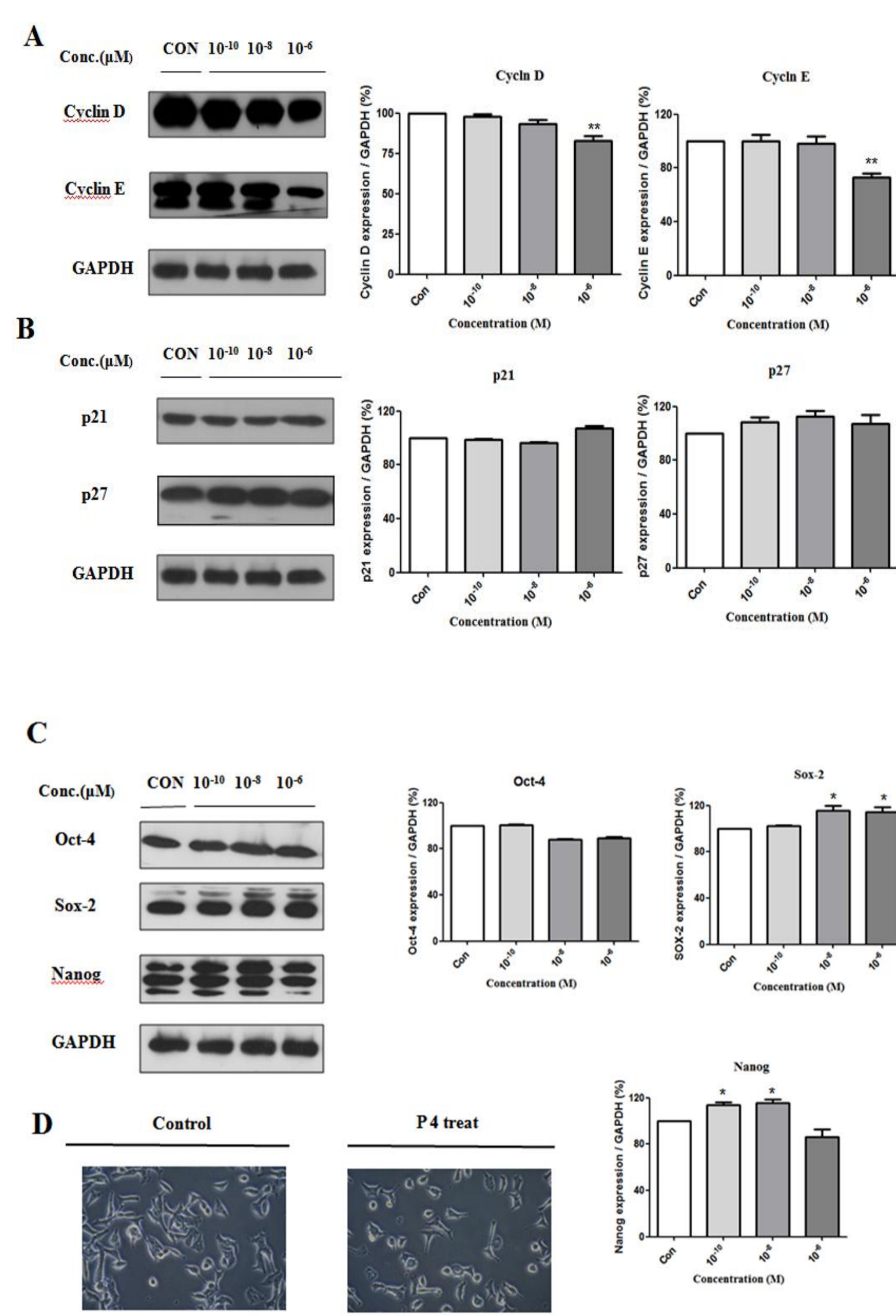


## RESULTS

**Figure 2. The functional role of E2 in EMT on BG-1 ovarian cancer cells.** BG-1 cells were treated with 0.1% DMSO as a vehicle. A, BG-1 cells were treated with vehicle (Cont) or $10^{-7}$ M E2 for 48 h. Morphological changes were observed by microscope. B, The effects of E2 ($10^{-8}$ M) was investigated on the expression of EMT markers in BG-1 cells by western blot. C, Confluent monolayers of BG-1 cells were wounded with a uniform scratch, washed to remove cell debris, and cultured for indicated times in the presence of vehicle (Cont) or E2 ($10^{-8}$ M)
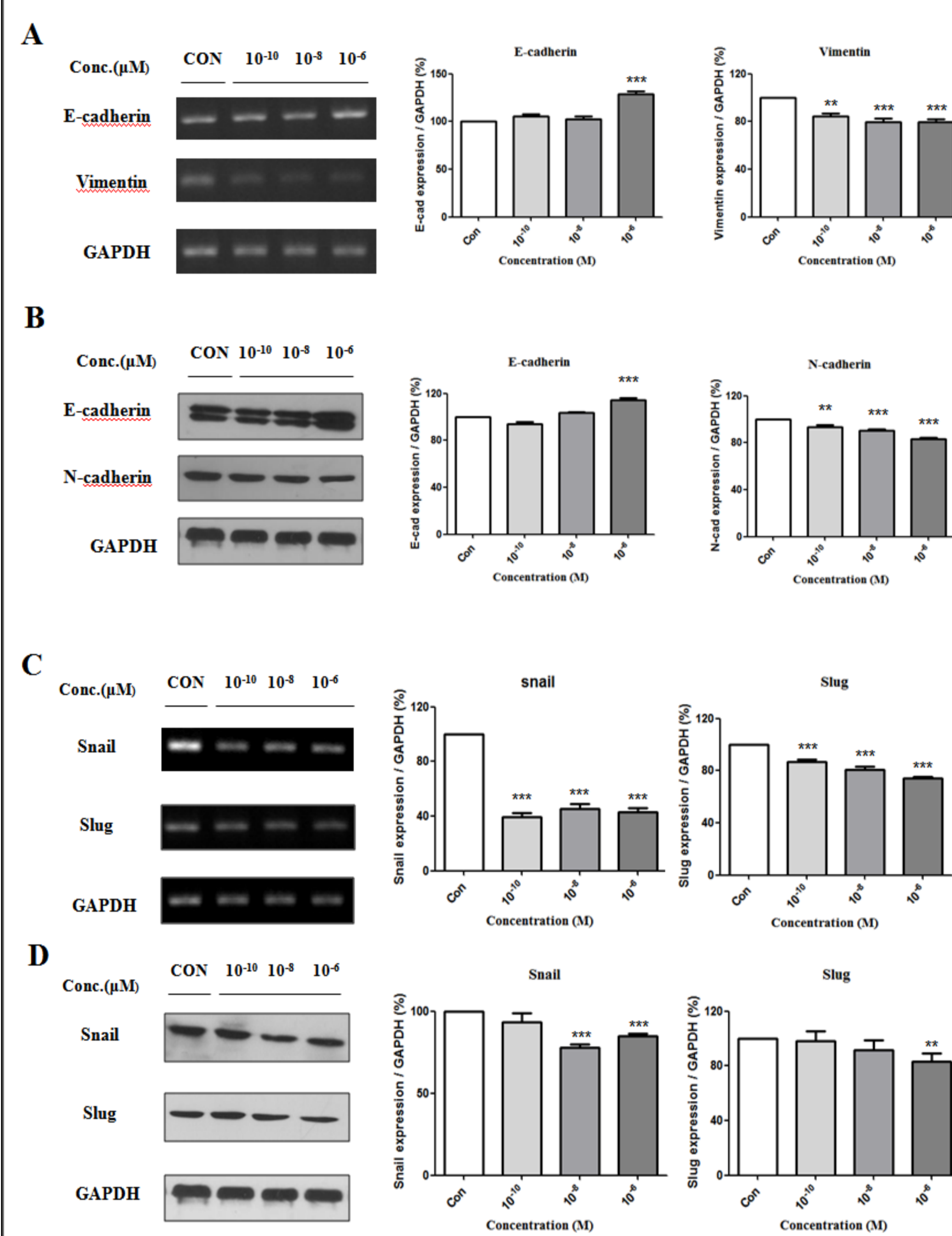


**Figure 3. The effects of Progesterone on the expression of EMT related genes.** BG-1 cells were treated with E2 ($10^{-8}$ M) as a vehicle. The effects of Progesterone ($10^{-10}$ M, $10^{-8}$ M, $10^{-6}$ M ) was investigated on the expression of (A, B) EMT specific marker (E-cadherin and N-cadherin) in BG-1 cells by western blot and RT-PCR. (C, D) EMT regulator genes (snail and slug) in BG-1 cells by western blot and RT-PCR.



## RESULTS

**Figure 4. The effects of Progesterone on the expression of Cell cycle related genes and un-differentiation cell marker.** BG-1 cells were treated with E2 ($10^{-8}$ M) as a vehicle. The effects of Progesterone ($10^{-10}$ M, $10^{-8}$ M, $10^{-6}$ M) was investigated on the expression of (A, B) cell cycle related genes (Cyclin D, Cyclin E, p27, p27) in BG-1 cells by western blot (C, D) un-differentiation cell marker (Oct-4, Sox-2 and Nanog) in BG-1 cells by western blot.
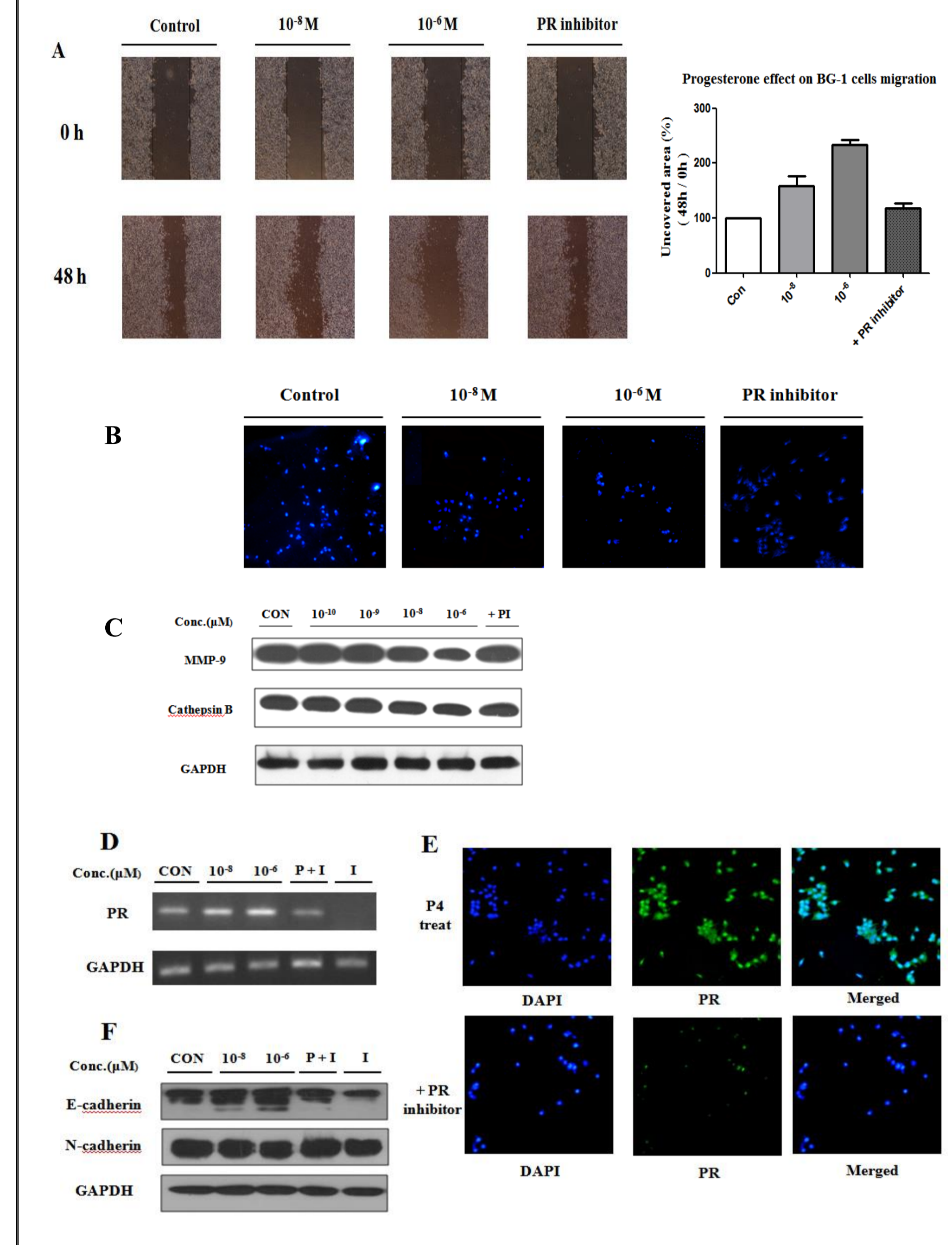


**Figure 5. The functional role of PR in EMT on BG-1 ovarian cancer cells.** A, B The effects of P4 was investigated on the migratory and invasive ability in BG-1 cells by wound healing assay and invasion assay. C, The effects of P4 was investigated on the expression of (C) invasion related marker (MMP-9 and Cathepsin B) in BG-1 cells by western blot. BG-1 cells were co-treated with E2+P4 and RU-486, (D) PR expression in BG-1 cells was investigated by (E) RT-PCR and (F) Immunocytochemistry.

## CONCLUSIONS

The results showed that P4 inhibited the migration and invasion activity of BG-1 ovarian cancer cells, which was increased by E2 via its receptor interaction. These alterations were also related with changes in the epithelial-mesenchymal transition (EMT) markers such as E-cadherin, Vimentin, and N-cadherin and EMT-associated transcriptional factors, Snail and Slug, too. Upon P4 stimulation, the expression of the epithelial marker E-cadherin was strikingly increased, whereas the expression of mesenchymal makers like N-cadherin and Vimentin was decreased. EMT-associated transcriptional factors, Snail and Slug, were also significantly down-regulated. These results indicate that P4 can inhibit the migration of BG-1 ovarian cancer cells by reducing EMT. Consequently, the present results represent that P4 is a potent substance which may inhibit the growth of human ovarian cancer cells and metastasis via regulation of PR. Therefore, the hormone therapy using P4 may be a clinically effective tool for the treatment of human epithelial ovarian cancer.

## REFERENCES

[1] Kenny HA ea al. (2009) MMP-2 functions as an early response protein in ovarian cancer metastasis. Cell cycle 12009;8 : 683-8

[2] Ovarian tumorigenesis : A proposed model based on morphological and molecular genetic analysis. AM J Pathol 12004; 164 : 1511-8