# 1α,25-dihydroxyvitamin D3 restrains stem-like properties of ovarian cancer cells by enhancing VDR and suppressing CD44

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*The establishment of the spontaneous neoplastic transformation of MOSE cells in vitro*. We first isolated primary mouse ovarian surface epithelium MOSE cells from mouse ovary and cultured them *in vitro*. The MOSE showed typical cobblestone-like phenotype and maintained contact inhibition of growth. The culture media used in the first 20 passages consisted of a 1:1 mixture of fresh and conditioned medium of existing cultures, which was different from previous report (1,2) but proved to be crucial to maintain the cultures. MOSE cells maintained proliferation and underwent spontaneous neoplastic transformation when subcultured for more than 80 passages *in vitro*. The capacity of anchorage-independent growth was determined, which in an *in vitro* hallmark of neoplastic transformation of cells (1). Early (<20 passages) and intermediate (21-80 passages) MOSE cells were unable to form colonies in soft agar. Notably, late-passage (>81) MOSE cells were capable of forming >30 mm colonies. Compared with MOSE cells at earlier stages, late-passage MOSE cells exhibited an increased plating efficiency and growth rate, another proliferative parameter that is often associated with neoplastic change (2,3). Furthermore, the tumor formation rates were significantly increased *in vivo*. M-L cells formed tumors up to 100%, whereas M-E and M-I cells did not form tumors. Therefore, three sequential stages of transformed MOSE cells were defined as M-E (≤20 passages; Early), M-I (21-80 passages; Intermediate) and M-L (≥81 passages; Late) cells, respectively.

*Detection of mycoplasma contamination*. Mouse ovarian surface epithelium (MOSE) cells were tested for mycoplasma contamination using the PCR Mycoplasma Detection Set (Takara Bio, Inc., Otsu, Japan). Briefly, liquid supernatant of the MOSE cells was used to test for mycoplasma contamination. In the first round of polymerase chain reaction (PCR), 25 $\mu$l reactions were set up containing 2.5 $\mu$l cell supernatant, 12.5 $\mu$l MIX (TaKaRa Taq; RR001A), 0.25 $\mu$l MCGp F1 primer, 0.25 $\mu$l MCGp R1 primer and 9.5 $\mu$l $ddH_2O$. Thermocycling conditions were as follows: 94˚C for 30 sec; followed by 35 cycles of 94˚C for 30 sec, 55˚C for 2 min and 72˚C for 1 min. A second round of PCR was subsequently performed using 0.25 $\mu$l of the first round PCR product, 12.5 $\mu$l MIX, 0.25 $\mu$l MCGp F2 primer, 0.25 MCGp R2 primer and 1.75 $\mu$l $ddH_2O$. Thermocycling conditions for the second round PCR were as follows: 94˚C for 30 sec; followed by 30 cycles of 94˚C for 30 sec, 55˚C for 2 min, 72˚C for 1 min. PCR products were separated by 1% agarose electrophoresis and were stained by 0.5 $\mu$g/ml ethidium bromide (Fig. S1).

*Detection of human DNA contamination*. MOSE cells also were tested for human DNA contamination. The DNA of $1x10^5$ MOSE cells was extracted using E.Z.N.A.™ DNA/RNA/Protein Isolation kit (OMEGA Bio-Tek, Inc., Norcross, GA, USA) and dissolved in Nuclease free water, following the manufacturer's protocol. Human and mouse DNA in the extracts were detected by PCR, using DreamTaq PCR Master Mix (cat. nos. K1071; Fermentas; Thermo Fisher Scientific, Inc., Waltham, MA, USA) in a 50 $\mu$l reaction set up using the components listed in Table SI. Human DNA PCR Primer (Invitrogen; Thermo Fisher Scientific, Inc.): Forward 5'-TGTGGAAGTCAGTGTGGCGA-3', reverse 5'-AGGGACATGGATGAAATTGG-3'. Mouse DNA PCR primer (Invitrogen; Thermo Fisher Scientific, Inc.): Forward 5'-GTCTTCACCACCATGGAGAAGGCT-3', reverse 5'-TGTAGCCCAGGATGCCCTTTAGTG-3'. Thermocycling parameters were as follows: 95˚C for 15 min; followed by 30 cycles of 94˚C for 30 sec, 60˚C for 45 sec and 72˚C for 45 sec, and a final extension at 72˚C for 5 min. PCR products were separated by 1% agarose electrophoresis and were stained by 0.5 $\mu$g/ml ethidium bromide and the product results were compared to both positive [Control Human genomic DNA (cat. no. G3041; Promega Corporation, Madison, WI, USA); control mouse genomic DNA (cat. no. G3091; Promega Corporation) and negative controls (no DNA). The results demonstrated that there was no human DNA contamination in MOSE cells (Fig. S2).

## References

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Figure S1. Detection of mycoplasma contamination for MOSE cells.

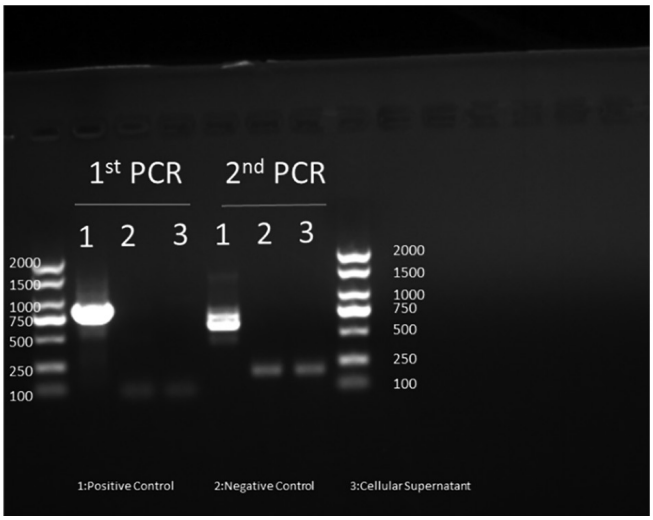

Figure S2. Detection of human DNA contamination for MOSE cells.

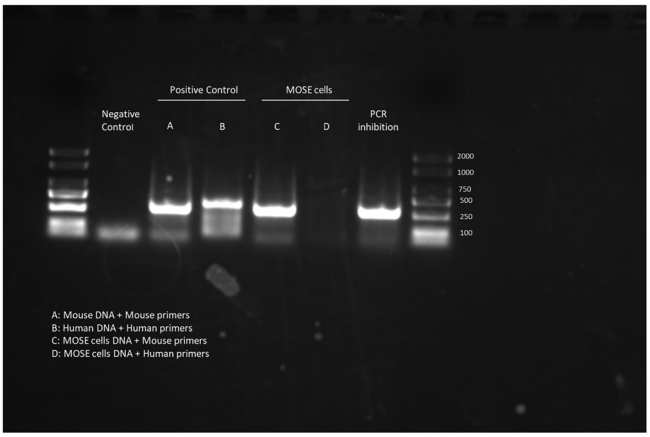

Table SI. The PCR reaction system for human DNA contamination detection.

| Reaction | 2X PCR Master Mix | Human primers[a] | Mouse primers[a] | DNA-free water | Sample DNA | Human DNA | Mouse DNA |
|---|---|---|---|---|---|---|---|
| Negative control | 25 $\mu$l | 0.2 $\mu$M | 0.2 $\mu$M | 10 $\mu$l | - | - | - |
| Positive control (1) | 25 $\mu$l | - | 0.2 $\mu$M | - | - | - | 30 pg |
| Positive control (2) | 25 $\mu$l | 0.2 $\mu$M | - | - | - | 30 pg | - |
| MOSE cell | 25 $\mu$l | - | 0.2 $\mu$M | - | 10 $\mu$l | - | - |
| MOSE cell | 25 $\mu$l | 0.2 $\mu$M | - | - | 10 $\mu$l | - | - |
| PCR inhibition | 25 $\mu$l | 0.2 $\mu$M | 0.2 $\mu$M | - | 10 $\mu$l | 30 pg | 30 pg |

[a]The same concentration of forward and reverse primers was used.