Materials and methods

Normoxia, Hypoxia and Mimic Hypoxia

For cell culture conditions, cells were grown in a humidified atmosphere containing either 5% CO_2 plus 20% O_2 (Normoxia) or 5% CO_2 plus 2% O_2 with the balanced N_2 (Hypoxia). For mimic Hypoxia drug treatment, cells were grown in a humidified atmosphere containing either 5% CO_2 plus 20% O_2 (Normoxia). Once cells reached 100% conflunce, they were treated with Cobalt(II) chloride hexahydrate 200uM for different time points.

Antibodies

Rabbit anti-human b-actin (#AV40173), and goat anti-human Vimentin (no.V4630) antibodies were purchased from Sigma-Aldrich (St Louis, MO, USA). Mouse anti-human MyD88 antibody (sc-74532), Mouse anti-human TWIST1 (sc-81417), and Rabbit anti-human E12 (sc-349) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Rabbit anti-human beta-Catenin (no. 9582), Rabbit anti-human Slug (no. 9585) antibodies were purchased from Cell Signaling Technology (Danvers, MA, USA). Mouse anti-human Vimentin antibody (no.550513) was purchased from BD Bioscience (San Diego, CA, USA). Rabbit anti-human HIF1a antibody (no. NB100-134) was purchased from Novus Biologicals (Littleton, CO, USA). Cobalt(II) chloride hexahydrate (sc-203004) was purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). CD44

antibody (#11-0441) for flow cytometry was purchased from eBioscience (San Diego, CA, USA), and CK18 antibody (no. 4548) for flow cytometry was purchased from Cell Signaling Technology (Danvers, MA, USA).

SDS–polyacrylamide gel electrophoresis and western blots

A quantity of 20 ug of each protein sample was denatured in sample buffer and subjected to 10%-12% SDS–polyacrylamide gel electrophoresis as previously described (Kamsteeg et al 2003, Mor et al 2002, Sapi et al 2004). The following antibody dilutions were used: mouse anti-human MyD88 (1:1000), rabbit anti-human beta-Catenin (1:1000), mouse anti-human TWIST1 (1:500), goat anti-human Vimentin (1:1000), mouse anti-human Vimentin antibody (1:1000), Rabbit anti-human E12 (1:1000) , rabbit anti-human Slug (1:1000), rabbit anti-human HIF1a (1:1000), rabbit anti-human b-actin (1:10 000). Specific protein bands were visualized using enhanced chemiluminescence (Pierce Biotechnology).

Immunohistonchemistry and Immunofluorescence Staining

For immunohistochemistry experiments, Tumor tissue from nude mice were embedded in paraffin and 5-mm sections were cut. Sections were deparaffinized and stained with hematoxylin and eosin (H&E). GFP fluorescence was captured by fluorescent microscope. For immunoflurence experiments, epithelial ovarian cells were seeded on four-chamber tissue culture glass slide (BD, #354104), and wait for early (clumps formation) or late differentiation (spheroid formation). Cells were fixed for 15 min with 4% paraformaldehyde, permeabilized for 10 min with

ice-cold methanol, washed with PBS, and blocked with 10% goat serum in PBS for 30min. Immunostaining was performed using mouse anti-human TWIST1 (Santa Cruz, sc-81417) as a primary antibody, and Alexa fluor546 (Invitrogen, #A11003) as a secondary antibody. Images were acquired using a Zeiss flurence microscope and were prepared by Zeiss LSM Image Browser software.

mRNA quantitative RT–PCR

Total RNA was prepared from EOC stem cell lines, spheroids and mature EOC cells using the TRIZOL reagent (Invitrogen). Total RNA isolated from each samples was then used as a template for cDNA synthesis, prepared with a first-strand cDNA synthesis kit (Amersham Biosciences) for mRNA. The expression of various transcripts was assessed by real-time PCR amplification (95 ℃ for 10 min; (95 ℃ for 15 s, 60 ℃ for 1 min; 40 cycles); and 72℃ for 10m) with Real-Time SYBR Green/Rox PCR supermix (Invitrogen), using the BioRad CFX Cycler (BioRad, Hercules, CA, USA). The primer sets used in this study are list in supplemental table 1. All PCR reactions were carried out in triplicates and validated by the presence of a single peak in the melt curve analysis. Changes in gene expression were calculated relative to GAPDH using the $2^{-\Delta\Delta Ct}$ method (Schmittgen and Livak 2008).

Figure Legend

Supp. Figure 1. CD44+/MyD88+ EOC stem cells give origin to CD44-/MyD88- EOC cells. GFP+/CD44+/MyD88+ EOC stem cells before and after undergoing differentiation. The morphologic characteristics of the cells can be observed by florescence microscopy and phase contrast. Figures are representative of at least 3 independent experiments.

Supp. Fig. 2. Differentiation induces invasion capacity. Mesenchymal spheroid-forming cells (MSFCs) derived from CD44+/MyD88+ EOC stem cells acquire enhanced invasion potential compared to progenitor EOC stem cells.

Supp. Fig 3. Morphological analysis of CD44+/MyD88+ Type I EOC stem cells undergoing EMT.

(A) CD44+/MyD88+ EOC stem cells acquire characteristics of fibroblast/mesenchymal cells; (B) eventually rounding-up and losing attachement; (C) finally forming viable spheroids similar to those observed in patient ascites; *E*, epithelial; *M*, mesechymal; MSFCs, mesenchymal-forming spheroid cells; yellow arrows point to cells losing epithelial morphology and acquiring fibroblast-like morphology. Figures are representative of at least six independent experiments

Supp. Fig. 4. EMT and MET process in CD44+/MyD88+ EOC stem cells. GFP+/CD44+/MyD88+ EOC stem cells can give origin to MSFCs via EMT, which can undergo MET to form GFP+/CD44-/MyD88- EOC cells. The linage is demonstrated by the maintenance of GFP through the differentiation process.

Supp. Fig. 5. Histological evaluation of tumors originated from CD44+/MyD88+ EOC stem cells or MSFCs. Resulting xenografts have identical morphology.

Supp. Fig. 6. MSFCs form secondary monolayer cells with epithelial morphology. MSFCs were plated on regular tissue culture plates and observed (A) 12 hours; (B) 24h; (C) 48h; and (D) 72h.

Supp. Table.1. Primers for pFUIGW-*TWIST1* construction and real-time PCR.

Restriction enzyme recognition sites and cutting protect nucleotides were underlined, and the open reading frame were shown in uppercase letters. The real-time PCR primers are shown in lowercase letters.

Supp. Table.2. Gene expression profile of EMT array

Red shows up-regulation; Green shows down-regulation.

Reference.

Kamsteeg M, Rutherford T, Sapi E, Hanczaruk B, Shahabi S, Flick M *et al* (2003). Phenoxodiol--an isoflavone analog--induces apoptosis in chemoresistant ovarian cancer cells. *Oncogene* **22:** 2611-2620.

Mor G, Straszewski S, Kamsteeg M (2002). Role of the Fas/Fas ligand system in female reproductive organs: survival and apoptosis. *Biochem Pharmacol* **64:** 1305.

Sapi E, Chen W, O'Malley D, Hao X, Dwipoyono B, Garg M *et al* (2004). Resistance of Ovarian Cancer Cells to Docetaxel is XIAP Dependent and Reversible by Phenoxodiol. *Anti-Cancer Drugs* **14:** 567-578.

Schmittgen TD, Livak KJ (2008). Analyzing real-time PCR data by the comparative C(T) method. *Nat Protoc* **3:** 1101-1108.