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Aberrant expression of genes associated with stemness and cancer in endometria and endometrioma in a subset of women with endometriosis

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STUDY QUESTION: Is there molecular evidence for a link between endometriosis and endometriosis-associated ovarian cancers (EAOC)?

STUDY ANSWER: We identified aberrant gene expression signatures associated with malignant transformation in a small subgroup of women with ovarian endometriosis.

WHAT IS KNOWN ALREADY: Epidemiological studies have shown an increased risk of EAOC in women with ovarian endometriosis. However, the cellular and molecular changes leading to EAOC are largely unexplored.

STUDY DESIGN, SIZE, DURATION: *CD73*$^+$*CD90*$^+$*CD105*$^+$ multipotent stem cells/progenitors (SC cohort) were isolated from endometrium (n = 18) and endometrioma (n = 11) of endometriosis patients as well as from the endometrium of healthy women (n = 14). Extensive phenotypic and functional analyses were performed *in vitro* on expanded multipotent stem cells/progenitors to confirm their altered characteristics. Aberrant gene signatures were also validated in paired-endometrium and -endometrioma tissue samples from another cohort (Tissue cohort, n = 19) of endometriosis patients.

PARTICIPANTS/MATERIALS, SETTINGS, METHODS: Paired-endometrial and -endometriotic biopsies were obtained from women with endometriosis (ASRM stage III–IV) undergoing laparoscopic surgery. Control endometria were obtained from healthy volunteers. Isolated *CD73*$^+$*CD90*$^+$*CD105*$^+$ SC were evaluated for the presence of known endometrial surface markers, colony forming efficiency, multi-lineage differentiation, cell cycle distribution and 3D-spheroid formation capacity. Targeted RT-PCR arrays, along with hierarchical and multivariate clustering tools, were used to determine both intergroup and intragroup gene expression variability for stem cell and cancer-associated markers, in both SC$^+$ and tissue cohorts.

MAIN RESULTS AND THE ROLE OF CHANCE: Isolated and expanded SC$^+$ from both control and patient groups showed significantly higher surface expression of *W5C5*$^+$, clonal expansion and 3D-spheroid formation capacity ($P < 0.05$) compared with SC$^-$. The SC$^+$ cells also undergo mesenchymal lineage differentiation, unlike SC$^-$. Gene expression from paired-endometriosis samples showed significant down-regulation of *PTEN*, *ARID1A* and *TNFα* ($P < 0.05$) in endometrioma compared with paired-endometrium SC$^+$ samples. Hierarchical and multivariate clustering from both SC$^+$ and tissue cohorts together identified 4 out of 30 endometrioma samples with aberrant expression of stem

cell and cancer-associated genes, such as *KIT*, *HIF2α* and *E-cadherin*, altered expression ratio of *ER-β/ER-α* and downregulation of tumour suppressor genes (*PTEN* and *ARID1A)*. Thus, we speculate that above changes may be potentially relevant to the development of EAOC.

LARGE-SCALE DATA: N/A.

LIMITATIONS, REASON FOR CAUTION: As the reported frequency of EAOC is very low, we did not have access to those samples in our study. Moreover, by adopting a targeted gene array approach, we might have missed several other potentially-relevant genes associated with EAOC pathogenesis. The above panel of markers should be further validated in archived tissue samples from women with endometriosis who later in life developed EAOC.

WIDER IMPLICATIONS OF THE FINDINGS: Knowledge gained from this study, with further confirmation on EAOC cases, may help in developing screening methods to identify women with increased risk of EAOC.

STUDY FUNDING/COMPETING INTEREST(S): The study is funded by the Swedish Research Council (2012-2844), a joint grant from Stockholm County and Karolinska Institutet (ALF), RGD network at Karolinska Institutet, Karolinska Institutet for doctoral education (KID), Estonian Ministry of Education and Research (IUT34-16), Enterprise Estonia (EU48695), Horizon 2020 innovation program (WIDENLIFE, 692065), European Union's FP7 Marie Curie Industry-Academia Partnerships and Pathways funding (IAPP, SARM, EU324509) and MSCA-RISE-2015 project MOMENDO (691058). All authors have no competing interest.

Key words: endometriosis / ovarian cancer / multipotent stem cells / gene expression regulation / cancer-associated gene / multicellular spheroids

Introduction

Endometriosis is a common, oestrogen-dependent, gynaecological disorder reported in ~10% of women in reproductive age and associated with chronic pelvic pain and infertility. Endometriosis is generally considered a benign condition, although these lesions sometimes exhibit aberrant characteristics such as enhanced growth, neo-angiogenesis, local deep invasion and cell migration (Munksgaard and Blaakaer, 2012).

A small group of women with endometriosis (~0.7–2.5%) has an increased risk for developing certain ovarian cancer subtypes; namely, clear cell, endometrioid invasive and low-grade serous ovarian cancers (Pearce *et al.*, 2012; Gadducci *et al.*, 2014). Likewise, pooled meta-analysis from several epidemiological studies on the above types of endometriosis-associated ovarian cancer (EAOC) show an increased risk (OR: 1.32–1.92) for women with endometriosis (Kryczek *et al.*, 2012). A variety of cancer-related somatic mutations have been previously identified in endometrioma, resulting in silencing of tumour suppressor genes *TP53* (Sainz de la Cuesta *et al.*, 2004), *PTEN* and *ARID1A*; activation of oncogenes *KRAS*, *PIK3CA*, *CTNNB1* (Gadducci *et al.*, 2014) and *PPP2R1A* (Anglesio *et al.*, 2017) and downregulation of *BCL2* (Nezhat *et al.*, 2002). Nevertheless, the cellular and molecular changes that precede the above aberrations, and the potential risk towards EAOC, are not fully understood (Pollacco *et al.*, 2012).

The stem cell theory for development of endometriosis suggests that endometrial stem cells/progenitors (SC) with altered molecular properties reflux via retrograde menstruation into the abdominal cavity where they adhere and form ectopic lesions. The presence of SC in menstrual blood (Musina *et al.*, 2008), peritoneal fluid (Dorien *et al.*, 2017) and endometriotic lesions (Gargett *et al.*, 2014) in women with endometriosis has engaged researchers to explore the molecular link between actively regenerating endometrial SC and endometriosis (Cheng *et al.*, 2017). Nevertheless, the direct evidence for the role of SC in endometriosis or malignant transformation of endometrial cells, which might lead towards the onset of EAOC, is still missing.

Herein, we hypothesize that a subpopulation of multipotent SC within endometrioma or endometrium of certain endometriosis patients may undergo aberrant alterations within cancer-associated genes, which may in turn increase the future risk of EAOC. Therefore, we aimed to screen the expression levels of a selected panel of endometrial or ovarian cancer-associated gene markers in isolated endometrial multipotent stem cells/progenitors as well as in a separate cohort of whole endometrial and endometrioma tissue biopsies.

Materials and Methods

Patient data

Endometrial samples (P-En, $n = 37$) and their paired-endometriotic lesions (Endo, $n = 30$) were collected from endometriosis patients during laparoscopic surgery from both Karolinska University Hospital, Sweden and Tartu University Hospital's Women's clinic, Estonia. All the patients recruited had not received any hormonal medications for at least 3 months before surgery and were verified by a pathologist for indications of moderate to severe endometriosis (stages III–IV, classified according to ASRM guidelines (American Society for Reproductive Medicine: Revised classification of endometriosis, 1997)). The patients' median age was 33.9 ± 5.6 years (years ± SD) and BMI was 22.29 ± 3.51 kg/m^2.

Endometrial biopsies (H-En) were collected from healthy women as controls at Karolinska University Hospital for both isolating SC (secretory phase, $n = 14$) as well as for whole tissue protein analysis (proliferative phase, $n = 5$). All above volunteers were within their fertile age (≤40 years), with normal BMI (within a range of 19–25), regular menstruation, had at least one live-born child and were clinically examined for the absence of hormonal diseases or uterine pathologies such as endometriosis, polycystic ovary syndrome and/or previous infertility records. In addition, control ovarian tissue biopsies ($n = 4$) were obtained from Tartu University Hospital's Women's clinic for evaluation of ovarian specific markers.

Ethical approval

The regional ethics committee at Stockholm, Sweden and at Tartu, Estonia approved the study. Written informed consent was obtained from all enrolled participants.

Endometrial stem cell isolation

Endometrial cell isolation from both patients and control samples (P-En, Endo and H-En; SC cohort) were performed according to our standard protocol, as previously described (Lalitkumar *et al.*, 2013). Briefly, endometrial tissues were homogenized, treated in sequential steps of pancreatin—0.05% trypsin enzymatic solution, collagenase 4 (0.1 U/ml) and DNaseI (16 µg/ml) solution in Ca^{2+}/Mg^{2+}-free PBS (Gibco® Thermo Fisher Scientific, Sweden) and incubated for 30 min at each step. However, thick endometriotic fibrous tissue (Endo) weighing ~200–300 mg required an altered enzymatic digestion mixture containing collagenase/dispase (Collagenase: 0.1 U/ml, Dispase: 0.8 U/ml, Roche Diagnostics) and DNaseI (16 µg/ml) with longer incubation time (60–90 min). Cell suspensions from both procedures were then expanded *in vitro* for two generations. Later, cells positive for mesenchymal stem cell markers namely CD73-APC (BD Pharmingen, USA), CD90-FITC (Abcam, UK) and CD105-PE (Abcam, UK) were sorted as SC^+ and populations negative for above markers as SC^- using MoFlow® XDP flow cell activated cell sorter (Beckman Coulter, USA). Extensive characterization was performed on both expanded SC^+ and SC^- cell populations to reveal their multipotent characteristics and anomalous proliferative behaviour among patients' SC^+ samples. The overall study design was presented in Fig. 1.

Colony formation and multi-lineage differentiation

Sorted and expanded SC^+ and SC^- cells from H-En, P-En and Endo were seeded as triplicates at a concentration of 100 cells/cm^2 in six-well plates and cultured for 2 or 3 weeks. Colonies were briefly fixed with acetic acid: methanol (1:7 vol/vol) and incubated with 0.5% Crystal violet solution (1:3 vol/vol). Colony forming efficiency was calculated as a percentage using the formula (number of colonies observed/number of cells seeded) × 100. Moreover, the above samples were plated as triplicates on cell culture slides (BD Falcon™) and induced to differentiate into mesenchymal lineages such as adipocytes, osteocytes and chondrocytes for 3 or 4 weeks, using commercially available Stem Pro® Adipogenesis, Osteogenesis and Chondrogenesis differentiation kits (Gibco® Thermo Fisher Scientific, Sweden), respectively. Later, culture slides were visualized via immunofluorescence using HCS LipidTOX™ reagent (Molecular Probes® Thermo Fisher Scientific, Sweden) or antibodies against osteocalcin or aggrecan proteins (R&D Systems, Sweden), respectively.

Cell cycle analysis

Expanded SC^+ cultures were evaluated for their proliferative activity and cell cycle distribution. Cells were pulsed with 10 µM bromodeoxyuridine (BrdU, Sigma Aldrich, USA) mixed in culture medium (DMEM/F12 and 10% MSC–FBS) and incubated at 37°C for 60 min, allowing BrdU to incorporate into the DNA of actively proliferating cells. SC^+ cultures were washed, fixed with 4% formaldehyde, washed in 95% ethanol and incubated with 200 ng/ml protease XXIV (Sigma Aldrich, USA). Later, cell distribution in different cell cycle phases were visualized with anti-BrdU-PE

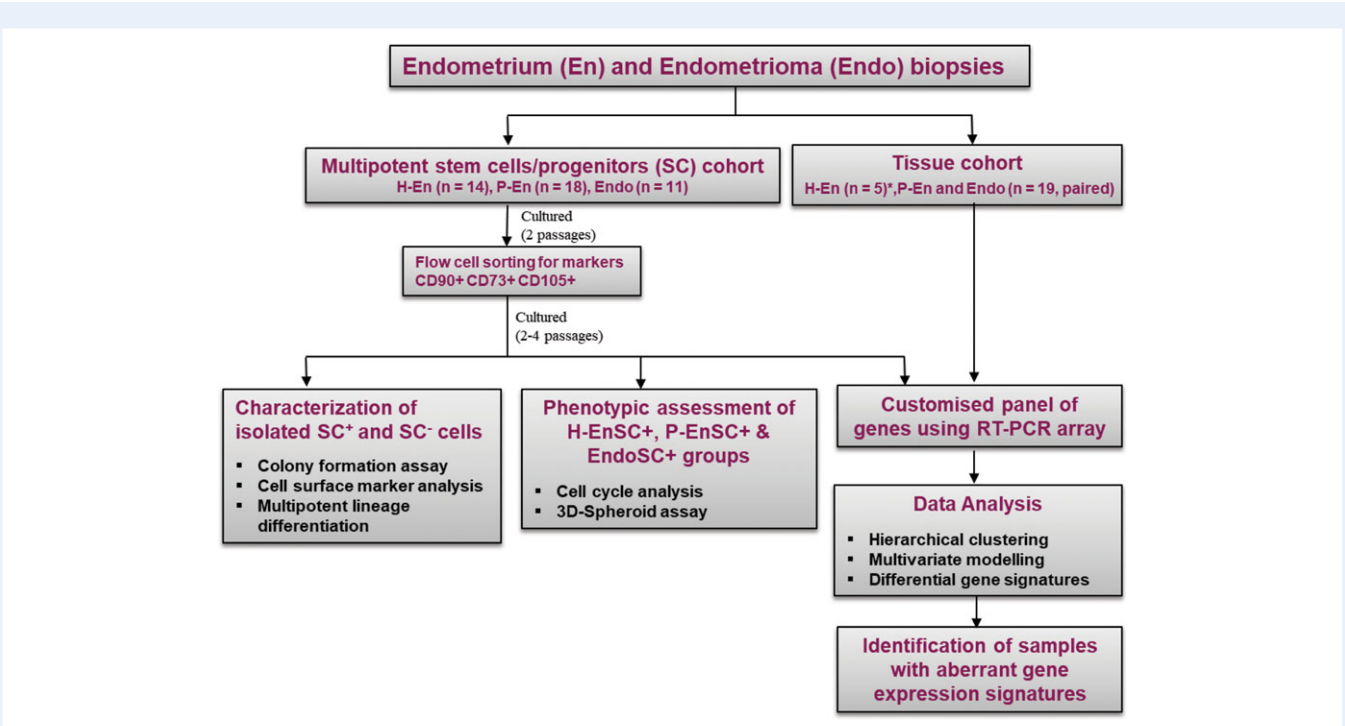

Figure 1 Study work-flow for identification and characterization of cancer-associated gene signatures in endometriosis patients. Healthy and patients' endometrial (H-En and P-En) and endometrioma (Endo) biopsies originated from two independent cohorts of endometriosis patients, named as multipotent stem cells/progenitors (SC) cohort and tissue cohort. SC^+ and SC^- denote positive and negative fractions from sorted endometrial/endometriotic cell populations using markers CD73, CD90 and CD105. Symbol *denotes control samples used only for protein analysis.

(BD Biosciences, USA) and 4′,6-diamidino-2-phenylindole (DAPI) using BD LSRII flow cytometry (BD Biosciences, USA). Ovarian (SKOV3) and endometrial (Ishikawa) cancer lines were used as positive control. Cell distributions were enumerated as percentages in different phases of the cell cycle using pseudo-dot plots generated by flow cytometry.

3D-spheroid SC^+ cultures

To assess self-renewal capacity, pluripotency and de-differentiation potential, expanded monolayer SC^+ cells (4000 cells/cm^2) from H-EnSC, P-EnSC and EndoSC groups were able to form suspension 3D-spheroids when plated on to ultra-low attachment six-well plates (Corning® Thermo Fischer Scientific Inc., USA) and cultured with sphere enrichment medium containing DMEM/F12, growth factors EGF, bFGF (10 ng/ml each, Invitrogen® Thermo Fisher Scientific, Sweden), B27 supplement and Insulin–Transferrin–Selenium (Gibco® Thermo Fisher Scientific, Sweden), as well as 2 nM progesterone (Sigma Aldrich, USA). 3D-Spheroid suspensions were allowed to grow up to a size larger than 50 µm and harvested 5–7 days after the onset of culture. Spheroids were dissociated, single cells were reseeded with the above conditions and later harvested for downstream applications.

Differential gene expression and data analysis

To evaluate the changes in gene expression at the cellular and, more broadly, tissue level, patients' samples were sub-divided into two independent cohorts: (i) the multipotent stem cell cohort (SC^+ cohort, paired-endometrium and -endometrioma samples collected at secretory phase, $n = 11$ and unpaired endometria, $n = 7$) for cell culture experiments and (ii) whole tissue biopsies from paired-endometrium and -endometrioma (named as Tissue cohort; proliferative phase, $n = 19$). All samples were stored in RNA*later* for gene expression analysis. RNAs were extracted using either miRNeasy Mini kit (Qiagen) or Purelink® RNA isolation kit (Invitrogen®, Thermo Fisher Scientific, Sweden), respectively. cDNAs were synthesized with SuperScript® VILO™ kit (Invitrogen®, Thermo Fisher Scientific, Sweden). Pre-diluted cDNA were loaded into customized RT-PCR array using 42 pre-selected TaqMan™ gene expression probes (Applied Biosystems™ Thermo Fisher Scientific, Sweden) known to represent stem cell and cancer-associated markers, based on previous publications (Supplementary Table S1) and quantified using StepOne Plus real time PCR (Applied Biosystems™ Thermo Fisher Scientific, Sweden). Fold changes (FC) between the groups were calculated using the formula $2^{-\Delta CT\ (\text{P-EnSC or EndoSC})}/2^{-\Delta CT\ (\text{H-EnSC})}$ in accordance to comparative C_T method (Schmittgen and Livak, 2008). Heat-maps with hierarchical clustering were generated with the Morpheus online tool (Broad Institute Inc.) using gene expression patterns from a panel of genes and applying Euclidean neighbouring distance method. Later, group variability was assessed with multivariate modelling, orthogonal partial least squares-descriptive analysis (OPLS-DA) using SIMCA 14 software (Umetrics AB, Umea, Sweden).

Flow cytometry analysis

Phenotypic characterization of sorted SC^+ and SC^- fractions were performed using cell surface markers, anti-human antibodies CD44-PE, CD146-PerCP-cy5.5, PDGFRβ/CD140b-PE and SUSD2/W5C5-APC (Biolegend, USA), EPCAM-PE (Miltenyi Biotec, Germany), ABCG2-PerCP-cy5.5 (Biolegend, USA), SSEA1-Alexa Fluor 488 (Santa Cruz Biotec.) and CD45-APC (BD Pharmingen, USA). Also, *in vitro* monolayer SC^+ cultures and respective 3D-spheroid cultures from H-En, P-En and Endo groups were compared for expression of ALDH1 enzyme by ALDEfluor assay (Stem cell technologies, Canada) and anti-human antibodies CD133-1-APC (Miltenyi Biotec, Germany), CD44-PE (Biolegend, USA) and CD117-PE-cy7 (Biolegend, USA). Percentage expression of these surface markers as well as their co-expression were calculated with respect to total live cells and presented using FlowJo data analysis software (LLC, Oregon).

Confocal imaging

Spheroid SC+ cultures were checked for co-expression of stem cell markers using dual colour immunofluorescence. Spheres were fixed with 4% paraformaldehyde, washed, blocked and permeabilized with 2% BSA in 0.1% Triton. Representative samples from each group were stained with primary antibodies anti-human OCT3/4 antibody (Santa Cruz Biotechnology, USA), CD44v6-Alexa Fluor 488 (Biolegend, USA) and rabbit polyclonal PROM1/CD133 (Biorbyt, UK) overnight at 4°C and later tagged with secondary antibodies, donkey anti-mouse Alexa Fluor 488 (Molecular Probes® Life technologies) and goat anti-rabbit Abberior® STAR633 (Abberior). The SKOV3 ovarian cancer line was used as positive control. Images were captured using Zeiss LSM 700 confocal microscopy (Carl Zeiss, Japan).

Automated western blot

Protein levels were studied in both cohorts (SC and Tissue cohorts) along with the positive control MCF7 breast cancer line (kind gift from Johan Hartman, Karolinska Institutet) using automated western blot (Protein Simple, San Jose, CA). Briefly, 0.4 mg/ml of protein from either multipotent SC^+ or tissue lysates were mixed with fluorescent master-mix (Protein Simple) and loaded into pre-designed Wes assay plates along with primary antibodies, mouse anti-human E-cadherin (MAB1838, R&D systems), ER-alpha/NR3A1 (clone:68118) and ER-beta/NR382 (Clone:733930) (both from Novus Biologicals), beta actin (Cell signalling) and other components as per the manufacturer's instructions. Samples were drawn automatically by Simple Wes machine into pre-designed capillaries and proteins separated by capillary electrophoresis. Detections were made with either streptavidin or secondary anti-mouse-HRP followed by luminol peroxide mix (Protein Simple, USA). Image analysis was performed using Compass software (Protein Simple, USA).

Statistical analysis

GraphPad Prism 6 and 7 softwares (GraphPad Software Inc., USA), were used for statistical analysis and illustrations. Datasets were checked for Gaussian distribution and homogeneity of variance using Shapiro–Wilk's normality test. *T*-test (paired/unpaired, parametric), Wilcoxon signed *T*-test (paired, non-parametric) or Mann–Whiteney test (unpaired, non-parametric) were used for comparisons involving two groups, while one- or two-way Anova (paired, parametric) or Kruskal–Wallis test (unpaired, non-parametric) were used for multiple group comparisons. *P*-values were adjusted for false discovery using the Benjamini, Krieger and Yekutieli method.

Results

Evaluating endometriosis tissues for expression of Mullerian marker

To assess whether the endometrioma samples used in this study are free from ovarian cells, we looked into the gene expression levels between paired-endometrium (P-En) and -endometrioma (Endo) samples from endometriosis women (tissue cohort, $n = 16$) as well as healthy ovarian tissues ($n = 4$) for the well-known ovarian tissue marker, anti-Mullerian hormone receptor-II (*AMHRII*). Previously, *AMHRII* was reported to be highly expressed in endometriotic tissues compared with endometrium both with and without endometriosis

(Carrarelli *et al.*, 2014). In line with this observation, Endo tissue samples showed significant upregulation compared with P-En (FC: 852.37; $P < 0.0001$; Fig. 2A). Interestingly, Endo showed a significantly lower expression level compared with ovarian tissues (FC: 3.36; $P < 0.05$), thus indicating a unique cellular phenotype for Endo with respect to both P-En and ovarian tissues.

Characterization of multipotent SC$^+$ from healthy endometria and patient endometria and endometrioma

Multipotent SC$^+$ from P-En, Endo and H-En were isolated, after a brief *in vitro* expansion, using widely-known mesenchymal stem cell markers *CD73*, *CD90* and *CD105* suggested by International Society for Cell therapy (Dominici *et al.*, 2006) (Fig. 2B). We explored whether the above sorted populations (SC$^+$ and SC$^-$) fulfil the set criteria for multipotent stem cells/progenitors, by phenotypic evaluation for markers of stem/stromal lineage (*W5C5/SUSD2*$^+$, *PDGFRβ/CD140b*$^+$, *CD146*$^+$, *CD44*$^+$), epithelial lineage (*SSEA1*$^+$, *EpCAM*$^+$) and side population (*ABCG2*$^+$), as well as the absence of a hematopoietic lineage *CD45*$^-$ (Fig. 2C-i–iii). W5C5 showed significant differences in both pooled group analysis (SC$^+$ 81.90% and SC$^-$ 65.80%, $P = 0.01$) as well as within each respective group, H-EnSC (SC$^+$ 96.30% and SC$^-$ 64.98%; $P < 0.0001$), EndoSC (SC$^+$ 80.50% and SC$^-$ 61.27%; $P < 0.05$; Fig. 2C-i). However, other markers did not show any differences in both pooled analysis and within the groups. Surprisingly, we detected significant differences in populations co-expressing SSEA1$^+$W5C5$^+$ in a pooled group analysis (SC$^+$ 22.90% and SC$^-$ 12.80%; $P < 0.05$; Fig. 2C-iv), implying an existence of a minor fraction of epithelial progenitors within sorted SC$^+$ groups.

Additionally, we also assessed the functional characteristics of SC$^+$ and SC$^-$ populations by analysing gene expression for stem cell factors

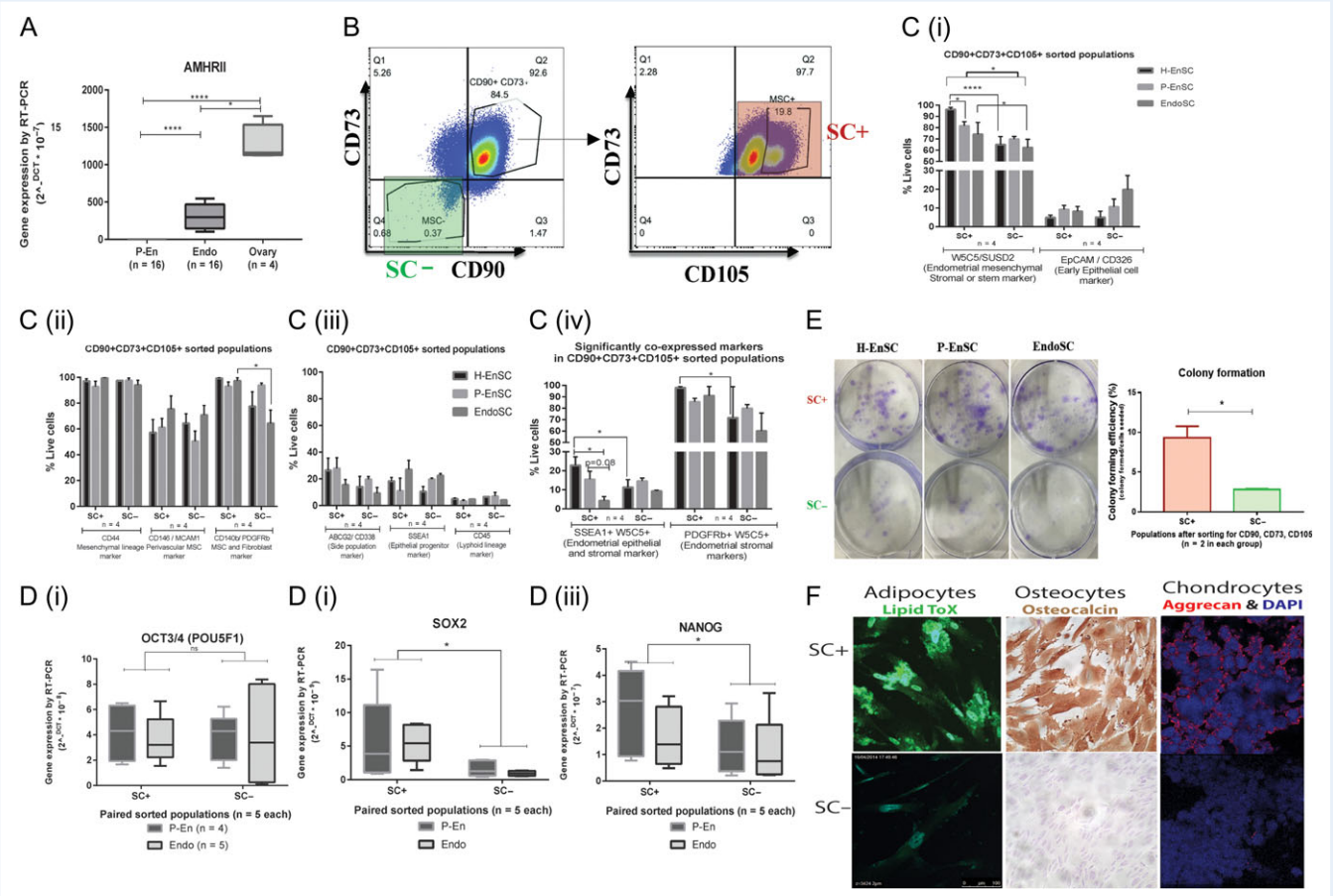

Figure 2 Isolation and characterization of multipotent cells/progenitors from endometriosis patients. **(A)** Differences in the expression levels of *AMHRII* between paired-samples from endometriosis patients (tissue cohort) endometrium (P-En, $n = 16$) and endometrioma (Endo, $n = 16$), compared with healthy ovarian tissues ($n = 4$). **(B)** Cell isolation strategy for sorting SC from *in vitro* expanded H-EnSC ($n = 14$), P-EnSC ($n = 18$) and EndoSC ($n = 11$) by flow cell sorter using markers CD73, CD90 and CD105. **(C-F)** Characterization of sorted triple positive-(SC$^+$) and triple-negative-(SC$^-$) fractions in groups H-EnSC, P-EnSC and EndoSC. **(C)** Percentage distribution of endometrial markers analysed by flow cytometry (i) W5C5$^+$ and EpCAM$^+$ (ii) CD44, CD146 and PDGFRb, (iii) ABCG2, SSEA1 and CD45 (iv) SSEA1$^+$W5C5$^+$; PDGFRb$^+$W5C5$^+$, Mean ± SE. (D) Gene expression levels for pluripotency and de-differentiation markers (i) *OCT3/4*, (ii) *SOX2*, (iii) *NANOG*. (E-i–ii) Colony forming capacity (bottom side of plate with seeding density of 100 cells/mm^2) and efficiency calculated using pooled analysis from all groups, Mean ± SE. (F) Fluorescence image shows multi-lineage mesenchymal differentiation ($n = 4$, each group and four technical replicates) into adipocytes (lipid granules in green, left panel), osteocytes (osteocalcin in brown, middle panel) and chondrocytes (aggrecan in red, right panel), visualized using confocal microscopy (bar: 100 μm).

OCT3/4, *SOX2* and *NANOG* (Fig. 2D-i–iii), oestrogen receptors *ER-α* and *ER-β* (Supplementary Figure S1.A-i,-ii) and proliferation marker *ki67* (Supplementary Figure S1.B-iii). SC^+ showed high expression of *SOX2* (FC: 4.3 ± 0.33, $P < 0.05$), *NANOG* (FC: 1.80 ± 0.83; $P < 0.05$) and *ER-α* (FC: 1.8 ± 0.43; $P < 0.05$) compared with SC^- in pooled analysis from both P-En and Endo groups. Also, SC^+ showed higher colony forming efficiency (SC^+ 9.3 ± 1.5% and SC^- 2.8 ± 0.2%; $P < 0.05$; Fig. 2E-i,-ii) when seeded at sub-optimally low dilutions (100 cells/cm^2). Subsequently, SC^+ alone showed mesenchymal multi-lineage potential as they were able to differentiate into adipocytes, osteocytes and chondrocytes (Fig. 2F).

Furthermore, we assessed the proliferation and cell cycle distribution among paired-endometriosis SC^+ and healthy SC^+. DAPI staining revealed the presence of single G1 and G2 peaks, indicating no sign of aneuploidy in any of the sample groups (Supplementary Figure S1B). Upon BrdU pulse treatment, P-EnSC in comparison with H-EnSC samples showed significant increase in $BrdU^+$ cells (S-phase cells) (9.34 ± 6.42% and 4.11 ± 2.04%, $P < 0.05$, Supplementary Figure S1C, S1D-i). Additionally, within total S-phase, both P-EnSC and EndoSC presented a significant increase of cells in early-S phase in comparison with H-EnSC (P-EnSC: 4.33 ± 2.71%, EndoSC: 4.47 ± 2.56 and H-EnSC: 1.91 ± 1.22%, $P < 0.01$, Supplementary Figure S1D-ii).

De-differentiation, stem and ovarian cancer-related markers enriched in certain endometriotic SC

3D-multicellular spheroid technique was adopted to assess markers of pluripotency or de-differentiation potential (*OCT3/4*, *SOX2*, *NANOG*)

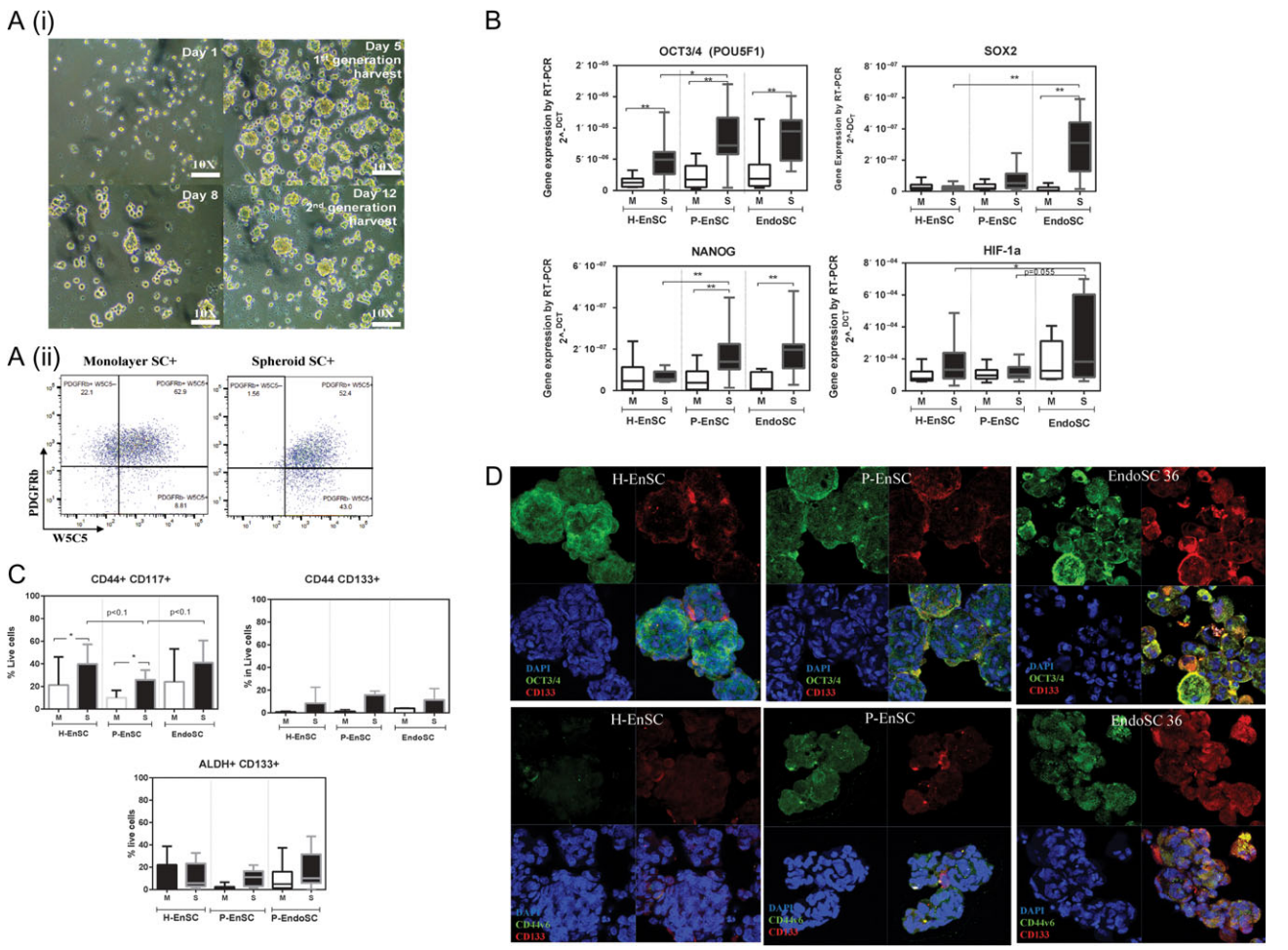

Figure 3 Phenotypic characterization and functional assessment of multipotent stem cells/progenitors from endometriosis patients using 3D-spheroid enrichment assay. (**A-i**) Illustrative figure for 3D-spheroid culture (10–12 days) in serum free, stem cell enriching conditions with growth factors. (**A-ii**) Representative flow cytometry plot for co-expression of surface markers $W5C5^+PDGFR\beta^+$ compared between monolayer and spheroid SC^+ cultures ($n = 4$, pooled analysis) (**B–C**) Characterization of P-EnSC ($n = 18$), EndoSC ($n = 11$) and H-EnSC ($n = 14$) groups under monolayer (M) and spheroid (S) culturing conditions. (B) Pluripotency, de-differentiation and hypoxia markers (*OCT3/4*, *SOX2, NANOG* and *HIF1α*), analysed by RT-PCR (C) Cancer-associated surface markers ($CD44^+CD117^+$, $CD44^+CD133^+$ and $ALDH1^+CD133^+$) analysed by flow cytometry. (**D**) Co-localization of markers associated with pluripotency (OCT3/4, green, top row), cancer-associated markers CD44v6 (green, bottom row) and CD133 (red, for both top and bottom row) for H-EnSC, P-EnSC and EndoSC groups ($n = 4$, each group), visualized by confocal microscopy (bar: 100 μm).

and hypoxia (*HIF1α*) by gene expression as well as assess ovarian stem cell and cancer-associated cell surface markers (*ALDH1*$^+$, *CD44*$^+$, *CD117* and *CD133*$^+$) by flow cytometry. All three sample groups successfully formed 3D-spheroids in vitro, with an enrichment of *OCT3/4* expression and *W5C5*$^+$*PDGFRb*$^+$ populations in second generation spheroids compared with monolayer cultures (95.40 ± 5.32% and 69.80 ± 3.45%; Figs 3A-i,-ii, 3B-i). Interestingly, H-EnSC and P-EnSC spheroid cultures showed significant gene expression differences for genes *OCT3/4* (FC: 1.80 ± 0.76; $P < 0.05$) and *NANOG* (FC: 22.12 ± 1.84, $P < 0.01$) (Fig. 3B), while H-EnSC and EndoSC spheroids showed a significant difference for gene *SOX2* (FC: 13.26 ± 0.84, $P < 0.01$) and a strong trend for *HIF1α* (FC: 21.07 ± 4.34; $P = 0.055$). However, there were no significant differences in ovarian cancer-associated markers between healthy and patient or between paired-patients' groups, assessed by flow cytometric analysis (Fig. 3C). Further, we investigated protein levels for co-expression of stem cell and ovarian cancer-related markers (OCT3/4 and CD133 as well as CD44v6 and CD133) between H-EnSC, P-EnSC and EndoSC. Surprisingly, one of the endometrioma samples (EndoSC36) showed co-existence for ovarian cancer-associated markers CD44v6, OCT3/4 and CD133 compared with other EndoSC, all H-EnSC and P-EnSC samples (Fig. 3D).

Gene deregulation in a subset of *in vitro* expanded endometriosis SC$^+$ cultures

Firstly, we explored the intrinsic differences within endometriosis SC$^+$ samples by selecting a panel of 42 stem cell and cancer-related genes that were previously reported for their association with endometrial or ovarian cancer (Supplementary Table S1), using customized RT-PCR array. A significant downregulation in *PTEN*, *ARID1A* and *TNFα* ($P < 0.05$) was observed in EndoSC compared with P-EnSC. Alternatively, P-EnSC showed downregulation of *ARID1A*, *BCL2* and *ALDH1A1* ($P < 0.05$) and upregulation of *PTEN, TNFα* and *MMP3* ($P < 0.05$, Table I) in comparison with H-EnSC.

Further, we applied a combination of bio-informatics tools such as hierarchical cluster analysis and multivariate OPLS-DA models to explore patient specific gene expression variability between H-EnSC and P-EnSC groups (Fig. 4A-i, B-i) as well as between paired-P-EnSC and -EndoSC groups (Fig. 4A-ii, B-ii). Comparison between P-EnSC and H-EnSC groups revealed clear distinct clusters, attributing to the disease pathophysiology. Moreover, P-EnSC intragroup analysis with multivariate OPLS-DA model identified three high-expression samples (En29, En24 and En36) forming a separate cluster with high gene expression variability (P-EnSC-hi) from other homogenous low-expression samples (P-EnSC-lo). Further, we discriminated differentially expressed genes between the above subgroups and observed upregulation of genes such as *MMP3*, *ERα/ESR1*, *CDH1/E-cadherin* ($P < 0.01$), *TGF-beta*, *Ki67*, *ARID1A*, *KRAS*, *FOS/USF2* and *BMI1* ($P < 0.05$; Supplementary Figure S2A-i). On other hand, three-paired-endometriosis P-EnSC and EndoSC samples (#24 and #36 and #18) showed aberrant gene expression pattern forming a separate cluster (referred as high-expression variability pairs) from rest of the homogenous paired samples (low-expression variability pairs). Further, we searched for discriminating genes between the above two groups by comparing gene expression ratios of EndoSC/P-EnSC and observed upregulation of genes *KIT*, *E-Cadherin*, *HIF2α* and downregulation of *NOTCH3*, *VEGFα*, *PTEN* and *ARID1A* ($P < 0.05$; Table II and Supplementary Figure S2A-ii).

Table I Differentially expressed genes in multipotent stem cells/progenitors isolated from endometrioma and endometrium from women with and without endometriosis.

Gene names	P-EnSC ($n = 18$) vs H-EnSC ($n = 14$)		EndoSC vs P-EnSC ($n = 11$, paired)	
	Fold change	*P*-value	Fold change	*P*-value
PTEN	1.34 ± 0.98	0.008	−1.31 ± 0.95	0.007
TNFα	5.28 ± 0.12	0.045	−8.61 ± 1.49	0.028
ARID1A	−1.48 ± 0.91	0.048	−1.43 ± 1.6	0.034
MMP3	2.40 ± 1.84	0.048	−46.95 ± 102.64	ns
BCL2	−1.87 ± 1.15	0.026	1.00 ± 1.85	ns
ALDH1A1	−8.92 ± 0.91	0.003	−1.04 ± 2.15	ns

ns: non-significant.

Moreover, we compared the gene expression ratio and protein levels of oestrogen receptors -α and β (ESR1, ESR2). We detected aberrant gene expression ratio of *ER-β/ER-α* in two patients (#24 and #36; Fig. 4C). However, protein validation revealed higher levels of ER-β in EndoSC-hi (#24) compared with all other samples (Supplementary Figure S2B-i,-ii). Also, *E-cadherin* protein fragments were detected in one of the high-variability samples EndoSC36 (Supplementary Figure S2B-iii).

Validation of aberrant gene expression profile in whole endometriosis tissues

We measured the gene expression of 25 out of 42 pre-selected markers based on the results from SC cohort (Supplementary Table S1) and 17 genes were differentially expressed between paired-endometrium (P-En) and -endometrioma (Endo) tissue groups. Intragroup heterogeneity within P-En (blue) and Endo (green) samples was explored using hierarchical heat-map clustering (Fig. 5A) and OPLS-DA multivariate model analysis (Fig. 5B). We identified only one endometrioma sample (Endo238) which had a highly-altered gene expression profile, ~95% CI. The top deregulated genes in E238 compared with other paired samples are presented in Table III.

Similar to the SC cohort, we observed significant differences in *ER-β/ER-α* mRNA transcript ratio in whole tissues of P-En and Endo groups ($P < 0.0001$; Fig. 5C). In line with gene expression trend, Endo groups showed high ER-β protein levels compared with ER-α, while P-En and H-En both showed moderate levels of ER-α and ER-β (Supplementary Figure S2C-i,-ii). Conversely, the *E-cadherin/N-cadherin* gene expression ratio was higher in P-En compared with Endo ($P < 0.0001$) and E-cadherin protein levels (120 kDa and its cleaved 98 kDa fragment) were also higher in P-En groups (Fig. 5D and Supplementary Figure S2C-iii). Interestingly, patient E238 alone showed altered characteristics with equal *E-cadherin/N-cadherin* gene expression ratios between P-En and Endo samples in contrast to the downregulated pattern observed in other samples. Also, E-cadherin protein fragments (98 kDa) were detected only in E238 patient's endometrioma.

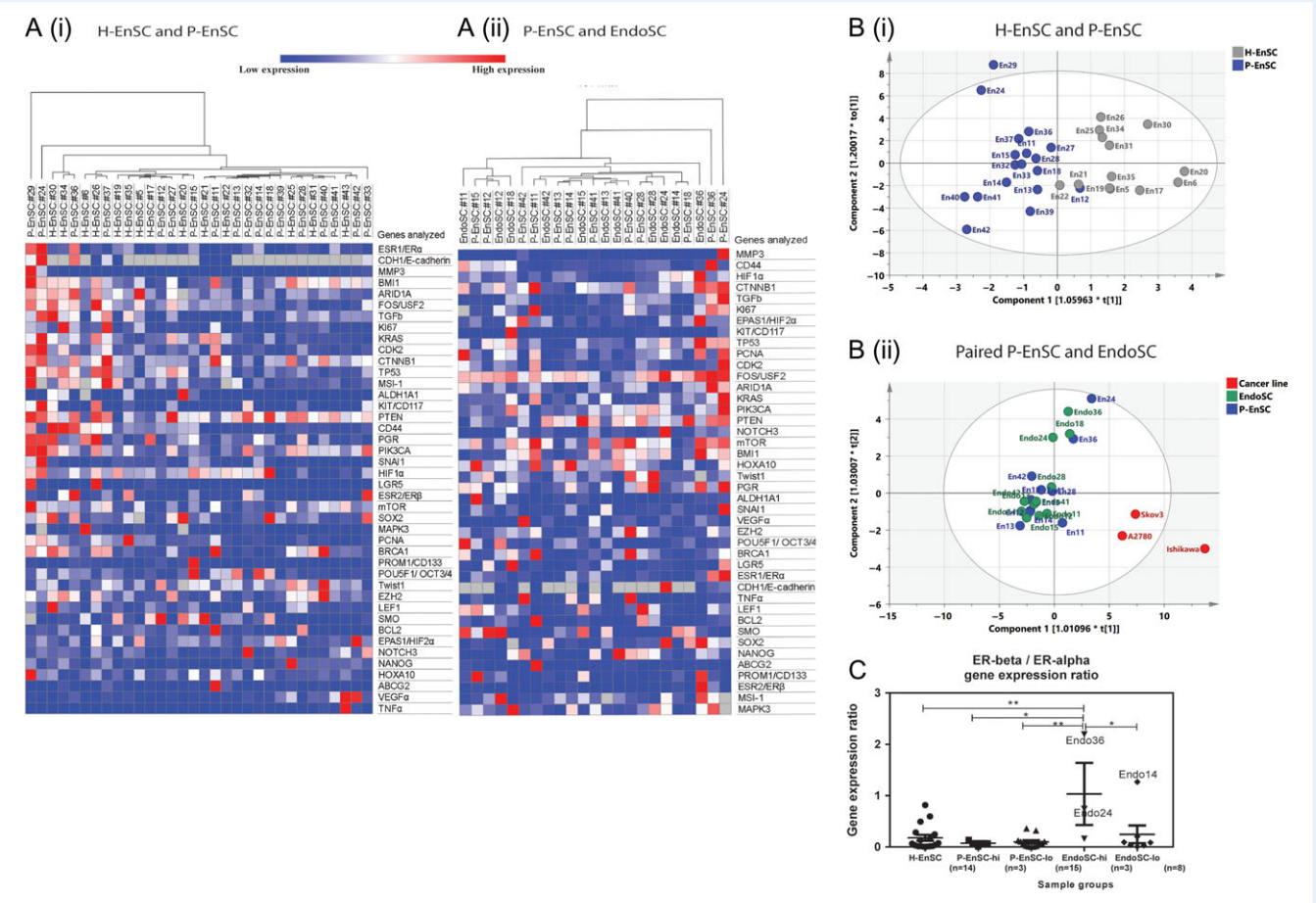

Figure 4 Differences in gene expression of cancer-associated markers in the *in vitro* expanded multipotent stem cells/progenitors from endometrium and endometrioma. (**A**) Heat-map of gene expression in SC^+ obtained by supervised hierarchical cluster analysis between (i) healthy (H-EnSC, $n = 14$) and patients' endometrium (P-EnSC, $n = 18$) and (ii) paired-P-EnSC and -EndoSC of endometriosis patients ($n = 11$). (**B**) Scatter plot from multivariate OPLS-DA model for above samples, compared between (i) H-EnSC and P-EnSC and (ii) paired-P-EnSC and -EndoSC in reference to cancer lines (Ishikawa, SKOV3, A2780). (**C**) Gene expression ratios of ER-β/ER-α compared between H-EnSC ($n = 14$), P-EnSC–hi ($n = 3$), P-EnSC–lo ($n = 15$), EndoSC–hi ($n = 3$) and EndoSC–lo ($n = 8$). *$P < 0.05$; **$P < 0.01$.

Table II Gene expression variability in multipotent stem cells/progenitors, in paired-endometrioma and -endometrium from the stem cell (SC) cohort.

Gene names	Mean fold change between paired low-expression (lo) variability samples* ($n = 8$)	Fold change between paired high-expression (hi) variability samples** ($n = 3$)			High- vs Low- expression variability group	
		#36	#24	#18	*P*-value	Gene regulation
EPAS1 (HIF2α)	−1.29 ± 0.27	2.90	1.38	1.42	0.003	↑
CDH1 (E-cadherin)	NA	10.26	4.06	NA	0.034	↑
KIT (CD117)	−1.06 ± 1.01	3.89	10.00	455.09	0.045	↑
VEGFα	22.89 ± 0.42	1.51	5.55	−2.72	0.040	↓
NOTCH3	12.31 ± 0.83	3.52	1.24	2.29	0.041	↓
PTEN	1.01 ± 0.34	−1.48	−1.04	−2.13	0.047	↓
ARID1A	−1.17 ± 0.49	−2.35	−2.10	−1.66	0.05	↓

*EndoSC-lo vs P-EnSC-lo; **EndoSC-hi vs P-EnSc-hi; NA- expression values undetermined.

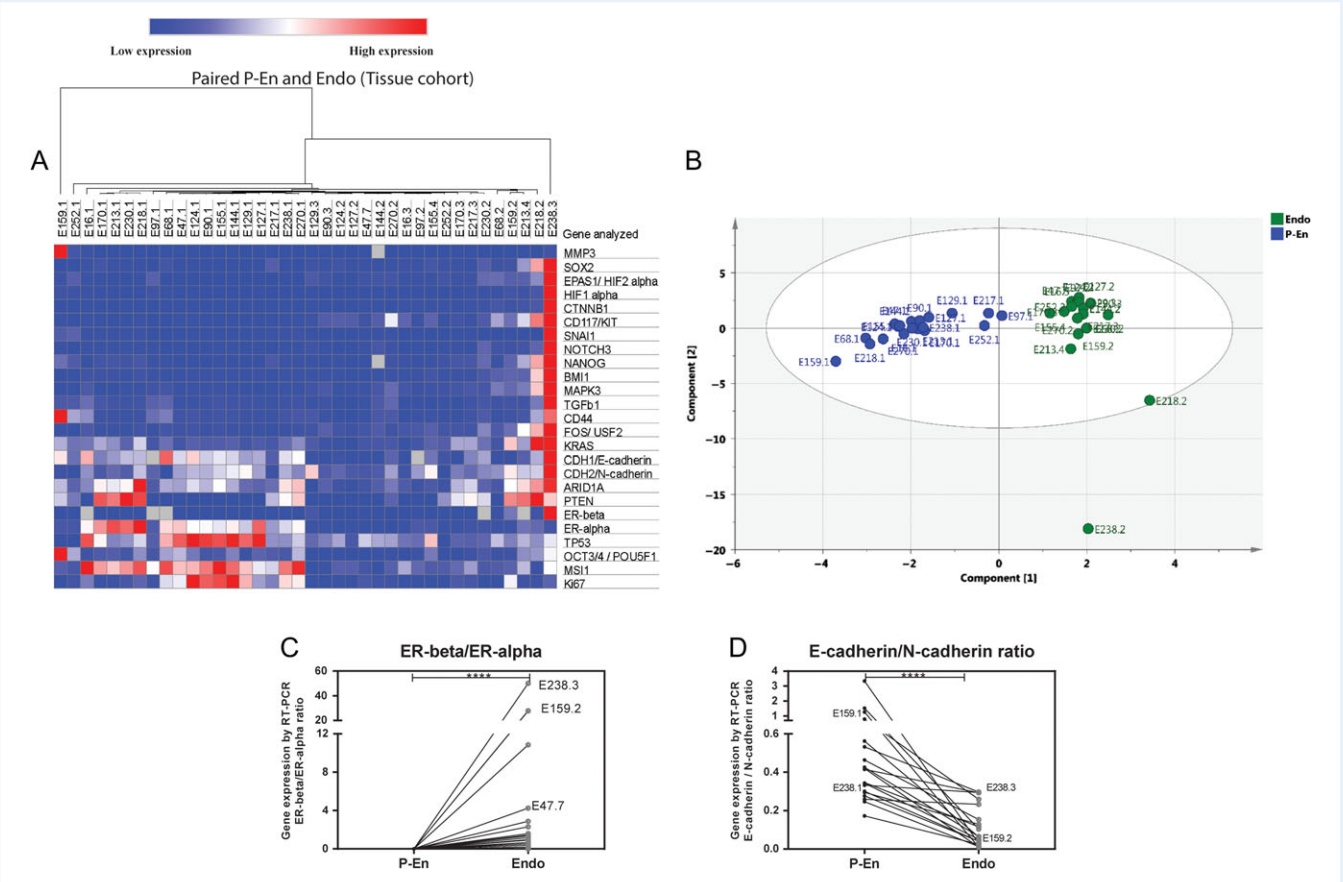

Figure 5 Differences in gene expression of cancer-associated markers in paired-endometrium and -endometrioma tissues from women with endometriosis. (**A**) Hierarchical cluster analysis comparing the gene expression of 25 pre-selected genes between paired-endometrium (P-En) and -endometrioma (Endo) tissues ($n = 19$). (**B**) Scatter plot from multivariate OPLS-DA analysis comparing paired tissues to investigate both intra- and intergroup variability. Plot shows one of the Endo-hi sample E238.2 segregated outside ellipse (95% confidence interval). (**C–D**) Graph illustrating gene expression ratios of (C) *ER-beta/ER-alpha* and (D) *E-cadherin/N-cadherin* compared between paired-P-En and -Endo tissues ($n = 19$).

Discussion

Although several theories have emerged about the biological processes behind malignant transformation of endometrial cells leading into EAOC, we still lack a deeper knowledge of the molecular changes or mechanisms behind disease development. In this study, we investigated one of the most challenging questions that prevail on the aetiology of EAOC: do adult multipotent stem cells/progenitors in endometrioma of higher cancer risk patients contain a subpopulation of cells with a cancer-associated gene signature? For the first time, we report a highly-altered molecular profile in a small subgroup of women with ovarian endometriosis, having overexpression of genes *KIT*, *HIF2α* and *E-cadherin*, altered *ER-β/ER-α* expression ratio and downregulation of tumour suppressor genes *PTEN* and *ARID1A*; and hypothesize that these changes may be potentially relevant to the development of EAOC. We believe that the observed aberrant expression of stem- and cancer-associated markers could reflect early molecular events prior to or in parallel with deleterious gene mutations/deletions, which may trigger de-differentiation, and/or alter epithelial versus mesenchymal phenotype in benign endometriotic multipotent SC, leading to increased risk of EAOC. The postulated theory and findings from this study are summarized in Fig. 6.

Previous studies have shown a key role for stem cells/progenitors (SC^+) in aetiology of endometriosis (Cheng *et al.*, 2017). Although SC have been identified in most of the vascularized tissues throughout the body (Crisan *et al.*, 2008) including endometriosis (Tanaka *et al.*, 2003), still the composition of cell surface markers in SC within endometrium and endometrioma is not clearly defined. Hence, we opted for widely-reported criteria for mesenchymal stem cells such as ≥95% of the sorted population expressing markers CD73, CD90 and CD105, and ≤2% of the sorted population expressing markers CD14, CD34 and CD45 (Dominici *et al.*, 2006). In our study, all SC^+ sorted cells showed stem cell characteristics such as the abilities to self-renew, form higher number of 3D-spheroids and express high levels of stem cell markers (*SOX2, NANOG*) as well as ability to differentiate into adipocytes, chondrocytes and osteoblasts. Interestingly, SC^+ also express *ER-α* and *ER-β* along with known endometrial surface markers $W5C5^+$, $CD146^+$, $PDGFR\beta^+$, $SSEA1^+$, $ABCG2^+$, $CD44^+$ and lack hematopoietic marker CD45 (Gargett *et al.*, 2016). Interestingly, a

Table III Inter- and intragroup variability between paired-endometrial (P-En) and -Endometrioma (Endo) from the tissue cohort.

Genes analysed	Gene expression fold change (Endo vs P-EnSC)		*P*-value between groups (Endo vs P-EnSC)	Gene regulation (E238 vs other paired tissues)
	Average of all other samples ($n = 18$)	Patient E238		
ER-beta (ESR2)	1169.99 ± 2518.1	8947.57	<0.0001****	↑
MMP3	4.78 ± 11.46	5989.59	0.05*	↑
SOX2	7.79 ± 9.8	212.77	0.009**	↑
EPAS1 (HIF2 alpha)	7.08 ± 5.74	90.22	0.002**	↑
CTNNB1	2.58 ± 1.88	56.63	0.001**	↑
CD117 (KIT)	7.34 ± 6.46	39.20	<0.0001****	↑
SNAI1	−4.29 ± 0.34	30.66	0.002**	↑
NOTCH3	1.82 ± 1.32	28.11	0.018*	↑
BMI1	1.92 ± 1.59	17.08	0.040*	↑
MAPK3	2.35 ± 1.62	12.57	0.0002***	↑
FOS (USF2)	1.8 ± 0.95	8.18	<0.0001****	↑
OCT3/4 (POU5F1)	−2.31 ± 0.5	3.21	0.026*	↑
CDH1 (E-cadherin)	−3.73 ± 0.46	2.25	0.002**	↑
TP53	−1.82 ± 0.39	1.45	0.004**	↑
MSI1	−3.42 ± 0.52	−1.48	<0.0001****	↑
ER-alpha (ESR1)	−7.99 ± 0.26	−5.05	<0.0001****	↑
ARID1A	1.38 ± 0.26	−1.42	0.007**	↓
PTEN	1.82 ± 0.76	−1.45	ns	↓
Ki67	2.23 ± 0.33	−3.18	0.001**	↓

ns $P > 0.05$; *$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$; ****$P \leq 0.0001$.

small fraction among them still expressed early epithelial markers such as EPCAM$^+$ and W5C5$^+$SSEA1$^+$. This was in concurrence with a previous report where a small fraction of W5C5$^+$ endometrial stromal cells expressed epithelial cell adhesion molecule (Masuda *et al.*, 2012). Therefore, we suggest that sorted SC$^+$ in our study may have some already characterized and some uncharacterized surface markers and include minor progenitor subsets displaying epithelial markers.

Epidemiological data show 0.7–2.5% of endometriosis patients have a potential risk for EAOC (Gadducci *et al.*, 2014). To investigate if endometriosis patients undergo alterations in key cellular pathways facilitating the onset of EAOC, we checked a panel of previously reported 42 genes associated with stem cell dysfunction or cancer-related properties on SC$^+$ populations. Although a majority of our SC$^+$ samples expressed the studied markers at moderate expression levels, our inter- and intragroup analyses identified patients (#36, #24 and #18) with higher gene expression profile, in comparison with the eight other paired samples. To further strengthen these findings, we also validated a selective panel of significant genes in another independent cohort of 19 paired tissue samples and identified one endometrioma (E238) exclusively showing highly-altered profile with more than 95% CI for the studied molecular markers. Remarkably, endometrioma of the above four high-variability samples (#24, #36, #18 from SC cohort and #E238 from tissue cohort) compared with other low variability samples showed upregulation of genes relevant to potential risk for cancer, *KIT, HIF2α*, *E-cadherin* (Xu *et al.*, 2012; Liu *et al.*, 2014; Tang *et al.*, 2014) and downregulation of tumour suppressor genes *PTEN* and *ARID1A*. Inactivation or reduced expression of both *PTEN* (Sato *et al.*, 2000) and *ARID1A* (Wiegand *et al.*, 2010) were previously identified as part of early events towards the onset of EAOC. *E-cadherin*, is generally not considered to be directly associated with cancer progression; however, recent studies have demonstrated that *E-cadherin* upregulation may have relevance with mesenchymal-to-epithelial transitions during metastatic seeding of primary cancers (Wells *et al.*, 2008). Furthermore, the above high-variability endometrioma samples exclusively displayed aberrant *ER-β* mRNA and protein levels. This key observation is in concordance with previous reports on a tumour suppressive role of ER-β, controlling the onset of ovarian cancer (Pujol *et al.*, 1998; Lazennec, 2006). Hence, we postulate that the high-variability group of endometriotic SC might be involved in de-differentiation of mesenchymal SC into a more epithelial stem-like phenotype, leading to the increased risk for development of epithelial ovarian cancer.

As only a few studied SC and whole tissue samples showed significant alterations in gene expression signatures, the clinical history of these patients were also carefully inspected. Patient E238 had been reported previously with melanoma. Though it is a non-gynaecological cancer, there are convincing epidemiological and clinical reports in favour of an association between endometriosis and the elevated risk for melanoma (Farland *et al.*, 2017); however, a molecular link between the two diseases is currently unknown. We acknowledge

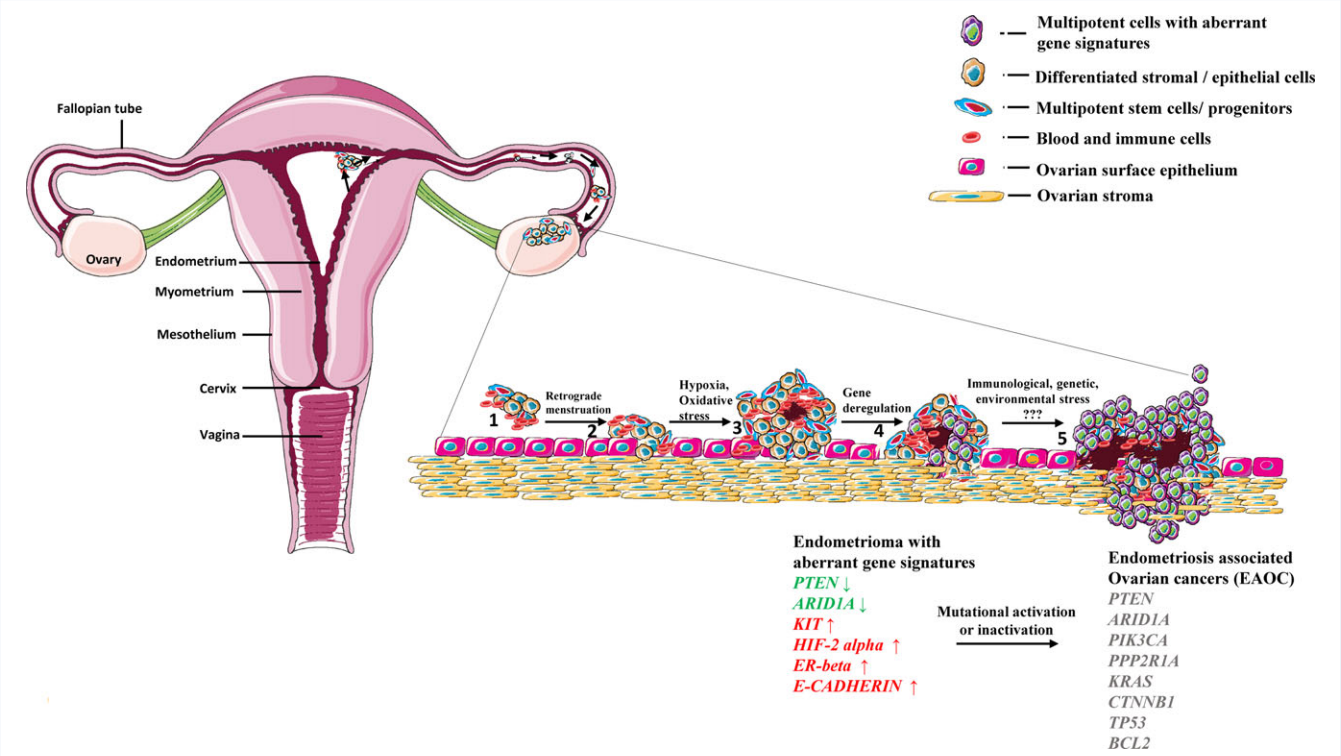

Figure 6 Hypothesis for the development of endometriosis-associated ovarian cancers (EAOC). (1) Initiation: Endometrial differentiated epithelial/stromal cells, multipotent stem cells/progenitors (SC^+) along with red blood cells and immune cells are shed into the peritoneal cavity during menstruation. (2) The cells attach onto the ovaries and form endometrioma. (3) SC^+ in endometrioma (EndoSC) may remain quiescent for several years. Repeated menstrual cycles lead to the accumulation of cell toxic factors including reactive oxygen species, inflammatory cytokines and hypoxia inducing factors; the cells in endometrioma are subjected to mutational stress. (4) As a consequence, EndoSC may undergo gene dysregulation leading to aberrant cellular phenotype forming EAOC precursor lesions. Aberrant expression may include elevated activity of cancer-associated genes (e.g. *KIT*, *HIF2α* and *E-cadherin*), altered *ER-β/ER-α* ratio and downregulation of tumour suppressor genes (*PTEN* and *ARID1A*). (5) Gene dysregulation within EndoSC may create a favourable peritoneal environment to directly or indirectly support the accumulation of several stochastic mutations causing inactivation of tumour suppressor genes *PTEN*, *TP53* and *ARID1A*, and activation of oncogenes *KRAS*, *CTNNB1* and *PIK3CA*, collectively leading to the development of EAOC. Genes marked in red and green were respectively upregulated and downregulated on comparing high and low-expression variability subgroups in this study. Genes marked in grey were previously identified cancer driver gene mutations causing EAOC.

that the observed molecular signatures indicating a potential risk of EAOC should be validated among a larger cohort of endometriosis patients.

Despite having stringent patient inclusion criteria, certain limitations need to be considered in the present study. Firstly, by adopting a pre-selected panel of genes, we might have missed several other key differentially regulated genes associated with EAOC. However, we believe that above approach is advantageous compared to whole transcriptome analysis as it would specifically focus on smaller gene expression changes for the above pre-selected genes leading to EAOC (Pollacco *et al.*, 2012). Secondly, women displaying highly-altered gene signatures observed among potential risk patients with endometriosis may or may not have EAOC later in life and thus our findings need to be confirmed on archived tissue samples from women with endometriosis who later in life developed ovarian cancer. Thirdly, cell culture *per se* may have some impact on the gene expression levels. However, multipotent SC^+ from endometrioma and endometrium were isolated and handled exactly in the same way for the entire cohort, thus the alteration observed in gene expression levels in some patients' samples likely present true alterations.

In conclusion, we observed an aberrant gene expression profile in a small subset of women with ovarian endometriosis, which could be a part of preceding steps towards malignant conversion of endometriotic cells to EAOC phenotype. The information gained from this study, with further confirmation, may help us to screen women with higher risk for developing EAOC, thus providing an opportunity to take prophylactic measures to prevent a life-threatening disease.

Supplementary data

Supplementary data are available at *Human Reproduction* online.

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Conflict of interest

The authors have no potential conflicts of interest.

Authors' roles

SPS: conception, study design data collection and analysis, interpretation and manuscript writing; KLA: conception, interpretation and manuscript writing; MN: critical comments, results interpretation and review on manuscript; MS, HAH, SJV, MP and AS: material collection, data analysis and manuscript reviewing; KGD and PGLL: conception, study design, sample collection, data interpretation, critical comments, manuscript writing and funding. All authors approved the final version of the manuscript.

References

American Society for Reproductive Medicine: Revised classification of endometriosis. American Society for Reproductive Medicine: revised classification of endometriosis. *Fertil Steril* 1997;**67**:817–821.

Anglesio MS, Papadopoulos N, Ayhan A, Nazeran TM, Noe M, Horlings HM, Lum A, Jones S, Senz J, Seckin T *et al*. Cancer-associated mutations in endometriosis without cancer. *N Engl J Med* 2017;**376**:1835–1848.

Aviel-Ronen S, Soriano D, Shmuel E, Schonman R, Rosenblatt K, Zadok O, Vituri A, Seidman D, Barshack I, Cohen Y. Surgically treated ovarian endometriosis association with BRCA1 and BRCA2 mutations. *Pathol Res Pract* 2014;**210**:250–255.

Bapat SA. Human ovarian cancer stem cells. *Reproduction* 2010;**140**: 33–41.

Barragan F, Irwin JC, Balayan S, Erikson DW, Chen JC, Houshdaran S, Piltonen TT, Spitzer TL, George A, Rabban JT *et al*. Human endometrial fibroblasts derived from mesenchymal progenitors inherit progesterone resistance and acquire an inflammatory phenotype in the endometrial niche in endometriosis. *Biol Reprod* 2016;**94**:118.

Bath LE, Critchley HO, Chambers SE, Anderson RA, Kelnar CJ, Wallace WH. Ovarian and uterine characteristics after total body irradiation in childhood and adolescence: response to sex steroid replacement. *Br J Obstet Gynaecol* 1999;**106**:1265–1272.

Bierie B, Moses HL. Tumour microenvironment: TGFbeta: the molecular Jekyll and Hyde of cancer. *Nat Rev Cancer* 2006;**6**:506–520.

Bossard C, Busson M, Vindrieux D, Gaudin F, Machelon V, Brigitte M, Jacquard C, Pillon A, Balaguer P, Balabanian K *et al*. Potential role of estrogen receptor beta as a tumor suppressor of epithelial ovarian cancer. *PLoS One* 2012;**7**:e44787.

Carrarelli P, Rocha AL, Belmonte G, Zupi E, Abrao MS, Arcuri F, Piomboni P, Petraglia F. Increased expression of antimullerian hormone and its receptor in endometriosis. *Fertil Steril* 2014;**101**:1353–1358.

Cervello I, Mas A, Gil-Sanchis C, Peris L, Faus A, Saunders PT, Critchley HO, Simon C. Reconstruction of endometrium from human endometrial side population cell lines. *PLoS One* 2011;**6**:e21221.

Chan RW, Gargett CE. Identification of label-retaining cells in mouse endometrium. *Stem Cells* 2006;**24**:1529–1538.

Chelariu-Raicu A, Wilke C, Brand M, Starzinski-Powitz A, Kiesel L, Schuring AN, Gotte M. Syndecan-4 expression is upregulated in endometriosis and contributes to an invasive phenotype. *Fertil Steril* 2016; **106**:378–385.

Chen J, Wang J, Zhang Y, Chen D, Yang C, Kai C, Wang X, Shi F, Dou J. Observation of ovarian cancer stem cell behavior and investigation of potential mechanisms of drug resistance in three-dimensional cell culture. *J Biosci Bioeng* 2014;**118**:214–222.

Chen Y, Zhang J, Wang H, Zhao J, Xu C, Du Y, Luo X, Zheng F, Liu R, Zhang H *et al*. miRNA-135a promotes breast cancer cell migration and invasion by targeting HOXA10. *BMC Cancer* 2012;**12**:111.

Cheng JC, Auersperg N, Leung PC. TGF-beta induces serous borderline ovarian tumor cell invasion by activating EMT but triggers apoptosis in low-grade serous ovarian carcinoma cells. *PLoS One* 2012;**7**: e42436.

Cheng Y, Li L, Wang D, Guo Q, He Y, Liang T, Sun L, Wang X, Cheng Y, Zhang G. Characteristics of human endometrium-derived mesenchymal stem cells and their tropism to endometriosis. *Stem Cells Int* 2017;**2017**: 4794827.

Cheng L, Sung MT, Cossu-Rocca P, Jones TD, MacLennan GT, De Jong J, Lopez-Beltran A, Montironi R, Looijenga LH. OCT4: biological functions and clinical applications as a marker of germ cell neoplasia. *J Pathol* 2007; **211**:1–9.

Crisan M, Yap S, Casteilla L, Chen CW, Corselli M, Park TS, Andriolo G, Sun B, Zheng B, Zhang L *et al*. A perivascular origin for mesenchymal stem cells in multiple human organs. *Cell Stem Cell* 2008;**3**:301–313.

Curley MD, Therrien VA, Cummings CL, Sergent PA, Koulouris CR, Friel AM, Roberts DJ, Seiden MV, Scadden DT, Rueda BR *et al*. CD133 expression defines a tumor initiating cell population in primary human ovarian cancer. *Stem Cells* 2009;**27**:2875–2883.

Davidson B, Zhang Z, Kleinberg L, Li M, Florenes VA, Wang TL. and Shih Ie M. Gene expression signatures differentiate ovarian/peritoneal serous carcinoma from diffuse malignant peritoneal mesothelioma. *Clin Cancer Res* 2006;**12**:5944–5950.

Do TV, Kubba LA, Du H, Sturgis CD, Woodruff TK. Transforming growth factor-beta1, transforming growth factor-beta2, and transforming growth factor-beta3 enhance ovarian cancer metastatic potential by inducing a Smad3-dependent epithelial-to-mesenchymal transition. *Mol Cancer Res* 2008;**6**:695–705.

Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, Krause D, Deans R, Keating A, Prockop D, Horwitz E. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. *Cytotherapy* 2006; **8**:315–317.

Dorien F, Roskams T, Van den Eynde K, Vanhie A, Peterse DP, Meuleman C, Tomassetti C, Peeraer K, D'Hooghe TM, Fassbender A. The presence of endometrial cells in peritoneal fluid of women with and without endometriosis. *Reprod Sci* 2017;**24**:242–251.

Er TK, Su YF, Wu CC, Chen CC, Wang J, Hsieh TH, Herreros-Villanueva M, Chen WT, Chen YT, Liu TC *et al*. Targeted next-generation sequencing for molecular diagnosis of endometriosis-associated ovarian cancer. *J Mol Med (Berl)* 2016;**94**:835–847.

Esteller M, Catasus L, Matias-Guiu X, Mutter GL, Prat J, Baylin SB, Herman JG. hMLH1 promoter hypermethylation is an early event in human endometrial tumorigenesis. *Am J Pathol* 1999;**155**:1767–1772.

Farland LV, Lorrain S, Missmer SA, Dartois L, Cervenka I, Savoye I, Mesrine S, Boutron-Ruault MC, Kvaskoff M. Endometriosis and the risk of skin cancer: a prospective cohort study. *Cancer Causes Control* 2017; **28**:1011–1019.

Feng YZ, Shiozawa T, Miyamoto T, Kashima H, Kurai M, Suzuki A, Ying-Song J, Konishi I. Overexpression of hedgehog signaling molecules and its involvement in the proliferation of endometrial carcinoma cells. *Clin Cancer Res* 2007;**13**:1389–1398.

Ferrell CM, Dorsam ST, Ohta H, Humphries RK, Derynck MK, Haqq C, Largman C, Lawrence HJ. Activation of stem-cell specific genes by HOXA9 and HOXA10 homeodomain proteins in CD34+ human cord blood cells. *Stem Cells* 2005;**23**:644–655.

Flesken-Nikitin A, Hwang CI, Cheng CY, Michurina TV, Enikolopov G, Nikitin AY. Ovarian surface epithelium at the junction area contains a cancer-prone stem cell niche. *Nature* 2013;**495**:241–245.

Forte A, Schettino MT, Finicelli M, Cipollaro M, Colacurci N, Cobellis L, Galderisi U. Expression pattern of stemness-related genes in human endometrial and endometriotic tissues. *Mol Med* 2009;**15**:392–401.

Gadducci A, Lanfredini N, Tana R. Novel insights on the malignant transformation of endometriosis into ovarian carcinoma. *Gynecol Endocrinol* 2014;**30**:612–617.

Gargett CE, Schwab KE, Brosens JJ, Puttemans P, Benagiano G, Brosens I. Potential role of endometrial stem/progenitor cells in the pathogenesis of early-onset endometriosis. *Mol Hum Reprod* 2014;**20**:591–598.

Gargett CE, Schwab KE, Deane JA. Endometrial stem/progenitor cells: the first 10 years. *Hum Reprod Update* 2016;**22**:137–163.

Ginestier C, Hur MH, Charafe-Jauffret E, Monville F, Dutcher J, Brown M, Jacquemier J, Viens P, Kleer CG, Liu S *et al*. ALDH1 is a marker of normal and malignant human mammary stem cells and a predictor of poor clinical outcome. *Cell Stem Cell* 2007;**1**:555–567.

Govatati S, Kodati VL, Deenadayal M, Chakravarty B, Shivaji S, Bhanoori M. Mutations in the PTEN tumor gene and risk of endometriosis: a case-control study. *Hum Reprod* 2014;**29**:324–336.

Groeneweg JW, DiGloria CM, Yuan J, Richardson WS, Growdon WB, Sathyanarayanan S, Foster R, Rueda BR. Inhibition of notch signaling in combination with Paclitaxel reduces platinum-resistant ovarian tumor growth. *Front Oncol* 2014;**4**:171.

Gun BD, Bahadir B, Bektas S, Barut F, Yurdakan G, Kandemir NO, Ozdamar SO. Clinicopathological significance of fascin and CD44v6 expression in endometrioid carcinoma. *Diagn Pathol* 2012;**7**:80.

Gupta N, Xu Z, El-Sehemy A, Steed H, Fu Y. Notch3 induces epithelial–mesenchymal transition and attenuates carboplatin-induced apoptosis in ovarian cancer cells. *Gynecol Oncol* 2013;**130**:200–206.

He QZ, Luo XZ, Wang K, Zhou Q, Ao H, Yang Y, Li SX, Li Y, Zhu HT, Duan T. Isolation and characterization of cancer stem cells from high-grade serous ovarian carcinomas. *Cell Physiol Biochem* 2014;**33**:173–184.

Heard ME, Simmons CD, Simmen FA, Simmen RC. Kruppel-like factor 9 deficiency in uterine endometrial cells promotes ectopic lesion establishment associated with activated notch and hedgehog signaling in a mouse model of endometriosis. *Endocrinology* 2014;**155**:1532–1546.

Herreros-Villanueva M, Zhang JS, Koenig A, Abel EV, Smyrk TC, Bamlet WR, de Narvajas AA, Gomez TS, Simeone DM, Bujanda L *et al*. SOX2 promotes dedifferentiation and imparts stem cell-like features to pancreatic cancer cells. *Oncogenesis* 2013;**2**:e61.

Hu L, McArthur C, Jaffe RB. Ovarian cancer stem-like side-population cells are tumourigenic and chemoresistant. *Br J Cancer* 2010;**102**:1276–1283.

Jabbour HN, Kelly RW, Fraser HM, Critchley HO. Endocrine regulation of menstruation. *Endocr Rev* 2006;**27**:17–46.

Kim TH, Yu Y, Luo L, Lydon JP, Jeong JW, Kim JJ. Activated AKT pathway promotes establishment of endometriosis. *Endocrinology* 2014;**155**: 1921–1930.

Kobayashi H, Sumimoto K, Kitanaka T, Yamada Y, Sado T, Sakata M, Yoshida S, Kawaguchi R, Kanayama S, Shigetomi H *et al*. Ovarian endometrioma–risks factors of ovarian cancer development. *Eur J Obstet Gynecol Reprod Biol* 2008;**138**:187–193.

Kozera B, Rapacz M. Reference genes in real-time PCR. *J Appl Genet* 2013; **54**:391–406.

Kryczek I, Liu S, Roh M, Vatan L, Szeliga W, Wei S, Banerjee M, Mao Y, Kotarski J, Wicha MS *et al*. Expression of aldehyde dehydrogenase and CD133 defines ovarian cancer stem cells. *Int J Cancer* 2012;**130**:29–39.

Kurman RJ, Shih Ie M. Molecular pathogenesis and extraovarian origin of epithelial ovarian cancer–shifting the paradigm. *Hum Pathol* 2011;**42**: 918–931.

Lalitkumar S, Boggavarapu NR, Menezes J, Dimitriadis E, Zhang JG, Nicola NA, Gemzell-Danielsson K, Lalitkumar LP. Polyethylene glycated leukemia inhibitory factor antagonist inhibits human blastocyst implantation and triggers apoptosis by down-regulating embryonic AKT. *Fertil Steril* 2013;**100**:1160–1169.

Lamouille S, Xu J, Derynck R. Molecular mechanisms of epithelial–mesenchymal transition. *Nat Rev Mol Cell Biol* 2014;**15**:178–196.

Lazennec G. Estrogen receptor beta, a possible tumor suppressor involved in ovarian carcinogenesis. *Cancer Lett* 2006;**231**:151–157.

Lee GH, Choi YM, Kim SH, Hong MA, Oh ST, Lim YT, Moon SY. Association of tumor necrosis factor-{alpha} gene polymorphisms with advanced stage endometriosis. *Hum Reprod* 2008;**23**:977–981.

Lee SH, Oh SY, Do SI, Lee HJ, Kang HJ, Rho YS, Bae WJ, Lim YC. SOX2 regulates self-renewal and tumorigenicity of stem-like cells of head and neck squamous cell carcinoma. *Br J Cancer* 2014;**111**:2122–2130.

Li MQ, Li HP, Meng YH, Wang XQ, Zhu XY, Mei J, Li DJ. Chemokine CCL2 enhances survival and invasiveness of endometrial stromal cells in an autocrine manner by activating Akt and MAPK/Erk1/2 signal pathway. *Fertil Steril* 2012;**97**:919–929.

Liang D, Ma Y, Liu J, Trope CG, Holm R, Nesland JM, Suo Z. The hypoxic microenvironment upgrades stem-like properties of ovarian cancer cells. *BMC Cancer* 2012;**12**:201.

Liu L, Chen X, Wang Y, Qu Z, lu Q, Zhao J, Yan X, Zhang H, Zhou Y. Notch3 is important for TGF-β-induced epithelial–mesenchymal transition in non-small cell lung cancer bone metastasis by regulating ZEB-1. *Cancer Gene Ther* 2014;**21**:364–372.

Liu T, Cheng W, Lai D, Huang Y, Guo L. Characterization of primary ovarian cancer cells in different culture systems. *Oncol Rep* 2010;**23**: 1277–1284.

Lopez J, Valdez-Morales FJ, Benitez-Bribiesca L, Cerbon M, Carranca AG. Normal and cancer stem cells of the human female reproductive system. *Reprod Biol Endocrinol* 2013;**11**:53.

Machado DE, Berardo PT, Palmero CY, Nasciutti LE. Higher expression of vascular endothelial growth factor (VEGF) and its receptor VEGFR-2 (Flk-1) and metalloproteinase-9 (MMP-9) in a rat model of peritoneal endometriosis is similar to cancer diseases. *J Exp Clin Cancer Res* 2010;**29**:4.

Mak VC, Siu MK, Wong OG, Chan KK, Ngan HY, Cheung AN. Dysregulated stemness-related genes in gynecological malignancies. *Histol Histopathol* 2012;**27**:1121–1130.

Makker A, Goel MM, Das V. and Agarwal A. PI3K-Akt-mTOR and MAPK signaling pathways in polycystic ovarian syndrome, uterine leiomyomas and endometriosis: an update. *Gynecol Endocrinol* 2012;**28**:175–181.

Masuda H, Anwar SS, Buhring HJ, Rao JR, Gargett CE. A novel marker of human endometrial mesenchymal stem-like cells. *Cell Transplant* 2012; **21**:2201–2214.

McConechy MK, Ding J, Senz J, Yang W, Melnyk N, Tone AA, Prentice LM, Wiegand KC, McAlpine JN, Shah SP *et al*. Ovarian and endometrial endometrioid carcinomas have distinct CTNNB1 and PTEN mutation profiles. *Mod Pathol* 2014;**27**:128–134.

Meng X, Ichim TE, Zhong J, Rogers A, Yin Z, Jackson J, Wang H, Ge W, Bogin V, Chan KW *et al*. Endometrial regenerative cells: a novel stem cell population. *J Transl Med* 2007;**5**:57.

Meng Q, Sun W, Jiang J, Fletcher NM, Diamond MP, Saed GM. Identification of common mechanisms between endometriosis and ovarian cancer. *J Assist Reprod Genet* 2011;**28**:917–923.

Mertens HJ, Heineman MJ, Evers JL. The expression of apoptosis-related proteins Bcl-2 and Ki67 in endometrium of ovulatory menstrual cycles. *Gynecol Obstet Invest* 2002;**53**:224–230.

Montserrat N, Mozos A, Llobet D, Dolcet X, Pons C, de Herreros AG, Matias-Guiu X, Prat J. Epithelial to mesenchymal transition in early stage endometrioid endometrial carcinoma. *Hum Pathol* 2012;**43**:632–643.

Morsch DM, Carneiro MM, Lecke SB, Araujo FC, Camargos AF, Reis FM, Spritzer PM. c-fos gene and protein expression in pelvic endometriosis: a local marker of estrogen action. *J Mol Histol* 2009;**40**:53–58.

Munksgaard PS, Blaakaer J. The association between endometriosis and ovarian cancer: a review of histological, genetic and molecular alterations. *Gynecol Oncol* 2012;**124**:164–169.

Musina RA, Belyavski AV, Tarusova OV, Solovyova EV, Sukhikh GT. Endometrial mesenchymal stem cells isolated from the menstrual blood. *Bull Exp Biol Med* 2008;**145**:539–543.

Nezhat F, Cohen C, Rahaman J, Gretz H, Cole P, Kalir T. Comparative immunohistochemical studies of bcl-2 and p53 proteins in benign and malignant ovarian endometriotic cysts. *Cancer* 2002;**94**:2935–2940.

Park JT, Shih Ie M, Wang TL. Identification of Pbx1, a potential oncogene, as a Notch3 target gene in ovarian cancer. *Cancer Res* 2008;**68**:8852–8860.

Pearce CL, Templeman C, Rossing MA, Lee A, Near AM, Webb PM, Nagle CM, Doherty JA, Cushing-Haugen KL, Wicklund KG *et al*. Association between endometriosis and risk of histological subtypes of ovarian cancer: a pooled analysis of case-control studies. *Lancet Oncol* 2012;**13**:385–394.

Peng S, Maihle NJ, Huang Y. Pluripotency factors Lin28 and Oct4 identify a sub-population of stem cell-like cells in ovarian cancer. *Oncogene* 2010;**29**:2153–2159.

Penumatsa K, Edassery SL, Barua A, Bradaric MJ, Luborsky JL. Differential expression of aldehyde dehydrogenase 1a1 (ALDH1) in normal ovary and serous ovarian tumors. *J Ovarian Res* 2010;**3**:28.

Ping S, Ma C, Liu P, Yang L, Yang X, Wu Q, Zhao X, Gong B. Molecular mechanisms underlying endometriosis pathogenesis revealed by bioinformatics analysis of microarray data. *Arch Gynecol Obstet* 2016;**293**:797–804.

Pizzo A, Salmeri FM, Ardita FV, Sofo V, Tripepi M, Marsico S. Behaviour of cytokine levels in serum and peritoneal fluid of women with endometriosis. *Gynecol Obstet Invest* 2002;**54**:82–87.

Pollacco J, Sacco K, Portelli M, Schembri-Wismayer P, Calleja-Agius J. Molecular links between endometriosis and cancer. *Gynecol Endocrinol* 2012;**28**:577–581.

Prud'homme GJ. Cancer stem cells and novel targets for antitumor strategies. *Curr Pharm Des* 2012;**18**:2838–2849.

Pujol P, Rey JM, Nirde P, Roger P, Gastaldi M, Laffargue F, Rochefort H, Maudelonde T. Differential expression of estrogen receptor-alpha and -beta messenger RNAs as a potential marker of ovarian carcinogenesis. *Cancer Res* 1998;**58**:5367–5373.

Raspaglio G, Petrillo M, Martinelli E, Li Puma DD, Mariani M, De Donato M, Filippetti F, Mozzetti S, Prislei S, Zannoni GF *et al*. Sox9 and Hif-2alpha regulate TUBB3 gene expression and affect ovarian cancer aggressiveness. *Gene* 2014;**542**:173–181.

Rizzino A. Sox2 and Oct-3/4: a versatile pair of master regulators that orchestrate the self-renewal and pluripotency of embryonic stem cells by functioning as molecular rheostats. *Wiley Interdiscip Rev Syst Biol Med* 2009;**1**:228–236.

Rutella S, Bonanno G, Procoli A, Mariotti A, Corallo M, Prisco MG, Eramo A, Napoletano C, Gallo D, Perillo A *et al*. Cells with characteristics of cancer stem/progenitor cells express the CD133 antigen in human endometrial tumors. *Clin Cancer Res* 2009;**15**:4299–4311.

Sainz de la Cuesta R, Izquierdo M, Canamero M, Granizo JJ, Manzarbeitia F. Increased prevalence of p53 overexpression from typical endometriosis to atypical endometriosis and ovarian cancer associated with endometriosis. *Eur J Obstet Gynecol Reprod Biol* 2004;**113**:87–93.

Saliminejad K, Memariani T, Ardekani AM, Kamali K, Edalatkhah H, Pahlevanzadeh Z, Khorram Khorshid HR. Association study of the TNF-alpha −1031T/C and VEGF +450G/C polymorphisms with susceptibility to endometriosis. *Gynecol Endocrinol* 2013;**29**:974–977.

Sato N, Tsunoda H, Nishida M, Morishita Y, Takimoto Y, Kubo T, Noguchi M. Loss of heterozygosity on 10q23.3 and mutation of the tumor suppressor gene PTEN in benign endometrial cyst of the ovary: possible sequence progression from benign endometrial cyst to endometrioid carcinoma and clear cell carcinoma of the ovary. *Cancer Res* 2000;**60**:7052–7056.

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

Schuring AN, Braun J, Wullner S, Kiesel L, Gotte M. mRNA-expression of ERalpha, ERbeta, and PR in clonal stem cell cultures obtained from human endometrial biopsies. *ScientificWorldJournal* 2011;**11**:1762–1769.

Shi J, Zhou Z, Di W, Li N. Correlation of CD44v6 expression with ovarian cancer progression and recurrence. *BMC Cancer* 2013;**13**:182.

Silveira CG, Abrao MS, Dias JA Jr., Coudry RA, Soares FA, Drigo SA, Domingues MA, Rogatto SR. Common chromosomal imbalances and stemness-related protein expression markers in endometriotic lesions from different anatomical sites: the potential role of stem cells. *Hum Reprod* 2012;**27**:3187–3197.

Stewart CJ, Leung Y, Walsh MD, Walters RJ, Young JP, Buchanan DD. KRAS mutations in ovarian low-grade endometrioid adenocarcinoma: association with concurrent endometriosis. *Hum Pathol* 2012;**43**:1177–1183.

Strzalka W, Ziemienowicz A. Proliferating cell nuclear antigen (PCNA): a key factor in DNA replication and cell cycle regulation. *Ann Bot* 2011;**107**:1127–1140.

Sun Y, Jia X, Wu X. High expressions of Lgr5 and ALDH1 in primary epithelial ovarian cancer correlate with advanced tumor stage and grade as well as poor prognosis of the patients. *Gynecol Obstet Invest* 2016;**81**:162–168.

Takai M, Terai Y, Kawaguchi H, Ashihara K, Fujiwara S, Tanaka T, Tsunetoh S, Tanaka Y, Sasaki H, Kanemura M *et al*. The EMT (epithelial–mesenchymal-transition)-related protein expression indicates the metastatic status and prognosis in patients with ovarian cancer. *J Ovarian Res* 2014;**7**:76.

Takeda T, Banno K, Okawa R, Yanokura M, Iijima M, Irie-Kunitomi H, Nakamura K, Iida M, Adachi M, Umene K *et al*. ARID1A gene mutation in ovarian and endometrial cancers (Review). *Oncol Rep* 2016;**35**:607–613.

Tanaka T, Nakajima S, Umesaki N. Cellular heterogeneity in long-term surviving cells isolated from eutopic endometrial, ovarian endometrioma and adenomyosis tissues. *Oncol Rep* 2003;**10**:1155–1160.

Tang YL, Fan YL, Jiang J, Li KD, Zheng M, Chen W, Ma XR, Geng N, Chen QM, Chen Y *et al*. C-kit induces epithelial-mesenchymal transition and contributes to salivary adenoid cystic cancer progression. *Oncotarget* 2014;**5**:1491–1501.

Uzan C, Cortez A, Dufournet C, Fauvet R, Siffroi JP, Darai E. Endometrium from women with and without endometriosis, and

peritoneal, ovarian and bowel endometriosis, show different c-kit protein expression. *J Reprod Immunol* 2005;**65**:55–63.

Wang ML, Chiou SH, Wu CW. Targeting cancer stem cells: emerging role of Nanog transcription factor. *Onco Targets Ther* 2013;**6**: 1207–1220.

Wells A, Yates C, Shepard CR. E-cadherin as an indicator of mesenchymal to epithelial reverting transitions during the metastatic seeding of disseminated carcinomas. *Clin Exp Metastasis* 2008;**25**:621–628.

Wiegand KC, Shah SP, Al-Agha OM, Zhao Y, Tse K, Zeng T, Senz J, McConechy MK, Anglesio MS, Kalloger SE *et al*. ARID1A mutations in endometriosis-associated ovarian carcinomas. *N Engl J Med* 2010;**363**: 1532–1543.

Wiehle R, Lantvit D, Yamada T. and Christov K. CDB-4124, a progesterone receptor modulator, inhibits mammary carcinogenesis by suppressing cell proliferation and inducing apoptosis. *Cancer Prev Res (Phila)* 2011;**4**:414–424.

Wu R, Hu TC, Rehemtulla A, Fearon ER, Cho KR. Preclinical testing of PI3K/AKT/mTOR signaling inhibitors in a mouse model of ovarian endometrioid adenocarcinoma. *Clin Cancer Res* 2011;**17**: 7359–7372.

Xiao W, Awadallah A, Xin W. Loss of ARID1A/BAF250a expression in ovarian endometriosis and clear cell carcinoma. *Int J Clin Exp Pathol* 2012;**5**:642–650.

Xu Y, Li Y, Pang Y, Ling M, Shen L, Yang X, Zhang J, Zhou J, Wang X, Liu Q. EMT and stem cell-like properties associated with HIF-2alpha are involved in arsenite-induced transformation of human bronchial epithelial cells. *PLoS One* 2012;**7**:e37765.

Young VJ, Brown JK, Saunders PT, Duncan WC, Horne AW. The peritoneum is both a source and target of TGF-beta in women with endometriosis. *PLoS One* 2014;**9**:e106773.

Zambon AC. Use of the Ki67 promoter to label cell cycle entry in living cells. *Cytometry A* 2010;**77**:564–570.

Zannoni GF, Monterossi G, De Stefano I, Gargini A, Salerno MG, Farulla I, Travaglia D, Vellone VG, Scambia G, Gallo D. The expression ratios of estrogen receptor alpha (ERalpha) to estrogen receptor beta1 (ERbeta1) and ERalpha to ERbeta2 identify poor clinical outcome in endometrioid endometrial cancer. *Hum Pathol* 2013;**44**:1047–1054.

Zeng J, Ruan J, Luo L, Shi J, Cui Q, Yang J, Shen K. Molecular portraits of heterogeneity related to cancer stem cells in human ovarian cancer. *Int J Gynecol Cancer* 2014;**24**:29–35.