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Biological Differences Between Ovarian Cancer-associated Fibroblasts and Contralateral Normal Ovary-derived Mesenchymal Stem Cells

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Abstract. *Background/Aim: The aim of this study was to clarify the biological differences between ovarian cancer-associated fibroblasts (OCa-CAFs) and normal ovary-derived mesenchymal stem cells (NO-MSCs). Materials and Methods: Surgically resected ovarian cancer and contralateral normal ovarian tissue samples were cut into small pieces for culture as "explants". The number of outgrown cells, their proliferative kinetics, and expression levels of cell surface markers of CAFs, as well as three miRNAs in OCa-CAFs and NO-MSCs were compared directly. Differentially expressed genes between both groups were also investigated. Results: Comparable numbers of outgrown cells were harvested from both groups. Significantly higher expression of α-smooth muscle actin and miR-142 was found in OCa-CAFs, which decreased significantly during ex vivo cell expansion. A total of 21 differentially expressed genes were identified between both groups. Conclusion: OCa-CAFs showed different biological properties in direct comparison with NO-MSCs, which might play major roles in the pathogenesis of ovarian cancer.*

Malignant tumours are comprised of not only cancer cells, but also various extracellular matrix proteins and interstitial stromal cells that include fibroblasts and immune/inflammatory cells. These interstitial stromal cells form a distinct microenvironment and play major roles in supporting cancer progression through interactions with cancer cells (1-4). It is well documented that cancer cells secrete transforming growth factor-β or platelet-derived growth factor (PDGF) to induce activation of fibroblasts (5-9). The activated fibroblasts (also called myofibroblasts) in epithelial malignant tumours are referred to as cancer-associated fibroblasts (CAFs) (10-12). CAFs are a major constituent of stromal cells in cancer with various origins, which mainly include mesenchymal stem cells and normal fibroblasts, and play major roles in tumour growth, angiogenesis, therapeutic resistances, and metastasis (10, 13, 14). CAFs are generally spindle-shaped cells similar to fibroblasts and smooth muscle cells, which are considered to have the properties of mesenchymal cells. Therefore, mesenchymal markers such as vimentin are used as positive markers of CAFs and epithelial markers such as cytokeratin are used as negative CAF markers. The most widely used marker for CAFs is α-smooth muscle actin (α-SMA), which is a specific marker for myofibroblasts (10). Several other markers, such as platelet-derived growth factor receptor α (PDGFR-α) and fibroblast-specific protein-1 (FSP1), have also been reported (10, 15, 16). CAFs are a heterogeneous cell population with various origins (10, 13, 14), which largely vary depending on the type/stage of tumours and the systemic pathophysio-logical conditions of patients. Furthermore, their biological properties in malignant tumours are not yet fully understood and their potential roles and relevant mechanisms in regulating tumour progression require further elucidation.

Ovarian cancer is one of the most common malignant tumours in women. Although the biological properties of CAFs in various types of cancer have been reported, there are few reports about ovarian cancer-associated fibroblasts (OCa-CAFs) because of the lack of a defined method for cell isolation. We focused on the fact that many ovarian cancer patients who undergo surgical treatment also have their contralateral normal ovary resected. If we could isolate primary OCa-CAFs, it would be useful to uncover the biological properties of OCa-CAFs by direct comparison

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with normal ovary-derived mesenchymal stem cells (NO-MSCs) from the same patient.

Several microRNAs (miRNAs), a class of non-coding RNAs that inhibit expression of target genes, are expressed in CAFs (17). MiRNAs play major roles in not only cancer cells, but also the tumour microenvironment that includes CAFs (18). Although there are few reports about miRNAs expressed in OCa-CAFs, three miRNAs (miR-142, miR-152 and miR-200a) have been reported to be expressed in epithelial ovarian cancer cells, which regulate the progression of ovarian cancer. MiR-142 inhibits the proliferation of epithelial ovarian cancer cells by targeting sirtuin 1 (19) and miR-152 mediates the proliferation and metastasis of ovarian cancer cells by repressing ERBB3 expression (20). Decreased expression of miR-200a is also significantly associated with poor outcomes of ovarian cancer patients (21).

In this study, ovarian cancer-derived mesenchymal stem cells were used to analyse OCa-CAFs. To clarify the biological differences between OCa-CAFs and NO-MSCs, we isolated and expanded ovarian cancer-derived mesenchymal stem cells from surgically resected ovarian cancer tissues as OCa-CAFs and NO-MSCs from the contralateral normal ovary of the same patients. The numbers of outgrown cells, their proliferative kinetics and expression levels of cell surface markers were compared between OCa-CAFs and NO-MSCs. Moreover, we evaluated the expression levels of three miRNAs (miR-142, miR-152, and miR-200a) known to regulate the development and progression of ovarian cancer in OCa-CAFs and NO-MSCs. Finally, to clarify the pathological differences between OCa-CAFs and NO-MSCs, differentially expressed genes between OCa-CAFs and NO-MSCs were identified by microarray-based expression analysis.

Materials and Methods

Ethics. This study was approved by the Institutional Review Boards for Ethical, Legal and Social Issues at Nagasaki University Graduate School of Biomedical Sciences [19012122]. All tissue samples were obtained for study after receiving written informed consent. All experiments were performed in accordance with the institutional and national guidelines.

Isolation and ex vivo expansion of NO-MSCs and OCa-CAFs. We collected tissue samples soon after resection of the normal ovary (n=6) and ovarian cancer (n=6: one high grade serous, three clear cell, one mucinous, and one endometrioid) and placed them in ice-cold Hank's balanced salt solution (Life Technologies, Carlsbad, CA, USA). The mean (±SD) age of the patients was 56.2±5.7 years. The tissue samples were transported to the laboratory and used for isolation and *ex vivo* expansion of mesenchymal stem cells as described previously (22-25). Briefly, after washing in Dulbecco's phosphate-buffered saline (PBS), the tissue samples were minced into small fragments (approximately 1 mm^3) and cultured as explants on 6-cm culture dishes coated with 10 μg/ml human fibronectin (Corning, Corning, NY, USA). Fibroblast-like stromal cells grew out from these tissue fragments in 5 days and became confluent at approximately 3 weeks. Outgrown cells were collected at 3 weeks after initiation of culture using 0.25% trypsin-EDTA (Gibco, Waltham, MA, USA) and then expanded further by general cell passaging. All cell culture was performed in Dulbecco's modified Eagle's medium (Wako, Osaka, Japan) supplemented with 10% foetal bovine serum (Hyclone Laboratories, Logan, UT, USA), 10 ng/ml human recombinant basic fibroblast growth factor (Wako), and a 1% penicillin (100 U/ml)/streptomycin (100 U/ml) solution (Life Technologies) in a 5% CO_2 incubator at 37˚C.

Immunocytochemistry. The phenotypes of *ex vivo*-expanded NO-MSCs and OCa-CAFs were evaluated by immunocytochemistry as described previously (24, 25) with minor modifications. Briefly, twice-passaged cells were cultured on micro glass coverslips (Matsunami, Osaka, Japan) for 3 days and then fixed in 4% paraformaldehyde. After blocking with 0.3% Triton X-100 and 1% bovine serum albumin in PBS for 20 minutes, the cells were incubated with primary antibodies against vimentin (D21H3), keratin-7 (D1E4), α-SMA (D4K9N), PDGFR-α (D1E1E), and FSP1 (D9F9D) (Cell Signaling Technology, Danvers, MA, USA) for 60 minutes at room temperature. Appropriate secondary antibodies conjugated with Alexa fluorochromes (Invitrogen, Carlsbad, CA, USA) were used to detect positive staining. Nuclei were counterstained with 4',6-diamidino-2-phenylindole and positively stained cells were visualized under a fluorescence microscope.

Western blotting. To confirm expression of α-SMA, PDGFR-α, and FSP1 at the protein level, western blotting was performed following protocols described previously (24). Briefly, total proteins were purified from cells, separated on SDS-PAGE gels, and then transferred to 0.22-μm PVDF membranes (Bio-Rad Laboratories, Hercules, CA, USA). After blocking, the membranes were incubated with rabbit polyclonal antibodies specific for α-SMA (D4K9N) or β-actin (D6A8) (Cell Signaling Technology), followed by the appropriate horseradish peroxidase-conjugated secondary antibody (Agilent Technologies, Santa Clara, CA, USA). The labelled proteins were visualized using an enhanced chemiluminescence detection kit. Semiquantitative analysis was performed by measuring the band density using an ImageQuant LAS 4000 mini (GE Healthcare Life Sciences).

qRT-PCR. MiRNA expression in NO-MSCs and OCa-CAFs was evaluated by quantitative reverse transcription-polymerase chain reaction (qRT-PCR). Total RNA was extracted from NO-MSCs and OCa-CAFs using a mirVana miRNA Isolation Kit (Ambion, Waltham, MA, USA) in accordance with the manufacturer's instructions. Total RNA concentrations were measured using a NanoDrop 2000 (Thermo Fisher Scientific, Waltham, MA, USA). A total of 100 ng RNA was used for the following steps. Four specific primers and TaqMan probes for test and control miRNAs [miR-142-3p (assay ID 000464), miR-152-3p (assay ID 000475), miR-200a-3p (assay ID 000502), and U6 snRNA (assay ID 001973)] were used for TaqMan MicroRNA Assays (Applied Biosystems). Absolute qRT-PCR of miRNAs was performed as described previously (25, 26). For each miRNA assay, a calibration curve was prepared by 10-fold serial dilution of single-stranded cDNA oligonucleotides that corresponded to each miRNA sequence from 1×10^2 to 1×10^8 copies/ml. Each sample and calibration dilution

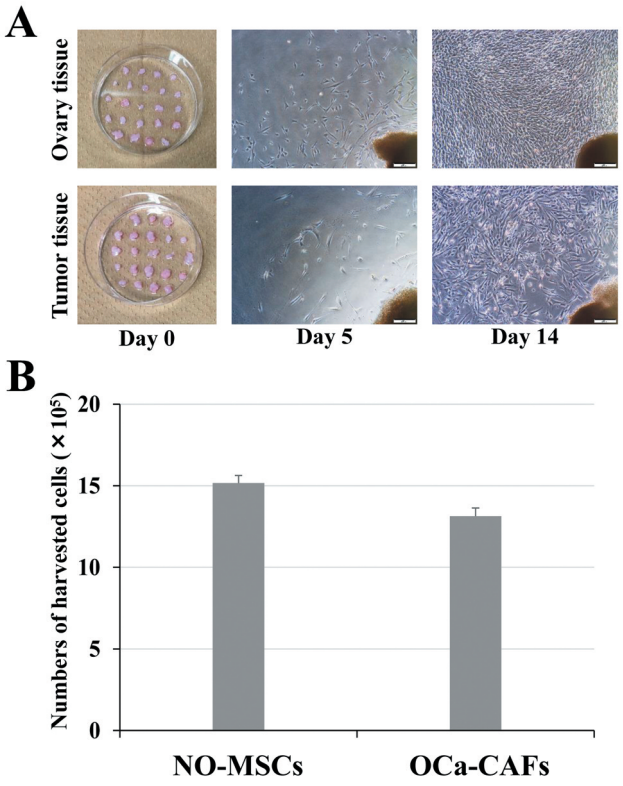

Figure 1. *Primary isolation of stromal cells from ovarian cancer and normal ovary. (A) Surgically resected tissues were minced into small pieces (approximately 1 mm³) and seeded on culture dishes. Fibroblast-like stromal cells grew out from the tissue explants. Scale bars: 200 μm. (B) Numbers of outgrown cells collected from the culture dishes at 14 days after initiation of culture.*

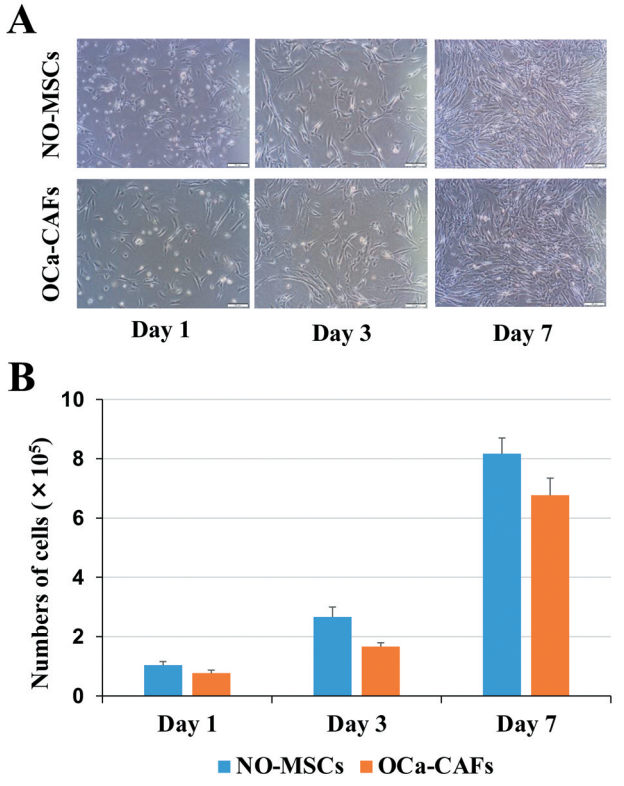

Figure 2. *Ex vivo expansion of stromal cells outgrown from ovarian cancer and normal ovary. (A) Representative images of stromal cells at an early passage (p2). Scale bars: 200 μm. (B) Proliferative potency of ovarian cancer-associated fibroblasts (OCa-CAFs) and normal ovary-derived mesenchymal stem cells from (NO-MSCs).*

were analysed in triplicate. Each amplification run included three water blanks as negative controls for each of the reverse transcription and PCR steps. All data were collected and analysed using a LightCycler® 480 real-time PCR system (Roche, Basel, Switzerland). Expression levels are represented as the relative ratio to U6 snRNA as the endogenous control for normalization.

Microarray analysis. To investigate differentially expressed genes between NO-MSCs and OCa-CAFs, microarray-based gene expression assessment was performed. Total RNAs were collected from twice-passaged NO-MSCs and OCa-CAFs using the mirVana miRNA Isolation Kit in accordance with the manufacturer's instructions. The integrity of total RNAs was estimated using a 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA). After performing background correction and quartile data normalization, the differentially expressed genes between NO-MSCs and OCa-CAFs were screened using a SuperPrint G3 human Microarray 8×60 ver. 3 (Agilent Technologies) with the criteria of | log fold change | >2 and p-value<0.05. Microarray expression profile datasets were downloaded from the National Center of Biotechnology Information Gene Expression Omnibus database (http://www.ncbi.nlm.nih.gov/geo/). The R software package was used to convert and reject unqualified data. The data were calibrated, standardized, and transformed by $\log_2$.

Statistical analyses. All quantitative data are presented as the mean±SD. Statistical significance was determined by the Mann–Whitney U-test (SPSS ver.23, IBM, Armonk, NY, USA). Differences were considered significant at $p<0.05$.

Results

Outgrowth and proliferative kinetics of NO-MSCs and OCa-CAFs. After seeding explants from the normal ovary and ovarian cancer tissues on culture dishes, we observed that fibroblast-like stromal cells grew out from the explants in 5 days and became confluent at approximately 2 weeks (Figure 1A). Comparable numbers of outgrown cells were harvested from both groups ($15.17\pm0.46\times10^5$ *vs* $13.13\pm0.50\times10^5$, $p=0.127$; Figure 1B).

The outgrown cells proliferated very well and showed very similar morphologies under a microscope during cell passaging (Figure 2A). The average population-doubling times of twice-passaged cells were not significantly different (37.3±2.9 h *vs*. 40.6±6.6 h, $p=0.827$; Figure 2B), which suggested similar proliferative kinetics of the groups.

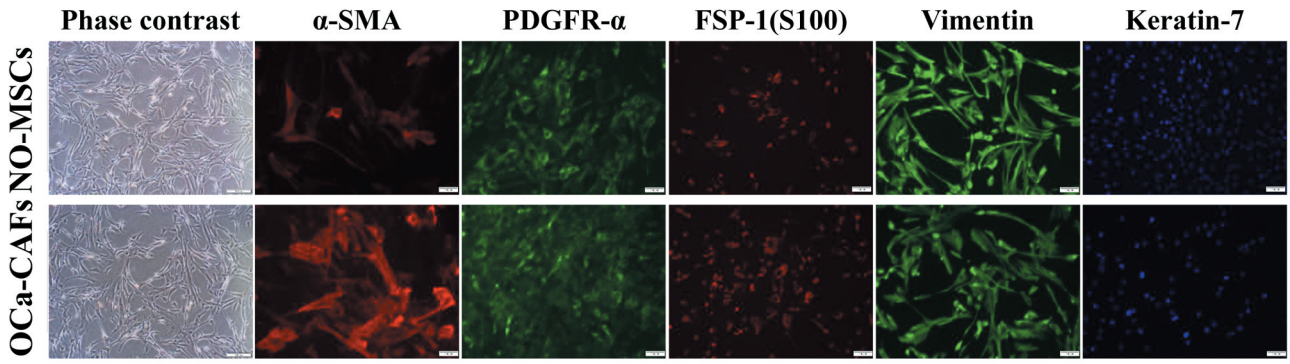

Figure 3. *Expression of cell surface markers in early passage (p2) NO-MSCs and OCa-CAFs. Representative images are shown. Scale bars: 50 μm.*

Different expression levels of CAF markers in NO-MSCs and OCa-CAFs. Immunocytochemical analysis showed that fibroblast-like stromal cells derived from the normal ovary and ovarian cancer expressed mesenchymal marker vimentin but did not express epithelial marker keratin-7 (Figure 3). The negative expression of keratin-7 indicated that there were no contaminating epithelial cancer cells among twice-passaged cells. Interestingly, expression of α-SMA was stronger and more extensive in twice-passaged fibroblast-like stromal cells derived from ovarian cancer than in those derived from the normal ovary, although there was no obvious difference in the expression of PDGFR-α or FSP1 between the groups (Figure 3).

To confirm the findings of immunocytochemical analysis, we evaluated the expression levels of α-SMA, PDGFR-α, and FSP-1 by western blotting. Semiquantitative data showed that the expression of α-SMA was significantly higher in twice-passaged OCa-CAFs than in NO-MSCs (Figure 4A, B). Interestingly, α-SMA expression was significantly decreased by cell passaging of OCa-CAFs, but was maintained at a very stable level in NO-MSCs, which resulted in comparable expression levels in late passage (p10) cells of both groups (Figure 4A, B). There was no significant difference in the expression of PDGFR-α or FSP1 between early passage (p2) NO-MSCs and OCa-CAFs or late passage (p10) cells of both groups (Figure 4C-F).

Differential expression of miRNAs in NO-MSCs and OCa-CAFs. Because miRNAs have been demonstrated to play a crucial role in the interaction between cancer cells and surrounding stromal cells (27), we investigated the expression levels of three miRNAs (miR-142, miR-152, and miR-200a) known to regulate the development and progression of ovarian cancer. Expression of miR-152 was very similar in OCa-CAFs and NO-MSCs and did not change with cell passaging (Figure 5). Although comparable levels of miR-200a expression were found in early passage OCa-CAFs and NO-MSCs, only OCa-CAFs showed a significant decrease with cell passaging ($p<0.01$ at p10 *vs*. p2, Figure 5). Interestingly, expression of miR-142 was significantly higher in early passage (p2) OCa-CAFs than in NO-MSCs ($p<0.01$, Figure 5). Moreover, expression of miR-142 was decreased by cell passaging of OCa-CAFs, but was maintained at a stable level in NO-MSCs, which resulted in comparable levels in late passage (p10) cells of both groups (Figure 5).

Identification of differentially expressed genes between NO-MSCs and OCa-CAFs. A total of 21 differentially expressed genes were identified between NO-MSCs and OCa-CAFs, of which 17 genes were upregulated and four genes were downregulated (Table I). The top 10 most significantly upregulated genes were calcium-dependent secretion activator (*CADPS*), matrix metallopeptidase 3 (*MMP3*), CD163 molecule (*CD163*), collagen type X alpha 1 chain (*COL10A1*), interleukin 7 receptor (*IL7R*), forkhead box F2 (*FOXF2*), colony-stimulating factor 1 receptor (*CSF1R*), S100 calcium-binding protein A4 (*S100A4*), forkhead box Q1 (*FOXQ1*), and transglutaminase 2 (*TGM2*). The four most significantly downregulated genes were alpha-2-macroglobulin (*A2M*), intelectin 1 (*ITLN1*), GATA-binding protein 5 (*GATA5*), and DEPP1 autophagy regulator (*DEPP1*). Furthermore, the target genes of miR-142, which showed significantly higher expression in early passage OCa-CAFs compared to NO-MSCs, were investigated by searching an online database for prediction of miRNA targets (http://mirdb.org/mirdb/index.html). Interestingly, *FOXF2* that was identified as a significantly upregulated gene between the groups was a target gene of miR-142.

Discussion

This study was conducted to isolate and expand OCa-CAFs and directly compare the biological properties of OCa-CAFs and NO-MSCs from the same patient. We isolated and *ex vivo* expanded enough fibroblast-like mesenchymal stromal

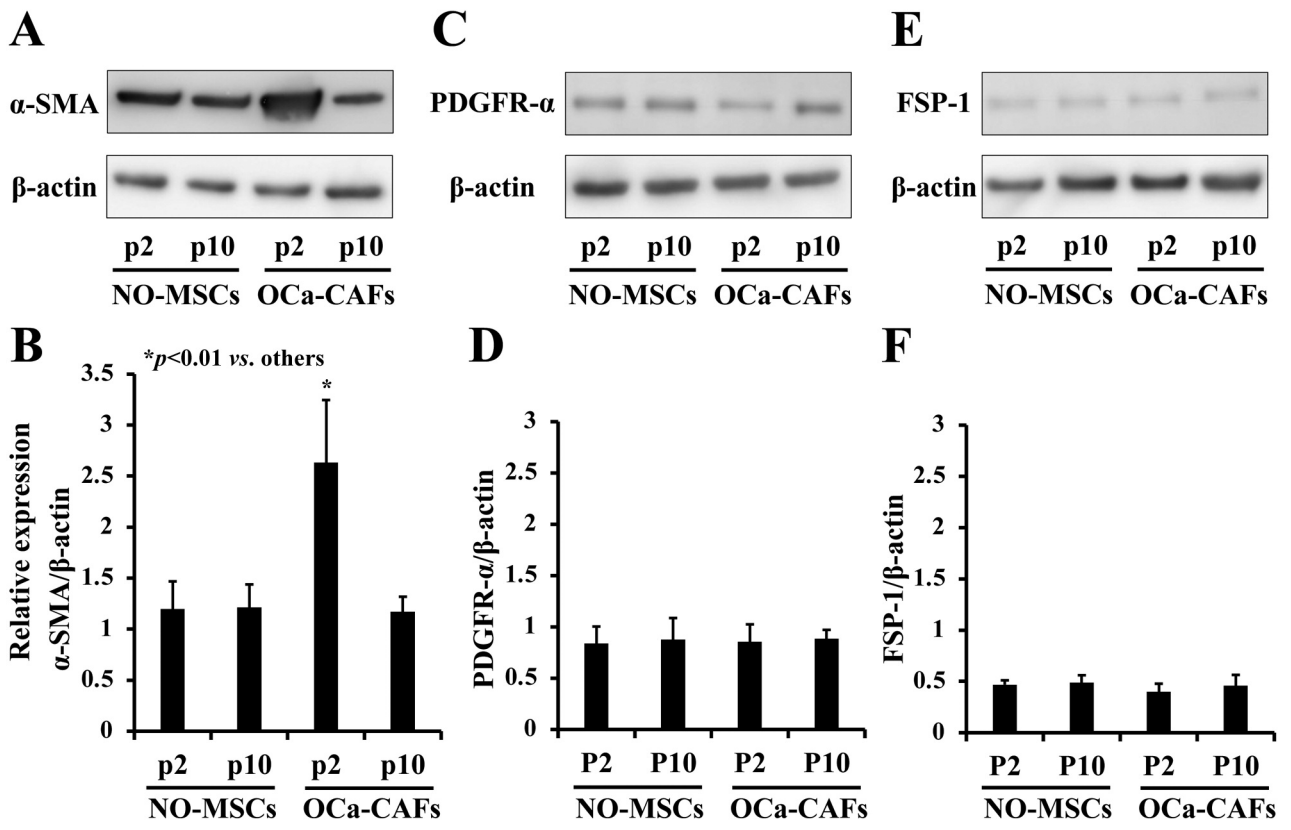

Figure 4. *Western blot analysis of α-SMA, PDGFR-α, and FSP-1 expression. Representative images (A, C, E) and semiquantitative data (B, D, F) of each marker in early (p2) and late (p10) passage NO-MSCs and OCa-CAFs are shown.*

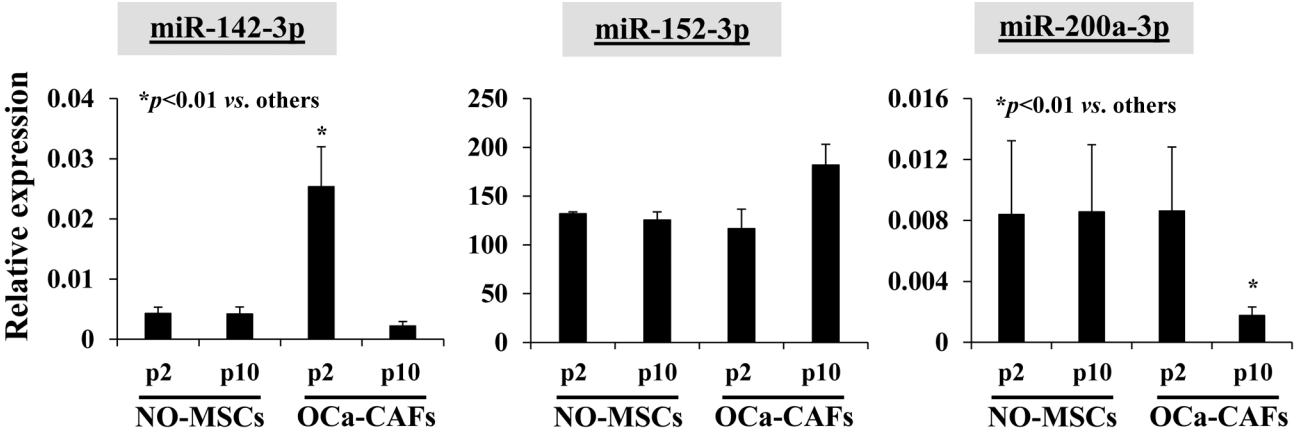

Figure 5. *Quantitative RT-PCR analysis of microRNA expression. Expression of miR-142, miR-152, and miR-200a in early (p2) and late (p10) passage NO-MSCs and OCa-CAFs is shown.*

cells from ovarian cancer and normal ovarian samples by well-defined culture of the tissues as "explants" on dishes. Although these primarily expanded cells showed similar morphologies and proliferative activities, enhanced expression of α-SMA and miR-142 was found in OCa-CAFs, which decreased significantly during *ex vivo* cell expansion. A total of 21 differentially expressed genes were identified between OCa-CAFs and NO-MSCs by microarray-based

Table I. *List of genes up and down-regulated over 2-fold in OCa-CAFs.*

Gene symbol	Gene name	Location	log2 fold change	*p*-Value
Up-regulated genes				
CADPS	Calcium dependent secretion activator	3p14.2	3.7012	0.0102
MMP3	Matrix metallopeptidase 3	11q22.2	3.4766	0.0281
CD163	CD163 molecule	12p13.31	2.7106	0.0091
COL10A1	Collagen type X alpha 1 chain	6q22.1	2.6669	0.0014
IL7R	Interleukin 7 receptor	5p13.2	2.5608	0.0445
FOXF2	Forkhead box F2	6p25.3	2.4582	0.0002
CSF1R	Colony stimulating factor 1 receptor	5q32	2.4540	0.0113
S100A4	S100 calcium binding protein A4	1q21.3	2.3888	0.0030
FOXQ1	Forkhead box Q1	6p25.3	2.3468	0.0097
TGM2	Transglutaminase 2	20q11.23	2.3133	0.0113
BEX1	Brain expressed X-linked 1	Xq22.1	2.1508	0.0202
ATP8A2	ATPase phospholipid transporting 8A2	13q12.13	2.1471	0.0113
lnc-RP11-597K23.2.1-2	Lnc-RP11-597K23.2.1-2	15q25.2	2.1342	0.0303
LOC105369586	LOC105369586	11q25	2.1082	0.0001
HLA-DMB	Major histocompatibility complex, class II, DM beta	6p21.32	2.0550	0.0353
DUXAP9	Double homebox A pseudogene 9	14q11.2	2.0205	0.0030
SLAMF7	SLAM family member 7	1q23.3	2.0010	0.0227
Down-regulated genes				
A2M	Alpha-2-macroglobulin	12p13.31	–2.6867	0.0263
ITLN1	Intelectin 1	1q23.3	–2.4642	0.0335
GATA5	GATA binding protein 5	20q13.33	–2.3150	0.0134
DEPP1	DEPP1 autophagy regulator	10q11.21	–2.1594	0.0465

gene expression assessment, of which 17 genes were upregulated and four genes were downregulated. *FOXF2* identified as a significantly upregulated gene between the groups was a target gene of miR-142.

CAFs interact with cancer cells and are responsible for cancer cell proliferation, metastasis, and invasion (28, 29). Previous studies have already demonstrated that various growth factors and miRNAs released from cancer cells may activate surrounding stromal cells, which contributes to supporting the growth and therapeutic resistance of malignant tumours (10, 27). Although previous studies have used various markers and methods to isolate stromal cells from tumours for experiments (30, 31), we obtained whole mixed populations of stromal cells by simply culturing the normal ovarian and ovarian cancer tissue samples as explants. The fibroblast-like morphology and extensive expression of mesenchymal markers indicated that these *ex vivo*-expanded cells were mesenchymal stromal cells. Moreover, these stromal cells maintained a good proliferative potency *in vitro* for a long time (>10 passages). There was no significant difference in proliferative kinetics between the groups. Further purification of these stromal cells was not performed because there is no unique specific marker for CAFs. Therefore, the primarily *ex vivo*-expanded cells from ovarian cancer represented several subpopulations of interstitial stromal cells in ovarian cancer tissues. The negative expression of epithelial marker keratin-7 in the twice-passaged stromal cells suggested that the primarily expanded stromal cells from ovarian cancer tissues were not contaminated by epithelial cancer cells. These stromal cells were isolated under several other culture conditions, but enough cells were obtained using only the culture conditions described here.

Numerous studies have demonstrated enhanced expression of α-SMA, a common marker of CAFs, in various types of cancer (12, 13, 32). Activated fibroblasts within the tumour stroma which were identified by their expression of α-SMA are called CAFs (33). In ovarian cancer, OCa-CAFs contribute to epithelial ovarian carcinoma metastasis by promoting angiogenesis, lymphangiogenesis and tumour cell invasion (33). Furthermore, patients with high expression levels of α-SMA in epithelial ovarian cancer tissues showed poor survival time, compared to those with low α-SMA expression levels (34). We also confirmed enhanced expression of α-SMA in the primarily *ex vivo*-expanded stromal cells from ovarian cancer tissues at an early passage (p2). These findings suggested that we could regard the cell population derived from ovarian cancer as OCa-CAFs because it showed representative biological properties of CAFs. Moreover, the enhanced expression of α-SMA in OCa-CAFs disappeared during cell passaging *in vitro*. This indirectly indicates that the interaction with cancer cells is critical to maintain an activated phenotype of stromal cells. However, PDGFR-α and FSP1 were not

considered to be suitable specific markers of CAFs in ovarian cancer because there was no obvious difference in their expression between OCa-CAFs and NO-MSCs.

MiRNAs are a class of non-coding RNAs that inhibit expression of target genes to regulate the development and progression of various cancers (35). Furthermore, miRNAs also play major roles in the tumour microenvironment (18). As there are few reports about miRNAs expressed in OCa-CAFs, we selected and investigated the expression levels of three miRNAs (miR-142, miR-152, and miR-200a) in OCa-CAFs and NO-MSCs, which have been reported to be expressed in epithelial ovarian cancer cells and regulate the progression of ovarian cancer. Interestingly, only miR-142 in OCa-CAFs showed dynamic changes in parallel with the expression levels of α-SMA. Although there is a report about an association between α-SMA and miR-142 (36), further experiments are required to investigate whether miR-142, but not miR-152 or miR-200a, is a crucial mediator of the interactions between ovarian cancer cells and OCa-CAFs.

Microarrays can be used to measure the expression levels of genes and explore complicated disease pathogeneses. In this study, 21 differentially expressed genes were identified between OCa-CAFs and NO-MSCs, of which 17 genes were upregulated and four were downregulated. Interestingly, *FOXF2,* a member of the FOX family, identified as a significantly upregulated gene between the groups, was a target gene of miR-142. *FOXF2* can both promote and inhibit proliferation, invasion, and metastasis in tumours, depending on the tumor type (37). In addition, the expression levels of *FOXF2* can be downregulated by a variety of miRNAs (37). In ovarian cancer, there is a report that *FOXF2* expression was decreased in ovarian cancer tissues, and the lncRNA ADAMTS9-AS2 could inhibit ovarian cancer progression by inhibiting the expression of miR-182-5p to improve the expression of FOXF2 (38). Although our data suggest that OCa-CAFs and NO-MSCs have different molecular backgrounds and interactions between OCa-CAFs and epithelial ovarian cancer cells, further studies are required to demonstrate a causal relationship between differentially expressed genes and the progression of ovarian cancer.

This study demonstrated that OCa-CAFs can be isolated and expanded with good proliferative potencies by our simple method as well as specific biological properties with dynamic changes in expression of α-SMA and miR-142 during cell passaging of OCa-CAFs for direct comparison with NO-MSCs of the same patient. A total of 21 differentially expressed genes were identified between NO-MSCs and OCa-CAFs, and a target gene of miR-142 was included among the significantly upregulated genes. However, the molecular mechanisms of the interaction between stromal and cancer cells require further studies. Coculture of stromal cells from the normal ovary with cancer cells may facilitate understanding the mechanisms of the phenotypic plasticity of the former. CAFs have been historically considered to play major roles in the development of tumours (10, 13, 14). However, recent studies indicate that tumor-derived fibroblasts may also restrain tumour progression in some circumstances (39, 40). Although further studies are required to clarify how fibroblasts show both tumour-promoting and tumour-restraining functions, a deep characterization of different subpopulations of CAFs would be important for the design of therapeutic strategies targeting CAFs (40). Therefore, identification of differences between OCa-CAFs derived from ovarian cancer tissues and NO-MSCs from contralateral normal ovary in the same patient may lead to novel therapeutic strategies targeting CAFs in ovarian cancer.

In conclusion, we successfully isolated OCa-CAFs with good proliferative potencies. OCa-CAFs and NO-MSCs showed very similar morphologies and proliferative kinetics. Moreover, different biological properties with dynamic changes during cell passaging were clearly observed in OCa-CAFs, which suggest interactions of stromal cells with cancer cells. On the basis of our experience and the preliminary data in this study, we believe that stromal cells isolated and expanded primarily from surgical resected ovarian cancer tissues may be a very useful tool for future studies to understand the roles and molecular mechanisms of OCa-CAFs in regulating ovarian cancer progression.

Conflicts of Interest

The Authors declare that they have no conflicts of interest.

Authors' Contributions

KO, TL, and KM conceived and designed the experiments. KO and TL performed the experiments and analysed the data. SM and YH provided study materials and technical support. KO, TL, and KM wrote the main manuscript text. All Authors reviewed the manuscript.

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