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Loss of SMARCA4 induces sarcomatogenesis through epithelial–mesenchymal transition in ovarian carcinosarcoma

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Abstract

Ovarian carcinosarcoma (OCS) is a rare and aggressive tumor, and the development of its sarcomatous component is believed to be due to epithelial–mesenchymal transition (EMT). The SWIch/sucrose nonfermentable chromatin remodeling factor (CRF) is closely related to EMT; however, the relationship between CRF and EMT in OCS remains unclear. In this study, we analyzed the protein expression of CRFs, including ARID1A and SMARCA4, and their downstream mRNA expression in 28 OCS cases, two fallopian tube CS cases, and one peritoneal CS case. ARID1A and SMARCA4 exhibited a histological type-specific loss of protein expression in 5 of 11 (45%) endometrioid cases and all 5 serous/homologous OCS cases, respectively. The mRNA analysis suggested that sarcomatogenesis is induced by the transforming growth factor- β and Hippo signaling pathways, both of which regulate YAP1. Immunostaining for YAP1 suggested YAP1-associated sarcomatogenesis in the CRF-retained group, whereas YAP1-unassociated sarcomatogenesis was suggested in the CRF-reduced group. High-grade serous carcinoma cell line experiments showed that the transcriptome of the SMARCA4-knockdown group showed lower expression of the epithelial gene *CDH1* and higher expression of mesenchymal genes such as *VIM*, *ZEB1*, and *SNAIL1* than the control group. Moreover, cell adhesion disappeared and cell morphology changed to a spindle shape, indicating sarcomatogenesis. In conclusion, this study reveals a mechanism for sarcoma development in OCS and provides novel therapeutic possibilities.

KEYWORDS

carcinosarcoma, CRF, epithelial–mesenchymal transition, ovarian neoplasm, sarcomatogenesis

Abbreviations: Ab, antibody; CRF, chromatin remodeling factor; CS, carcinosarcoma; EMT, epithelial–mesenchymal transition; FFPE, formalin-fixed, paraffin-embedded; GO, Gene Ontology; GSEA, Gene Set Enrichment Analysis; KD, knockdown; KEGG, Kyoto Encyclopedia of Genes and Genomes; miRNA, microRNA; OCS, ovarian carcinosarcoma; PCA, principal component analysis; PFS, progression-free survival; SWI/SNF, SWIch/sucrose nonfermentable; TGF β , transforming growth factor β ; UCS, uterine carcinosarcoma.

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1 | INTRODUCTION

Ovarian carcinosarcoma is a rare tumor histologically composed of carcinoma and sarcoma components and accounts for 1%–4% of ovarian carcinomas.¹ It has a poor prognosis, with a median overall survival of 8–26 months.^{2,3} The epithelial component is classified as serous, endometrioid, clear cell, or undifferentiated, whereas the sarcomatous component is classified as homologous and heterologous, depending on whether the tissue is derived from the ovary.

Three theories have been proposed for the mechanism of sarcomatogenesis in gynecologic CS, namely, collision, combination and conversion theories, although the monoclonal origin of development is favored.^{4–9} Furthermore, various mechanisms have been proposed for sarcomatogenesis in recent years, including EMT and miRNA methylation.^{9–13} It is also widely known that EMT is induced by abnormalities in the CRF, including SWI/SNF proteins such as SMARCA4 and ARID1A.^{14–18} Moreover, high YAP1 expression and nuclear migration, which are regulated by the Hippo signaling pathway, have been reported to cause EMT.^{18,19} Nonetheless, there is limited research on the relationship between sarcomatogenesis and CRF or YAP1 in OCS.

Therefore, we undertook this study to identify associations between sarcomatogenesis and CRF or YAP1 abnormalities in OCS through genetic analysis and protein expression.

2 | MATERIALS AND METHODS

2.1 | Patients and samples

We collected 31 specimens from patients diagnosed with ovarian, fallopian tube, and peritoneal CS between 2000 and 2020 at Kyushu University, Red Cross Fukuoka Hospital, Kitakyushu Municipal Medical Center, Japan Community Healthcare Organization Kyushu Hospital, Red Cross Matsuyama Hospital, and NHO Shikoku Cancer Center. The specimens were prepared as FFPE samples, after which they were classified for histology by immunostaining with p53, WT1, ER, PgR, and HNF1 β Abs and evaluated by two pathologists (Y.K. and T.I.). There were no excluded cases.

Clinicopathologic findings such as age, FIGO stage, and postoperative adjuvant chemotherapy, obtained by reviewing the medical records, were evaluated.

This study was approved by the Ethics Committee of Kyushu University (IRB: 21120-00).

2.2 | DNA alteration profiling

A public exome sequence dataset was used to evaluate the frequency of DNA alterations in 14 gynecologic carcinosarcoma cases (9 UCS cases and 5 OCS cases) from the study of Gotoh

et al.¹⁰ Epigenome-associated genes, including CRFs, were extracted from exome sequencing data and analyzed for their alteration frequency.

2.3 | Immunohistochemistry

Surgically resected sections of both carcinoma and sarcoma components were selected in each case. The FFPE blocks were cut into 3 μ m-thick slices, dewaxed in xylene, and rehydrated in a graded series of ethanol. The samples were treated with 3% hydrogen peroxide in methanol for 30 min to block endogenous peroxidase activity and then washed twice with PBS. The primary Abs used in this study are listed in Table S1.

The expression of ARID1A and SMARCA4 was considered “loss” or “partial loss” of nuclear staining compared with that of surrounding normal stromal cells or endothelium. The complete loss of protein expression was defined as “loss,” while focally positive case was classified as “partial loss.”

CHD4 and YAP1 were evaluated for nuclear staining using the Histo-score (H-score), which analyzes four staining intensities, namely, negative (0), weak (1+), moderate (2+), and strong (3+), multiplied by the percentage of stained cells for a total value (range 0–300).

Chromatin remodeling factors were assessed in two groups: carcinoma (Ca.) and sarcoma (Sa.). YAP1 was analyzed among the three groups: Ca., Sa. (SWI/SNF-retained), and Sa. (SWI/SNF-reduced), as well as in terms of homologous versus heterologous components in sarcoma.

2.4 | Dissection and RNA extraction

Carcinomas and sarcomas were separated by macrodissection and laser capture microdissection depending on their localization. For laser capture microdissection, 8 μ m-thick sections cut from the FFPE block were placed on membrane slides (MembraneSlide 1.0 PEN; Carl Zeiss), stained with hematoxylin for 10 s, and then dehydrated using ascending concentrations of ethanol solutions. The stained specimens were microdissected by laser microdissection (LMD6500; Leica Microsystems), collected into 0.5 mL PCR tubes, and immediately processed for subsequent RNA extraction, as described previously.^{20,21}

2.5 | mRNA expression and bioinformatics analysis

Comprehensive mRNA expression was evaluated using the nCounter Gene Expression Assay (NanoString Technologies Inc.). A total of 20 tissue sections (carcinoma and sarcoma components from 10 cases) were extracted from the FFPE blocks, and the target regions were enriched using the nCounter Pan Cancer Progression panel (<https://nanosttring.com/products/ncounter-assays-panels/>)

[oncology/pancancer-progression/](#)) and evaluated using the nCounter Analysis System (NanoString Technologies Inc.). Raw data were normalized using the nSolver analysis software (version 4.0; NanoString Technologies Inc.). All procedures were carried out according to the manufacturer's protocol. The PCA was undertaken using the prcomp package of R. Gene Set Enrichment Analysis was carried out using the GSEA software for comparing the two groups of carcinomas and sarcomas and extracting gene sets with $p < 0.05$. Differentially expressed genes with $|\log_2(\text{FC} [\text{fold change}])| > 1$ and $p < 0.05$ were extracted using the edge R package of R. The GO analysis was carried out using the DAVID online tool with the KEGG database. We further compared the SWI/SNF-retained sarcoma group and the SWI/SNF-reduced sarcoma group to identify genes with varying expressions according to the presence or absence of SWI/SNF expression.

2.6 | Cell culture

The human high-grade serous ovarian cancer cell line OVSAHO was purchased from the JRCB Cell Bank. The OVSAHO cell line has been confirmed to retain SMARCA4.²² The cell line was grown in DMEM (Nacalai Tesque) supplemented with 10% FBS (Sigma-Aldrich), 100 µg/mL streptomycin, and 100 U/mL penicillin (Nacalai Tesque) and was maintained at 37°C in a humidified incubator with 5% CO₂.

2.7 | Electroporation of cells with siRNA

The siRNA oligos siSMARCA4 (forward, 5'-GCAUUUCAAGGAAUAUCAC-3' and reverse, 5'-GUGAUUCCUUGAAAUGC-3') and control siScramble (Silencer Select Negative Control #1; Thermo Fisher Scientific) were used for KD of SMARCA4. Electroporation was carried out using the Neon Transfection System (Invitrogen). Cells transfected with siRNA were re-spread into a 6-well plate, with each well containing 2 mL antibiotic-free media, and incubated under standard conditions (37°C, 5% CO₂, air humidity) for 48 h, followed by rapid extraction of RNA for RNA sequencing and proteins for western blotting.

2.8 | Western blot analysis

Cells were washed twice with PBS, centrifuged, resuspended in SDS sample buffer, and then denatured at 97°C for 3 min as described previously.²³ The samples were separated by SDS-PAGE (Mini-PROTEAN TGF; Bio-Rad) and electrotransferred onto a PVDF membrane using the Trans-Bot Turbo Transfer System (Bio-Rad Laboratories). The details of blocking, primary Abs, secondary Abs, and detection were the same as those used previously.²⁴ The primary Abs used for western blotting are listed in Table S1.

2.9 | mRNA sequencing and mRNA sequencing data analysis

Total RNA was extracted from the cell line under each condition using Sepasol-RNA I Super G (Nacalai Tesque). Library preparation was performed using 1 µg total RNA and the NEBNext Ultra Directional RNA Library Prep Kit (New England Biolabs). mRNA sequencing was carried out using Illumina NovaSeq 6000 at Relixa for 150-bp paired ends. More than 4-GB reads were obtained in each sample. The reads were mapped against human genome hg19 using HISATS (version 2.1.0), and count data were obtained using featureCounts (version 1.6.3).

2.10 | Immunofluorescence staining

Cell immunofluorescence staining was undertaken on coverslips. Cells plated onto coverslips were fixed with 4% paraformaldehyde in PBS for 10 min, followed by permeabilization with 0.5% Triton X-100 in PBS for 5 min. After blocking with Blocking One-P for 10 min, the cells were incubated with anti-SMARCA4, anti-YAP1, anti-E-cadherin, and anti-vimentin Abs prepared in a blocking solution for 2 h at room temperature. After washing in PBS, the cells were incubated with CF488A-labeled goat anti-mouse or CF568-labeled goat anti-rabbit Ab (1:800; Biotium) prepared in PBS for 30 min at room temperature under shading. Finally, the coverslips were washed in PBS and mounted using ProLong Gold Diamond Antifade Reagent with DAPI (Thermo Fisher Scientific). Immunofluorescence images were obtained using a DMi8 THUNDER imaging system (Leica). Triton X-100 was not used in E-cadherin staining to evaluate its membrane expression.

2.11 | Statistical analysis

All statistical analyses were carried out using a statistical software package (JMP Pro 16.1.0; SAS Institute Inc.). Survival curves were generated using the Kaplan–Meier method, and comparisons between the two groups were undertaken using the Cox proportional hazards model. $p < 0.05$ was considered statistically significant. Statistical analyses were conducted exclusively on OCS cases.

3 | RESULTS

3.1 | Clinicopathologic characteristics

The clinicopathological characteristics of the 31 cases of CS, including fallopian tube and peritoneal CS, are shown in Table 1. The median age of the patients was 63.5 years (interquartile range, 34–85). The carcinoma component was serous carcinoma in the majority (61.2%) of cases, and the sarcoma component was heterologous

TABLE 1 Characteristics of patients with gynecologic carcinosarcoma ($n=31$).

Factor	Group	n
Age, years	<50/≥50	6/24 (1 NA)
	Median (IQR)	63.5 (34–85)
Location	Ovary	28
	Fallopian tube	2
	Peritoneum	1
Stage	I, II	14
	III, IV	16 (1 NA)
Histological type of carcinoma	Serous	19
	Endometrioid	11
	Clear	1
Histological type of sarcoma	Homologous	10
	Heterologous	21
	Chondro	15
	Rhabdo	6

Abbreviations: Chondro, chondrosarcoma; Clear, clear cell carcinoma; Endometrioid, endometrioid carcinoma; IQR, interquartile range; NA, not available; Rhabdo, rhabdomyosarcoma.; Serous, serous carcinoma.

in the majority (67.7%) of cases, with chondrosarcoma being more common than rhabdomyosarcoma (71.4% in the heterologous type).

3.2 | Analysis of alterations

The alteration frequencies of epigenome-associated genes extracted from public next-generation sequencing data are summarized in Figure S1. We identified alterations in various epigenome-associated genes, including CRF and histone modification-related genes. A high alteration frequency was found for *ARID1A* (UCS, 55%; OCS, 20%), *CHD4* (UCS, 55%; OCS, 0%), and *SMARCA4* (UCS, 33%; OCS, 20%) among the CRFs.

3.3 | Immunohistochemical results of CRFs

ARID1A, *CHD4*, and *SMARCA4* are predominantly expressed in the nucleus of tumor cells. Figures 1A and S2 show representative images of hematoxylin-stained OCS samples and the immunohistochemistry results of respective CRFs. *ARID1A* showed loss of both carcinoma and sarcoma components in 5 of 11 endometrioid cases (45%). *SMARCA4* demonstrated loss or partial loss in only the sarcoma component in all five serous/homologous OCS cases. The only serous/homologous case with retained *SMARCA4* was a peritoneal CS. The results of CRF immunostaining are summarized in Figure 1B. Protein expression of *CHD4* showed no significant difference between carcinomas and sarcomas (Figure 1C).

3.4 | Prognostic analysis

The results of the prognostic analysis are presented in Table S2 and Figure S3. This study found no prognostic differences based on age, adjuvant chemotherapy, histological features of carcinoma and sarcoma components, or the presence or absence of SWI/SNF dysfunction.

3.5 | Comprehensive mRNA expression analysis

In the PCA, PC1 component with a 47% contribution was separated between carcinoma and sarcoma (Figure 2A); hence, we decided to perform the analysis between the carcinoma and sarcoma groups. The results of GSEA are depicted in Figure 2B. The enriched adherens junction, cell–cell junction, tight junction, and Wnt signaling pathway contained several EMT-related genes, indicating an association between sarcomatogenesis and EMT. A total of 248 EMT-related genes were extracted from the genes included in nCounter by referring to the Epithelial–Mesenchymal Transition Gene Database (<https://dbemt.bioinfo-minzhao.org>). A total of 69 DEGs were extracted, and the sarcoma component showed increased expression of mesenchymal markers such as *TGFβ1*, *SNAI2*, and *ZEB1*, suggesting an association with EMT (Figure 2C). GO analysis was performed using the KEGG database focusing on the *TGFβ* signaling pathway and Hippo signaling pathway, which are closely associated with EMT (Figure 2D). These analyses indicate that *YAP1* is located downstream of these pathways.

3.6 | Immunohistochemical analysis of YAP1 expression

Figure 3A–C shows representative images of *YAP1* staining for each component. Nuclear expression of *YAP1* was higher in the SWI/SNF-retained sarcoma group than in the carcinoma group, whereas it was lower in the SWI/SNF-reduced sarcoma group (Figure 3D). Furthermore, nuclear *YAP1* expression was greater in the heterologous Sa. component compared to the homologous Sa. component (Figure 3E).

3.7 | Effects of SMARCA4 KD in OVSAHO cell line

OVSAHO cell lines were prepared for analyzing the changes in *YAP1* expression and EMT associated with *SMARCA4* KD. This was done because a reduced protein expression of *SMARCA4* is a specific change in serous homologous OCS. Our results confirmed that *SMARCA4* was downregulated by si*SMARCA4* at mRNA and protein expression levels.

In the OVSAHO cell line, si*SMARCA4* significantly reduced the *CDH1* mRNA expression levels. In contrast, the mRNA expression levels of *VIM*, *ZEB1*, *SNAI1*, and *TGFβ1* were significantly upregulated (Figure 4A). Furthermore, the protein expression of vimentin was upregulated (Figure 4B). *YAP1* expression showed no significant changes at either mRNA or protein level (Figure 4A,B).

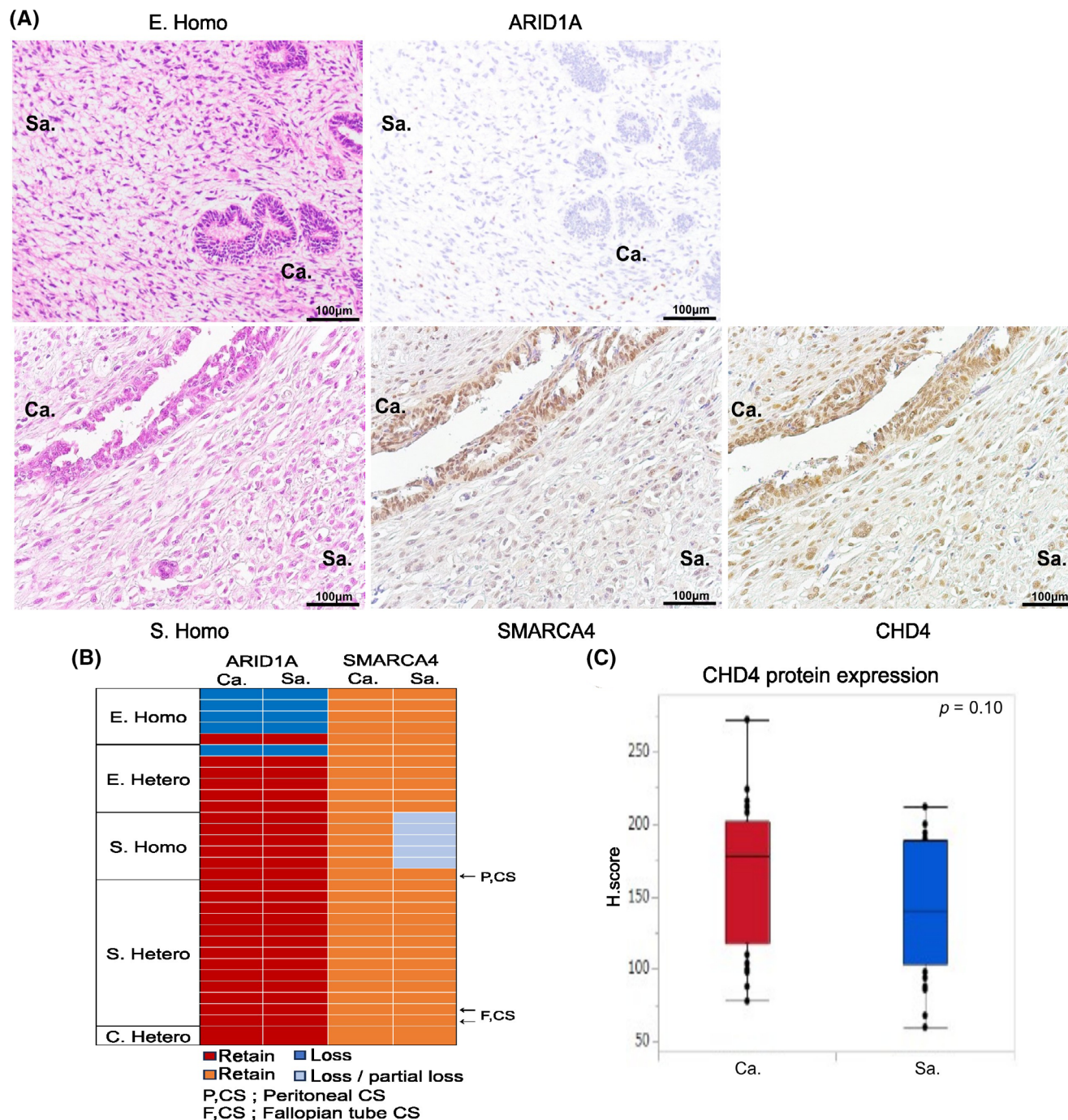


FIGURE 1 Chromatin remodeling factor (CRF) expression analysis by immunohistochemistry. Normal stromal cells and endothelium demonstrated consistently preserved nuclear staining, serving as a positive internal control. (A) Representative images of H&E staining of endometrioid homologous ovarian carcinosarcoma (OCS) and serous homologous OCS and immunostaining of the respective CRFs. (B) Summary of ARID1A and SMARCA4 immunostaining results for each case. Histological types are shown on the left. (C) Boxplot of the Histo-score (H-score) in CHD4. Mann–Whitney *U*-test was used for comparison. C. Hetero, clear cell heterogeneous OCS; Ca., OCS carcinoma component; E. Homo, endometrioid homologous OCS; F,CS, fallopian tube CS; P,CS, peritoneal CS; S. Homo, serous homologous OCS; Sa., OCS sarcoma component.

Immunofluorescence experiments revealed that siSMARCA4 reduced the membrane expression of E-cadherin and increased the cytoplasmic expression of vimentin. The localization of YAP1 moved from the nucleus to the cytoplasm. Moreover, cell adhesion disappeared, and cell morphology changed to a spindle shape (Figure 4C).

3.8 | Expression of *TGFβ1*

TGFβ1 was the EMT-related gene that showed an extreme difference in expression. SWI/SNF reduction was associated with a higher expression of *TGFβ1* in both nCounter data and OVSCHO cell line RNA

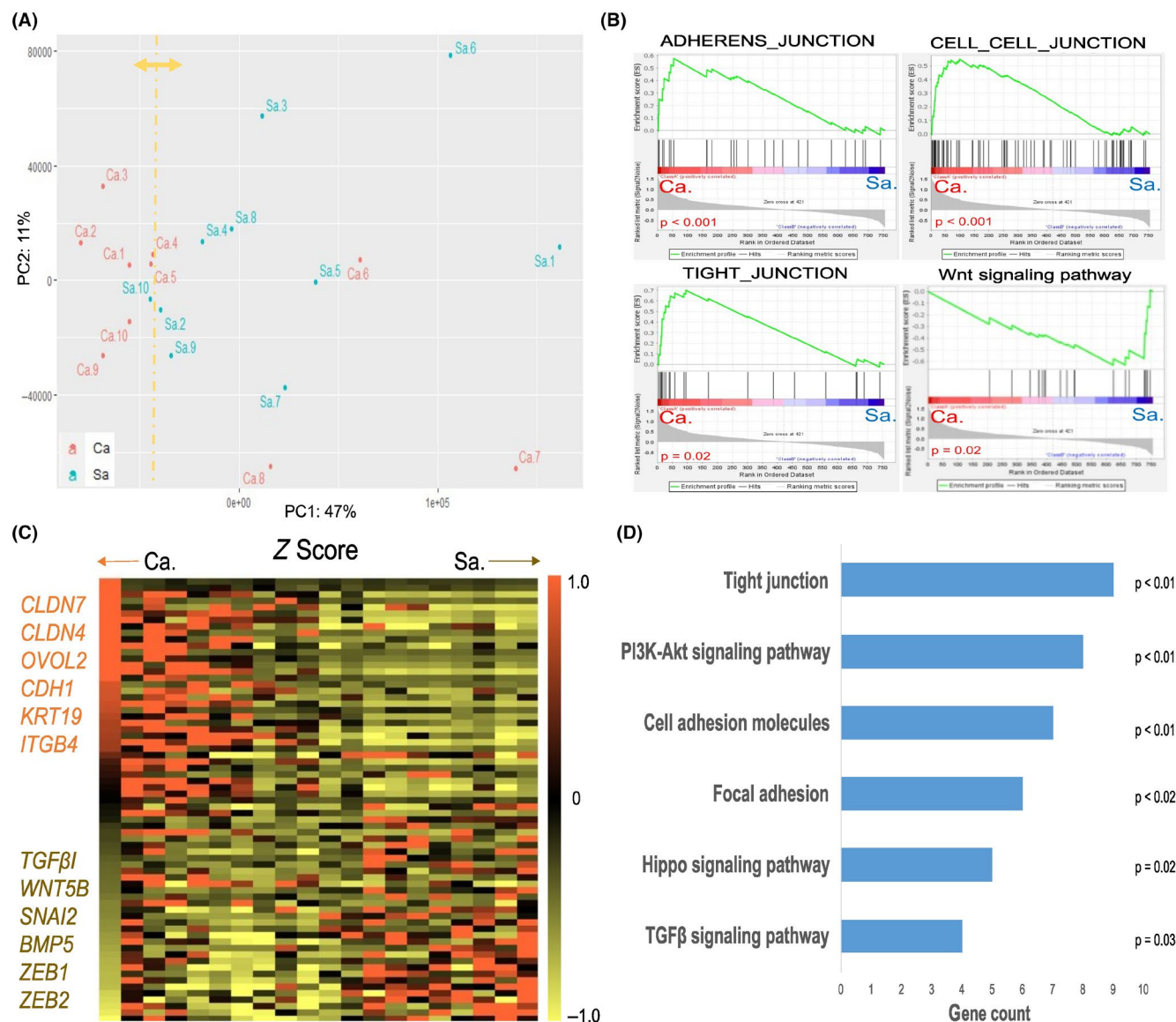


FIGURE 2 Summary of gene expression analysis using nCounter. (A) Principal component (PC) analysis of tumor components in each patient with ovarian carcinosarcoma (OCS). Red and blue colors indicate the OCS carcinoma (Ca.) and OCS sarcoma (Sa.) components, respectively. Yellow line separates the Ca. and Sa. components. x- and y-axes indicate PC1 and PC2, respectively. (B) Gene Set Enrichment Analysis. Significantly enriched oncological signatures in the carcinoma and sarcoma groups ($p < 0.05$). (C) Heatmap of epithelial-mesenchymal transition (EMT)-related genes among differentially expressed genes. A total of 69 EMT-related genes were standardized and mapped using Z-score. Orange and yellow indicate high and low expression of genes in the heatmap, respectively. Representative epithelial and mesenchymal genes are shown to the left of the heatmap in orange and yellow, respectively. (D) Gene Ontology analysis. Bar plot showing the results in order of p value. TGFβ, transforming growth factor β.

sequencing data (Figure 5). In particular, nCounter data revealed higher TGFβ1 expression in the sarcoma group than in the carcinoma group, with the SWI/SNF-reduced group also showing higher expression (Figure 5A).

4 | DISCUSSION

To our knowledge, this is the first study to identify the relationship among SWI/SNF CRF, YAP1, and EMT in OCS. SMARCA4 showed a

histology-specific change among OCS cases, with the loss of protein expression observed only in the sarcoma component in all serous/homologous cases. In cell line experiments, the transcriptome and protein expression of the SMARCA4 KD group suggested sarcomatoid differentiation, as well as a change to a spindle-shaped, sarcomatoid morphology.

SWI/SNF CRF is a subfamily of ATP-dependent chromatin remodeling complexes, whose alterations contribute to resistance to DNA damage repair, DNA replication stress, senescence, angiogenesis, metastasis, and tumor immunity.²⁵ In ovarian cancer, ARID1A mutation is

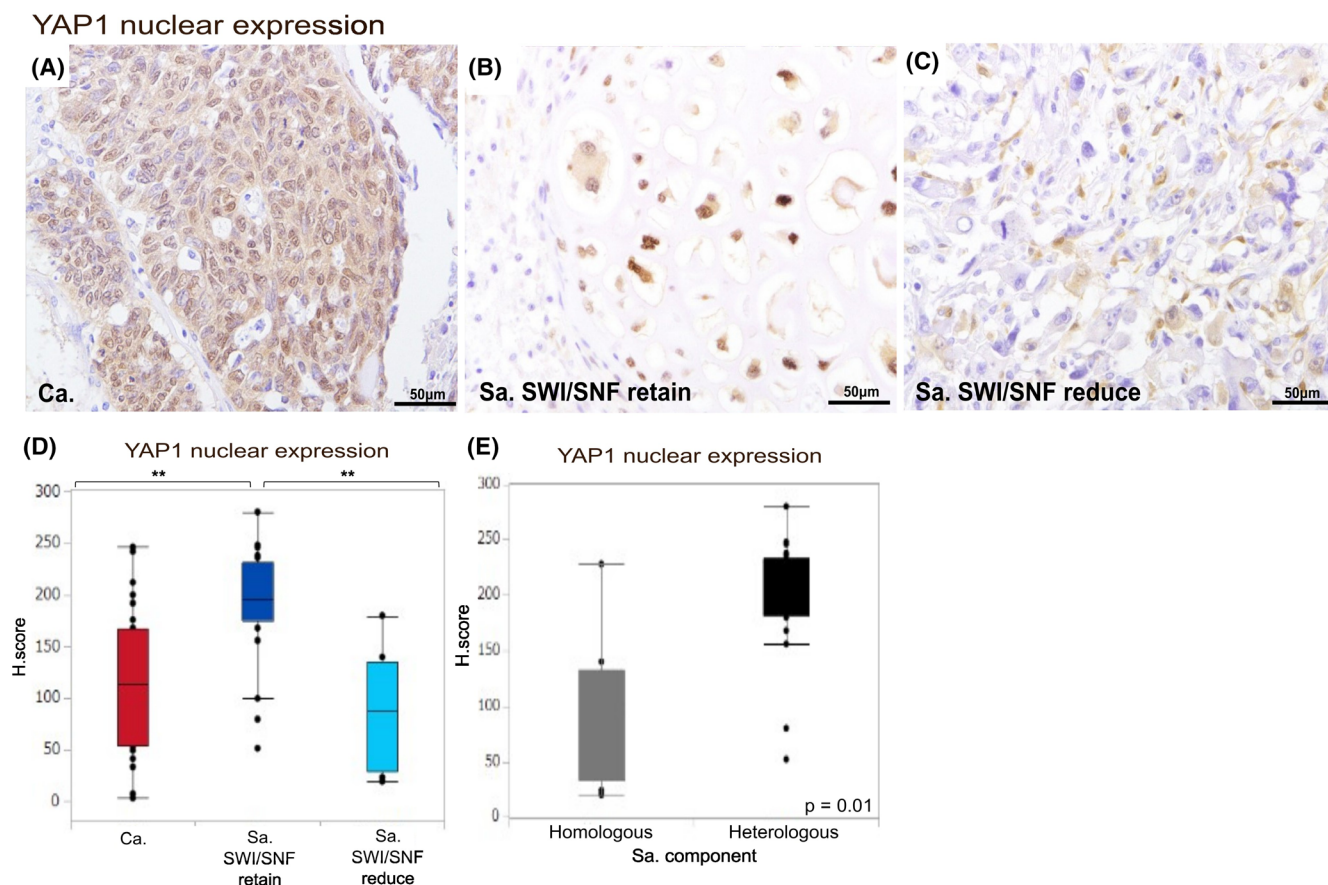


FIGURE 3 YAP1 expression analysis by immunohistochemistry. (A–C) Representative images of YAP1 for (A) ovarian carcinosarcoma (OCS) carcinoma component (Ca.), (B) OCS sarcoma component (Sa.) (SWI/SNF-retained), and (C) Sa. (SWI/SNF-reduced), respectively. (D, E) Boxplot of YAP1. Mann-Whitney *U*-test was used for comparison. ***p* < 0.001. H-score, Histo-score.

found in 46%–57% of clear cell carcinoma and 30% of endometrioid carcinoma cases.^{26,27} Studies have also reported that SWI/SNF CRF, including ARID1A and SMARCA4, induce EMT through the TGFβ and Wnt signaling pathways,^{16,18,28} suggesting that changes in SWI/SNF CRFs are closely associated with EMT. In this study, the loss of ARID1A protein expression was observed in both carcinoma and sarcoma components. Therefore, ARID1A is considered a factor in carcinoma development rather than a driver gene in sarcomatogenesis. Nonetheless, a high frequency of protein loss was detected in endometrioid cases (5/11 cases, 45%), suggesting that the loss of ARID1A is an endometrioid-specific change and is susceptible to sarcomatogenesis. Conversely, in serous/homologous OCS, the loss of SMARCA4 protein was detected only in the sarcoma component, indicating that this is a serous/homologous-specific change.

YAP1 is a gene regulated by the Hippo signaling pathway and plays a vital role in tumor invasion and metastasis. YAP1 is associated with EMT in multiple organs, including the mammary gland,²⁹ pancreas,³⁰ colon,³¹ and liver.³² YAP1 is also closely associated with SWI/SNF; it forms a complex with ARID1A, and the loss of ARID1A contributes to EMT by allowing YAP1 and TEAD to bind and act as a transcription factor.³³ In a study of triple-negative breast cancer cell lines, the loss of function of SMARCA4 resulted in high expression of YAP1 and induced EMT.¹⁸ However, in the present study, YAP1 showed high

nuclear expression in the sarcoma component with retained SWI/SNF, whereas its nuclear expression was reduced or absent in the sarcoma component with reduced SWI/SNF, in both patient specimens and the cell line. Furthermore, nuclear expression of YAP1 was also elevated in heterologous versus homologous patient specimens. These results indicate that heterologous component may be more prone to YAP1-induced EMT in the SWI/SNF-retained group. Conversely, in the SWI/SNF-reduced group, specifically with SMARCA4 loss of function, the homologous component may undergo EMT that is not linked to YAP1. We infer that the localization of YAP1 moved from the nucleus to the cytoplasm as feedback for EMT that is not a Hippo signaling pathway. Our focus in this study was on TGFβ1 as an important signal for EMT in the SWI/SNF-reduced group. The results of mRNA expression in both the SWI/SNF-reduced group in nCounter data and in the SMARCA4 KD group in cell line experiments revealed extremely high levels of TGFβ1 expression. Overexpression of TGFβ1 could be a specific alteration in the loss of function of SWI/SNF and may be responsible for EMT.

Transforming growth factor β1 is defined as different from TGFβ1 or TGFβ2. It is a 68 kDa ECM protein involved in various pathological conditions, including cancer, diabetes, nephropathy, wound healing, and atherosclerosis.^{34–37} In cholangiocarcinoma, TGFβ1 is highly expressed in carcinoma cells that show

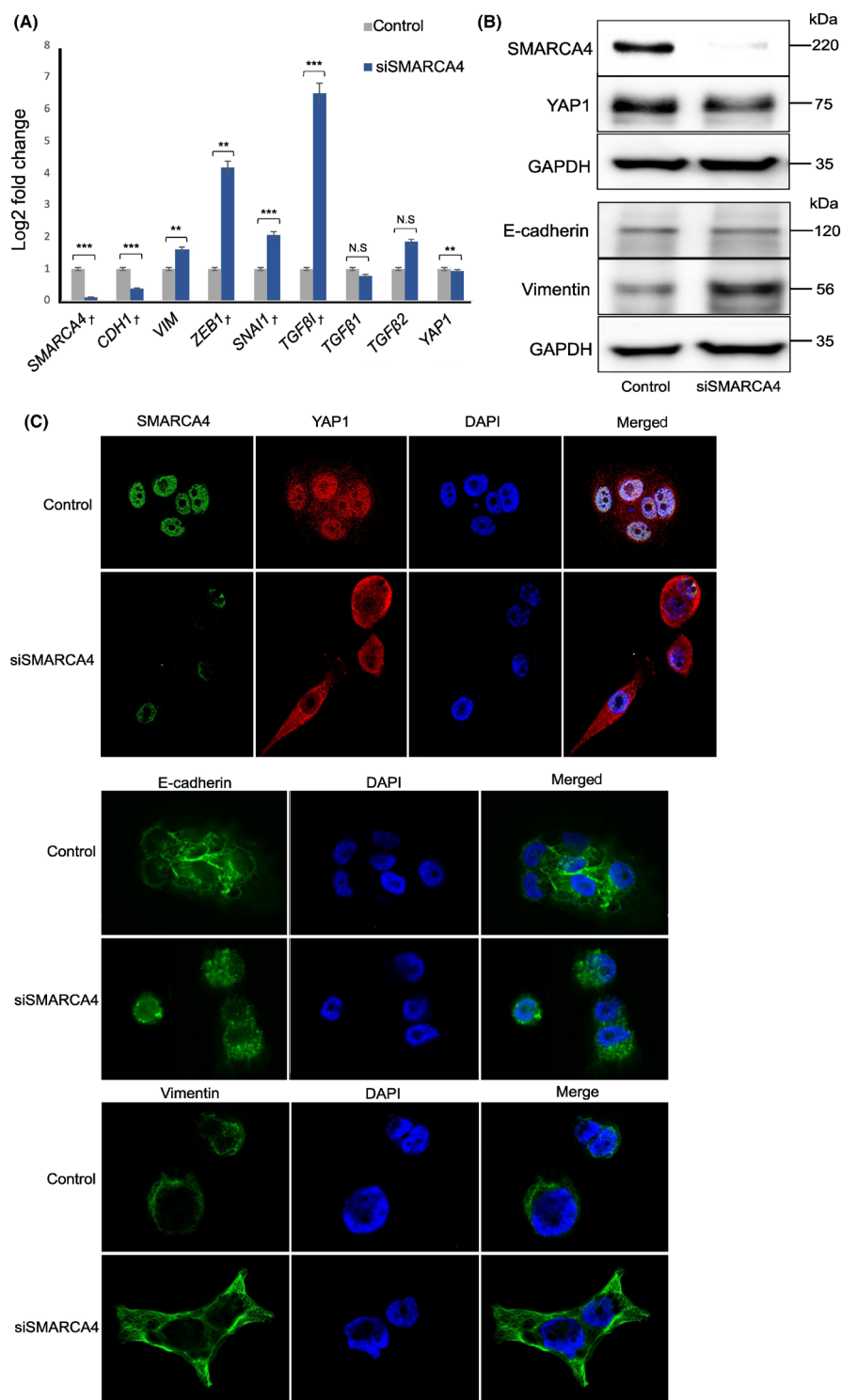


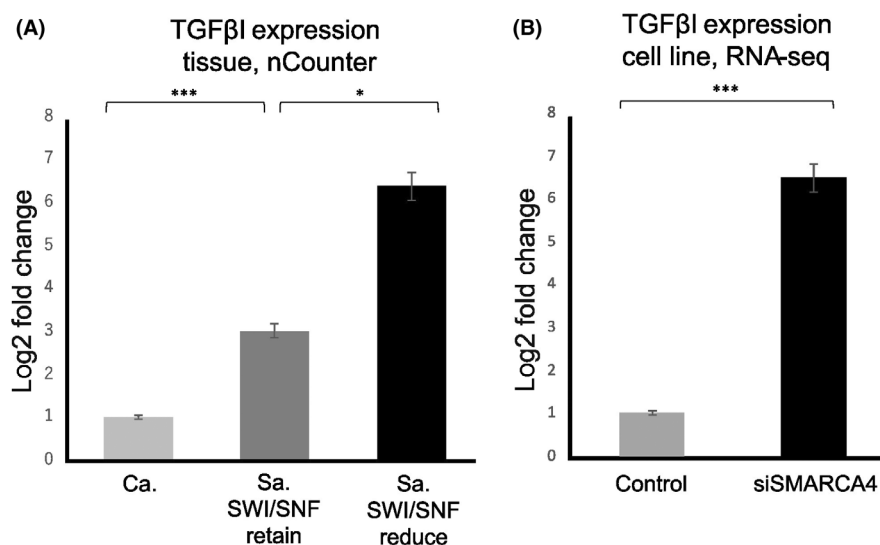
FIGURE 4 SMARCA4 knockdown (KD) analysis using the OVSAHO cell line. (A) mRNA expression analysis of epithelial-mesenchymal transition markers by RNA sequencing. The y-axis indicates the log2 fold change between control and SMARCA4 KD cells. (B) Protein expression of YAP1, epithelial marker (E-cadherin), and mesenchymal marker (vimentin). (C) Immunofluorescence staining of YAP1, epithelial marker (E-cadherin), and mesenchymal marker (vimentin) ** $p < 0.01$, *** $p < 0.001$. [†]Differentially expressed gene (DEG). N.S, not significant.

sarcomatoid changes in contrast to epithelial carcinoma cells and was emphasized as a candidate EMT regulator.³⁸ TGFβ1 is over-methylated in ovarian carcinoma.³⁹ In this study, the expression of TGFβ1 in the carcinoma component was also suppressed, whereas its expression in the sarcoma component of the SWI/SNF-reduced group was extremely increased (Figure 5A), suggesting that SWI/SNF reduction induces high TGFβ1 expression. TGFβ1 is downstream TGFβ1,2, but it is also affected by epigenomic regulation such as miRNAs. Corneal studies have reported that miRNA21

and miRNA181a positively correlated with TGFβ1 expression.^{40,41} The difference in TGFβ1 expression may not be due to TGFβ1,2 but to other factors, including epigenomic abnormalities such as miRNAs, because there was no change in TGFβ1,2 expression in the SMARCA4 KD group. Epithelial-mesenchymal transition may be induced by the loss of function of SWI/SNF CRF, affecting the regulator of TGFβ1 methylation.

Interestingly, siSMARCA4 did not lead to significant differences in E-cadherin protein levels, despite a decrease in mRNA expression.

FIGURE 5 mRNA expression of transforming growth factor β I (TGF β I) using nCounter and RNA sequencing (RNA-seq). (A) nCounter data of formalin-fixed, paraffin-embedded samples. The y-axis indicates the log2 fold change between ovarian carcinosarcoma (OCS) carcinoma component (Ca.), OCS sarcoma component (Sa.) (SWI/SNF-retained), and Sa. (SWI/SNF-reduced). (B) RNA-seq data of OVSCHO cell line. The y-axis indicates the log2 fold change between control and SMARCA4 KD cells. * $p < 0.05$; *** $p < 0.001$.



Localization or stability of mRNA and protein may have caused the discrepancy between mRNA and protein expression. However, membrane expression of E-cadherin was reduced, suggesting that siSMARCA4 induced EMT.

No differences in prognosis were observed in the multivariate analysis, including the protein expression of SWI/SNF, in this study. However, OCS has an extremely poor prognosis, which may be due to treatment resistance. We believe that EMT contributes to the acquisition of therapeutic resistance in OCS. Therefore, clarifying the mechanism underlying EMT could provide a link to the identification of novel therapeutic targets.

In conclusion, this study suggests that the regulation of the Hippo signaling pathway is a potential therapeutic target for SWI/SNF-retained OCS. Conversely, OCS with reduced SWI/SNF expression is not considered a type of sarcomatogenesis mediated by the Hippo signaling pathway, and further studies are required on sarcomatogenesis to identify novel therapeutic targets.

AUTHOR CONTRIBUTIONS

Yoshihiro Katayama: Conceptualization; data curation; formal analysis; funding acquisition; investigation; methodology; resources; validation; visualization; writing – original draft. **Takeshi Iwasaki:** Conceptualization; data curation; formal analysis; funding acquisition; investigation; methodology; resources; supervision; validation; visualization; writing – review and editing. **Takeo Yamamoto:** Investigation; methodology; validation; writing – review and editing. **Naomi Shimada:** Resources. **Miya Nakashima:** Resources. **Masato Toya:** Resources. **Fumiya Narutomi:** Formal analysis. **Takumi Tomonaga:** Formal analysis. **Kiyoko Kato:** Conceptualization; project administration; supervision; writing – review and editing. **Yoshinao Oda:** Conceptualization; project administration; resources; supervision; writing – review and editing.

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CONFLICT OF INTEREST STATEMENT

Yoshinao Oda and Kiyoko Kato are editorial board members of *Cancer Science*. The other authors declare no conflict of interest.

ETHICS STATEMENT

Approval of the research protocol by an institutional review board: This study was approved by the Ethics Committee of Kyushu University (IRB: 21120-00).

Informed Consent: N/A.

Registry and the Registration No. of the study/trial: N/A.

Animal Studies: N/A.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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