ALDH1-High Ovarian Cancer Stem-Like Cells Can Be Isolated from Serous and Clear Cell Adenocarcinoma Cells, and ALDH1 High Expression Is Associated with Poor Prognosis

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Abstract

Cancer stem-like cells (CSCs)/cancer-initiating cells (CICs) are defined as a small population of cancer cells that have high tumorigenicity. Furthermore, CSCs/CICs are resistant to several cancer therapies, and CSCs/CICs are therefore thought to be responsible for cancer recurrence after treatment and distant metastasis. In epithelial ovarian cancer (EOC) cases, disease recurrence after chemotherapy is frequently observed, suggesting ovarian CSCs/CICs are involved. There are four major histological subtypes in EOC, and serous adenocarcinoma and clear cell adenocarcinoma are high-grade malignancies. We therefore analyzed ovarian CSCs/CICs from ovarian carcinoma cell lines (serous adenocarcinoma and clear cell adenocarcinoma) and primary ovarian cancer cells in this study. We isolated ovarian CSCs/CICs as an aldehyde dehydrogenase 1 high (ALDH1$^{\text{high}}$) population from 6 EOC cell lines (3 serous adenocarcinomas and 3 clear cell adenocarcinomas) by the ALDEFLUOR assay. ALDH1$^{\text{high}}$ cells showed greater sphere-forming ability, higher tumorigenicity and greater invasive capability, indicating that ovarian CSCs/CICs are enriched in ALDH1$^{\text{high}}$ cells. ALDH1$^{\text{high}}$ cells could also be isolated from 8 of 11 primary ovarian carcinoma samples. Immunohistochemical staining revealed that higher ALDH1 expression levels in ovary cancer cases are related to poorer prognosis in both serous adenocarcinoma cases and clear cell adenocarcinoma cases. Taken together, the results indicate that ALDH1 is a marker for ovarian CSCs/CICs and that the expression level of ALDH1 might be a novel biomarker for prediction of poor prognosis.

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Introduction

Ovarian cancer is a malignant disease with high mortality and is the fourth most common cause of cancer-related death in women worldwide. [1,2] Obscure and unclear symptoms make detection of ovarian cancer in the early stage difficult. [3] Generally, ovarian cancer is relatively sensitive to first-line chemotherapy based on platinum/taxane. [4] Clinical complete response (CR) can often be achieved by cytoreductive surgery and chemotherapy in advanced ovarian cancer patients; however, the majority of patients with advanced stage have disease recurrence which is the reason for the high mortality of this disease. [5] Some therapeutic candidates of molecular target drugs for ovarian cancer had been tested, but notable improvements in prognosis were not achieved. [2,6].

Recent progress in cancer research has revealed that cancers are composed of a heterogeneous population of cells and that only a small population of cells called cancer stem-like cells (CSCs)/cancer-initiating cells (CICs) have high tumor-initiating potential (cancer stem cell hypothesis). CSCs/CICs are defined as a small population of cells that have (1) high tumorigenicity, (2) multiple differentiation ability and (3) self-renewal capability. [7–9] Results of recent study have also shown that CSCs/CICs are related to cancer recurrence and resistance to radiation or chemotherapy. [10,11] Therefore, CSCs/CICs are thought to be responsible for cancer recurrence and distant metastasis, and elimination of CSCs/CICs is therefore indispensable for curing cancer.

There are several approaches for identifying CSCs/CICs from cancers in a variety of organ tissues. [12] These approaches include (1) use of cell surface marker such as CD44$^+$CD24$^-$lowESA$^+$ [13], CD133$^+$ [14], CD44$^+$CD117$^+$ [15], and CD166$^+$ [16], (2) side population (SP) assay [17], in which the cell population that has the ability to pump out a drug (Hoechst33342
[18] or Dye Cycle Violet [19]) through the ATP-binding cassette transporter is regarded as CSCs/CICs, and (3) ALDEFLUOR assay based on the level of aldehyde dehydrogenase 1(ALDH1) enzyme activity. [20].

The function of intracellular ALDH is to catalyze the oxidation of aldehyde, and ALDH therefore plays an important role in cellular homeostasis. Recent studies have revealed that both normal and cancer cells with high levels of ALDH1 activity have the potential to function as stem cells and potentials for self-renewal capability and stress-resistant properties. [20–22].

Also in epithelial ovarian cancer (EOC), some researches have suggested the usefulness of ALDH1 activity to identify CSCs/CICs. The existence of cells with high ALDH activity (ALDH1-high cells, compared with ALDH1-low cells) has also been shown in EOC cell lines and in clinical specimens. [23–25] The correlation between ALDH1 activity and prognosis of patients, however, is still controversial in EOC. [26] At the same time, the relevance to ALDH1 expression for each histological subtype of EOC has not been clarified yet.

In this study, we identified and evaluated the stemness of ALDH1-high cells in serous adenocarcinoma and clear cell adenocarcinoma of the ovary, not only from established cell lines but also from primary ovarian cancer cells. We also statistically analyzed the association between ALDH1 expression and clinical outcome for ovarian cancer patients.

Figure 1. ALDEFLUOR assay for 6 epithelial ovarian cancer cell lines. ALDH1-high cells were detected in 3 serous adenocarcinoma cell lines (AMOC-2, HUOA and OVCAR-3) and in 3 clear cell adenocarcinoma cell lines (ES-2, RMG-1 and TOV-21G). SSC-A: single strand conformation analysis. BAAA: boron-dipyrromethene- aminoacetaldehyde. FITC-A: fluorescein isothiocyanate analysis. Percentages in boxes indicate ALDH1high cell ratios. Diethylaminobenzaldehyde (DEAB), an ALDH-specific inhibitor, was used as a negative control.

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Figure 2. Comparison of sphere-forming ability and invasion capability of ALDH1<sup>high</sup> cells and ALDH1<sup>low</sup> cells. A Sphere-forming assay One thousand ALDH1<sup>high</sup> cells and ALDH1<sup>low</sup> cells were cultured in a floating condition. The spheres were counted on day 10. Magnification of images: ×100. Criterion for spheroid size: over 100 μm. Each value is the mean number of spheroids ± SD. *P values. B Single cell sphere-forming
**Materials and Methods**

**Ethics Statement**
Mice were maintained and experimented on in accordance with the guidelines of and after approval by the Committee of Sapporo Medical University School of Medicine, Animal Experimentation Center under permit number 08-006. Any animal found unhealthy or sick was promptly euthanized. All studies were approved by Institutional Review Boards (IRB) of Sapporo Medical University Hospital and the IRB of Hakodate Goryokaku Hospital. Written Informed consent was obtained from all patients according to the guidelines of the Declaration of Helsinki.

**Cell Lines and Culture Conditions**
In this study, 3 ovarian serous adenocarcinoma cell lines (AMOC-2, HUOA and OVCAR-3) and 3 ovarian clear cell adenocarcinoma cell lines (ES-2, RMG-1 and TOV-21G) were used. AMOC-2 (kindly provided by Dr. Yabushita, Department of Obstetrics and Gynecology, Aichi Medical University, Aichi, Japan) [27], HUOA (kindly provided by Dr. Ishiwata, Obstetrics and Gynecologic Hospital, Ibaraki, Japan) [28], OVCAR-3 and TOV-21G (ATCC, Manassas, VA, USA) were cultured in RPMI1640 (Sigma-Aldrich, St Louis, MO, USA). ES-2 cells (ATCC) were cultured in Dulbecco’s Modified Eagle’s medium (DMEM, Sigma-Aldrich). RMG-1 cells (JCRB Cell Bank, Osaka, Japan) were cultured in DMEM/F-12 (Life Technologies, Grand Island, NY, USA). Each medium was supplemented with 10% fetal bovine serum (FBS). Cells were incubated in a humidified 5% CO₂ incubator at 37°C.

**Isolation of Primary Cancer Cells from Clinical Specimens**
All studies were approved by Institutional Review Boards (IRB) of Sapporo Medical University Hospital and the IRB of Hakodate Goryokaku Hospital. Written Informed consent was obtained from all patients according to the guidelines of the Declaration of Helsinki.

Solid tumors were cut into fragments, washed in phosphate buffered saline (PBS), and centrifuged at 2000 rpm for 10 minutes. Then cell aggregates were incubated at 37°C for about 30 min with 2 mg Liberase™ research grade (Roche, Basel, Switzerland) in 10 ml Iscove’s Modified Dulbecco’s Medium (IMDM, Life

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**Figure 3. Comparison of cell cycle and growth of ALDH1<sup>high</sup> cells and ALDH1<sup>low</sup> cells. A Cell cycle analysis** ALDH1<sup>high</sup> and ALDH1<sup>low</sup> cells were analyzed by a cell cycle assay. The graph indicates the ratios of cells in G0/G1 phase, S phase and G2/M phase. **B Cell growth analysis** The growth capabilities of ALDH1<sup>high</sup> cells and ALDH1<sup>low</sup> cells were investigated. Each values is the mean number of cells ± SD. doi:10.1371/journal.pone.0065158.g003
AMOC-2 cells, which tumors observed on both sides of their back. Histological images: Hematoxylin-Eosin staining (H-E, left panel), ALDH1 immunohistochemical staining (middle panel), Ki-67 immunohistochemical staining (right panel) of ALDH1high/low tumors. Magnification of images: × 400. C Growth curves of ALDH1high/low tumors derived from AMOC-2 and RMG-1 cells. Left column: AMOC-2 cells, 1.0×10⁴ injection. Middle column: AMOC-2 cells, 1.0×10³ injection. Right column: RMG-1 cells, 1.0×10³ injection. Each value is the mean tumor volume ± SD. *P values. D Immunoreactivity to ALDH1 or Ki-67 of ALDH1high/low tumors derived from AMOC-2 and RMG-1 cells. Each value is the mean positive percentage ± SD. *P values.

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Table 1. Tumor-initiation incidence of ALDH1high/low cells derived from AMOC-2 and RMG-1 cells.

| Number of cells injected into mice | AMOC-2 | RMG-1 |
|-----------------------------------|--|--|
| 1.0×10⁴ | ALDH1high | ALDH1low | ALDH1high | ALDH1low |
| 5/5 | 3/5 | 4/5 | 2/5 |
| 1.0×10³ | 4/5 | 2/5 | 3/5 | 2/5 |
| 1.0×10² | 2/5 | 0/5 | 0/5 | 0/5 |

The number indicates the incidence of tumor-initiation of NOD/SCID Mice.

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Table 2. Patient list of clinical specimen that were examined by ALDEFLUOR assay.

| Pt.No. | Age | Histological subtype | Stage | ALDH1<sup>high</sup> | CD326<sup>+</sup> [%] |
|--------|-----|----------------------|-------|----------------------|---------------------|
| 1      | 42  | Clear                | IIc   | 7.2                  | 62.7                |
| 2      | 67  | Mucinous             | IIIc  | 0                    | 16.5                |
| 3      | 52  | Endometrioid         | Ic    | 12                   | 78.7                |
| 4      | 45  | Clear                | IIc   | 8.1                  | 8.1                 |
| 5      | 71  | Serous               | IIIc  | 2.2                  | 80                  |
| Mean   | 55.4|                      |       | ±13.0                | ±5.05               |

±SD: ±13.0; ±5.05; ±34.5

Cancer cells from solid cancer tissue

| Pt.No. | Age | Histological subtype | Stage | ALDH1<sup>high</sup> |
|--------|-----|----------------------|-------|-----------------------|
| 1      | 65  | Unknown              | IIc   | 0                     |
| 2      | 62  | Serous               | IIc   | 2.2                   |
| 3      | 67  | Mucinous             | IIc   | 0                     |
| 4      | 58  | Serous               | IIc   | 4.2                   |
| 5      | 52  | Serous               | IIc   | 4.1                   |
| 6      | 59  | Clear-Endometrioid   | IIIb  | 1.7                   |
| Mean   | 60.5|                      |       | 2.03                  |

±SD: ±5.39; ±1.86

Cancer cell from primary cancer tumor: 6 cases; Cancer cell from ascites: 5 cases.

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with mouse anti-ALDH1 monoclonal antibody (1:250, Sigma-Aldrich) or mouse anti-Ki-67 monoclonal antibody (1:100, DAKO, Glostrup, Denmark) for 1 hour followed by incubation with biotinylated anti-mouse IgG (Nichirei) for 30 min. Subsequently, the sections were stained with streptavidin-biotin complex (Nichirei Biosciences, Tokyo, Japan), followed by incubation with 3,3′-diaminobenzidine used as a chromogen and counter-staining with hematoxylin. Cytoplasmic staining was regarded as positive for ALDH1, and nuclei staining were regarded as positive for Ki-67. For evaluation of ALDH1 staining, the cases were divided into two groups (ALDH1<sup>high</sup> group and ALDH1<sup>low</sup> group) by medians (medians for serous adenocarcinoma the median and clear cell adenocarcinoma being 20% and 15%, respectively).

Matrigel Invasion Assay

The invasive capability of cells was evaluated using matrigel invasion chambers (BD Biosciences). Isolated ALDH1<sup>high</sup> and ALDH1<sup>low</sup> cells (1.0×10<sup>3</sup>) were plated in each upper chamber in serum-free DMEM. The outer chambers were filled with DMEM including 10% FBS as a chemoattractant. Cells were incubated for 48 hours, and invasive cells were stained with Hematoxylin, mounted on slides, and counted at 400-fold upper field by light microscopy.

Characterization of ALDH1<sup>high</sup> Cells

Since CSCs/CICs are known to form spheres in floating culture conditions [29], we analyzed ALDH1<sup>high</sup> cells by a sphere-forming assay. A total of 10<sup>4</sup> cells per well were sorted and incubated into a 6-well plate in an anchorage-independent environment. At day 10, ALDH1<sup>high</sup> cells derived from AMOC-2 and RMG-1 showed greater sphere-forming ability than that of ALDH1<sup>low</sup> cells. Similar results were obtained from ES-2 cells, RMG-1 cells and TOV-21G cells (Figure 2A). Since the sphere-forming assay is not suitable for quantification of the ratios of CSCs/CICs in ALDH1<sup>high</sup> cells, we performed a single cell sphere-forming assay. Sphere formation was observed in 4.86% of the wells of ALDH1<sup>high</sup> cells derived from AMOC-2 cells and in 3.13% of the wells of ALDH1<sup>high</sup> cells derived from ES-2 cells (Figure 2B). On the other hand, wells of ALDH1<sup>low</sup> cells derived from both AMOC-2 and ES-2 cells did not show any sphere formation.

We then investigated the invasion ability of ALDH1<sup>high</sup> and ALDH1<sup>low</sup> cells by the matrigel invasion assay. ALDH1<sup>high</sup> cells derived from all four lines showed greater matrigel invading ability than did ALDH1<sup>low</sup> cells (Figure 2C).

The cell cycle status of ALDH1<sup>high</sup> cells and that of ALDH1<sup>low</sup> cells were analyzed. The ratios of ALDH1<sup>high</sup> cells in S phase and G2/M phase were greater than ratio of ALDH1<sup>low</sup> cells in S phase and G2/M phase (Figure 3A). Cell grow analysis revealed that ALDH1<sup>high</sup> cells derived from AMOC-2 and RMG-1 had higher grow rates than those of ALDH1<sup>low</sup> cells (Figure 3B).

Isolation of ALDH1<sup>high</sup> Cells from EOC Cell Lines

Several methods to isolate CSCs/CICs have already been described [12], and an aldehyde dehydrogenase 1 high population (ALDH1<sup>high</sup>) identified by the ALDEFLUOR assay was described to be enriched with CSCs/CICs. [20] We therefore analyzed ovarian carcinoma cell lines by the ALDEFLUOR assay to isolate ovarian CSCs/CICs. We investigated 3 human ovarian serous adenocarcinoma cell lines (AMOC-2, HUOA and OVCAR-3) and 3 human clear cell adenocarcinoma cell lines (ES-2, RMG-1 and TOV-21G) (Figure 1). ALDH1<sup>high</sup> population was identified in all ovarian carcinoma line cells, and the ratio of ALDH1<sup>high</sup> cells ranged from 0.7% for ES-2 cells to 7.9% for TOV-21G cells. We could isolate ALDH1<sup>high</sup> cells stably from 4 cell lines (AMOC-2, ES-2, RMG-1 and TOV-21G), and we therefore used these cell lines for further analysis.

Higher Tumorigenicity of ALDH1<sup>high</sup> Cells than that of ALDH1<sup>low</sup> Cells

To address the in vivo tumorigenicity of ALDH1<sup>high</sup> and ALDH1<sup>low</sup> cells from EOC cell lines, xenograft transplantation into NOD/SCID mice by ALDH1<sup>high</sup> and ALDH1<sup>low</sup> cells derived from AMOC-2 and RMG-1 was performed (Figure 4A, 4B and 4C). As shown in Table 1, tumors were observed in 5 of 5 mice (10<sup>4</sup> cells injection), 4 of 5 mice (10<sup>3</sup> cells injection) and 2 of 5 mice (10<sup>2</sup> cells injection) in which xenotransplantation of ALDH1<sup>high</sup> cells derived from AMOC-2 cells was performed. On the other hand, tumors were observed in only 5 of 5 mice (10<sup>4</sup> cells injection) and 2 of 5 mice (10<sup>3</sup> cells injection) in which xenotransplantation of ALDH1<sup>high</sup> cells derived from RMG-1 cells was performed.
Table 3. Correlation between ALDH1 expression and clinical factors in 123 epithelial ovarian cancer patients.

| Characteristic | ALDH1<sup>high</sup> | ALDH1<sup>low</sup> | Total | P Value |
|----------------|---------------------|--------------------|--------|---------|
| Patient No.(%)  | 72(58.5)            | 51(41.5)           | 123    |         |
| Mean Age ± SD (years) | 53.8 ± 7.86 | 50.6 ± 10.7 | 54.8 ± 10.9 |         |
| Pathological subtype No.(%) | 0.004 |
| Serous           | 34(54.8)            | 28(45.2)           | 62     |         |
| Clear cell      | 18(48.6)            | 19(51.4)           | 37     |         |
| Endometrioid    | 14(77.8)            | 4(22.2)            | 18     |         |
| Mucinous        | 6(100)              | 0(0)               | 6      |         |
| FIGO stage No.(%) | 0.706               |
| Stagel I        | 26(61.9)            | 16(38.1)           | 42     |         |
| Stagell II      | 2(33.3)             | 4(66.7)            | 6      |         |
| Stagell III     | 39(58.2)            | 28(41.6)           | 67     |         |
| Stagell IV      | 5(62.5)             | 3(37.5)            | 8      |         |
| Serous adenocarcinoma Characteristic | ALDH1<sup>high</sup> | ALDH1<sup>low</sup> | Total | P Value |
| Patient No.(%)  | 34(54.8)            | 28(45.2)           | 62     |         |
| Mean Age ± SD (years) | 53.8 ± 7.86 | 50.6 ± 10.7 | 54.8 ± 10.9 |         |
| FIGO stage No.(%) | 0.991               |
| I-II            | 6(60.0)             | 4(40.0)            | 10     |         |
| III-IV          | 28(53.8)            | 24(46.2)           | 52     |         |
| T No.(%)        | 0.589               |
| T1-2            | 7(53.8)             | 6(46.2)            | 13     |         |
| T3              | 27(55.1)            | 22(44.9)           | 49     |         |
| Lymphadenectomy | 0.537               |
| Case No.(%)     | 23(53.5)            | 20(46.5)           | 43     |         |
| L/N Meta (+)    | 12(48.0)            | 13(52.0)           | 25     |         |
| L/N Meta (-)    | 11(61.1)            | 7(38.9)            | 18     |         |

Identification of ALDH1<sup>high</sup> Cells in Primary EOC Samples

To detect ALDH1<sup>high</sup> cells in primary ovarian cancer, we analyzed eleven clinical materials using the ALDEFLUOR assay (5 cases of solid ovarian cancer cells and 6 cases of malignant ascites of ovarian cancer cases, summarized in Table 2). CD326-positive epithelial cells were identified in solid ovarian cancer tissues, and the positive rates ranged from 8.1% to 81.0% (Figure 5A, upper panels). ALDH1<sup>high</sup> cells were detected in 4 of the 5 cases, and positive ALDH1<sup>high</sup> cell rates ranged from 0.9% to 12.0% (Figure 5A, lower panels). Furthermore, tumors derived from AMOC-2 ALDH1<sup>high</sup> cells and RMG-1 ALDH1<sup>low</sup> cells showed significantly higher positive rates for Ki-67 (MIB-1) than those of tumors derived from AMOC-2 ALDH1<sup>high</sup> cells and RMG-1 ALDH1<sup>low</sup> cells (Figure 4D).

Association of High Expression Level of ALDH1 is with Poor Prognosis

A total of 123 epithelial ovarian cancer tissues were immunohistochemically stained with anti-ALDH1 antibody (Table 3, Figure 6A). The medians of ALDH1-positive rates in serous adenocarcinoma cases and clear cell adenocarcinoma cases were 20% and 15%, respectively. We therefore divided the cases into
two groups, ALDH1\textsuperscript{high} group and ALDH1\textsuperscript{low} group, according to the medians. As shown in Table 3, there was no significant correlation of the expression level of ALDH1 with age or with each FIGO clinical stage. High expression level of ALDH1 showed no significant correlation with advanced stages (stage III+IV), T factor or lymph node metastasis in both serous adenocarcinoma cases and clear cell adenocarcinoma cases. The log-rank test revealed that higher expression level of ALDH1 is associated with poorer prognosis with a significant difference in OS of patients with serous adenocarcinoma ($P = 0.006$) and OS of patients with clear cell adenocarcinoma ($P = 0.047$) than those of lower expression level of ALDH1 (Figure 6B). Higher expression level of ALDH1 showed a tendency for shorter PFS than that of lower expression level of ALDH1, but the differences were not significant (serous adenocarcinoma: $P = 0.062$; clear cell adenocarcinoma: $P = 0.058$).

**Discussion**

In this study, we isolated ovary CSCs/CICs with high tumorigenicity by the ALDEFLUOR assay. An ALDH1\textsuperscript{high} population could be isolated not only from ovarian cancer cell

![Image](image_url)
lines but also from primary ovarian cancer samples. Several methods for isolation of CSCs/CICs have been reported. Indeed, ovarian CSCs/CICs have been successfully isolated by using various methods including the ALDEFLUOR assay [25], side population (SP) analysis [32,33], and use of CD133+ [34], CD44+CD24− [35] and CD24+ cells. [36] However, there is a controversial report showing that a CSC/CIC marker does not work in some types of cells. [37,38] Therefore, it is essential to validate the cell population isolated by CSC/CIC markers by several types of analysis. In this study, we confirmed that the ALDH1high population had higher tumorigenicity and greater sphere-forming ability. These observations indicate that the ALDH1high cells we used in this study are enriched with CSCs/CICs. There have been few reports on successful isolation of CSCs/CICs from primary ovarian cancer cells. We isolated ALDH1high cells from 8 of 11 primary ovarian cancer cases. We could not analyze ALDH1high cells from primary ovarian cancers because of the limitation of cell numbers; however, our approach is a possible and promising method for isolating CSCs/CICs from primary ovarian cancers.

Major histological subtypes of EOC are serous adenocarcinoma, clear cell adenocarcinoma, endometrioid adenocarcinoma and mucinous adenocarcinoma, and these subtypes are known to have different characteristics in risk factor of carcinogenesis, molecular biological aspects and so on. More information about individual subtypes is needed to improve the survival of patients. Serous adenocarcinoma is the histology subtype with worst prognosis; however, serous adenocarcinoma cases are often diagnosed at advanced stage. Clear cell adenocarcinoma is gradually increasing up to nearly 12% of EOC in Asian countries, and comparison of the cases in the same stage of 3 histological subtypes (clear cell adenocarcinoma, endometrioid adenocarcinoma and mucinous adenocarcinoma) revealed that clear cell adenocarcinoma is the poorest prognosis in every stages. Therefore, clear cell adenocarcinoma is thought to be the highest grade EOC, recently. [39,40] Serous adenocarcinoma cases were mainly analyzed in previous studies, and there have been few studies in which clear cell adenocarcinoma cases were analyzed. [23-25] In this study, we analyzed not only serous adenocarcinoma cases but also clear cell adenocarcinoma cases. Therefore, these information will bring insight into not only for serous adenocarcinoma cases but also clear cell adenocarcinoma cases. Further investigations on molecular aspects of ALDH1high cells in ovarian cancers are needed.

In summary, we successfully isolated an ALDH1high cell population with high tumorigenicity not only from serous adenocarcinoma but also from clear cell adenocarcinoma. ALDH1high cells have higher tumorigenicity and greater sphere-forming ability. ALDH1-positive immunohistochemical staining is related to poor prognosis in both serous adenocarcinoma and clear cell adenocarcinoma. These findings indicate that ovarian CSCs/CICs are positive for ALDH1, and further analysis of ALDH1-positive ovarian CSCs/CICs will lead to the establishment of novel strategies for treating ovarian CSCs/CICs.

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Author Contributions

Conceived and designed the experiments: TK YH TT TS NS. Performed the experiments: TK KY AT HA RM. Analyzed the data: TK YH TT NS. Contributed reagents/materials/analysis tools: TK TM TA MM TS. Wrote the paper: TK YH TT NS.
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