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Original Article

Suppression of ARID1A associated with decreased CD8 T cells improves cell survival of ovarian clear cell carcinoma

Un Suk Jung,[1] **Kyueng-Whan Min**,[2] **Dong-Hoon Kim**,[3] **Mi Jung Kwon**,[4] **HoHyun Park**,[5] **Hyung Seok Jang**[6]

[1]Department of Obstetrics and Gynecology, Hanyang University Guri Hospital, Hanyang University College of Medicine, Guri, Korea
[2]Department of Pathology, Hanyang University Guri Hospital, Hanyang University College of Medicine, Guri, Korea
[3]Department of Pathology, Kangbuk Samsung Hospital, Sungkyunkwan University School of Medicine, Seoul, Korea
[4]Department of Pathology, Hallym University Sacred Heart Hospital, Hallym University College of Medicine, Anyang, Korea
[5]Department of Biomedical Laboratory Science, Mokpo Science University, Mokpo, Korea
[6]Department of Clinical Laboratory Science, Ansan University, Ansan, Korea

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Correspondence to
Kyueng-Whan Min
Department of Pathology, Hanyang University Guri Hospital, Hanyang University College of Medicine, 153 Gyeongchun-ro, Guri 11923, Korea.
E-mail: kyueng@gmail.com

ORCID iDs
Un Suk Jung
https://orcid.org/0000-0003-0421-4148
Kyueng-Whan Min
https://orcid.org/0000-0002-4757-9211
Dong-Hoon Kim
https://orcid.org/0000-0001-5722-6703
Mi Jung Kwon
https://orcid.org/0000-0002-2441-0448
HoHyun Park
https://orcid.org/0000-0001-9698-7302
Hyung Seok Jang
https://orcid.org/0000-0002-3313-6540

Conflict of Interest
No potential conflict of interest relevant to this article was reported.

ABSTRACT

Objective: AT-rich interactive domain 1A (ARID1A) plays an important role as a tumor suppressor gene in ovarian clear cell carcinoma (OCCC), but the clinical application of ARID1A remains unclear. The aim of this study was to analyze clinicopathological parameters, molecular interactions and immune-infiltration in patients with low ARID1A expression and to provide candidate target drugs.
Methods: We investigated the clinicopathologic parameters, specific gene sets/genes, and immunological relevance according to ARID1A expression in 998 OCCC patients from 12 eligible studies (using meta-analyses); 30 OCCC patients from the Hanyang University Guri Hospital (HYGH) cohort; and 52 OCCC patients from gene set enrichment (GSE) 65986 (25 patients), 63885 (9 patients), and 54809 (6 patients and 12 healthy people) of the Gene Expression Omnibus (GEO). We analyzed network-based pathways based on gene set enrichment analysis (GSEA) and performed in vitro drug screening.
Results: Low ARID1A expression was associated with poor survival in OCCC from the meta-analysis, HYGH cohort and GEO data. In GSEA, low ARID1A expression was related to the tumor invasion process as well as a low immune-infiltration. In silico cytometry showed that CD8 T cells were decreased with low ARID1A expression. In pathway analysis, ARID1A was associated with angiogenic endothelial cell signaling. In vitro drug screening revealed that cabozantinib and bicalutamide effectively inhibited specific hub genes, such as vascular endothelial growth factor-A and androgen receptor, in OCCC cells with low ARID1A expression.
Conclusions: Therapeutic strategies making use of low ARID1A could contribute to better clinical management/research for patients with OCCC.

Keywords: ARID1A Protein; Ovary; Adenocarcinoma; Clear Cell; Human

Author Contributions
Conceptualization: M.K.W.; Data curation: J.U.S., M.K.W., K.D.H., K.M.J., P.H., S.J.H.; Investigation: J.U.S., M.K.W.; Methodology: M.K.W.; Project administration: J.U.S.; Supervision: M.K.W.; Visualization: M.K.W.; Writing - original draft: M.K.W.; Writing - review & editing: M.K.W.

INTRODUCTION

Ovarian cancers that have spread beyond the ovary at the time of diagnosis have first-lined chemotherapy response (60%–80%), even in patients with advanced-stage disease [1]. However, most of these patients (about 70%) will later have disease progression and thus be candidates for second-line chemotherapy [2]. Others ultimately recur and develop chemoresistance [3,4]. Ovarian clear cell carcinoma (OCCC) comprises approximately 4% to 12% of epithelial ovarian carcinomas. The incidence of OCCC with unique biological features differs among races. Among Asian women, OCCC is diagnosed twice as frequently as among Caucasian women [5]. OCCC is known to be a highly aggressive and chemotherapy-resistant neoplasm with poor prognosis, as demonstrated in a study of Gynecologic Oncology Group trial [6]. In general, the prognostic parameters for determining a treatment plan are peritoneal seeding with ruptured capsule, histological subtypes (clear, hobnail, cuboid, oxyphilic, signet), tumor susceptibility based on ethnicity, and predictive biomarkers [7].

The AT-rich interactive domain 1A gene (ARID1A), encoding a BAF250a protein, is an accessory subunit of the Switch/Sucrose Non-Fermentable chromatin remodeling complex that modulates the accessibility of promoters to transcriptional activation or repression [8]. Previous studies using breast and lung cancer cell lines demonstrated that the loss of ARID1A could promote cancer progression [9], which suggested that ARID1A is a tumor suppressor gene [10]. Another study demonstrated that low ARID1A expression was related to worse survival for several different types of cancer, including ovarian, endometrial, stomach, colon, and kidney cancer [11-15]. The role of ARID1A as a tumor suppressor is supported by the nature and pattern of its mutations in OCCC [16].

Recently, a study using RNA sequencing revealed that the incidence of ARID1A mutations in patients with OCCC and ovarian endometrioid carcinomas (OECs) was 46% and 30%, respectively, while there were none in patients with high-grade ovarian serous carcinomas (OSCs) [17]. Increasing interest has been focused on determining whether the suppression of ARID1A is associated with poor prognosis in patients with OCCC. A study by Previous studies reported that ARID1A deletion was related to worse survival in OCCC [12,18]. In other studies, no significant difference in survival was found between the absence and presence of ARID1A [19,20]. Controversy still exists regarding the relationship between ARID1A and the survival rate in OCCC.

The present study aimed to assess whether ARID1A correlates with clinicopathological parameters and the survival rate of patients with OCCC in meta-analyses, our Hanyang University Guri Hospital (HYGH) cohort and the GEO database. We focused on evaluating ARID1A-associated specific gene sets and genes, different types of immune cells and pathway analyses in OCCC as well as in vitro drug screening tests in ovarian cancer cell lines.

MATERIALS AND METHODS

1. Patient selection from HYGH and immunohistochemistry

This study included 30 patients with OCCC who underwent surgery at HYGU in Korea between 1999 and 2015. The Reporting Recommendations for Tumor Marker Prognostic Studies (REMARK) criteria were followed throughout this study [21]. The inclusion criteria were as follows: 1) patients with microscopic features of primary OCCC confirmed by pathologists and with known medical records, and 2) patients who did not undergo systemic

therapy. Patients with missing paraffin blocks of tumor samples or incomplete clinical outcomes were excluded. In our HYGH cohort, 30 patients with OCCC were enrolled to assess the associations between ARID1A and survival rate.

Immunohistochemistry procedures were carried out on 3 μm tissue sections using the Bond Polymer Refine Red Detection System (Leica Biosystems Newcastle Ltd. Newcastle, UK) according to the manufacturer's instructions with minor modifications. Immunostaining for ARID1A (1:100; Cell Marque, Rocklin, CA, USA) was performed using the Bond-Max automatic slide stainer (Leica Biosystems Melbourne Pty., Ltd., Mount Waverley, VIC, Australia). ARID1A expression was graded according to the intensity and proportion of nucleus-stained tumor cells [22]. The immunoreactive score (IRS) was calculated (intensity×proportion). To determine the optimal cut-off values of ARID1A, receiver operating characteristic (ROC) curves plotting sensitivity versus 1–specificity were used. The cut-off value calculated by the ROC was used to evaluate the relationship between overall survival (OS) rate and ARID1A expression. ROC values exhibited good discriminatory power for ARID1A expression considering the disease-specific survival (DSS; area under the ROC=0.651) ARID1A expression was determined as either low (IRS <1) or high (IRS ≥1).

2. Data sources, literature search and selection criteria for the meta-analysis

Relevant articles were obtained by searching the PubMed and MEDLINE databases through August 31, 2019. The search terms used in PubMed included combinations of the following keywords: 'ARID1A' OR 'BAF250' AND 'ovarian clear cell carcinoma'. The titles and abstracts of all searched articles were screened for exclusion. Review articles were also screened to find additional eligible studies. Articles were included if the study was performed in human ovarian cancer and if there was information about the clinicopathological characteristics of CCC. Articles were excluded if they were case reports or nonoriginal articles or if the article was not written in English. In a meta-analysis of 13 eligible studies, 1,104 patients with OCCC were analyzed to validate the relationships between ARID1A and clinicopathological parameters (**Supplementary Table 1**).

Data from all eligible studies were extracted by 2 independent authors (M.K.W., J.U.S.). The following information was extracted from the included studies: the first author's name, year of publication, country of origin, sample size, ARID1A and/or BAF250 immunohistochemical expression, International Federation of Gynecology and Obstetrics (FIGO) stage, number of patients receiving chemotherapy, association of endometriosis, association of adenofibroma and disease-free survival (DFS) or OS data. The characteristics of the 12 eligible studies are described in detail in the supplementary information.

3. Gene set enrichment (GSE) analysis, in silico cytometry and network-based pathway analysis

In the Gene Expression Omnibus (GEO), patients with OCCC were included to analyze the survival rate, ARID1A mRNA expression/mutation, specific gene sets, antitumoral immune-infiltration and network-based pathway analysis. We obtained bioinformation of 52 OCCC patients from GSE 65986 (25 patients, RNA expression profiling by array, Affymetrix Human Genome U133 Plus 2.0 Array) [23], 63885 (9 patients, RNA expression profiling by array, Affymetrix Human Genome U133 Plus 2.0 Array) [24], and 54807 (6 patients and 12 healthy people, RNA expression profiling by array, Affymetrix Human Gene 1.0 ST Array) [25] from the GEO database. To determine the optimal cut-off values of ARID1A mRNA from GSE 65986, median value (284.2) was used.

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We analyzed significant gene sets using gene set enrichment analysis (GSEA, version 4.3) from the Broad Institute at MIT [26]. The oncogenic, curated and immunological gene sets (189, 5,529 and 4,872 sets, respectively) were used to identify the gene sets associated with low ARID1A expression. For this analysis, 1,000 permutations were used to calculate the p-values, and the permutation type was set to phenotype. The following cut-offs were used: $p<0.05$ and false discovery rate <0.25.

We applied CIBERSORT as known in silico cytometry to determine the proportions of 22 subsets of immune cells using 547 genes [27]. Gene expression datasets were prepared using standard annotation files, the data were uploaded to the CIBERSORT web portal, and the algorithm was run using the default signature matrix at 1,000 permutations [27].

The network-based pathway analysis was visualized using Cytoscape (version 3.8.0) software. To interpret the biological relevance of ARID1A and its relevant elements in OCCC, we performed functional enrichment analysis to clarify functionally grouped gene ontology and pathway annotation networks using ClueGO (version 2.5.6) [28,29].

4. Data extraction from the Genomics of Drug Sensitivity (GDSC) in Cancer database

We investigated anticancer drug effects in ovarian cancer cell lines with low ARID1A using the GDSC dataset. We analyzed the relationship between anticancer drug sensitivity and ARID1A expression based on the GDSC version 1 dataset, which contains data on the response of approximately 5 OCCC cell lines to 285 anticancer drugs. In OCCC cells with high ARID1A expression (3 cell lines >−0.2 expression based on z-score: ES-2, OVTOKO, RMG-I) or low expression (2 cell lines <−0.2: OVISE, TOV-21G), the drug response was defined as the natural log of the half-maximal inhibitory concentration (LN IC50). A drug was identified as an effective drug when the calculated LN IC50 value was decreased in cell lines with low ARID1A expression and increased in those with high ARID1A expression, i.e., when an inverse correlation was observed. Pearson's correlation analysis between LN IC50 values and ARID1A expression was performed. Student's t-test was performed to determine the difference in LN IC50 between wild-type and mutated ARID1A. In other words, when the LN IC50 and ARID1A expression were inversely related, it was defined as the most effective drug.

5. Statistical analysis

For meta-analyses, we calculated a pooled hazard ratio (HR) and 95% confidence interval (CI) using the generic inverse variance method to estimate the effects of the suppression of ARID1A on DSS, DFS, advanced FIGO stage, chemoresistance, and endometriosis/adenofibroma. An observed meta-HR >1 implied a worse prognosis for the low ARID1A group if its 95% CI did not overlap 1 ($p<0.05$). The heterogeneity among the included studies was evaluated using the T^2 (tau-square, between-study variance) value, I^2 (proportion of true variance) value and Cochran Q (difference between the observed weighted sum of squares) test. If p was ≤0.10 in the Cochran Q test or I^2 was ≥50% in the I^2 test, the heterogeneity was regarded as statistically significant: 50%–75%, moderate heterogeneity; and 75%–100%, high heterogeneity. If there was no significant heterogeneity, fixed-effects models were used. Otherwise, random-effects models were performed. Publication bias was quantified using Begg's and Egger's regression tests and visualized using funnel plot analyses. If a publication bias did exist, its influence on the overall effect was assessed by Duval and Tweedie's trim and fill method.

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In the HYGH cohort and GEO data, Student's t-test and/or Pearson's correlation analysis were used to examine the differences among continuous variables. DFS was defined as the time from the date of diagnosis to recurrence/new distant metastasis, and DSS was defined as the time from the date of diagnosis to cancer-related death. Overall survival time was defined as the time from the date of diagnosis to all-cause death. Progression-free survival (PFS) time was defined as the time from the date of diagnosis to no evidence of death or alive with disease. Survival analyses using the log-rank test and Cox proportional hazards model were performed. A 2-tailed p-value of <0.05 was considered statistically significant. All data were analyzed using R packages and SPSS statistics (version 25.0; SPSS Inc., Chicago, IL, USA).

6. Ethics approval

This study (involving human participants) was approved by the Ethics Committee of the Hanyang University Guri Hospital, Seoul, Korea (2019-04-021-002), and was performed according to the ethical standards of the Declaration of Helsinki, as revised in 2008. A review conducted by our institutional review board confirmed that informed consent was not necessary for this study.

RESULTS

1. Meta-analysis of relationships between ARID1A deficiency and clinicopathological parameters

The proportion of patients with low ARID1A expression in the eligible studies varied from 15% to 68.6% (mean proportion: 47.9%). Low ARID1A expression was significantly associated with poor OS (random-effects model, HR=1.28; 95% CI=1.12–1.47; p=0.003) and the presence of endometriosis (random-effects model, HR=1.47; 95% CI=1.18–1.83; p=0.006). There was no significant heterogeneity (OS, I^2=0, T^2=0, p=0.632; endometriosis, I^2=0, T^2=0, p=0.688) among these studies, which suggested good consistency and that the results from the 12 studies can be pooled together (**Supplementary Table 2**). When we compared the HRs between DFS and low ARID1A expression, patients with low ARID1A expression were associated with poor DFS (random-effects model, HR=1.3; 95% CI=1.03–1.65; p=0.026). The heterogeneity test showed moderate heterogeneity, but it was not significant (I^2=37.6, T^2=0.032, p=0.155). Low ARID1A expression was not related to the presence of adenofibroma (random-effects model, HR=0.917; 95% CI=0.51–1.66; p=0.774) or chemoresistance (random-effects model, HR=1.25; 95% CI=0.74–2.12; p=0.402). In a fixed-effects model comparing advanced FIGO stages, including stages 3 and 4, and low ARID1A expression, there was a good relationship between them (HR=1.23; 95% CI=1.01–1.48; p=0.036), but it was not significant after applying a random-effects model (HR=1.34; 95% CI=0.93–1.92; p=0.119) (**Fig. 1**).

2. Characteristics of ARID1A expression

In the HYGH cohort, 12 patients had low ARID1A expression (40%), and 18 had high ARID1A expression (60%) (**Fig. 2A**). In analysis of ARID1A mutations, ARID1A mRNA expression was elevated in wild-type compared to mutants samples from ovarian cancer cell lines of the GDSC dataset (p=0.001) (**Fig. 2B**). In analysis reverse phase protein array, low ARID1A mRNA expression was associated ARID1A protein expression (p<0.001) (**Fig. 2C**) [30]. In GSE 54809, OCCC tissues showed lower ARID1A expression than normal tissues (**Fig. 2D**). Among different types of ovarian carcinoma, ARID1A expression was decreased in patients with OCCC and OEC compared with OSC in GSE 65986 (p=0.015 and 0.044, respectively) (**Fig. 2E**).

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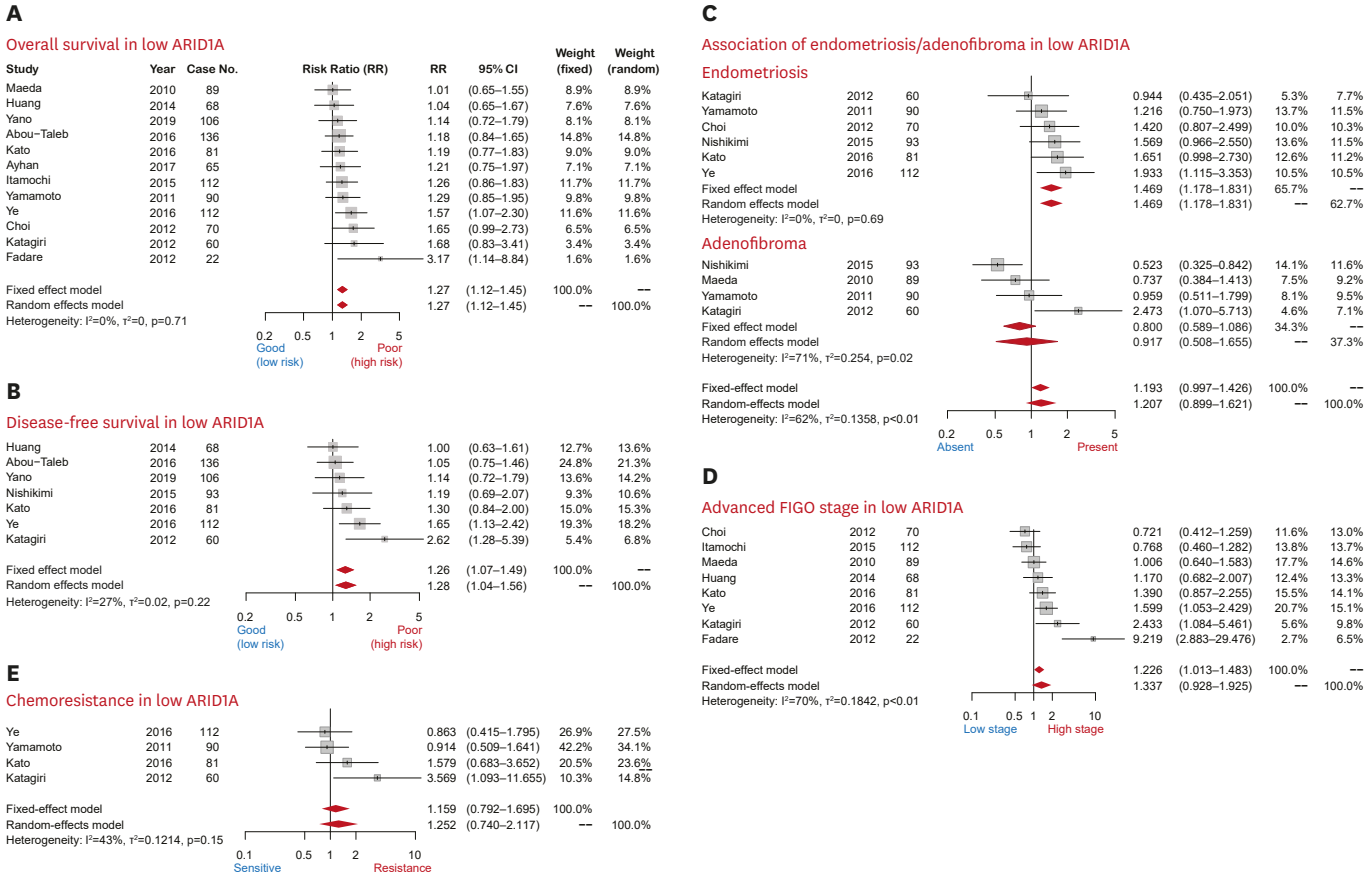

Fig. 1. Forest plots for the differences in the survival rates between patients with low ARID1A expression and those with high ARID1A expression: (A) overall survival (12 studies), (B) disease-free survival (7 studies), (C) association of endometriosis (6 studies) and adenofibroma (4 studies), (D) advanced FIGO stage (stage 3 or 4) (8 studies), (E) chemoresistance (4 studies).
ARID1A, AT-rich interactive domain 1A; CI, confidence interval; FIGO, International Federation of Gynecology and Obstetrics; RR, risk ratio.

In the HYGH cohort, low ARID1A expression was significantly correlated with worse DFS and DSS (DFS, HR=5.85; 95% CI=1.22–28.03; p=0.013 and DSS, HR=6.31; 95% CI=0.79–50.57; p=0.043, respectively) (**Fig. 2F and G**) (**Supplementary Table 3**). After adjusting confounders including FIGO stage, old age, histological grade, tumor size, low ARID1A expression was still associated with poor DFS and DSS (DFS, HR=7.91; 95% CI=1.45–43.08; p=0.02 and DFS, HR=11.67; 95% CI=1.08–126.73; p=0.05, respectively). In the GSE 65986 data, low ARID1A expression was significantly associated with poor PFS (p=0.037) (**Fig. 2H**).

3. GSEA, immune cell fraction and pathway network analyses in low ARID1A expression

In the GSE 65986 database, we conducted GSEA to identify the gene sets associated with low ARID1A expression. We found 4 significantly enriched gene sets related to the invasion process of ovarian cancer: downregulated CD8 T cells and B cells and activated CD4 T cells (**Fig. 3A-D**). In CIBERSORT analysis, low ARID1A expression was related to decreased CD8 T cells (p=0.021). Low ARID1A expression showed a tendency to reduce plasma cells, but it was not statistically significant (p=0.689). Unexpectedly, CD274 encoding programmed death-ligand 1 (PD-L1) was decreased with low ARID1A expression (p<0.001). Activated CD4 T cells were elevated with low ARID1A expression compared with high ARID1A expression, but this elevation was not significant (p=0.058) (**Fig. 3E**).

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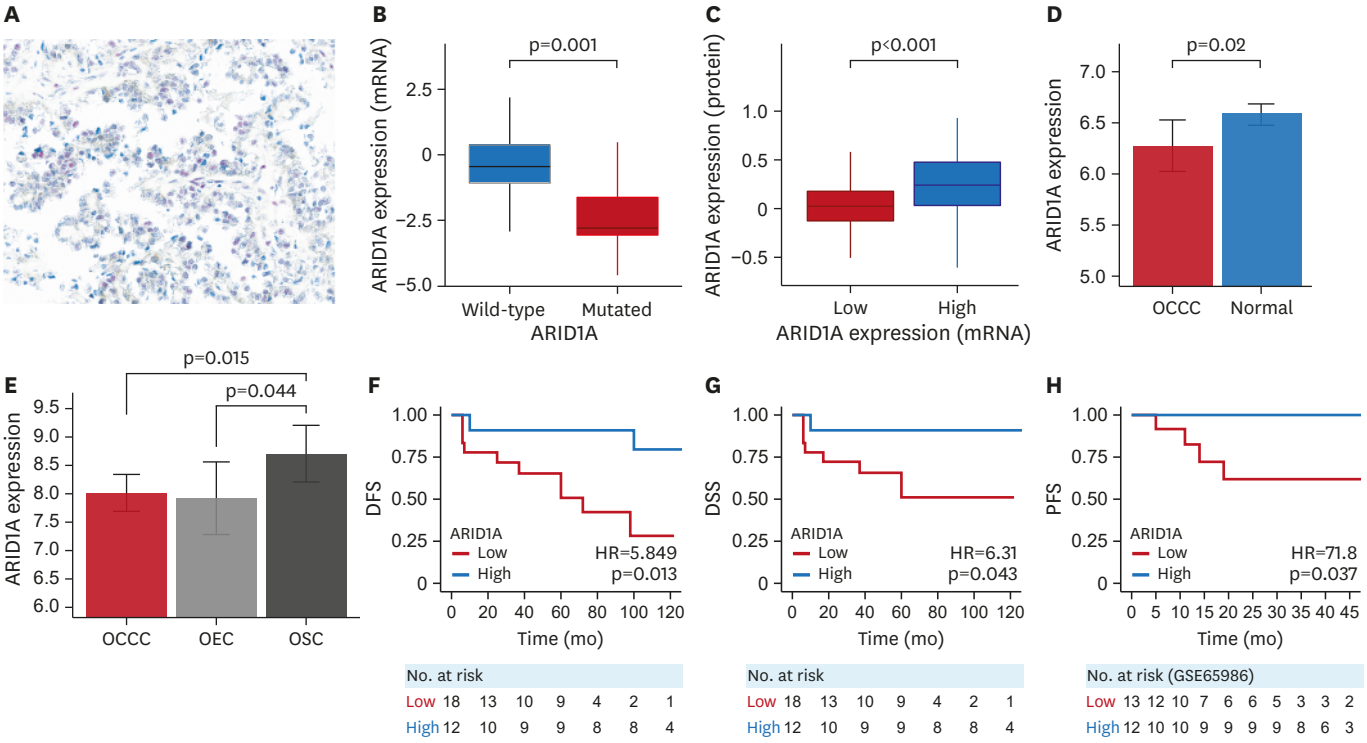

Fig. 2. (A) Representative microphotographs showing ARID1A expression (red) in OCCC (original magnification ×200). (B) Low ARID1A mRNA expression was associated with mutated ARID1A rather than wild-type ARID1A (p=0.001). (C) Low ARID1A protein expression was related with low ARID1A mRNA expression (p<0.001) (D) ARID1A expression was decreased in OCCC compared with normal tissue (p=0.02). (E) ARID1A was elevated in OSC compared with OCCC and OEC (p=0.015 and 0.044, respectively) (error bars: standard errors of the mean). In the Hanyang University Guri Hospital cohort, low ARID1A expression was associated with (F) poor disease-free survival and (G) disease-specific survival (p=0.013 and 0.043, respectively). (H) In the GSE 65986 database, low ARID1A expression was related to progression-free survival (p=0.037).
ARID1A, AT-rich interactive domain 1A; DFS, disease-free survival; DSS, disease-specific survival; OCCC, ovarian clear cell carcinoma; OEC, ovarian endometrioid carcinoma; OSC, ovarian serous carcinoma; PFS, progression-free survival.

In network-based pathway analysis, we found that ARID1A was associated with blood vessel endothelial cell migration, androgen receptor (AR) signaling pathway, DNA damage response/detection of DNA damage and embryo implantation.

In the analysis of ARID1A in a network-based pathway, we identified that tumor angiogenesis was related to vascular endothelial growth factor-A (VEGF-A) and AR signaling pathway (**Fig. 4**).

4. Drug screening in OCCC cell lines with low ARID1A expression

In GDSC data, we analyzed drug sensitivity patterns in 35 ovarian cancer cell lines with ARID1A expression based on 316 drugs. Using Pearson's correlation, we considered drugs exhibiting a high negative correlation between low ARID1A expression and high LN IC50 value as effective drugs. On the basis of the network-based pathway, 2 drugs most effectively inhibited the growth of OCCC cells with low ARID1A expression: cabozantinib (r=−0.941, p=0.017 [Pearson's correlation]) and bicalutamide (r=−0.878, p=0.05) (**Fig. 5A**). In the comparison of the effects of 2 drugs using Student's t-test, cabozantinib and bicalutamide suppressed cell growth in OCCC cells with high ARID1A expression (p=0.05 and 0.481, respectively) (**Fig. 5B**).

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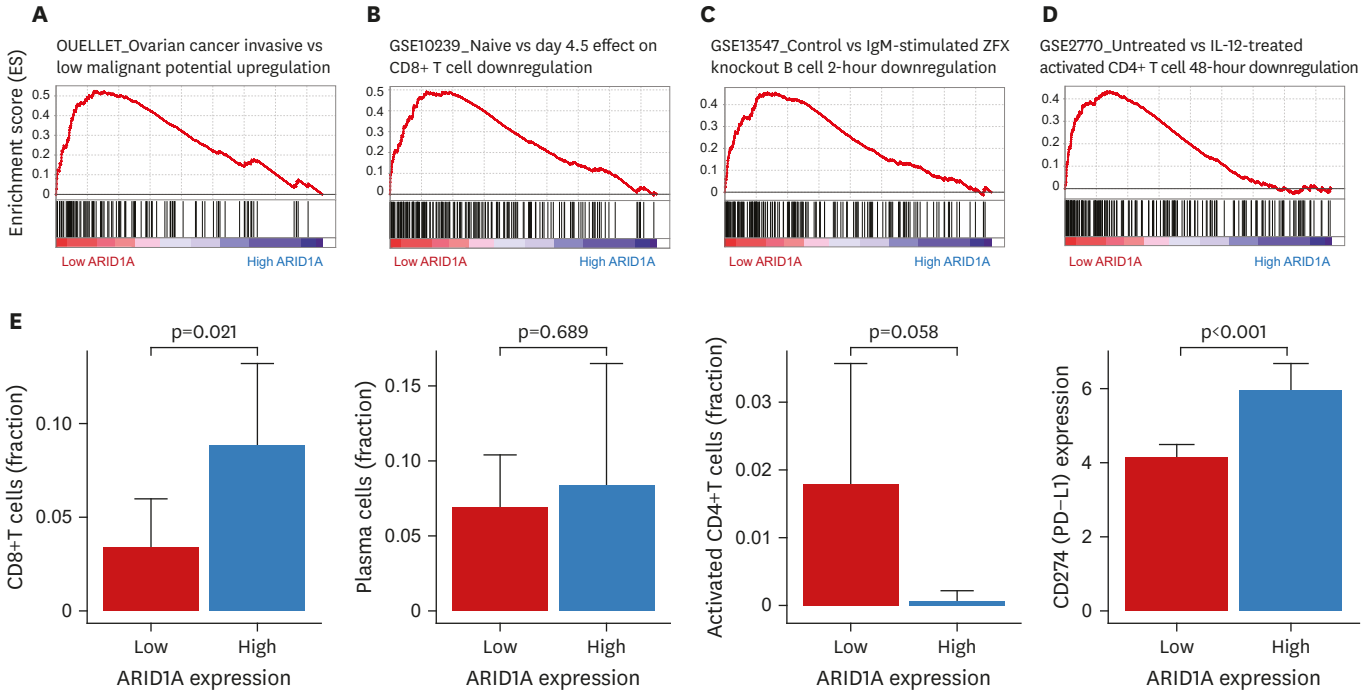

Fig. 3. (A-D) Gene set enrichment analysis of 4 low ARID1A-dependent gene sets reveals the upregulation of the invasion process of ovarian cancer and the downregulation of CD8 T cells, B cells and CD4 T cells. (E) Bar plots of low ARID1A expression and the following parameters: decreased CD8 T cells and plasma cells; increased CD4 T cells; and decreased CD274 (p=0.02, 0.689, 0.058 and <0.001, respectively) (error bars: standard errors of the mean).
ARID1A, AT-rich interactive domain 1A; GSE, gene set enrichment; Ig, immunoglobulin; IL, interleukin; PD-L1, programmed death-ligand 1.

DISCUSSION

ARID1A protein loss of expression may be limited to OCCC among major ovarian carcinomas, but this is still neither well unknown nor widely available. This study was performed using bioinformatic analyses with a meta-analysis exploring the clinicopathological parameters of OCCC with low ARID1A expression. In a meta-analysis of patients with OCCC, low ARID1A expression showed worse OS and DFS and an increased presence of endometriosis than high ARID1A expression. This effect is estimated as a relative increase in the pooled HR of OS, DFS and endometriosis of 28%, 30% and 47%, respectively. The results from heterogeneity testing, sensitivity analysis and publication bias confirmed the reliability of the OS and endometriosis. Notably, endometriosis was associated with CCC in 321 (50.2%) of 642 patients and was associated with low ARID1A expression. A previous study reported that the mutation of ARID1A had already developed from the endometriosis [17]. Thus, low ARID1A may be an early event in the cancer development of endometriosis-associated ovarian cancer [31].

To further reinforce the implications of survival rate, we analyzed the association between ARID1A with DFS/DSS and PFS in the HYGH cohort and GSE 65986 to improve the reproducibility of the findings. As seen previously in our study, low ARID1A expression was statistically related to poor DFS, DSS and PFS.

A study by Huang et al. [10] suggested that ARID1A could function as a tumor suppressor. Other studies have reported a decreased survival rate in OCCC patients with low ARID1A expression [32,33]. Another study demonstrated that ARID1A collaborates with p53 and regulates several downstream target genes, including CDKN1A and SMAD3, to arrest the

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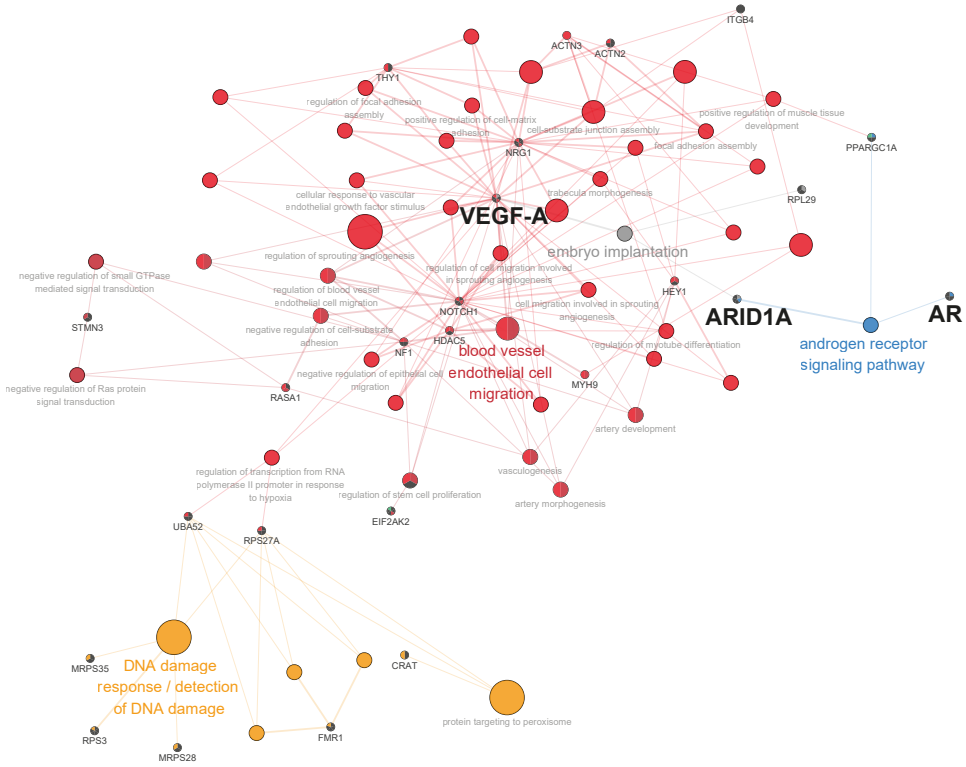

Fig. 4. Grouping of networks based on functionally enriched GO terms and pathways. ARID1A is associated with blood vessel endothelial cell migration, androgen receptor signaling pathway, DNA damage response. The specific hub genes indirectly associated with ARID1A included VEGF-A and androgen receptor (black color, in bold).
AR, androgen receptor; ARID1A, AT-rich interactive domain 1A; GO, Gene Ontology; VEGF-A, vascular endothelial growth factor-A.

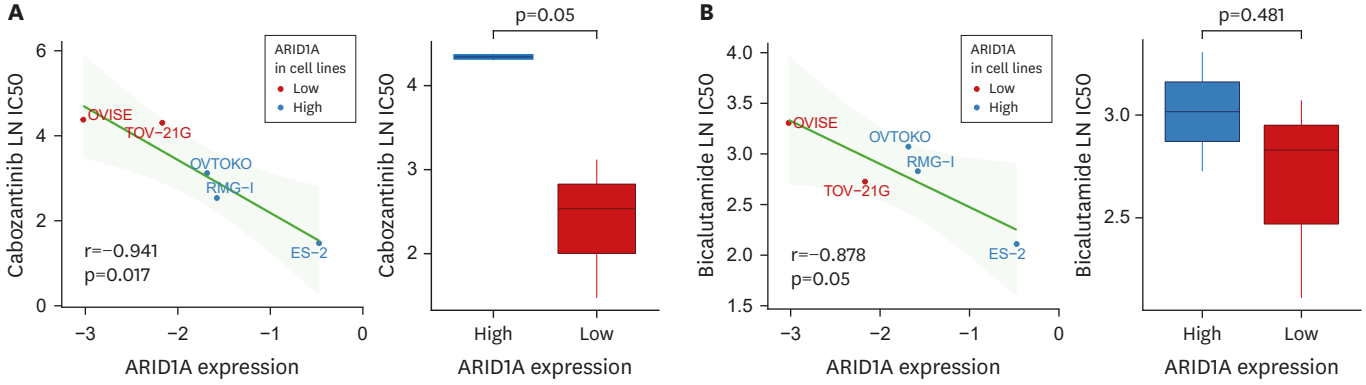

Fig. 5. Genomics of Drug Sensitivity in Cancer database analysis: Pearson's correlations showing the natural LN IC50 values of (A) cabozantinib as vascular endothelial growth factor inhibitor and (B) bicalutamide as antiandrogen agent in ovarian clear cell carcinoma cells (cabozantinib: r=−0.941, p=0.017, boxplot, p=0.05; bicalutamide: r=−0.878, p=0.05, boxplot, p=0.481) (error bars: standard errors of the mean).
ARID1A, AT-rich interactive domain 1A; LN IC50, log of the half-maximal inhibitory concentration.

cell cycle [33]. Furthermore, published data showed that ARID1A plays a pivotal role in the DNA damage checkpoint, preventing genomic instability [34], and that low ARID1A was associated with poor prognosis in the PI3K/AKT signaling and p53 tumor surveillance pathways [11,35].

In computational analyses such as GSEA, in silico cytometry and network-based pathways, our results revealed that low-ARID1A-related gene sets were associated with the invasion

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process of ovarian cancer and the downregulation of immune cells, such as CD8 T cells, B cells, and activated CD4 T cells. Low ARID1a expression was associated with decreased CD8 T cells, plasma cells and CD274, but it was related to increased activated CD4 T cells. This suggests that low ARID1A could unfavorably affect the anticancer immune-infiltration and promote tumor invasion. The results of decreased CD8 T cells and CD274 in OCCC patients with low ARID1A expression suggest that the lack of detectable immune reactions could be involved in the tumor microenvironment [36]. In this result, in OCCC, the need for peritumoral injections of immunostimulatory RNA (poly:IC) to initiate a cytotoxic inflammatory response for immunotherapy should be considered. Further experimental studies are necessary to prove these relationships among the various antitumoral immune factors associated with ARID1A.

The GDSC database, which includes data from drug screening in cancer cell lines, is available for analyses of drug sensitivity [37]. Given the association with ARID1A, we investigated the sensitivity to cabozantinib and bicalutamide between ovarian cancer cell lines with low ARID1A expression and those with high ARID1A expression. As seen previously in the network-based pathway, VEGF-A, as a hub gene, was suppressed by cabozantinib, an VEGF inhibitor, on the basis of reduced tyrosine kinase signaling, which regulates cell survival [38,39]. AR signaling pathway were reduced by bicalutamide [40]. In network-based pathway analysis, the above target genes/proteins, such as VEGF-A and AR, were associated with the ARID1A-associated pathways for cabozantinib and bicalutamide in ovarian cancer cell lines with low ARID1A expression. Two drugs with potential anticancer activities that is known as a synergistic therapeutic agent with chemotherapy. Along with in vivo studies, clinical trials based on cabozantinib and bicalutamide in OCCC with low ARID1A expression are needed in the future.

This study has potential limitations that should be acknowledged. First, the meta-analysis of the enrolled studies is retrospective in design and, therefore, has inherent selection bias, making it difficult to ascertain concrete conclusions. Second, experiments allowing novel biological interpretations into the relationship between ARID1A and immune cells could not be performed, and in vivo studies may be needed. Third, unlike the responses in ovarian cancer cell lines with low ARID1A expression, the therapeutic responses in patients with OCCC may be highly heterogeneous and affected by various microenvironments, which could have effects on clinical applications. Fourth, there are limitations in explaining the relationship between ARID1A mRNA expression and the survival rate, due to the small number of patients (25 cases) for ARID1A mRNA expression.

In summary, the study demonstrated that low ARID1A expression was associated with poor OS in the meta-analysis, and DFS/DSS and PFS in patients with OCCC, HYGH cohort and GSE65986 database. In OCCC patients with low ARIDA1 expression, the decreased CD8 T cell fraction and CD274 (PD-L1) expression are related to type II immunological ignorance. Because of the low numbers of CD8 T cells in OCCC, it is unlikely that blocking PD-L1 will lead to a T cell response [36]. Cabozantinib and bicalutamide could be candidate drugs for the treatment of patients with low ARID1A expression and resistance to conventional chemotherapy. To confirm the effect of cabozantinib and bicalutamide, in vitro experiment using OCCC cell lines should be repeated and in vivo animal study with human OCCC xenotransplantation will be required.

We believe that medical oncologists and researchers will be interested in the role of ARID1A as a growth inhibitor in the development of OCCC and that our results will facilitate further

studies. In addition, our analytic workflow for ARID1A will contribute to designing future experimental studies and future drug development for patients with OCCC.

SUPPLEMENTARY MATERIALS

Supplementary Data 1
Information

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Supplementary Table 1
Baseline characteristics of 13 eligible studies with 1,104 patients (low ARID1A, 530; high ARID1A, 574)

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Supplementary Table 2
Relationships between clinicopathological parameters and the loss of ARID1A expression in ovarian clear cell carcinoma

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Supplementary Table 3
ARID1A expression and clinicopathological parameters in 30 patients with ovarian clear cell carcinoma

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Supplementary References

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