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Original Research

The clinicopathologic significance of the ARID1A expression in ovarian epithelial tumors

D. Ayaz[1], G. Diniz[3], S. Sayhan[1], O. Kaya[1], D.S. Kahraman[1], T. Karadeniz[1], M. Sanci[2]

[1]Pathology Department, [2]Gynecological Oncology Clinics, Izmir Tepecik Education and Research Hospital, Izmir
[3]Pathology Department, Izmir Democracy University, Izmir (Turkey)

Summary

Objective: AT-rich interactive domain 1A (ARID1A) or BAF250a protein, which is encoded by tumor suppressor ARID1A gene, is involved in chromatin remodeling. The aim of this study is to investigate the association between tissue ARID1A expression and ovarian epithelial tumors. *Material and Methods:* ARID1A expression was studied in a total of 137 formalin-fixed, paraffin-embedded specimens of ovarian epithelial tumors. *Results:* Statistically, normal ARID1A expression was determined in borderline and malignant serous tumors ($p < 0.001$). On the contrary, the decreased levels of ARID1A expression were found in endometrioid and mucinous carcinomas, as well as in benign serous tumors (0.033). *Conclusions:* This study demonstrated the presence of a relationship between ARID1A expression deficiency and pathogenesis of mucinous and endometrioid carcinomas. Contrary to previous studies, ARID1A expression was normal in borderline serous tumors similar to serous carcinomas. Based on these results, it can be suggested that pathogenesis of borderline serous ovarian tumors is closer to high-grade than to low-grade serous carcinomas.

Key Words: Ovary; ARID1A; Serous tumors; Mucinous tumors; Endometrioid tumors.

Introduction

Ovarian cancer is the leading cause of death among gynecologic malignancies [1]. Due to lack of diagnostic tools for early detection, most of the patients are diagnosed when they have an advanced stage disease, not curable with existing therapies. Therefore a five-year survival rate is less than 30% in advanced ovarian carcinomas, whereas it reaches 90% in early ovarian carcinomas [1-3]. Recently a new model for ovarian carcinogenesis has been proposed [4]. According to this model, ovarian epithelial tumors are divided into two broad categories: type 1 tumors originate from precursor lesions which have been described comprehensively before, while type 2 tumors may develop *de novo* from tubal and/or ovarian surface epithelium [5, 6]. Type 1 tumors include low-grade serous, mucinous, endometrioid, clear cell, and transitional cell carcinomas, while type 2 tumors include high-grade serous, undifferentiated carcinomas, and carcinosarcomas [4].

The AT-rich interacting domain -1 alpha (ARID1A) protein, which is encoded by ARID1A gene, is a subunit of chromatin remodeler switch/sucrose non-fermentable (SWI/SNF) complex [7-9]. Mutations in ARID1A gene alter the expression of multiple genes due to chromatin remodeling dysfunction [9]. It has been shown that these mutations contribute to carcinogenesis, and cause transformation of cells mediated by PI3K/AKT pathway [9-11]. Various studies have previously emphasized the potential prognostic significance of loss of ARIDIA expression in various predominantly gynecological malignancies [12]. ARID1A mutations have been also detected in especially type 1 ovarian cancer [13].

The objective in this study is to examine the correlations between loss of ARID1A expression in tissues, and different clinicopathologic parameters, and to investigate the potential role of ARIDIA as a diagnostic tool or a target marker in the treatment of ovarian carcinomas.

Materials and Methods

All subjects signed informed consents. This study was approved by Ethics Committee of Tepecik Education and Research Hospital.

The resection specimens of 137 patients who were diagnosed and treated in Izmir Tepecik Education and Research Hospital between 2002 and 2013 were included in this study. The study was approved by the Local Ethics Committee of the Hospital. The staging system developed by the International Federation of Gynecology and Obstetrics (FIGO) was used to describe the extent of spread of these tumors. According to this system, Stage I diseases are confined to one or both ovaries. In Stage II, there is pelvic extension or implants, but tumor is still limited to the pelvis. Stage III diseases demonstrate extension to peritoneum or regional lymph nodes. There are distant metastases to the liver or outside the peritoneal cavity in Stage IV disease.

For immunhistochemistry (IHC), hematoxylin and eosin (HE) staining was used to select appropriate diagnostic paraffin blocks and to identify viable tumor areas. IHC was performed by the

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streptavidin- biotin peroxidase method. Serial 5-µm sections were obtained and placed on slides which were baked overnight at 60^{0}C, dewaxed in xylene, and hydrated with distilled water through decreasing concentrations of alcohol. All slides were treated with heat-induced epitope retrieval procedure in a microwave. In this procedure slides were left for 20 minutes in 10 mM/L citrate buffer at pH 6.0, cooled at room temperature for 20 minutes, and then blocked to retrieve endogenous peroxidase and biotin. Purified monoclonal mouse antibodies against ARID1A were used at a dilution of 1: 200. A pathologist blinded to clinical characteristics of the patient performed histopathological assessments. In the evaluation of immune reactivity for ARIDIA, percentage, and intensity (mild, moderate, strong) of nuclear staining in the tumoral area were evaluated. Since almost all cases demonstrated diffuse, and strongly positive nuclear ARID1A expression, a scoring system was not used, and only absence of presence of the expression was indicated. ARID1A expression was revealed as a strong nuclear staining and the authors estimated the percentage of cells demonstrating nuclear positivity. Accordingly, staining percentage of 66 % was calculated using ROC curve analyses, and below this limit was evaluated as loss of ARIDIA expression. In this study, the author also re-evaluated the histopathological classification of all tumors according to current classification system. *Chi*-square test was performed using SPSS v. 21.0 package program. *P* values less than 0.05 were considered to be statistically significant.

Results

Surgery and chemotherapy were the treatment modalities which were applied alone or in combination to the total of 137 patients according to their individual characteristic features. In this series, there were 89 (64.9%) ovarian epithelial carcinomas, 12 (8.7%) borderline serous tumors, and 36 benign serous tumors (26.7%). Among malignant tumors, 34 (38.2%) serous, 17 (19.1%) mucinous, and 38 (42.6%) endometrioid carcinomas were detected. All 12 patients with borderline serous tumors had Stage I disease (Stages IA: n=8, 66.6%; IB: n=3, 25 %, and IC, n=1, 8.3%). Most cases with serous carcinomas (n=24, 70.5%) had Stage IIIC, while most cases with mucinous (n=10, 58.8%) and endometrioid carcinomas (n=17, 44.7%) had Stage IC disease. Patients with carcinomas had Stage I (n=41, 46%), II (n=2, 2.2%), III (n=42, 47.1%), and IV (n=4, 4.4%) disease (Table 1).

Mean age of patients was 46.2±13.8 (range, 17-78) years. The cases with serous carcinoma (52.4±9 years/30-70 years) were significantly older than cases with both borderline (35.9±13.4 years/23-72 years) and benign serous tumors (38.5±14.8 years/17-62 years). Contrarily, there were no significant differences between the mean ages of the patients with carcinomas. The mean age of the patients with mucinous, and endometrioid carcinomas were 46.1±15 (18-78) years and 51.1±10.5 (34-77) years, respectively. All 48 cases with benign and borderline serous tumors were alive, while 17 cases with carcinoma died. The highest mortality rate was detected in cases with serous carcinoma (32.4%, n=11). Five (13.2%) cases with endometrioid carcinoma and one (5.9%) case with mucinous carcinoma also died. In addition, three endometrioid and one mucinous carcinoma cases were lost to follow-up. Mean overall survival times for cases with serous, endometrioid, and mucinous carcinomas were 15.2±11.8 (0-45), 63.1±60.6 (3-185), and 96.1±66.5 (12-203) months, respectively (Table 2).

Normal nuclear ARID1A expression was determined in most of the serous carcinomas (n=30, 88.2%) and borderline serous tumors (n=10, 83.3%). In contrast, decreased or negative expressions were determined in 21 (55.2%) endometrioid carcinomas, nine (52.9%) mucinous carcinomas, and 30 (83.3%) benign serous tumors (Figure 1). Statistically it was determined that the loss of ARID1A expression was more closely related with the type 1 tumors except for the borderline serous tumors ($p = 0.033$). All epithelial ovarian carcinomas have not demonstrated the same pattern of ARID1A expressions (Figure 2) and there was statistical differences between type of carcinoma and

Table 1: Characteristics of the cases according to the histological subtypes.

Tumor Type	Stage (N:%)	ARID1A (normal /decreased)	Status (Alive/ Deceased/ Lost)	Eventfree survive (Months)	Overall Survive (Months)
Benign Serous Tumor (N=36)	-	N=6; 16.7% D=30; 83.3%	A=36, 100%	-	-
Borderline serous tumor (N=12)	1a=8;66.7% 1b=3;25% 1c=1;8.3%	N=10; 83.3% D=2;16.7%	A=9; 75% L=3; 25%	54.2±31.8	54.2±31.8
Serous Carcinoma (usual/ High grade)(N=34)	1c=1;2.9% 2=1;2.9% 3a=5;14.7% 3b=1;2.9% 3c=24;70.6% 4=2;5.9%	N=31;91.2% D=3=8.8%	A=20; 58.8% D=11; 32.4% L=3; 8.8%	11.4±10	15.3±11.9
Endometrioid Carcinoma (N=38)	1a=5;14 % 1b=2;5.2% 1c=17;44.7% 2=1;2.6% 3a=2;5.2% 3b=2;5.2% 3c=8;21% 4=1;2.6%	N=17;44.7% D=21; 55.3%	A= 24; 63.3% D=2; 5.3% L=12; 31.6%	79±63.1	85.8±60.8
Mucinous Carcinoma (N=17)	1a=6;35.3% 1c=10;58.8% 4=1;5.9%	N= 8; 47.1% D=9; 52.9%	A=11; 64.7% D=1; 5.7% L=5;29.4%	97±64.7	126.1±55.1
P	-	0.032	0.059	<0.01	<0.01

Table 2: Features of the cases with ovarian tumors according to the ARID1Aexpression status.

	ARID1A Normal	ARID1A Absent or Decreased	P
Patients N	72	65	-
Mean ± SD age (years)	47.6 ± 13.8	43.9 ± 14.4	0.847
Histology type (%) Benign serous tumor Borderline seros tumor Serous carcinoma Endometrioid carcinoma Mucinous carcinoma	 6; 8.3% 10; 13.9% 31; 43.1% 17; 23.6% 8; 11.1%	 30; 46.2% 2; 3.1% 3; 4.6% 21; 32.3% 9; 13.8%	**0.035**
Stage (%) I II III IV	 34 - 32 4	 19 2 10 -	**<0.01**
Status (Alive/Deceased/Lost)	A= 47; 65.3% D=13; 18.1% L=12; 16.7%	A= 53; 81.5% D=1; 1.5% L=11; 16.9%	0.850
Overall Survive	41.6±25.1	91.3±61.4	**0.034**
Eventfree survive	40.09±24.9	77.3±60	**<0.01**

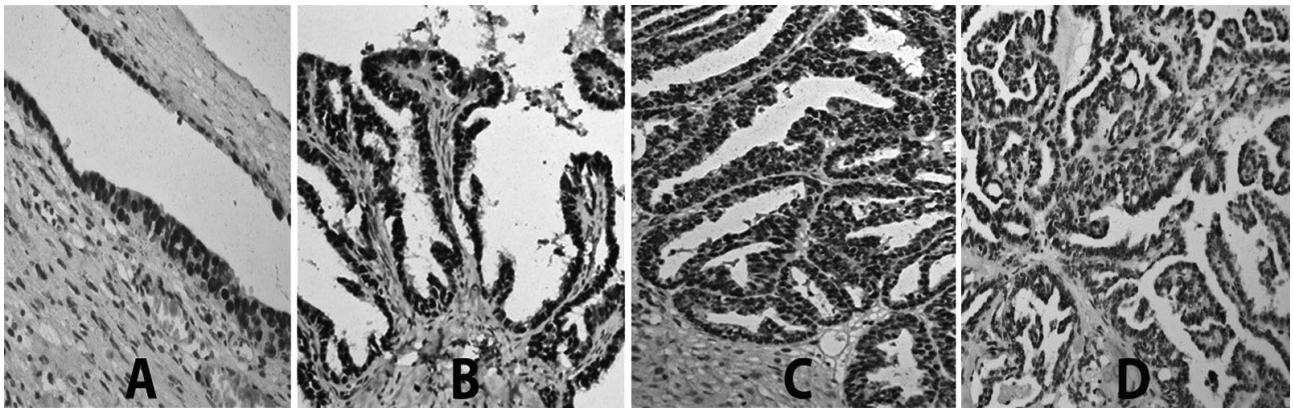

Figure 1. — Different ARID1A expressions in specimens with different ovarian serous tumors: A) Positive ARID1A expression in a benign serous tumor and adjacent normal ovarian stroma. B) ARID1A positivity in a borderline serous tumor. C) Diffuse strong ARID1A expression in a high- grade serous carcinoma. D) Decreased ARID1A expression in another high-grade serous carcinoma (DAB ×200).

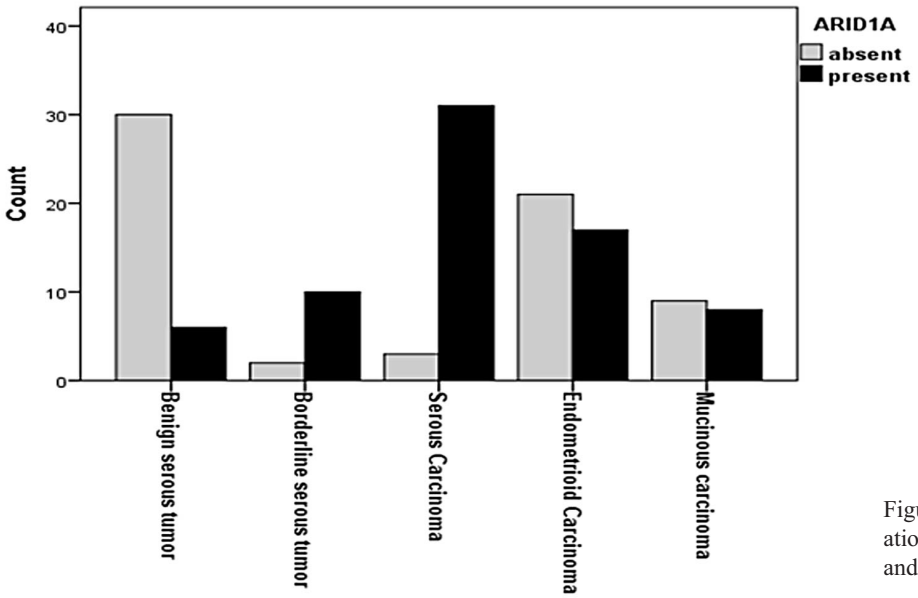

Figure 2. — There is a significant association between subtypes of ovarian tumors and expressions of ARID1A ($p < 0.01$).

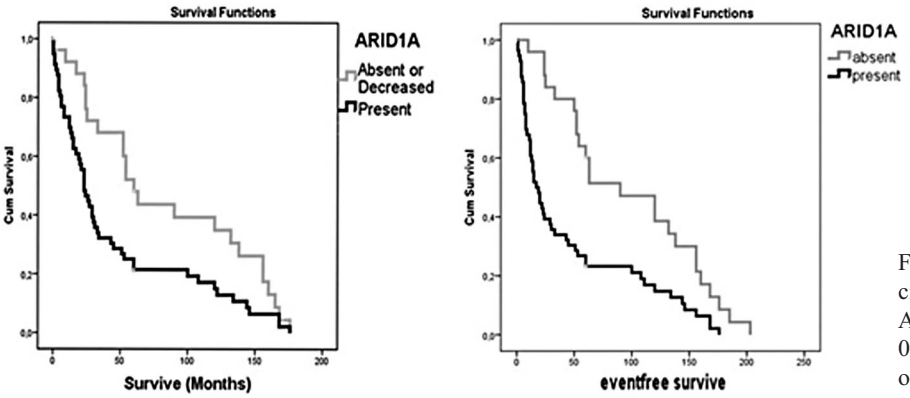

Figure 3. — There is a significant association between the expressions of ARID1A and overall survival ($p = 0.013$) or event-free survival ($p = 0.01$) of the patients with ovarian carcinomas.

ARID1A status ($p < 0.001$). In addition, an association was found between the type of carcinomas and survival time ($p = 0.013$). In the Kaplan-Meier survival analysis, statistically significant differences between overall survival rates of the patients were categorized according to the status of ARID1A expression (Figure 3). Interestingly, it was found that the lower ARID1A expression correlated with longer survival perhaps due to serous carcinomas have poor prognosis.

Discussion

It was previously reported that ARID1A is a tumor suppressor gene located in 1p36.11 region which is frequently deleted in human cancers [7-9, 12]. Although loss of ARID1A expression was demonstrated in many gynecological cancers, the functional and prognostic significance of ARID1A has not yet been fully elucidated [9-12]. Interestingly ARID1A mutation frequently coexists with activating mutations of PIK3CA which increase the intrinsic kinase activity of PI3K and/or loss of PTEN expression, which both lead to a downstream activation of the PI3K/AKT pathway [10, 12]. It was also suggested that ARID1A mutation and activation of the PI3K/AKT pathway occur during the early stage of carcinogenesis in ovary, especially during endometriosis-associated subtypes of ovarian carcinomas. Recently a new drug was used as inhibitor of PI3K/AKT pathway in targeted therapy [13]. Therefore, detection of ARID1A mutations which activate the PI3K/AKT pathway is very important in the treatment of advanced ovarian carcinomas. In the present study, the authors determined the expression defects of ARID1A protein in the most of benign serous tumors and more than half of endometrioid and mucinous carcinomas. This finding suggests that decreasing expression of ARID1A may play a role in tumorigenesis of some ovarian tumors, especially in type 1 ovarian tumors.

Each histologic subtype of ovarian epithelial tumors is further divided into three groups as benign, intermediate (borderline or low malignant potential), and malign tumors reflecting their clinical behavior [14, 15]. Tumors with low malignant potential constitute the most enigmatic group of ovarian neoplasms. Since their behavior is undefined, and their pathogenesis is unclear, therefore their clinical management is controversial. Borderline serous tumor is the most common type of ovarian borderline tumors which has a puzzling relationship with invasive carcinoma. Because their pathogenesis and behavior have not yet been fully elucidated. In the literature; borderline epithelial tumors are generally accepted as type 1 tumors [14-17]. Therefore loss of ARID1A expression may be expected in borderline tumors. Contrary to this expectation, the authors found normal ARID1A expression in most borderline serous tumors in this study. However the present study population did not have any patient with borderline endometrioid or borderline mucinous tumors which is a limitation of this study. Therefore the authors could not draw conclusive remarks regarding the relationship with ARID1A expressions and other type borderline tumors.

In light of recent molecular studies, a new model for pathogenesis of ovarian serous carcinomas was developed. In this model, serous carcinoma is subdivided into low- and high-grade types that have distinct pathways of tumorigenesis [14]. Low-grade serous carcinomas develop in a slow stepwise fashion from borderline serous tumors and intraepithelial carcinoma, whereas the majority of high-grade serous carcinomas develop rapidly, presumably from inclusion cysts or ovarian surface epithelium. In the dualistic model, the borderline serous tumor is a distinct entity that represents the putative precursor of invasive low-grade serous carcinoma which is unrelated to high-grade (usual type) serous carcinoma (14). In the present study, unlike to the previous reports, the authors found normal ARID1A expression in borderline serous tumors similar to the serous carcinomas. This result suggested that these serous carcinomas may develop from a preexisting borderline serous tumors or both tumors may have similar pathogenetic pathway.

Loss of BAF250a expression which is encoded by ARID1A gene and its potential correlation between various clinicopathologic parameters have recently been reported in several tumor types, including ovarian neoplasms [17-19]. In a recent study, it was determined that BAF250a expression gradually decreased in transitioning from normal to cervical carcinomas, and this loss of expression was significantly associated with several prognostic parameters such as tumor stage, tumor grade, tumor size, and lymph node metastasis. The authors reported that the overall survival in cervical cancer was significantly reduced in cases with ARID1A loss [18]. In the current study, the authors also found a statistically significant association between ARID1A expression and survival. However unlike previous reports, the overall survival in ovarian carcinomas was significantly reduced in cases without ARID1A loss.

Conclusions

In conclusion, the authors compared ARID1A expression pattern in several ovarian epithelial neoplasm. The results of this study demonstrate that altered expression of ARID1A proteins may involve in tumorigenesis of mucinous, endometrioid carcinomas, and also benign serous tumors. In contrast, most of the borderline serous tumors and carcinomas normally express ARID1A. However the present findings require further investigation to determine the mechanism of chromatin remodeling dysregulation induced by ARID1A mutation, and to examine the involvement of ARID1A mutations in the development and progression of ovarian cancers.

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Corresponding Author:
G. DINIZ, M.D., PhD
Department of Pathology
Izmir Democracy University
35220 Izmir (Turkey)
e-mail: gulden.diniz@idu.edu.tr