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# ARID1 and BRG1 Expression in Endometrial Cancer

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**Abstract.** *Background/Aim: Endometrial cancer (EC) is the predominant malignancy among gynecologic cancers and ranks fourth among all types of cancer. Recently, researchers have focused on the development of new prognostic biomarkers. Subunits of the SWI/SNF protein complex, like the ARID1 and BRG1, have been associated with the development of endometrial cancer. The present study aimed to evaluate the expression patterns of ARID1A and BRG1 in a collection of endometrioid adenocarcinomas of the uterus using immunohistochemistry. Patients and Methods: The study comprised a total of thirty-three individuals diagnosed with stage I endometrioid endometrial cancer, treated with radical hysterectomy. The histological material was then examined to assess the cytoplasmic and nuclear expression of the proteins. Results: ARID1A exhibited expression in both the cytoplasm and nucleus of cancer cells, whereas BRG1 was mainly expressed in the nuclei. In addition, ARID1A exhibited a notable decrease in expression in grade 3 histology, with no significant correlation with the depth of myometrial invasion. The reduced expression was highly related to tumor expansion into the endocervix. The findings demonstrated a total absence of ARID1A expression in 27% of endometrioid carcinomas, with a significant reduction in expression in an additional 51% of cancer cells. These findings align with the most recent published data. In contrast, in the current study, BRG1 was rarely down-regulated and was extensively expressed in the majority of endometrioid carcinomas, preventing the possibility of statistical analysis. Conclusion: In summary, ARID1A expression loss can be used as a biomarker to guide post-operative therapy; however, further investigation is needed, especially for early-stage endometrial cancer.*

Endometrial cancer (EC) is the most common malignancy of the female genital tract worldwide with 417.000 new cases and an estimated 97000 deaths reported per year (1). It is considered to be the fourth most common after breast, lung, and colorectal neoplasms. Most EC cases (90%) are sporadic, 10% of the cases are considered familial, being part of the hereditary non-polyposis colorectal cancer syndrome (2).

During the last decades, research studies have focused on molecular targets in order to improve diagnostic methods, develop new prognostic biomarkers and propose therapeutic interventions. Among them, an interesting target is the SWI/SNF protein complex. The SWI/SNF complex is composed of a variety of subunits: ARID1A or ARID1B, SMARCA4, SMARCC1 and 2, SMARCD1, 2 and 3, and ACTL6A and B. It has an important role in chromatin remodeling and participates in a variety of cellular processes, like cell differentiation, DNA repair, and tumor repression (3, 4).

Mutations and changes in the expression of its subunits have been documented in different types of cancer. Previous research studies have shown that ARID1A is a

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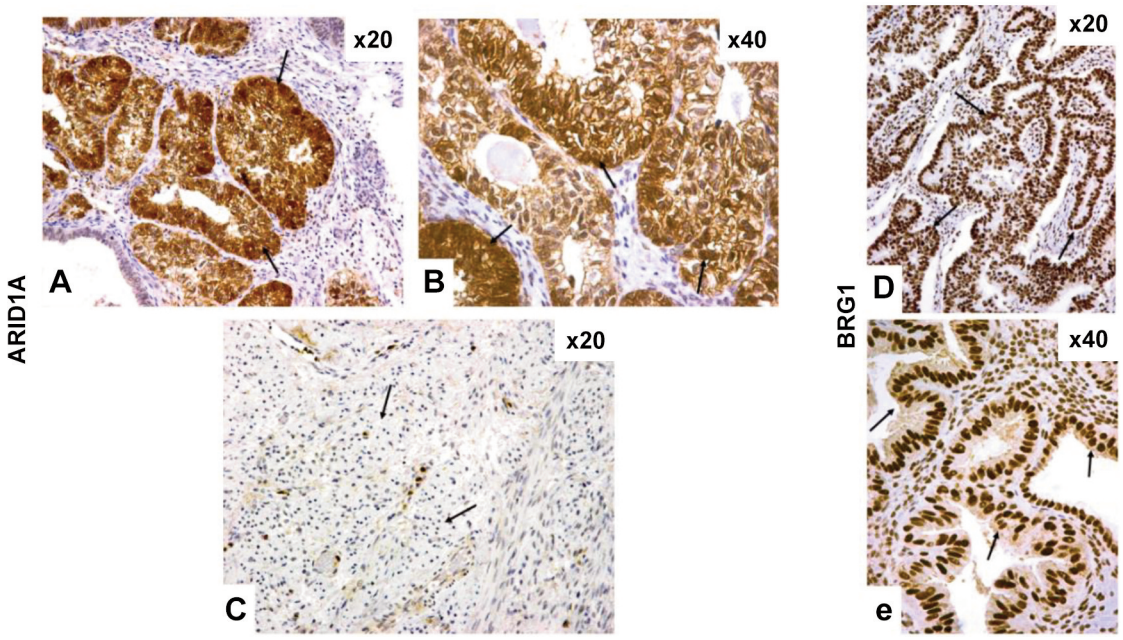


Figure 1. *Typical immunohistochemical images of ARID1A (A-C) and BRG1 (D, E) expression.*

potential tumor suppressor gene, and ARID1A mutations change its protein expression, while low expression levels have been documented in different types of tumors. *ARID1A* gene is located on chromosome 1p36.11 and encodes a protein that interacts with AT-rich DNA sequence. Furthermore, the C-terminus of the protein stimulates glucocorticoid receptor-dependent transcriptional activation (5, 6) . Mutations of the ARID1 have been linked with endometrial carcinogenesis (7).

Besides ARID1A, SMARCA4 (BRG1) is another interesting SWI/SNF subunit. BRG1 has helicase and ATPase functions, interacts with ARID1A, and controls the expression of a variety of genes. *In vitro* experiments confirmed that different cancer cell lines exhibit loss of BRG1, while lack of expression has been documented in ovarian cancer (8, 9).

In the current study, we assessed the expression patterns of ARID1A and BRG1 in a series of endometrioid adenocarcinomas of the uterus using immunohistochemistry to provide evidence of their involvement in endometrial carcinogenesis and clinical behavior.

## Patients and Methods

*Ethical considerations*. The Scientific and Ethics Research Committees of the University Hospital of Alexandroupolis granted approval for the conduct of this study (SC8/01-12-2016). All patients gave written informed consent and agreed to the anonymous analysis and publication of their clinical and laboratory data.

*Patients and disease characteristics*. Thirty-three patients with endometrial cancer, treated with radical hysterectomy for stage I endometroid carcinoma, were prospectively recruited in the study. Paraffin-embedded tissue material was retrieved from the archives of the Department of Pathology, Democritus University of Thrace, Greece. All cases were endometrioid carcinomas. Nineteen were of grade 1, while nine and five were of grade 2 and 3, respectively. Deep myometrial invasion exceeding 50% of the myometrium thickness was present in seven of the 33 patients, while invasion to the endocervix was noted in six of the 33 cases. The median age of patients was 64 years (range=33-82 years).

*Immunohistochemistry*. Three μm tissue sections were cut from formalin fixed paraffin-embedded cancer tissue material. The slides were, subsequently, deparaffinized in xylene and rehydrated in solutions of graded ethanol. Epitope retrieval was enhanced by heating in a microwave oven. The slides were placed in the Dako EnVision FLEX Target Retrieval Solution (pH 9.0) (Glostrup, Denmark). After washing twice for six minutes, the slides were incubated overnight with the rabbit anti-ARID1α primary antibody (HPA005456, dilution 1/100; Merck SA, Athens, Greece), incubation and rabbit anti-BRG1/SMARCA4 Antibody (SMARCA4, dilution 1/200; Merck SA). Endogenous peroxidase was quenched with EnVision Flex Peroxidase Block (DAKO) for 10 min. The color was developed after incubation with EnVsion Flex Chromogen (DAKO) and sections were counterstained with hematoxylin. For negative control, we replaced the primary antibody with normal species-specific immunoglobulin-G. Normal endometrial tissue was used as a positive control.

Assessment of cytoplasmic and nuclear expression of the proteins was performed in all-optical fields at ×200 magnification. The percentage of cancer cells expressing the proteins (whether in the nuclei or cytoplasm) was recorded per field, and the mean value was used to provide a score for each case.

*Statistical analysis*. Statistical analysis was conducted using the GraphPad Prism 7.0 (GraphPad Software Inc., Boston, MA, USA) statistical package. The unpaired two-tailed *t*-test or the Wilcoxon matched-pairs signed rank test was used to compare groups with continuous variables, as appropriate. Linear regression analysis was applied to assess the association between continuous variables. *p*-Values <0.05 were considered for significance.

## Results

*Expression patterns of ARID1A*. ARID1A was expressed in the cytoplasm and nuclei of cancer cells. This mixed pattern of expression ranged between 0-70% of cancer cells (median 10%, 25$^{th}$ and 75$^{th}$ percentiles 2.5% and 40%, respectively). Overall, extensive lack of expression (positive cancer cells <5%; negative) was noted in 9/33 (27.2%) cases, while partial expression (positive cancer cells 10-40%; low expression cases) was evident in 17/33 (51.5%) cases. Marked expression in >50% of cancer cells (high expression cases) were noted in 7/33 (21.2%) cases. Figure 1A-C shows typical immunohistochemical images of ARID1A expression. Figure 2A shows the distribution of cases according to the % of cancer cells expressing ARID1A in the three groups of negative, low, and high expression.

*Expression patterns of BRG1*. BRG1 showed a nuclear pattern of expression in cancer cells. The percentage of cancer cells expressing BRG1 was high, with a median of 90% (range=20-100%). Only three cases had reduced expression (each in 60%, 50%, and 20% of cancer cells). Figure 1D and E show typical immunohistochemical images of BRG1 expression. Figure 2B shows the distribution of cases according to the % of cancer cells expressing BRG1 in the three groups of expression (<50%, 60-80%, and 90-100%).

*Association with histopathological parameters*. A significantly reduced expression of ARID1A was noted in cases with grade 3 histology. The median value was 0% *vs*. 20% in grade 1,2 cases (Figure 3A; $p$=0.05). There was no significant association of ARID1A expression with the depth of myometrial invasion (Figure 3B; $p$=0.92), but lower ARID1A expression was significantly linked with the extension of tumor to the endocervix (Figure 3C; $p$=0.05). Due to the extensive expression of BRG1 in most cases analyzed, no statistical analysis was feasible.

## Discussion

ARID1A subunit is the most frequently mutated component of the SWI/SNF complex. Its C-terminal domain mediates the interactions of ARID1A with nuclear hormone receptors and, furthermore, is implicated in the regulation of the activity of multiple transcription factors (10). ARID1A is considered a tumor-suppressor gene regulating cell cycle progression and preventing genomic instability (11). Indeed,

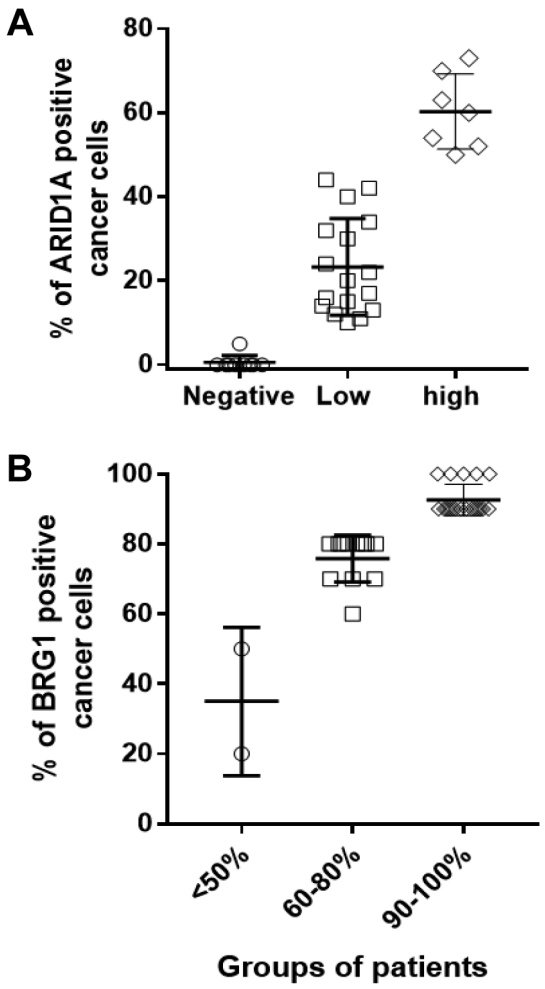


Figure 2. *Distribution of cases according to the percentage of cancer cells expressing ARID1A and BRG1. A) Distribution of cases according to the percentage of cancer cells expressing ARID1A in the three groups of negative, low, and high expression. B) Distribution of cases according to the percentage of cancer cells expressing BRG1 in the three groups of expression, <50%, 60-80%, and 90-100%.*

loss of ARID1A expression has been found in 35% of breast carcinomas, 50% of non-small cell lung cancers (NSCLCs), 33% of cervical carcinomas, 11-70% of gastric carcinomas, a feature that is linked with aggressive histopathological features and poor prognosis (12-15).

Loss of ARID1A expression seems to be an early event in endometrial cancer carcinogenesis (16). In 2017, a meta-analysis of 11 studies that included 1432 patients with endometrial cancer reported that loss of ARID1A expression was linked with advanced FIGO stage and shorter progression-free survival (17). In a combined analysis of the PORTEC cohorts, a panel of genes that included ARID1A improved the assessment of risk in early-stage endometrial cancer. It also provided a basis for grouping cases to low and

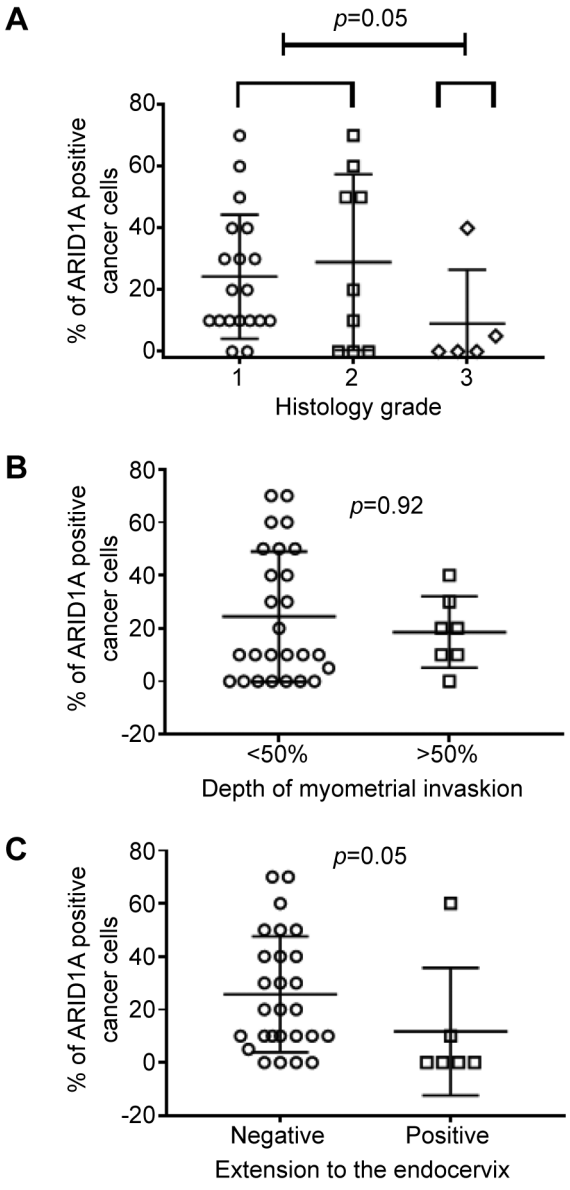


Figure 3. *The association of ARID1A expression with histological parameters. ARID1A revealed (A) decreased expression in grade 3 histology, (B) no significant correlation with the depth of myometrial invasion and (C) a weaker association with the expansion of the tumor to the endocervix.*

high risk of recurrence, to personalize the necessity of adjuvant radiotherapy and chemotherapy (18). ARID1A loss of expression in the subgroup of copy-number low endometrial carcinoma subtype, identified as 'no specific molecular subtype' (NSMP), defined the worst prognosis and was linked with early tumor recurrences (19, 20).

In our study, complete loss of ARID1A expression was evident in 27%, while prominent lack of expression in the majority of cancer cells was noted in an additional 51% of endometrioid carcinomas examined. Complete loss was significantly linked with high tumor grade and extension to the endocervix. In accordance with our findings, Korentzelos *et al*., confirmed loss of ARID1A expression in 75% of 20 undifferentiated endometrial carcinoma cases (21).

BRG1/SMARCA4 is another catalytic subunit of the SWI/SNF chromatin-remodeling complex, used by a variety of enzymes involved in the modification of the chromatin structure. Its function as a tumor-suppressor gene has also been postulated, although its function as a tumor promoter has also been suggested in recent studies (22, 23). The role of BRG1 in other malignancies has been investigated. In NSCLC, loss of BRG1 expression was very low (4% of cases) and occurred in undifferentiated tumors (24). Similarly, infrequent is its loss of expression in head neck carcinomas (25). In breast cancer, however, low expression of BRG1 has been documented in approximately half of cases, and this feature was linked with poor prognosis (26). Moreover, low BRG1 expression has been confirmed in 33% of colorectal carcinomas, but, in contrast to other studies, high BRG1 expression was linked with poorer prognosis (27). In the current study, BRG1 was expressed in the nuclei of cancer cells in the majority of endometrioid carcinomas and was seldomly down-regulated. This is in accordance with the study by Korentzelos *et al*. where BRG1 loss was noted in 20% of undifferentiated endometrial carcinomas (21).

It is concluded that ARID1A expression is completely lost in one-fourth of endometrial carcinomas, and partially in a significant proportion of cancer cells is further evident in an additional 50% of cases. Complete loss was linked with histological features of aggressive clinical behavior, like poor differentiation and extension to the endocervix. Such features are key factors that determine the necessity of post-operative radiotherapy. BRG1 seems, however, not to be involved in endometrial carcinogenesis and has no role in the clinical behavior of endometrial cancer. Whether ARID1A expression loss can be used as biomarker to guide post-operative therapy in early-stage endometrial cancer demands further investigation.

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## Conflicts of Interest

The Authors declare no conflicts of interest in relation to this study.

## Authors' Contributions

P.S., P.T., and A.G. contributed to conception and design. E.N.K., N.N. were responsible for overall supervision. K.N.; Drafted the manuscript, which was revised by P.P. All Authors read and approved the final manuscript.

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