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Original contribution

# Various ARID1A expression patterns and their clinical significance in gastric cancers☆,☆☆

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**Summary** AT-rich interactive domain 1A (ARID1A) is frequently mutated in gastric cancers, and loss of ARID1A expression is considered a poor prognostic factor in various cancers. However, in practice, ARID1A shows various expression patterns, and our understanding of its significance is limited. We performed immunohistochemistry for ARID1A, MLH1, and pS6 using whole tissue blocks of 350 gastric cancers and classified the ARID1A expression as follows: retained (63.7%), reduced (17.7%), complete loss (14.9%), and partial loss (3.7%). Complete/partial loss was more common in poorly differentiated histology ($P < .001$), and reduced or complete loss of ARID1A was frequent in cases with MLH1 loss ($P < .001$). The ARID1A-reduced group showed only slightly inferior disease-free survival (DFS; $P = .254$) and overall survival (OS; $P = .377$) compared to those of the ARID1A-retained group, whereas the group with complete loss showed significantly worse DFS (hazard ratio [HR], 1.732; $P = .015$) and OS (HR, 1.751; $P = .013$). Worse DFS (HR, 2.672; $P = .005$) and OS (HR, 2.531; $P = .002$) were also noted in the group with partial loss. High expression of pS6 was observed more frequently in groups showing altered ARID1A expression patterns ($P < .001$). In conclusion, reduced ARID1A expression is not a major prognostic determinant, although it may lead to AKT pathway activation. Tumor cells lacking ARID1A expression may influence the prognosis even if they constitute only a small proportion of the tumor sample. Our data provide an enhanced roadmap for understanding ARID1A with implications for future research and therapeutics.


## 1. Introduction

AT-rich interactive domain 1A (ARID1A) is a member of the ARID family of proteins that encodes a subunit of the Switch/Sucrose nonfermentable chromatin remodeling family [1]. Recent genome-wide sequencing studies have demonstrated that ARID1A is frequently mutated in some cancers including ovarian, endometrial, and gastric cancers [2-4]. The mutation rates are particularly high in gynecologic cancers, and *ARID1A* mutations have been detected in 46%

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of ovarian clear cell carcinomas and 30% of endometrioid carcinomas [3]. The frequency of *ARID1A* mutations in gastric cancers varies according to studies, ranging from 8% to 33% [4-6]. Interestingly, alterations in *ARID1A* are associated with microsatellite instability (MSI) and Epstein-Barr virus (EBV) infection in patients with gastric cancer [4].

ARID1A is a key component of the Switch/Sucrose nonfermentable chromatin remodeling complex and is involved in regulating diverse cellular processes including development, differentiation, proliferation, and DNA repair [7]. Previous studies have demonstrated that ARID1A knockdown enhances cellular proliferation in gastric cancer cell lines containing wild-type ARID1A but has no effect on ARID1A-null cells [6], indicating a tumor suppressive role for ARID1A. In addition, ARID1A directly represses E2F1, a master cell cycle regulator [8], and ARID1A knockdown in a leukemia cell line confers resistance to Fas-mediated apoptosis [9]. In contrast, growing evidence indicates that loss of ARID1A expression leads to the activation of the phosphatidylinositol 3-kinase (PI3K)/AKT pathway, particularly by AKT phosphorylation [10-12], which has not been assessed in gastric cancers.

Genetic alterations in *ARID1A* in gastric cancers are mostly insertion/deletion mutations, and these lead to truncation of the ARID1A protein [4]. Loss of ARID1A expression as assessed by immunohistochemistry (IHC) is closely related to the *ARID1A* mutations [4,13,14], although several other mechanisms including epigenetic modulations can induce loss of ARID1A expression as well. Loss of ARID1A expression has been correlated with poorer survival in patients with gastric cancer [15-17]. However, in practice, ARID1A shows various expression patterns in patients with gastric cancers. Some tumor samples show strong nuclear expression, whereas others show complete loss of expression. In addition, a significant decrease in ARID1A protein expression is noted in some tumors [4], whereas a subset of tumors has discrete components of ARID1A-positive and ARID1A-negative cells [16]. The clinicopathological and prognostic significance of these various ARID1A expression patterns has not been investigated.

To clarify the clinical significance of the various ARID1A expression patterns in gastric cancers, we performed ARID1A IHC using whole tissue blocks. Using MLH1 staining, we analyzed our data according to the MSI status. We also performed IHC for phospho-S6 kinase (pS6), a reliable marker of PI3K/AKT pathway activation to pursue a possible relationship between ARID1A expression and PI3K/AKT pathway activation in gastric cancers.

## 2. Materials and methods

### 2.1. Patient selection and data collection

A total of 473 patients with gastric cancer underwent curative surgical resection with standard lymphadenectomy from January 2005 to December 2006 at Ajou University Hospital in Korea. After excluding mucosal cancers (T stage 1a) and multiple simultaneous gastric cancers, 350 gastric cancer cases were enrolled in this study. Patients who were pathologically diagnosed with stage II or higher cancer were recommended to receive adjuvant chemotherapeutic regimens including 5-fluorouracil.. In addition, we planned to follow up the patients for at least 5 years after the surgery by computed tomographic scans, tumor markers, and gastroscopy 1 to 4 times per year. Clinical data were retrieved from the patient medical records. The median follow-up duration was 71 months. Overall survival (OS) time was measured from the date of surgery to death or the last follow-up visit. Disease-free survival (DFS) time was defined as the interval between the date of surgery until detection of a tumor recurrence or death.

The hematoxylin and eosin–stained sections of all the cases were reviewed by experienced gastrointestinal pathologists (D. L. and Y.-B. K.), and appropriate histologic diagnoses were made. The tumors were classified into differentiated type (well-differentiated and moderately differentiated adenocarcinoma) and undifferentiated type (poorly differentiated adenocarcinoma, signet ring cell carcinoma, and mucinous adenocarcinoma) for statistical analysis. The pathologic stages were adjusted based on the AJCC 7th edition [18]. This study was carried out in accordance with the code of ethics of the World Medical Association (Declaration of Helsinki) and was approved by the Institutional Review Board of Ajou University Hospital (approval no. BMR-SMP-14-155, August 14, 2014).

### 2.2. Immunohistochemistry

The IHC study was carried out on formalin-fixed, paraffin-embedded, 4-$\mu$m-thick tissue sections. The primary antibodies included rabbit polyclonal anti-ARID1A (HPA005456; 1:200, Sigma-Aldrich, St Louis, MO), rabbit polyclonal anti-pS6 (Ser235/236) (no. 2211; 1:300, Cell Signaling Technology, Beverly, MA), and mouse monoclonal anti-MLH1 (M1; Ventana Medical Systems, Tucson, AZ). The immunostaining was performed using a Ventana BenchMark XT autoimmunostainer (Ventana Medical Systems) with a cell conditioner for 60 minutes. The slides were incubated with the primary antibodies at 37°C for 32 minutes, followed by standard Ventana signal amplification, counterstaining with hematoxylin for 4 minutes, and staining with a bluing reagent for 4 minutes. The slides were then removed from the immunostainer, mounted, and examined by light microscopy. Slides processed without the primary antibodies were used as negative controls. All cases of improper staining were retested and properly examined.

### 2.3. Interpretation of IHC expression patterns for various proteins

ARID1A is strongly and uniformly expressed in the nuclei of normal cells, including gastric epithelial cells, lymphocytes, and