

ORIGINAL PAPER

CAN ARID1A EXPRESSION LEVELS IN GASTRIC CARCINOMAS PREDICT RESPONSE TO NEOADJUVANT THERAPY AND PROGNOSIS?

SEVINC HALLAC KESER¹, EZGI NURDAN YENILMEZ TUNOGLU^{2,3}, TUNAY DOĞAN^{4,5}, SIBEL SENSU¹, SELMA SENGİZ ERHAN¹, DIDEM CANOĞLU¹, ERHAN ÇONAY⁶, AYLIN EGE GÜL¹, NAGEHAN ÖZDEMİR BARIŞIK¹

¹Department of Pathology, Kartal Dr. Lutfi Kirdar City Hospital, Istanbul, Turkey

²Division of Medical Techniques and Services, Vocational School of Health Services, Demiroglu Science University, Istanbul, Turkey

³Experimental Medicine Center, R&D Laboratory, Demiroglu Science University, Istanbul, Turkey

⁴Istinye University Molecular Cancer Research Center (ISUMKAM), Istanbul, Turkey

⁵Department of Medical Laboratory Techniques, Istinye University Vocational School of Health Care Services, Istanbul, Turkey

⁶Department of Medical Oncology, Kartal Dr. Lutfi Kirdar City Hospital, Istanbul, Turkey

The study aims to evaluate the significance of ARID1A expression in gastric cancer (GC) patients receiving neoadjuvant therapy, using immunohistochemical methods and bioinformatics approaches.

Immunohistochemical analysis of an institutional clinical cohort ($n = 80$) was combined with bioinformatics analyses of independent public datasets. RNA-sequencing and clinical data from the TCGA-STAD cohort were retrieved and processed using TCGAbiolinks, differential expression analysis was performed using DESeq2, and overall survival (OS) was evaluated using Kaplan-Meier, log-rank, and univariate Cox regression analyses. STRING was used to construct an ARID1A-centered protein association network, while Human Protein Atlas and Cellosaurus data were used to evaluate ARID1A expression.

Loss of ARID1A protein expression rate was 41.3%. ARID1A loss and larger tumor size were correlated (median 6.0 cm vs. 4.0 cm; $p = 0.004$). ARID1A status and histopathological response to neoadjuvant therapy, pathological tumor stage and OS are not statistically related. Bioinformatics analyses demonstrated marked variation in ARID1A expression across GC cell lines, while TCGA-STAD analyses showed no significant tumor-normal difference or survival association at the transcript level.

While ARID1A loss is associated with increased tumor size, it is not a strong independent predictor of treatment response or OS in GC. These findings underscore the molecular complexity of gastric adenocarcinoma and the need for larger, multicenter studies to refine the clinical utility of ARID1A.

Key words: neoadjuvant therapy, ARID1A, bioinformatics, immunohistochemistry, gastric cancer.

Introduction

Gastric adenocarcinoma accounts for approximately 95% of gastric cancer (GC) cases and exhibits high morphological and molecular heterogeneity [1]. Despite therapeutic advances, the overall 5-year survival rate

for GC patients worldwide remains below 25%. Gastric carcinogenesis is a multistep process driven by the interaction of environmental factors, dietary habits, *Helicobacter pylori* infection, genetic predisposition, and epigenetic changes. In the model described by Correa, the progression involves a cascade from chronic gastritis

to invasive carcinoma necessitating an understanding of tumor behavior at the molecular level [1–5].

Interest in molecular markers that reflect the biological heterogeneity of GC – such as HER2 amplification, microsatellite instability, and Epstein-Barr virus-associated subtypes – has increased significantly [6–8]. Besides, chromatin remodeling complexes are gaining increasing importance in tumor biology. In this context, the AT-rich interaction domain 1A (*ARID1A*) gene emerges as a key epigenetic modulator. As a core component of the SWI/SNF complex, ARID1A regulates gene expression by controlling chromatin accessibility, influencing fundamental biological processes such as the cell cycle, DNA damage response, and apoptosis. Functional mutations or reduced expression of *ARID1A* disrupt tumor-suppressor functions in various solid tumors [1, 9–12], resulting in the loss of nuclear expression, as assessed by immunohistochemistry (IHC) [13–15]. Notably, a study on locally advanced rectal cancers suggested that tumor cells with low baseline ARID1A expression might exhibit higher sensitivity to neoadjuvant therapy [16].

ARID1A alterations are common in certain histological and molecular subtypes of GC and may predict responses to both chemotherapy and immune checkpoint inhibitors [1, 17–19]. However, *ARID1A* may exhibit different behaviors at various biological levels in GC. While ARID1A is primarily assessed in clinical practice immunohistochemically for protein expression, bioinformatics studies primarily reflect mRNA transcript levels. Due to post-transcriptional and translational regulation mRNA and protein levels do not always correlate [20, 21]. Indeed, significant discrepancies between ARID1A mutations, mRNA, and protein expression have been reported in GC [22]. Therefore, combining protein-level IHC with transcript-level bioinformatics analyses is crucial to fully comprehend its significance [1, 22, 23].

In this study, we evaluated ARID1A protein expression in GC using immunohistochemical methods and analyzed its association with clinical-pathological parameters, response to neoadjuvant therapy, and survival. Although perioperative chemotherapy is the standard for locally advanced GC, reliable predictive biomarkers remain an unmet need [24]. By integrating IHC with bioinformatics profiling of mRNA, we aim to comprehensively elucidate the role of ARID1A in GC.

Material and methods

The histopathologically examined cases of the study included the period between 2016 and 2024. Patients who had a biopsy performed prior to neoadjuvant therapy, a resection of a specimen following neoadjuvant chemotherapy, and who underwent total or partial gastrectomy due to adenocarcinoma of the stomach or the esophagogastric junction were included.

Demographic and clinicopathological data were retrieved from the medical records. Three-micrometer-thick sections were prepared from formalin-fixed, paraffin-embedded pretreatment biopsy specimens and immunohistochemically stained using a Ventana BenchMark XT automated staining system. After automated deparaffinization, heat-induced epitope retrieval was performed with cell conditioning 1 (CC1) for 64 minutes according to the manufacturer's protocol. Sections were incubated with a rabbit monoclonal antibody against ARID1A/BAF250a (clone EP303, Epitomics, catalog no. 407R-14-RUO) at a dilution of 1 : 200 for 120 minutes at 37°C. Immunoreactivity was visualized using the ultraView Universal DAB/OptiView DAB detection system, followed by counterstaining with hematoxylin for 16 minutes and bluing reagent for 4 minutes.

Nuclear ARID1A expression was independently evaluated by two pathologists (Sevinc Hallac Keser and Sibel Sensu) by comparing tumor cell staining with that of stromal, endothelial, lymphoid, metaplastic epithelial, and other non-neoplastic epithelial cells on the same slide, which served as internal positive controls [6, 25]. ARID1A expression was classified as retained, reduced, complete loss, or partial loss. Retained expression was defined as unequivocal nuclear staining in tumor cells comparable in intensity to the internal controls, whereas reduced expression was defined as diffuse and unequivocal nuclear staining that was consistently weaker than the controls without a distinct area of complete loss. In tumors showing both retained and reduced staining, the predominant pattern was recorded. Complete loss was defined as absent nuclear staining in all evaluable tumor cells despite preserved staining in internal controls. Partial loss was defined as a geographically distinct ARID1A-negative tumor cell population comprising more than 10% of the evaluable tumor area, with retained or reduced staining in the remaining tumor cells, according to Kim *et al.* [14]. For statistical analyses, retained and reduced cases were grouped as ARID1A-retained, and complete and partial loss cases as ARID1A-loss. Discrepant assessments were resolved by consensus.

The cases were re-staged using the American Joint Committee on Cancer tumor-node-metastasis staging system (8th edition, 2019). Overall survival (OS) was the time from diagnosis to death/the last follow-up (January 31, 2026). Histopathological response to neoadjuvant chemotherapy was assessed using the Ryan tumor regression grade (TRG) system [26] (No viable cancer cells (complete response) = 0; Single cells or rare small groups of cancer cells (near complete response) = 1; Residual cancer with evident tumor regression, but more than single cells or rare small groups of cancer cells (partial response) = 2; and Extensive residual cancer with no evident tumor regression (poor or no response) = 3 [26].

Statistical analysis

Analyses were performed using SPSS V20.0 (IBM Corp., Armonk, NY, USA). Continuous variables were reported as median (IQR) and compared using the Mann-Whitney *U* test. Categorical variables were reported as frequencies (%) compared using χ^2 or Fisher's exact test. Overall survival was estimated using the Kaplan-Meier method and compared *via* the log-rank test. Due to the limited outcome events, only univariate Cox regression analysis was performed to estimate hazard ratios (HRs) with 95% confidence intervals (CIs). $P < 0.05$ was defined as statistically significant.

Bioinformatics analysis

The institutional immunohistochemical cohort and the Cancer Genome Atlas-Stomach Adenocarcinoma (TCGA-STAD) cohort were independent and non-overlapping datasets. The 80 institutional cases were used for clinicopathological and protein-expression analyses, whereas TCGA-STAD data were used exclusively for transcript-level expression and survival analyses. A protein-protein association network centered on ARID1A was constructed using the STRING database, version 11.0 [27]. Interactions were restricted to a minimum required confidence score of ≥ 0.9 , and the resulting network was visualized to identify ARID1A-associated members of the SWI/SNF chromatin-remodeling complex. Gene-level RNA-sequencing count data and corresponding clinical information were retrieved from The Cancer Genome Atlas Stomach Adenocarcinoma cohort using the TCGAbiolinks R/Bioconductor framework. For the paired tumor-normal analysis, only patients with both a primary tumor sample and a matched solid tissue normal sample were included, resulting in 33 matched pairs. Differential expression analysis was performed using DESeq2 with a paired design incorporating patient identity and tissue condition. Variance-stabilizing transformation values were used for visualization, and differences between matched normal and tumor tissues were additionally assessed using the paired Wilcoxon signed-rank test. For OS analysis, 404 TCGA-STAD tumor cases with available expression and survival data were included. DESeq2 size-factor-normalized *ARID1A* counts were $\log_2$ -transformed after adding a pseudocount of 1. Patients were stratified into high- and low-expression groups according to the median *ARID1A* expression level, resulting in 202 patients per group. Overall survival was evaluated using Kaplan-Meier analysis, the log-rank test, and univariate Cox proportional hazards regression [28–30]. The gastric cancer cell lines were not experimentally cultured or tested in the present study. Publicly available normalized transcripts per million values for *ARID1A* were retrieved from the Human Protein Atlas for 33 GC cell lines [31]. Cell line-specific annotations, including patient age, sex, population ori-

gin, and histopathological classification, were obtained from Cellosaurus [32]. The median expression value of 42.6 normalized transcripts per million (nTPM) was used as a descriptive cutoff to categorize the cell lines into high- and low-expression groups.

RESULTS

Results of the bioinformatics analysis

The results of the *in silico* bioinformatics analysis conducted to better understand the molecular role of *ARID1A* and its relationship to our clinical findings are presented below.

STRING analysis demonstrated that ARID1A was embedded within a dense high-confidence interaction network composed of core SWI/SNF complex proteins, including SMARCA4, SMARCB1, SMARCD1, SMARCE1, PBRM1, SMARCA2, SMARCD3, DPF2, ACTL6A, and SMARCC1 (Figure 1). These findings support the central role of ARID1A in chromatin-remodeling machinery and indicate that its functional effects in GC are likely mediated through coordinated interactions with multiple SWI/SNF subunits.

In the paired TCGA-STAD subset, *ARID1A* expression showed a slight upward trend in gastric adenocarcinoma tissues relative to matched normal gastric tissues; however, the difference was not statistically significant (Figure 2A) (paired Wilcoxon signed-rank test, p -value = 0.214). DESeq2 analysis yielded

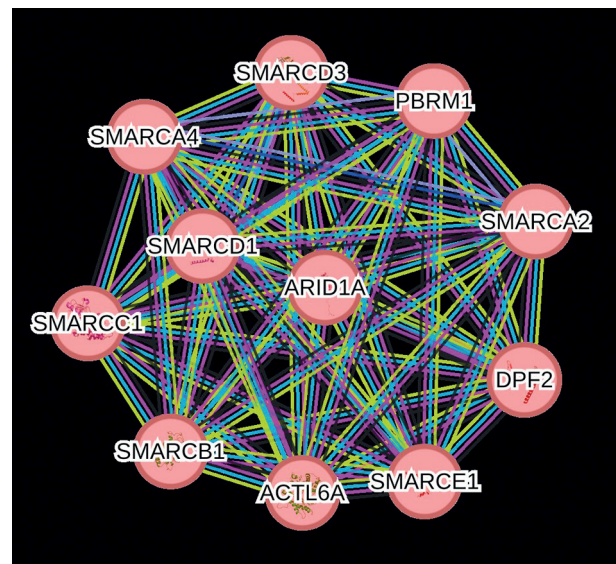


Figure 1. Protein-protein interaction network of ARID1A and its interacting partners within the SWI/SNF chromatin-remodeling complex, visualized using the STRING database with a confidence score of ≥ 0.9

The network displays high-confidence associations supported by evidence from experiments, curated databases, text mining, gene co-expression, and other sources. The central node, ARID1A, interacts with core members of the SWI/SNF complex, including SMARCA4, SMARCB1, SMARCD1, SMARCE1, PBRM1, and others. Edge colors represent the different types of supporting evidence [26].

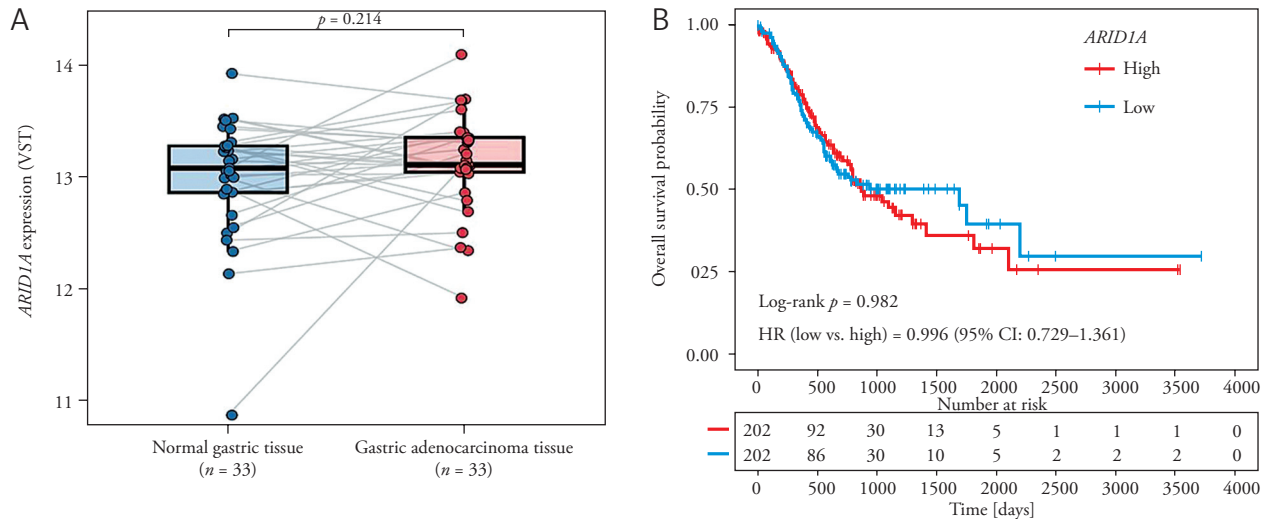


Figure 2. A) *ARID1A* expression in paired TCGA-STAD samples. B) Kaplan-Meier analysis of 404 TCGA-STAD patients stratified into high- and low-expression groups (n = 202 each) revealed no significant overall survival difference (log-rank $p = 0.982$)

HR – hazard ratio, VST – variance-stabilizing transformation

Variance-stabilizing transformation-transformed *ARID1A* expression values are shown for 33 matched normal gastric tissue samples and 33 gastric adenocarcinoma tissue samples. Each dot represents an individual sample, and gray connecting lines indicate paired normal and tumor tissues from the same patient. Boxplots summarize the distribution of VST-transformed expression values in each group. No statistically significant difference in *ARID1A* expression was observed between matched normal and tumor tissues (paired Wilcoxon signed-rank test, $p = 0.214$) (A).

Univariate Cox analysis confirmed no survival association (HR = 0.996, 95% CI: 0.729–1.361) [27–29] (B).

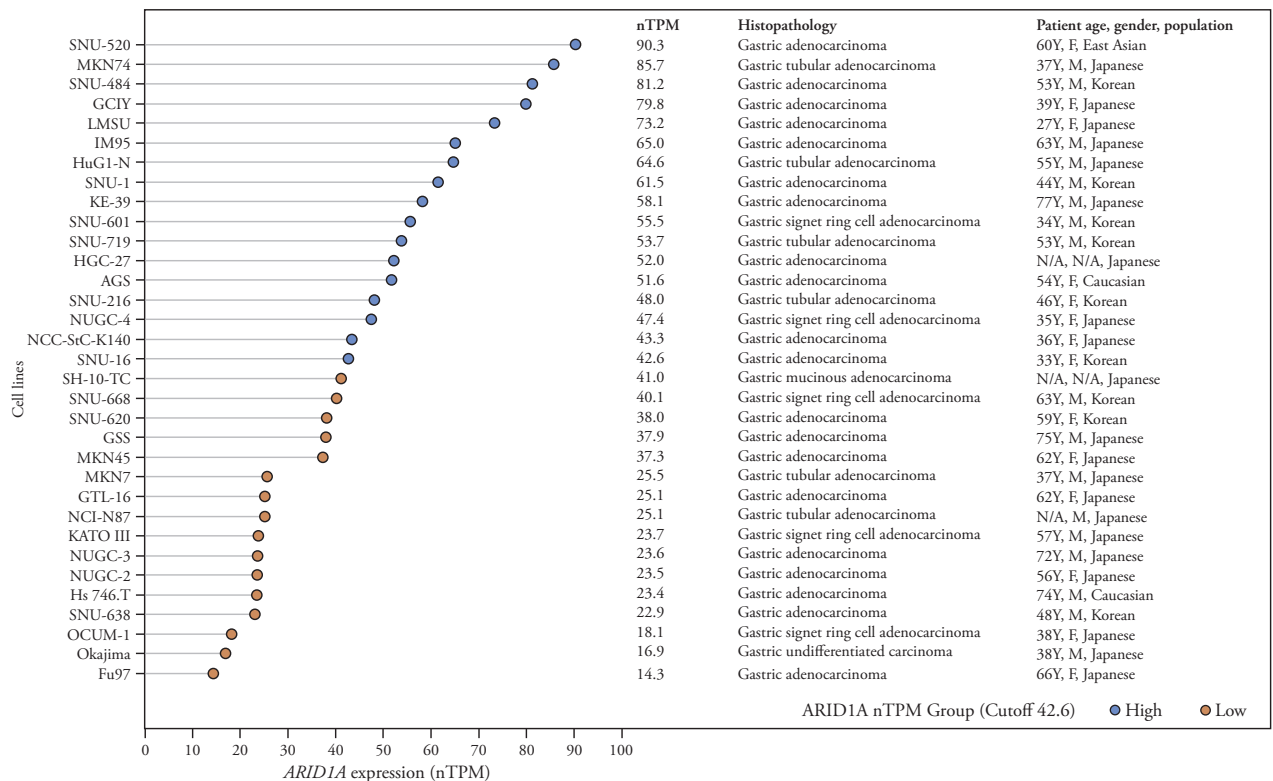


Figure 3. *ARID1A* expression levels in gastric cancer (GC) cell lines

nTPM – normalized transcripts per million

Normalized transcripts per million (nTPM) expression values for *ARID1A* were obtained from the Human Protein Atlas database for 33 GC cell lines, including gastric adenocarcinoma, gastric tubular adenocarcinoma, GC ring cell adenocarcinoma, gastric mucinous adenocarcinoma, and gastric undifferentiated carcinoma [30]. Cell line-specific information, including patient age, sex, population origin, and histopathological classification, were retrieved from the Cellosaurus database [31]. The cell lines were not experimentally cultured or tested in the present study. Instead, the figure represents a secondary analysis of publicly available database-derived expression and annotation data. Cell lines were stratified into high- and low-expression groups using the median *ARID1A* expression value as the cutoff (42.6 nTPM). Among the analyzed cell lines, *ARID1A* expression was highest in SNU-520 and lowest in Fu97. The broad range of expression values highlights the molecular heterogeneity among GC cell lines.

a log₂ fold change of 0.129 and an adjusted *p*-value of 0.344. Mean variance-stabilizing transformation (VST)-transformed expression values were 12.997 in normal tissues and 13.135 in tumor tissues, indicating only a modest numerical increase in tumors.

In the survival cohort, 404 tumor cases were analyzed, including 202 patients in the high-expression group and 202 patients in the low-expression group, with 158 observed events. Kaplan-Meier analysis demonstrated no significant difference in OS between the two groups (log-rank *p* = 0.982). Consistently, univariate Cox regression showed no significant association between *ARID1A* expression group and OS (HR for low vs. high = 0.996, 95% CI: 0.729–1.361) (Figure 2B).

ARID1A expression varied markedly across the 33 GC cell lines, with nTPM values ranging from 14.3 in Fu97 to 90.3 in SNU-520, indicating substantial inter-cell-line heterogeneity (Figure 3). Using the median expression level as the cutoff (42.6 nTPM), the cell lines were stratified into high- and low-expression groups. Relatively higher *ARID1A* expression was observed in several gastric adenocarcinoma and tubular adenocarcinoma models, whereas lower expression levels were seen in a subset of signet ring cell and other

GC cell lines, supporting the molecular diversity of GC models.

Taken together, the figures suggest that *ARID1A* is biologically relevant in GC but not strongly informative at the bulk mRNA level alone. The STRING network supports a central mechanistic role for *ARID1A* within the SWI/SNF chromatin-remodeling complex, whereas the TCGA-STAD analyses show that its transcript levels are neither significantly different between paired tumor and normal tissues nor associated with overall survival. At the same time, the marked variability observed across GC cell lines indicates substantial context-dependent heterogeneity, implying that the functional and clinical significance of *ARID1A* may depend more on tumor subtype, interacting molecular partners, or non-transcriptional alterations than on mRNA abundance alone.

Results of the clinicopathological analysis

Totally, 80 cases of gastric adenocarcinoma with gastrectomy specimens were included. Retained *ARID1A* expression was observed in 47 cases (58.7%) (Figure 4A). *ARID1A* loss was detected in 33 cases (41.3%)

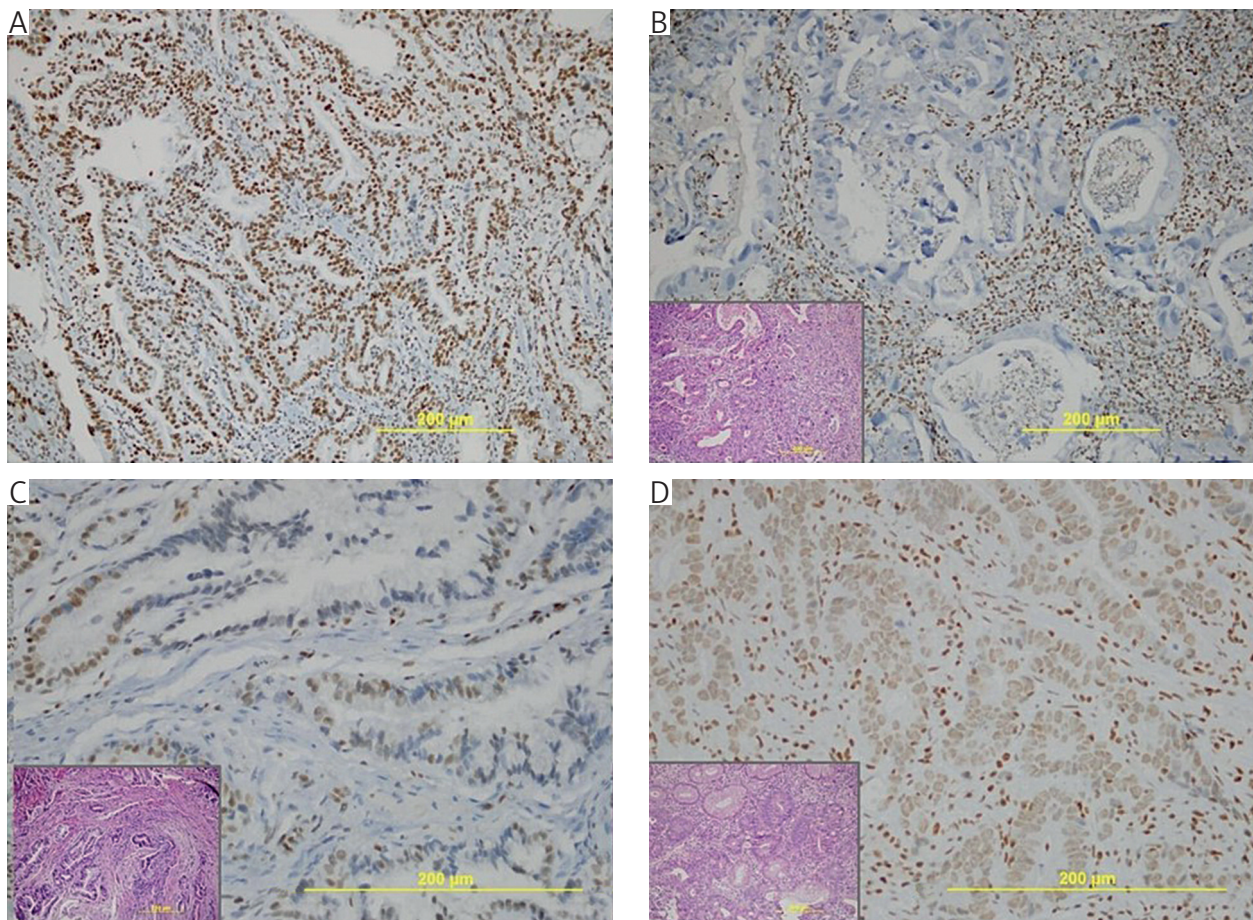


Figure 4. A) Intense *ARID1A* staining in all tumor cells (*ARID1A* 200 $\times$). B) Loss of *ARID1A* expression in all tumor cells (HE 200 $\times$, small figure, *ARID1A* 200 $\times$ large figure). C) Loss of *ARID1A* expression in some tumor cells (HE 200 $\times$, small figure, *ARID1A* 400 $\times$ large figure). D) Weak staining in tumor cells (HE 200 $\times$, small figure, *ARID1A* 400 $\times$, large figure)

which showed either complete ($n = 8$, 24.24%) (Figure 4B) or partial ($n = 25$, 75.75%) nuclear loss. In tumors with partial ARID1A loss, ARID1A-positive areas frequently exhibited heterogeneous staining intensity (Figure 4C). Cases classified as showing reduced expression demonstrated diffuse but unequivocal weak nuclear staining without geographically distinct areas of complete loss (Figure 4D). Stromal cells and lymphocytes, used as internal positive controls, showed strong nuclear staining. The clinical-pathological characteristics of the cohort according to ARID1A expression status are summarized in Table I.

The analysis revealed a statistically significant association between tumor size and ARID1A loss. The median tumor size was 6.00 cm (IQR: 4.00–9.50) in the ARID1A loss group and 4.00 cm (IQR: 2.50–5.25) in the ARID1A-positive group ($p = 0.004$). However, no statistically significant differences were observed between the groups in terms of gender, lymphovascular invasion, perineural invasion, or lymph node positivity (Table I).

The relationship between ARID1A expression and response to neoadjuvant therapy

Treatment protocols were available for 37 of the patients included in our study. Of these patients, three re-

ceived FOLFOX as neoadjuvant therapy, two received capecitabine plus oxaliplatin, one received carboplatin plus paclitaxel, and the remaining 31 patients received the FLOT regimen (5-fluorouracil, leucovorin, oxaliplatin, docetaxel).

The distribution of treatment response according to ARID1A expression status, as assessed by TRG, is presented in Table I. A good response to neoadjuvant therapy (TRG 0–1) was observed in 19.15% of patients in the ARID1A-positive group and in 12.12% of patients in the ARID1A-loss group. A poor response (TRG 2–3) was detected in 80.85% and 87.88% of cases, respectively. Although a difference in percentage rates was observed, this difference was not found to be statistically significant ($p = 0.595$).

Pathological tumor stage and ARID1A expression

Table I shows the distribution of post-neoadjuvant pathological tumor stages (ypT0–ypT4) according to ARID1A expression status. No statistically significant association was found between ARID1A loss and ypT stage ($p = 0.068$).

To evaluate the tumor stage distribution in greater detail, cases were grouped into early-stage tumors (ypT0–2) ($n = 15$) and advanced-stage tumors (ypT3–4) ($n = 65$). Although advanced-stage disease was more

Table I. Clinical and pathological characteristics, histopathological response to neoadjuvant therapy (tumor regression grade), post-neoadjuvant pathological tumor stages (ypT0–ypT4) and post-neoadjuvant pathological lymph node stages (ypN0–ypN3) in patients with gastric cancer according to ARID1A expression status

PARAMETERS	ARID1A POSITIVE ($N = 47$)	ARID1A LOSS ($N = 33$)	P-VALUE
Tumor size [cm], median	4.00 [2.50–5.25]	6.00 [4.00–9.50]	0.004
Female, n (%)	16 (34.04)	15 (45.45)	0.425
LVI (positive), n (%)	34 (72.34)	24 (72.73)	1.000
PNI (positive), n (%)	34 (72.34)	26 (78.79)	0.694
Lymph node positive (N+), n (%)	32 (68.09)	25 (75.76)	0.620
TRG, n (%)			
Good response (TRG 0–1)	9 (19.15)	4 (12.12)	0.595
Poor response (TRG 2–3)	38 (80.85)	29 (87.88)	
ypT stage			
ypT0	3	2	0.068
ypT1	3	2	
ypT2	4	1	
ypT3	23	15	
ypT4	14	13	
ypN stage			
ypN0	15	8	0.889
ypN1	9	7	
ypN2	8	7	
ypN3	15	11	

LVI – lymphovascular invasion, PNI – perineural invasion, TRG – tumor regression grade, ypT – pathological tumor stage after neoadjuvant therapy, ypN – pathological lymph node stage following neoadjuvant therapy

common in the ARID1A loss group, the difference was not statistically significant ($p = 0.736$).

Lymph node stage based on ARID1A expression

The detailed distribution of post-neoadjuvant lymph node stages (ypN0–ypN3) according to ARID1A expression status is presented in Table I. To facilitate interpretation, lymph node status was further categorized as lymph node-negative (ypN0, $n = 23$) and lymph node-positive (ypN+ (ypN1–3), $n = 57$). Accordingly, among ARID1A-positive cases, 15 were ypN0 and 32 were ypN+, whereas among cases with ARID1A loss, 8 were ypN0 and 25 were ypN+. No statistically significant association was observed between ARID1A loss and lymph node metastasis.

Survival analysis and cox regression

The Kaplan-Meier survival analysis showed that the median OS was 17 months in the ARID1A-loss group and 18 months in the ARID1A-positive group. The log-rank test revealed no statistically significant difference in OS between the two groups ($\chi^2 = 0.067$; $p = 0.795$). Univariate Cox regression analysis demonstrated that tumor size was significantly associated with mortality risk (HR = 1.09; 95% CI: 1.02–1.16; $p = 0.015$). This finding indicates that each 1 cm increase in tumor size is associated with an approximate 9% increase in the risk of death. In contrast, ARID1A loss (HR = 0.92; $p = 0.803$) and other clinicopathological parameters were not significantly associated with OS (Table II). The ypT stage showed a borderline association with survival ($p = 0.068$).

Discussion

This study evaluated the association between ARID1A expression, clinicopathological parameters, neoadjuvant treatment response, and survival in GC, integrating institutional IHC with bioinformatics analyses.

Previous studies show that ARID1A mutation rates in GC range is 8–51.3% [5, 33, 34], with TCGA datasets reporting a frequency of 31.1% [35]. The 41.3% ARID1A loss rate identified in our study is consistent with this literature range. In studies, ARID1A loss of function includes complete/partial loss or reduced expression, all might be linked to carcinogenesis [1, 33, 36]. The complete or partial loss might result from *ARID1A* mutations occurring in the early or late stages of gastric carcinogenesis, respectively; in GC where complete loss is detected, *ARID1A* mutations are found to be driver mutations, whereas in tumors showing partial loss, various other genetic alterations resulting in a heterogeneous tumor with complex genomic pathway changes are thought to be present [6]. Additionally, varying ARID1A staining intensities within the same tumor have been observed in the vast majority of GCs [6, 35]. In our study, since ARID1A expression was evaluated on endoscopic biopsies, due to the heterogeneity, the prognostic value of ARID1A loss may not be definitively established. The wide range of ARID1A expression levels observed in GC cell lines in bioinformatics analyses further supports the molecular heterogeneity of GCs. This variability may explain the heterogeneous expression patterns observed in immunohistochemical staining and may limit the prognostic accuracy of evaluations based on a single biopsy sample.

We aimed to clarify how ARID1A expression is related to neoadjuvant therapy response, with the aim of more successfully implementing personalized treatment in patients with GC. ARID1A loss affects various parameters that may influence treatment in different organ cancers, such as DNA damage repair, cell cycle regulation, and epithelial-mesenchymal transition [15]. Although ARID1A expression did not significantly correlate with TRG, the response rate was numerically higher in the ARID1A-positive group, suggesting our limited sample size may have obscured statistical significance. Similarly, Barisik *et al.* [16] found no significant association between ARID1A loss and response

Table II. The effect of ARID1A status and clinical-pathological variables on overall survival was evaluated using univariate Cox regression analysis

PARAMETERS	RISK RATIO (HR)	95% CI	P-VALUE
<i>ARID1A</i> IHC status (loss vs. retained expression)	0.92	0.49–1.72	0.803
Tumor size (per 1 cm increase)	1.09	1.02–1.16	0.015
Sex (female vs. male)	1.63	0.86–3.11	0.136
LVI (positive)	1.33	0.57–3.06	0.508
PNI (positive)	2.20	0.76–6.36	0.146
Lymph node positive (N+)	1.27	0.58–2.80	0.553
ypT stage	1.36	0.98–1.88	0.068
ypN stage	1.08	0.83–1.41	0.568
Tumor regression grade	1.35	0.90–2.04	0.150

IHC – immunohistochemistry, LVI – lymphovascular invasion, PNI – perineural invasion, ypT – pathological tumor stage after neoadjuvant therapy, ypN – pathological lymph node stage after neoadjuvant therapy

to neoadjuvant therapy in rectal carcinoma, indicating that the predictive role of ARID1A may depend on the tumor's molecular context.

Various studies have investigated whether ARID1A loss is associated with prognosis in patients with GC [5]. Although some studies report better outcomes [5, 15], some others are associated with poor prognosis [5, 37–41]. In our study there is a statistically significant association between ARID1A loss and tumor size. This finding suggests that loss of ARID1A function may be associated with tumor growth and progression. Protein-protein interaction network results obtained from bioinformatics analyses also provide biological support for this finding. STRING analysis has shown that ARID1A interacts extensively with key components of the SWI/SNF chromatin remodeling complex. This complex is known to play a significant role in cell cycle regulation, the DNA damage response, and proliferation control. Therefore, it is hypothesized that loss of ARID1A function may facilitate tumor growth by causing disruptions in chromatin regulation and may be associated with the increased tumor size observed in our study.

When the effect of ARID1A expression on survival was investigated, some studies found that loss of ARID1A was associated with shorter survival [5]. One study reported that loss of ARID1A expression was associated with poor OS in GC, independent of the tumor clinical stage, tumor differentiation, the rate of ARID1A deficiency, and sample size [5]. In contrast, there are also studies reporting no significant difference in survival [3]. In survival analyses, consistent with the results of our bioinformatics analyses, no statistically significant association was found between ARID1A expression status and overall survival. In the Kaplan-Meier survival analysis based on the TCGA-STAD dataset, no significant difference in OS was observed between the high- and low-expression groups. The parallel absence of a significant survival association at the protein and transcript levels suggests that ARID1A expression alone may have limited prognostic value. However, these analyses represent distinct biological levels and independent cohorts and should not be considered direct validation of one another.

Our bioinformatics analysis showed no tumor-normal differences in ARID1A mRNA levels, contrasting with the IHC protein loss and highlighting post-transcriptional regulation. Despite this mismatch, network analyses confirm ARID1A is central to the proliferation-regulating SWI/SNF complex. Combined findings suggest that ARID1A is linked to tumor growth but poorly predicts treatment response or survival.

One of the key strengths of this study is that the immunohistochemical findings were supported by bioinformatics analyses. The evaluation of clinical cohort data in conjunction with transcriptomic and molecular analyses derived from large-scale datasets has en-

abled a multifaceted examination of the biological role of ARID1A in GC. The consistency between clinical and bioinformatics findings enhances the reliability of the results.

One of the limitations of our study is that we assessed ARID1A expression in endoscopic biopsies and were unable to interpret tumor heterogeneity. The small size of our study group and the low number of events observed in the survival analysis prevented us from performing multivariate analyses. This is a preliminary study and should be expanded to include surgical resections and larger populations. The discrepancies between our results and findings in the literature may be explained by potential differences in immunohistochemical procedures, including intra-tumoral heterogeneity, limited sample sizes in existing studies, the sensitivity of detection methods, and the use of non-standardized interpretive criteria (including antibodies), staining platforms, cutoff values, and scoring systems.

Conclusions

In this study, ARID1A loss was prevalent in gastric adenocarcinomas and significantly associated with larger tumor size, though it did not correlate with neoadjuvant response or overall survival. Bioinformatics transcriptomic and survival data mirrored these clinical findings, suggesting ARID1A alone lacks strong prognostic or predictive biomarker utility. However, its central role in the SWI/SNF complex and link to tumor size support its involvement in GC progression. In conclusion, ARID1A is an insufficient standalone biomarker for predicting survival or treatment response but remains associated with tumor growth. Larger, multicenter studies evaluating molecular subtypes are needed to clarify its clinical significance.

Disclosures

1. Institutional review board statement: This study was approved by the Ethics Committee of the Kartal Dr. Lutfi Kırdar City Hospital (approval decision no. 2025/010.99/14/19, dated: 26.03.2025).
2. Assistance with the article: None.
3. Financial support and sponsorship: None.
4. Conflicts of interest: None.

References

1. Angelico G, Attanasio G, Colarossi L, Colarossi C, Montalbano M, Aiello E, et al. *ARID1A* mutations in gastric cancer: a review with focus on clinicopathological features, molecular background and diagnostic interpretation. *Cancers (Basel)* 2024; 16: 2062.
2. Bray F, Laversanne M, Sung H, Ferlay J, Siegel RL, Soerjomataram I, et al. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 2024; 74: 229–263.

3. Correa P. Human gastric carcinogenesis: a multistep and multifactorial process. *Cancer Res* 1992; 52: 6735-6740.
4. Salvatori S, Marafini I, Laudisi F, Monteleone G, Stolfi C. Helicobacter pylori and gastric cancer: pathogenetic mechanisms. *Int J Mol Sci* 2023; 24: 2895.
5. Yang L, Wei S, Zhao R, Wu Y, Qiu H, Xiong H. Loss of ARID1A expression predicts poor survival prognosis in gastric cancer: a systematic meta-analysis from 14 studies. *Sci Rep* 2016; 6: 28919.
6. Tober JM, Halske C, Behrens HM, Krüger S, Röcken C. Intratumoral heterogeneity and loss of ARID1A expression in gastric cancer correlates with increased PD-L1 expression in Western patients. *Hum Pathol* 2019; 94: 98-109.
7. Kim M, Kim WH. Molecular pathology of gastric cancer. *J Gastric Cancer* 2022; 22: e35.
8. Cheng J, Wang H, Jiang W, Li C, Zeng X, Li L, et al. HER2 becomes a novel survival biomarker for gastric cancer: updated systematic review and meta-analysis. *Ther Adv Med Oncol* 2024; 16: 17588359241271913.
9. Huang SC, Ng KF, Chang IY, Chang CJ, Chao YC, Chang SC, et al. The clinicopathological significance of SWI/SNF alterations in gastric cancer is associated with the molecular subtypes. *PLoS One* 2021; 16: e0245356.
10. Zhang X, Zhang Y, Zhao J, Wu Y, Zhang N, Shen W. ARID1A mutations in cancer development: mechanism and therapy. *Carcinogenesis* 2023; 44: 197-208.
11. Xu S, Tang C. The role of ARID1A in tumors: tumor initiation or tumor suppression? *Front Oncol* 2021; 11: 745187.
12. Lu S, Duan R, Cong L, Song Y. The effects of ARID1A mutation in gastric cancer and its significance for treatment. *Cancer Cell Int* 2023; 23: 296.
13. Fontana B, Gallerani G, Salamon I, Pace I, Roncarati R, Ferracin M. ARID1A in cancer: friend or foe? *Front Oncol* 2023; 13: 1136248.
14. Kim YB, Ham IH, Hur H, Lee D. Various ARID1A expression patterns and their clinical significance in gastric cancers. *Hum Pathol* 2016; 49: 61-70.
15. Sun M, Gu Y, Fang H, Shao F, Lin C, Zhang H, et al. Clinical outcome and molecular landscape of patients with ARID1A-loss gastric cancer. *Cancer Sci* 2024; 115: 905-915.
16. Barisik NO, Sensu S, Keser SH, Gurbuz YS, Ozdemir O, Yuceer RO, et al. Does loss of ARID1A expression affect neoadjuvant chemoradiotherapy response in rectal carcinomas? *Pol J Pathol* 2025; 76: 87-93.
17. Kadoch C, Crabtree GR. Mammalian SWI/SNF chromatin remodeling complexes and cancer: mechanistic insights gained from human genomics. *Sci Adv* 2015; 1: e1500447.
18. Mathur R, Roberts CWM. ARID1A loss in cancer: towards a mechanistic understanding. *Nat Rev Cancer* 2018; 18: 487-505.
19. Li H, Liu Y, Wang H, Wang Y, Guo Z, Xu J, et al. Somatic ARID1A mutation stratifies patients with gastric cancer to PD-1 blockade and adjuvant chemotherapy. *Cancer Immunol Immunother* 2023; 72: 1633-1647.
20. Liu Y, Chen S, Xu Z, Zhang D, Li W, Chen W, et al. Identification of the consistently differential expressed hub mRNAs and proteins in lung adenocarcinoma and construction of the prognostic signature: a multidimensional analysis. *Int J Surg* 2024; 110: 885-900.
21. Upadhyay SR, Ryan CJ. Experimental reproducibility limits the correlation between mRNA and protein abundances in tumor proteomic profiles. *Cell Rep Methods* 2022; 2: 100300.
22. Hwang I, Cho Y, Ahn S, Choi J, Park YS, Ahn SH, et al. Comparative analysis of ARID1A mutations with mRNA levels and protein expression in gastric carcinoma. *Pathol Res Pract* 2024; 255: 155097.
23. Ma Y, Li Q, Li A, Wei Y, Long P, Jiang X, et al. Current development of molecular classifications of gastric cancer and clinical implications. *Int J Oncol* 2024; 65: 67.
24. Lordick F, Carneiro F, Cascinu S, Fleitas T, Haustermans K, Piesen G, et al. Gastric cancer: ESMO Clinical Practice Guideline for diagnosis, treatment and follow-up. *Ann Oncol* 2022; 33: 1005-1020.
25. Guan B, Mao TL, Panuganti PK, Kuhn E, Kurman RJ, Maeda D, et al. Mutation and loss of expression of ARID1A in uterine low-grade endometrioid carcinoma. *Am J Surg Pathol* 2011; 35: 625-632.
26. Ryan R, Gibbons D, Hyland JM, Treanor D, White A, Mulcahy HE, et al. Pathological response following long-course neoadjuvant chemoradiotherapy for locally advanced rectal cancer. *Histopathology* 2005; 47: 141-146.
27. Szklarczyk D, Gable AL, Lyon D, Junge A, Wyder S, Huerta-Cepas J, et al. STRING v11: protein-protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. *Nucleic Acids Res* 2019; 47: D607-D613.
28. Cancer Genome Atlas Research Network. Comprehensive molecular characterization of gastric adenocarcinoma. *Nature* 2014; 513: 202-209.
29. Colaprico A, Silva TC, Olsen C, Garofano L, Cava C, Garolini D, et al. TCGAAbiolinks: an R/Bioconductor package for integrative analysis of TCGA data. *Nucleic Acids Res* 2016; 44: e71.
30. Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol* 2014; 15: 550.
31. Uhlén M, Fagerberg L, Hallström BM, Lindskog C, Oksvold P, Mardinoglu A, et al. Proteomics. Tissue-based map of the human proteome. *Science* 2015; 347: 1260419.
32. Bairoch A. Thecellousaurus, a cell-line knowledge resource. *J Biomol Tech* 2018; 29: 25-38.
33. Saito M, Kohno T, Kono K. Heterogeneity of ARID1A expression in gastric cancer may affect patient survival and therapeutic efficacy. *Hum Pathol* 2020; 101: 80-81.
34. Wang DD, Chen YB, Pan K, Wang W, Chen SP, Chen JG, et al. Decreased expression of the ARID1A gene is associated with poor prognosis in primary gastric cancer. *PLoS One* 2012; 7: e40364.
35. Sakuratani T, Takeuchi T, Yasufuku I, Iwata Y, Saigo C, Kito Y, et al. Downregulation of ARID1A in gastric cancer cells: a putative protective molecular mechanism against the Harakiri-mediated apoptosis pathway. *Virchows Arch* 2021; 478: 401-411.
36. Inada R, Sekine S, Taniguchi H, Tsuda H, Katai H, Fujiwara T, et al. ARID1A expression in gastric adenocarcinoma: clinicopathological significance and correlation with DNA mismatch repair status. *World J Gastroenterol* 2015; 21: 2159-2168.
37. Zhang Y, Qian HS, Hu G, Wang L, Zhu Y. ARID1A is involved in DNA double-strand break repair in gastric cancer. *J Gastrointest Oncol* 2024; 15: 862-872.
38. Wang X, Che K, Shi T, Liu Q, Xu X, Wu H, et al. Loss of ARID1A expression is associated with systemic inflammation markers and has important prognostic significance in gastric cancer. *J Cancer Res Clin Oncol* 2022; 148: 1583-1595.
39. Zhang Z, Li Q, Sun S, Ye J, Li Z, Cui Z, et al. Prognostic and immune infiltration significance of ARID1A in TCGA molecular subtypes of gastric adenocarcinoma. *Cancer Med* 2023; 12: 16716-16733.
40. Zhu YP, Sheng LL, Wu J, Yang M, Cheng XF, Wu NN, et al. Loss of ARID1A expression is associated with poor prognosis in patients with gastric cancer. *Hum Pathol* 2018; 78: 28-35.
41. Ashizawa M, Saito M, Min AKT, Ujiie D, Saito K, Sato T, et al. Prognostic role of ARID1A negative expression in gastric cancer. *Sci Rep* 2019; 9: 6769.

Address for correspondence

Sevinc Hallac Keser, MD
Department of Pathology
Kartal Dr. Lutfi Kirdar City Hospital
Istanbul, Turkey
e-mail: sskeser2@hotmail.com