Relationship between the protein expression of ARID1A, ARID1B and ARID2 with the clinicopathological characteristics of colorectal cancer

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Abstract. The SWitch/sucrose non-fermentable chromatin remodeling complex functions as a tumor suppressor by regulating gene expression and thus various cellular processes. Key subunits of this complex, including the AT-rich interactive domain (ARID)-containing proteins ARID1A, ARID1B and ARID2, are frequently mutated in cancer, including colorectal cancer (CRC). However, their protein expression and prognostic significance in CRC remain unclear. The present study investigated the ARID1A, ARID1B and ARID2 expression levels in CRC tissues and their relationship with the clinicopathological characteristics of patients. Bioinformatics analysis of The Cancer Genome Atlas-colon adenocarcinoma cohort revealed frequent mutations, positive mRNA correlations among the *ARID* genes and increased promoter methylation levels. Immunohistochemical analysis of 63 CRC tissue samples demonstrated significantly decreased ARID1A, ARID1B and ARID2 protein expression in cancerous areas compared with adjacent non-cancerous areas of the tissues. Low ARID1A and ARID1B expression levels were significantly associated with advanced clinicopathological characteristics in Thai patients with CRC. All three ARIDs showed a trend towards an association with the 5-year progression-free survival of Thai patients with CRC. Kaplan-Meier plotter database analysis further demonstrated that low expression of all three *ARID* genes was associated with a shorter overall survival time in patients with CRC; however, only *ARID1A* expression showed a statistically significant prognostic relevance. In conclusion, the ARID1A, ARID1B and ARID2 expression levels were shown to be positively correlated, and their reduced expression was associated with worse clinicopathological characteristics in patients with CRC. These findings suggest the potential of ARIDs as prognostic biomarkers, warranting further investigation to validate their clinical significance.

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Introduction

Colorectal cancer (CRC) is the third most diagnosed cancer worldwide, accounting for >10% of all cancer cases, and is the second leading cause of cancer-related mortality globally. Predictions indicate that the number of new CRC cases will rise by 20% by 2030 (1,2). In Thailand, CRC is a significant health issue and is the third most common cancer in men and the fourth in women. Unlike other cancer types, a steady increase in CRC cases has been observed for both sexes in Thailand (3,4). Studies have consistently highlighted the critical role of early diagnosis and intervention in improving the treatment outcomes of this disease (5,6). Therefore, the identification and development of predictive biomarkers are essential for optimizing treatment strategies for CRC.

The SWitch/sucrose non-fermentable (SWI/SNF) chromatin remodeling complex serves a crucial role as a tumor suppressor that regulates gene expression, transcription and DNA repair (7,8). Within this complex, the AT-rich interactive domain (ARID) proteins are key regulators of transcription, cell cycle control, growth and differentiation (9,10). Among the ARID family of proteins, ARID1A, ARID1B and ARID2 have demonstrated a strong association with cancer progression (11). *ARID1A* is a well-known

tumor suppressor gene that is frequently mutated in several cancer types and its loss is correlated with advanced cancer stages and metastasis (9,12,13). ARID1B, which shares 66% sequence similarity with ARID1A, has also been linked to cancer (9). Although *ARID1B* expression in human cancer remains unclear, its mutations have been identified and are considered to promote tumorigenesis in various cancer types, including breast, ovarian, pancreatic and bladder cancer (14-17). ARID2, another important SWI/SNF subunit, is essential for chromatin remodeling and tumor suppression (18). Loss of *ARID2* has been detected in several cancer types, including liver, melanoma, lung and CRC (18-20). Emerging evidence has highlighted the tumor-suppressive roles of ARID1A, ARID1B and ARID2 in various cancer types, including CRC (21-24). *ARID1A* upregulation has been revealed to suppress invasion and migration by modulating the expression of epithelial-mesenchymal transition (EMT)-related markers (24) and *ARID1A* inhibition has been shown to enhance metastatic potential (22,23). Similarly, *ARID1B* knockdown was shown to disrupt DNA repair and chromatin accessibility (25), while *ARID2* deficiency was demonstrated to promote cancer cell proliferation and metastasis (18). Despite these insights, the relationships among the ARID protein expression levels in CRC remain largely unexplored. Furthermore, to the best of our knowledge, no study has specifically investigated the expression patterns and prognostic implications of ARID1A, ARID1B and ARID2 in Thai patients with CRC. Given that genetic and environmental factors influence CRC development differently across populations (26), region-specific studies are essential for identifying novel prognostic markers and potential therapeutic targets.

The present study aimed to investigate the gene mutations in *ARID1A*, *ARID1B* and *ARID2* and explore the correlations in their expression in CRC through bioinformatics analysis. Additionally, the protein expression levels of these three ARIDs in CRC tissues and their association with the clinicopathological characteristics of Thai patients with CRC were assessed to gain insights into their role and prognostic value in CRC.

Materials and methods

Bioinformatics analysis of the ARID1A, ARID1B and ARID2 gene mutations and expression correlations in CRC. Genomic alterations of the *ARID1A*, *ARID1B* and *ARID2* genes were explored using The Cancer Genome Atlas-colon adenocarcinoma (TCGA-COAD) dataset through the Cancer Virtual Cohort Discovery Analysis Platform (CVCDAP) (https://omics.bjcancer.org/cvcdap/) (27). The expression correlations among the *ARID1A*, *ARID1B* and *ARID2* genes in TCGA-COAD dataset were assessed using the Pearson correlation coefficient in the Gene Expression Profiling Interactive Analysis 2 platform (28). The promoter methylation levels of the *ARID1A*, *ARID1B* and *ARID2* genes in TCGA-COAD dataset were examined through The University of ALabama at Birmingham CANcer data analysis portal (https://ualcan.path.uab.edu/) (29,30). The Kaplan-Meier (KM) plotter database (https://kmplot.com/analysis/) (31) was used to evaluate the association of *ARID1A* (Affymetrix probe ID: 218917_s_at; n=1,061), *ARID1B* (Affymetrix probe ID: 238043_at; n=814) and *ARID2* (Affymetrix probe ID: 225486_at; n=814) with overall survival (OS) in patients with CRC. Low- and high-expression groups were determined using the 'Auto select best cut-off' option, which is based on the median expression values. The hazard ratio and log-rank P-value were automatically computed by the database for survival analysis. The KM plots for overall survival were generated directly by the KM plotter tool without any additional statistical analysis or modification. A late-stage crossover was observed in the survival curves, as provided by the tool.

Patient samples. The present study was approved by the Human Research Ethics Committee of Sawanpracharak Hospital (Nakhon Sawan, Thailand; certificate of approval no. 53/2567) and the Naresuan University Human Research Ethics Committee (Phitsanulok, Thailand; approval no. P1-0107/2567; certificate of approval no. 139/2024), and conducted in accordance with the principles of the Declaration of Helsinki. Tissue biopsies from 63 patients diagnosed with CRC of varying pathological differentiation were submitted to the Pathology Unit at Sawanpracharak Hospital between 2017 and 2021. This patient cohort included 27 males and 36 females, with a median age of 66 years (range, 52-97 years). Formalin-fixed, paraffin-embedded (FFPE) blocks containing the CRC tissues, including both cancerous and adjacent non-cancerous areas, were obtained for each patient. The clinicopathological data, including age, sex, tumor location, tumor size, pathological differentiation, American Joint Committee on Cancer (AJCC) staging 8th edition (32), tumor invasion, metastasis, lymphovascular invasion, comorbidities and follow-up period post-operation, were comprehensively assessed by a clinical pathologist. The exclusion criteria included patients with incomplete data, patients diagnosed with hereditary CRC syndromes, those with cancer of unknown primary origin and cases where a pathologist or researcher was unable to clarify the findings of the histological and/or immunohistochemical investigation. To ensure anonymity, each FFPE block was labeled with a unique research code and sensitive patient information was carefully protected.

Immunohistochemistry (IHC). To assess the expression of the ARID proteins, IHC was performed using the following specific antibodies: Anti-ARID1A rabbit polyclonal antibody (1:400; cat. no. HPA005456; MilliporeSigma), anti-ARID1B mouse monoclonal antibody (1:200; cat. no. ab57461; Abcam) and anti-ARID2 rabbit polyclonal antibody (1:250; cat. no. ab113283; Abcam). CRC tissue samples were initially fixed in 10% neutral buffered formalin (NBF), and then processed into FFPE blocks. These blocks were sectioned into 3-μm-thick slices, followed by deparaffinization in xylene and rehydration using an ethanol gradient. Antigen retrieval was achieved at 97˚C for 35 min using the heat-induced epitope retrieval method in citrate buffer (pH 6.0). Endogenous peroxidase activity was blocked with 3% hydrogen peroxide/sodium azide (NaN_3) for 25 min at room temperature (RT). After washing the slides three times with PBS (5 min each), non-specific protein binding was blocked with 0.1% NaN_3 for 20 min at RT. The slides were then incubated with the specified primary antibodies at 4˚C overnight in a humidified chamber. As a negative control, the primary antibody

was replaced with PBS. Subsequent steps involved incubation with biotinylated goat anti-rabbit IgG (H+L) (from the Rabbit specific HRP/DAB Detection IHC Kit; cat. no. ab64261; Abcam) or goat anti-mouse IgG (H+L) secondary antibody [from the Mouse-specific HRP/DAB (ABC) Detection IHC Kit; cat. no. ab64259; Abcam] for 15 min at RT, followed by three washes with PBS (5 min each). The slides were then incubated with streptavidin peroxidase for 15 min at RT. After three additional washes with PBS, immunostaining was performed using the chromogen 3,3'-diaminobenzidine (DAB) substrate (1:50; cat. no. ab64238; Abcam) for 4 min at RT to detect specific antigen-antibody interactions, with the DAB reaction halted using distilled water. The slides were counterstained with Mayer's hematoxylin (C.V. Laboratories Co., Ltd.) by 3 dips at RT, washed in running tap water for 5 min, dehydrated with increasing concentrations of ethanol, cleared with xylene, mounted with mounting media (Permount; Thermo Fisher Scientific, Inc.) and covered with a cover slip.

Quantitative analysis of ARID1A, ARID1B and ARID2 protein expression. In total, five independent areas per slide, covering both cancerous and adjacent non-cancerous regions in the same CRC tissues, were analyzed using the ZEN program (Rushmore Precision Co., Ltd.) and an Axiocam 105 color ZEISS microscope (Carl Zeiss AG) at high power fields with x40 magnification. ImageJ software (version 1.53c; National Institutes of Health) was employed to detect and analyze the ARID-positive cells. All images were evaluated in a double-blind manner by both a pathologist and the investigators. IHC scoring utilized the modified histoscore (H-score), which combines staining intensity with the percentage of positively stained cells to assess the abundance and distribution of proteins in tissue samples (33). Staining intensity was classified as follows: Negative staining (0), weak positivity (1), moderate positivity (2) and strong positivity (3) (34). The H-score was calculated using the following formula: H-score=[(0 x % negative cells) + (1 x % weak positive cells) + (2 x % moderate positive cells) + (3 x % strong positive cells)] (33). The H-score values ranged from 0 to 300. The ARID protein expression levels were categorized into two groups based on the median value: Low (< median value) and high (≥ median value). This classification approach aligns with the previously established optimal cut-off for SWI/SNF component expression (35).

Statistical analysis. Descriptive statistics are expressed as the mean ± SD and the median. Quantitative data are presented as the mean ± SEM. Comparisons between two groups were performed using the Mann-Whitney U test. The correlation among the protein expression levels of ARID1A, ARID1B and ARID2 were determined using Spearman's rank correlation coefficient. Pearson's χ^2 test was used to analyze the association between the ARID1A, ARID1B and ARID2 protein expression levels and the clinicopathological characteristics of patients with CRC when the expected value in <20% of cells was <5. In cases where this condition was violated in a 2x2 table, Fisher's exact probability test was performed (36). The relationship between ARID protein expression and progression-free survival (PFS) was assessed using KM analysis and the log-rank test. Moreover, the PFS univariate and multivariate analyses were performed using Cox proportional hazards regression analysis. All statistical analyses were performed using IBM SPSS statistical software (version 25; IBM Corp.) and GraphPad Prism 9 (Dotmatics). P<0.05 was considered to indicate a statistically significant difference.

Results

Gene mutations, expression correlations and promoter methylation levels of ARID1A, ARID1B and ARID2 in CRC. Gene mutations in *ARID1A*, *ARID1B* and *ARID2* in TCGA-COAD dataset were investigated using the CVCDAP platform. The analysis revealed that ~20.75% of patients with CRC had mutations in at least one of these *ARID* genes. Among these genes, *ARID1A* was the most prevalent, accounting for 13% of all samples. Mutations in *ARID1B* and *ARID2* were each identified in 8% of the samples. Frameshift mutations were the most common in *ARID1A*, while missense mutations were the most frequently observed in both *ARID1B* and *ARID2* (Fig. 1A). Additionally, significant positive correlations were found among the expression of *ARID1A*, *ARID1B* and *ARID2*. A strong correlation was found between *ARID1A* and *ARID1B* expression (r=0.71, P<0.001), while moderate correlations were observed between *ARID1A* and *ARID2* (r=0.48, P<0.001) as well as between *ARID1B* and *ARID2* (r=0.43, P<0.001). Pearson correlation analysis revealed linear relationships between gene expression pairs, as visualized in the scatter plots with clear positive trend lines in each panel (Fig. 1B-D). Furthermore, promoter methylation analysis revealed that all three *ARID* genes exhibited significantly higher methylation levels in the COAD samples compared with the normal tissues (Fig. 1E-G). These findings suggest that genetic mutations and epigenetic regulation may contribute to the altered expression of ARID1A, ARID1B and ARID2 in CRC.

Demographic and clinical characteristics of patients with CRC. A total of 63 patients diagnosed with CRC were included in the present study, with ages ranging from 52 to 97 years, yielding a mean age of 67.17±8.83 years and a median age of 66 years. Among the cohort, 36 patients (57.14%) were women and 27 (42.86%) were men. The tumor location was predominantly in the rectum/sigmoid colon (47.62%), followed by the right-sided colon (38.09%) and the left-sided colon (14.29%). Tumor sizes varied from 2.00 to 12.50 cm, with a mean size of 5.52±2.07 cm and a median size of 5.00 cm. Pathological differentiation examination indicated that the majority of tumors were well-differentiated adenocarcinomas, found in 42 patients (66.67%), followed by moderately differentiated tumors in 15 patients (23.81%) and poorly differentiated tumors in 6 patients (9.52%). According to the AJCC staging, patients were classified as Stage I (12.17%), Stage II (26.98%), Stage III (36.51%) and Stage IV (23.81%). The depth of tumor invasion examination indicated that 80.95% of cases were categorized as late-stage (pT3-pT4). Lymph node involvement (pN) was absent in 58.73% of patients (pN0), while 41.27% had one or more positive lymph node (pN1-pN2). Distant metastasis (pM1) was identified in 23.81% of the cohort. Additionally, nearly half of the patients (49.21%) exhibited lymphovascular invasion. Lymph node metastasis was detected in 26 of the 63 patients

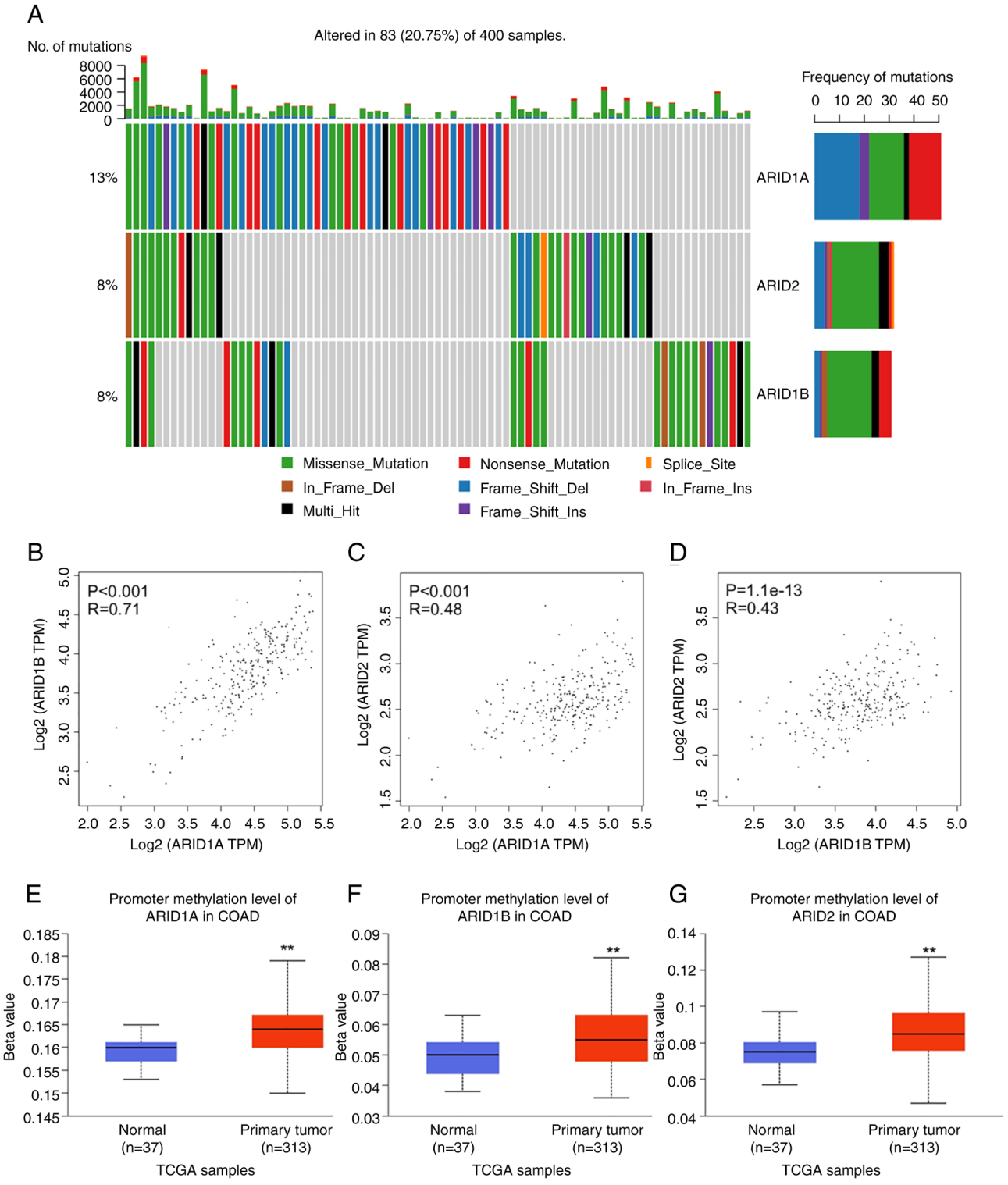


Figure 1. Gene mutations, expression correlations and promoter methylation levels of *ARID1A*, *ARID1B* and *ARID2* in COAD. (A) Genetic alterations of *ARID1A*, *ARID1B* and *ARID2* in patients from TCGA-COAD cohort, analyzed using the Cancer Virtual Cohort Discovery Analysis Platform. Expression correlations between (B) *ARID1A* and *ARID1B*, (C) *ARID1A* and *ARID2* and (D) *ARID1B* and *ARID2* in TCGA-COAD cohort, analyzed via the Gene Expression Profiling Interactive Analysis 2 platform. The promoter methylation level of (E) *ARID1A*, (F) *ARID1B* and (G) *ARID2* in TCGA-COAD samples, analyzed via The University of ALabama at Birmingham CANcer data analysis portal. **P<0.01. ARID, AT-rich interactive domain; COAD, colon adenocarcinoma; TCGA, The Cancer Genome Atlas.

(41.27%). Furthermore, 73.02% of patients had comorbidities, including diabetes mellitus, hypertension and dyslipidemia, highlighting the complexity of the patient population. The clinicopathological characteristics of the 63 patients with CRC are summarized in Table I.

ARID1A, ARID1B and ARID2 protein expression in adjacent non-cancerous vs. cancerous areas. The expression levels of ARID1A, ARID1B and ARID2 in the cohort of 63 patients with CRC were analyzed using IHC. The results indicated that nuclear ARID1A, ARID1B and ARID2 proteins were

Table I. Clinicopathological characteristics in 63 patient samples of CRC.

Clinicopathological characteristics	Value
Age (years)	
Age range (mean ± SD)	52-97 (67.17±8.83)
Median of age	66
Sex [n (%)]	
Men	27 (42.86)
Women	36 (57.14)
Location of tumor [n (%)]	
Left-sided colon	9 (14.29)
Right-sided colon	24 (38.09)
Rectum/sigmoid colon	30 (47.62)
Largest dimension of tumor (cm)	
Size range (mean ± SD)	2.0-12.5 (5.52±2.07)
Median of the largest dimension of tumor	5.0
Pathological differentiation [n (%)]	
Well	42 (66.67)
Moderate	15 (23.81)
Poor	6 (9.52)
AJCC CRC staging [n (%)]	
Stage I	8 (12.70)
Stage II	17 (26.98)
Stage III	23 (36.51)
Stage IV	15 (23.81)
Depth of tumor invasion (pT stage) [n (%)]	
Early stage (pT0-pT2)	12 (19.05)
Late stage (pT3-pT4)	51 (80.95)
No. of positive lymph nodes (pN stage) [n (%)]	
Not identified (negative; pNX-pN0)	37 (58.73)
1 node or >1 node (positive) (pN1-pN2)	26 (41.27)
Distant metastasis (pM stage) [(%)]	
Not identified (pM0)	48 (76.19)
Metastasized other organs (pM1)	15 (23.81)
Lymphovascular invasion [n (%)]	
Not identified	32 (50.79)
Presence	31 (49.21)
Lymph node metastasis [n (%)]	
Negative	37 (58.73)
Positive	26 (41.27)
Comorbidity [n (%)]	
Absence	17 (26.98)
Presence	46 (73.02)

CRC, colorectal cancer; AJCC, American Joint Committee on Cancer; pT, tumor; pN, lymph node; pM, metastasis.

predominantly found in the colonic epithelial cells that form the intestinal glands. Strong nuclear expression of these ARIDs was observed in the intestinal cells of adjacent non-cancerous areas, while cancerous regions exhibited weaker staining. Additionally, ARID2 also exhibited cytoplasmic localization (Fig. 2A-C).

Semi-quantitative analysis (Fig. 2D) revealed a significant decrease in ARID1A protein expression in cancerous areas (90.89±6.67) compared with adjacent non-cancerous areas (234.23±7.40) in all CRC cases (P<0.001). In well-differentiated tumors, ARID1A protein expression was significantly lower in cancerous areas (89.92±9.69) compared with adjacent non-cancerous areas (234.59±8.84) (P<0.001). Similarly, moderately differentiated cancerous areas exhibited reduced ARID1A levels (96.94±10.40) compared with adjacent non-cancerous areas (224.95±17.74) (P<0.001). Poorly differentiated tissues also displayed decreased ARID1A protein expression in cancerous areas (82.58±11.79) compared with adjacent non-cancerous areas (254.88±17.69) (P<0.01).

ARID1B protein expression was significantly decreased in cancerous areas (83.87±8.04) compared with adjacent non-cancerous areas (117.34±8.13) in all CRC cases (P<0.05). Well-differentiated cancerous areas had lower ARID1B levels (68.46±9.20) than adjacent non-cancerous areas (106.25±9.08) (P<0.01). However, no significant differences were observed in tissues with moderate or poor differentiation grades, although ARID1B expression tended to be lower in cancerous areas (P=0.351 and P=0.818, respectively) (Fig. 2E).

ARID2 expression was significantly lower in cancerous areas (50.51±4.43) than in adjacent non-cancerous tissues (114.26±14.45) in all CRC cases (P<0.001). In well-differentiated tumors, ARID2 expression was significantly lower in cancerous areas (51.83±5.80) compared with adjacent non-cancerous areas (104.44±6.11) (P<0.001). Moderately differentiated cancerous areas also had decreased ARID2 expression (43.94±8.94) compared with adjacent non-cancerous areas (85.66±12.53) (P<0.05). Similarly, ARID2 expression in poorly differentiated cancerous areas was significantly lower (57.69±6.02) than in adjacent non-cancerous areas (111.96±13.39) (P<0.01) (Fig. 2F).

Next, the H-score for the cancerous areas was used to categorize ARID expression as either low or high based on the median cut-off value. For ARID1A, scores <164 were categorized as 'low expression' and scores of ≥164 as 'high expression.' There were 57 cases with low ARID1A expression (90.48%) and 6 cases with high ARID1A expression (9.52%). The median H-score of ARID1B expression was 100, with 34 cases showing low ARID1B expression (53.97%) and 29 cases showing high ARID1B expression (46.03%). For ARID2, the median H-score was 78, with 15 cases showing high ARID2 expression (23.81%) and 48 cases showing low ARID2 expression (76.19%) (Fig. 2G).

Correlation between the ARID1A, ARID1B and ARID2 protein expression levels in CRC tissues. The Spearman's correlation coefficient was used to assess the linear correlation among the expression levels of ARID1A, ARID1B and ARID2 in CRC tissues (Fig. 3). The results showed a moderate correlation between the H-scores of ARID1A and ARID1B (ρ=0.350, P=0.005), but no significant correlation between ARID1A and ARID2 (ρ=0.189, P=0.139) (Fig. 3A and B). Additionally, ARID1B expression was moderately correlated with ARID2 expression (ρ=0.436, P=0.0004; Fig. 3C). The

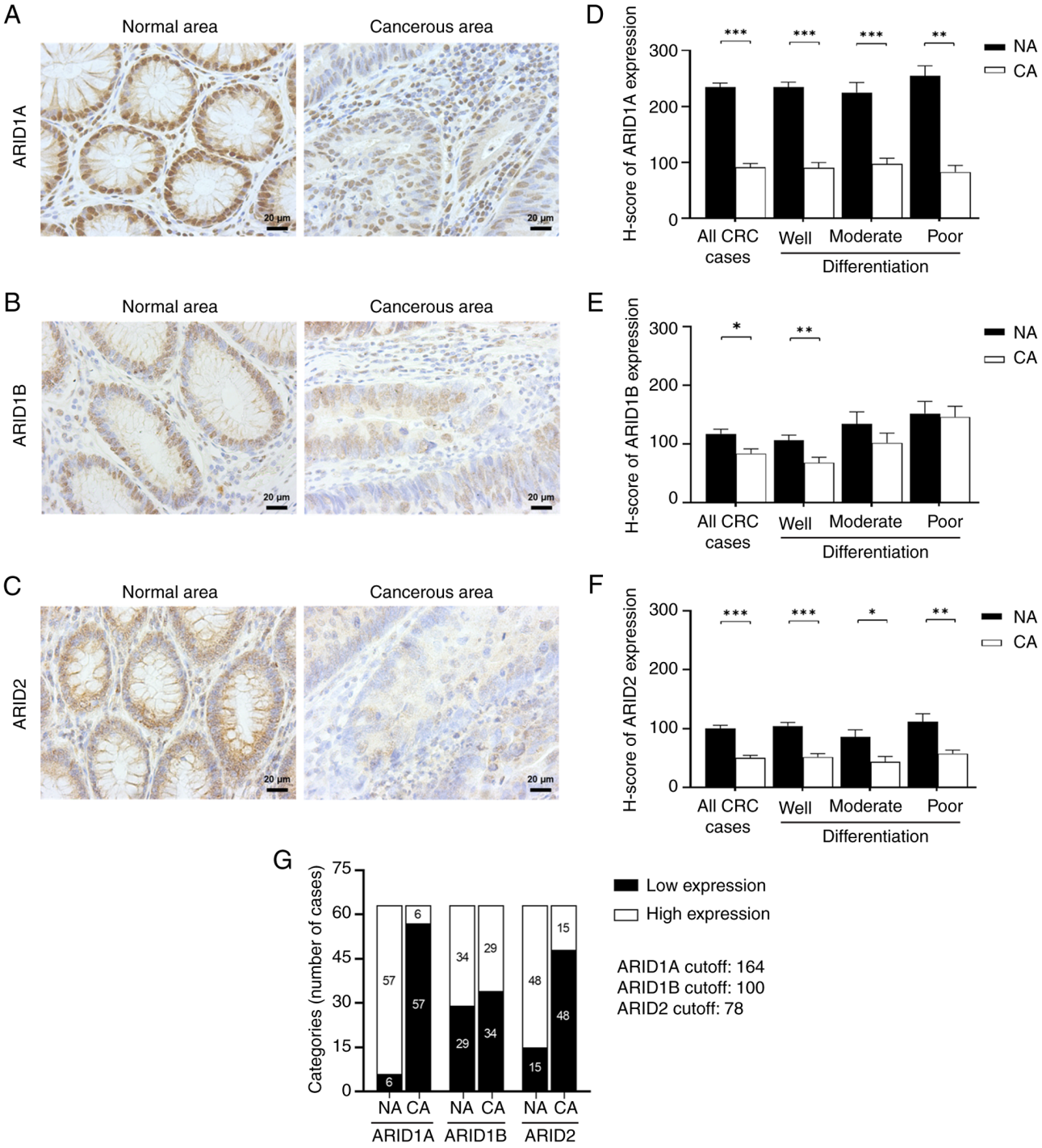


Figure 2. ARID1A, ARID1B and ARID2 protein expression in colorectal cancer tissues. Immunohistochemistry of (A) ARID1A, (B) ARID1B and (C) ARID2 proteins in the adjacent non-cancerous area compared with the cancerous area. ARID protein staining appears brown (magnification, x400; scale bar, 20 μm). The H-score of (D) ARID1A, (E) ARID1B and (F) ARID2 protein expression in the non-cancerous area (black bar) compared with the cancerous area (white bar). The data are presented as the mean ± SEM and analyzed by the Mann-Whitney U test. (G) Distribution of ARID1A, ARID1B and ARID2 expression categorization in adjacent non-cancerous and cancerous areas. Expression is classified as low or high depending on the median H-score. ARID1A cut-off, 164; ARID1B cut-off, 100; ARID2 cut-off, 78. *P<0.05, **P<0.01 and ***P<0.001. ARID, AT-rich interactive domain; H-score, histoscore; SEM, standard error of the mean; NA, adjacent non-cancerous area; CA, cancerous area.

ρ values of the correlation coefficients from the Spearman's correlation analysis are presented in a heatmap (Fig. 3D).

Association between ARID expression and the clinicopathological characteristics of patients with CRC. Fisher's exact and χ^2 analysis revealed that low ARID1A expression in patients with CRC was significantly associated with late-stage disease (P=0.049), a higher pN stage (P=0.038), the pM stage (P=0.025) and LNM (P=0.038) compared with those exhibiting high ARID1A expression. However, no significant differences were observed in age, sex, tumor location, tumor size, pathological differentiation, pT stage, lymphovascular invasion or comorbidities between the two expression groups. Furthermore, low ARID1B expression in patients with CRC

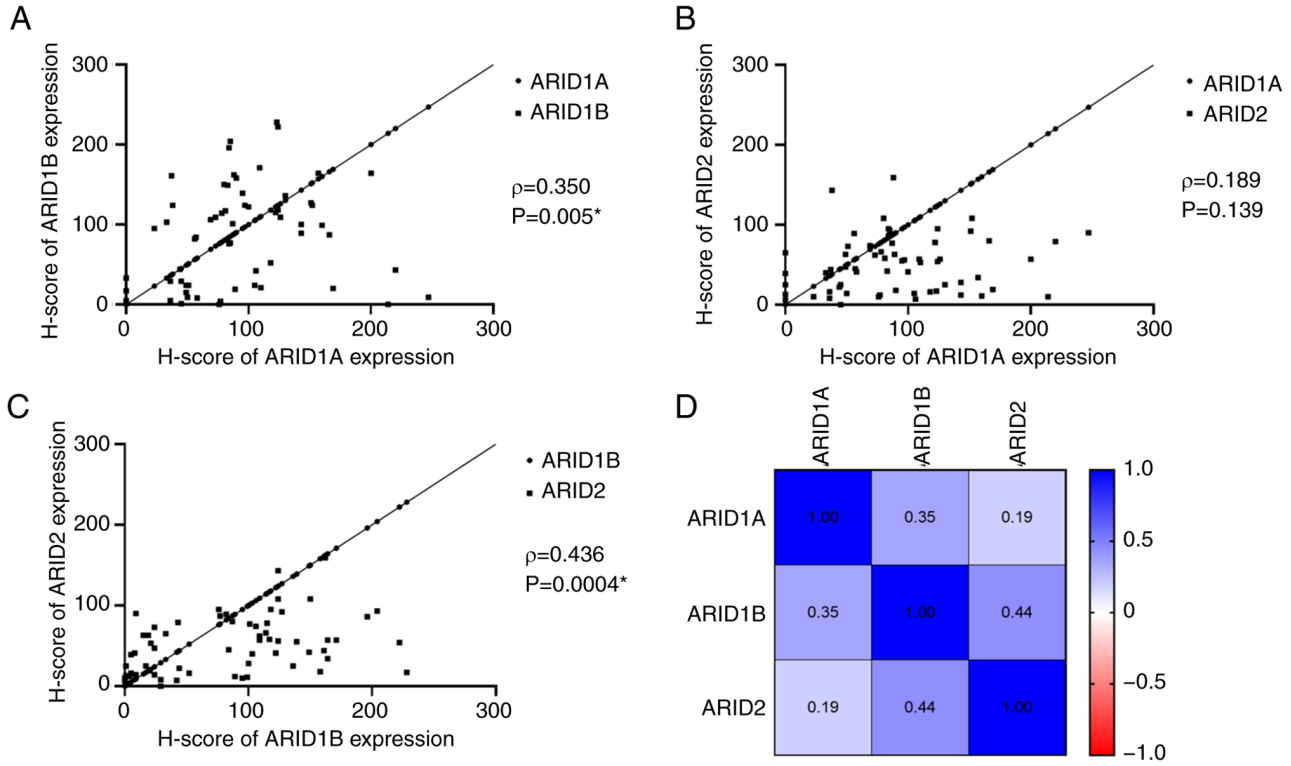


Figure 3. Expression correlations of ARID1A, ARID1B and ARID2 protein in colorectal cancer. Scatterplots showing the relationships between (A) ARID1A and ARID1B, (B) ARID1A and ARID2 and (C) ARID1B and ARID2. (D) Heatmap of Spearman's correlation coefficients among the ARID proteins. *P<0.05. ARID, AT-rich interactive domain; H-score, histoscore.

was significantly associated with pathological differentiation (P=0.004), late-stage disease (P=0.038), pN stage (P=0.010) and LNM (P=0.010) compared with those exhibiting high ARID1B expression. However, ARID2 expression did not show a significant association with the clinicopathological characteristics (Table II). After applying the Bonferroni adjustment (adjusted P=0.0014) to all ARID associations, none of these associations remained statistically significant.

Association between the protein expression levels of ARID1A, ARID1B and ARID2 with the survival outcomes of patients with CRC. The associations between the 5-year PFS of patients and the expression levels of ARID1A, ARID1B and ARID2 were analyzed using KM curve and log-rank test analysis (Fig. 4A-C). The results revealed that patients with CRC exhibiting high ARID1A expression had a significantly shorter PFS time compared with those exhibiting low ARID1A expression (P=0.036). Additionally, there was a trend towards a shorter PFS time in patients with low ARID1B and ARID2 expression compared with those with high expression; however, the differences were not statistically significant.

Cox proportional hazards regression analysis was conducted to assess the significance of potential prognostic factors in patients with CRC. Univariate analysis revealed that low ARID1A expression (P=0.005) was significantly associated with PFS. Furthermore, the multivariate analysis, which included ARID1A, ARID1B and ARID2 expression as well as LNM status, indicated that low ARID1A expression (P=0.015) was an independent prognostic factor related to PFS (Table III).

To further assess the prognostic significance of ARID1A, ARID1B and ARID2, additional analysis was conducted using the KM plotter database. The results demonstrated that lower expression of all three *ARID* genes showed a general trend towards a shorter OS time. However, only ARID1A expression showed prognostic significance, with patients with CRC exhibiting low ARID1A expression having a significantly shorter OS time compared with those exhibiting high expression (Fig. 4D-F).

Discussion

CRC is a prevalent cancer worldwide, with incidence rates that are increasing, including in Thailand (4). The SWI/SNF chromatin remodeling complex, particularly the ARID1A, ARID1B and ARID2 subunits, plays a critical role in CRC pathogenesis, affecting transcription regulation, cell differentiation, cell growth and cell progression (9,10). In the present study, bioinformatics analysis revealed frequent mutations in these *ARID* genes, with *ARID1A* predominantly exhibiting frameshift mutations and *ARID1B* and *ARID2* primarily exhibiting missense mutations. These findings align with a previous study reporting that certain cancers with a high frequency of *ARID1A* mutations also exhibit recurrent mutations in *ARID1B* and *ARID2* (37). *ARID1A* predominantly exhibited frameshift mutations, particularly at codons such as Gln456fs and Ser1315fs, which result in premature stop codons and loss of function (38). *ARID1B* and *ARID2* primarily exhibited missense mutations, including Arg1271Cys and Gly1973Arg in *ARID1B*, and

Table II. Association of ARID1A, ARID1B, and ARID2 expression with clinicopathology of CRC patients (n=63).

	ARID1A				ARID1B				ARID2			
Clinicopathological characteristics	Low n (%)	High n (%)	χ^2	P-value	Low n (%)	High n (%)	χ^2	P-value	Low n (%)	High n (%)	χ^2	P-value
Age			0.003	1.000[a]			0.501	0.479[b]			0.233	1.000[a]
<60 years old	10 (15.87)	1 (7.94)			7 (11.11)	4 (6.35)			9 (14.29)	2 (3.17)		
≥60 years old	47 (65.15)	5 (16.67)			27 (42.86)	25 (39.68)			39 (61.90)	13 (20.63)		
Sex			0.138	1.000[a]			0.048	0.827[b]			0.729	0.393
Men	24 (38.10)	3 (4.76)			15 (23.81)	12 (19.05)			22 (34.92)	5 (7.94)		
Women	33 (52.38)	3 (4.76)			19 (30.16)	17 (26.98)			26 (41.27)	10 (15.87)		
Location of tumor			4.716	0.095[b]			0.518	0.772[b]			1.260	0.533
Left-sided colon	7 (11.11)	2 (3.17)			4 (6.35)	5 (7.94)			6 (9.52)	3 (4.76)		
Right-sided colon	24 (38.10)	0 (0.00)			14 (22.22)	10 (15.87)			20 (31.75)	4 (6.35)		
Rectum/sigmoid colon	26 (41.27)	4 (6.35)			16 (25.40)	14 (22.22)			22 (34.92)	8 (12.70)		
Largest dimension of tumor			1.239	0.355[a]			0.925	0.336[b]			2.547	0.196[a]
<4.50 cm	16 (25.40)	3 (4.76)			12 (19.05)	7 (11.11)			12 (19.05)	7 (11.11)		
≥4.50 cm	41 (65.08)	3 (4.76)			22 (34.92)	22 (34.92)			36 (57.14)	8 (12.70)		
Pathological differentiation			3.316	0.191[b]			10.939	0.004[b,c]			2.074	0.355[b]
Well	36 (57.14)	6 (9.52)			28 (44.44)	14 (22.22)			31 (49.21)	11 (17.46)		
Moderate	15 (23.81)	0 (0.00)			6 (9.52)	9 (14.29)			11 (17.46)	4 (6.35)		
Poor	6 (9.52)	0 (0.00)			0 (0.00)	6 (9.52)			6 (9.52)	0 (0.00)		
AJCC CRC staging			7.881	0.049[b,c]			8.415	0.038[b,c]			1.763	0.623[b]
Stage I	7 (11.11)	1 (1.59)			5 (7.94)	3 (4.76)			5 (7.94)	3 (4.76)		
Stage II	16 (25.40)	1 (1.59)			11 (17.46)	6 (9.52)			12 (19.05)	5 (7.94)		
Stage III	23 (36.51)	0 (0.00)			7 (11.11)	16 (25.40)			19 (30.16)	4 (6.35)		
Stage IV	11 (17.46)	4 (6.35)			11 (17.46)	4 (6.35)			12 (19.05)	3 (4.76)		
pT stage			0.878	0.320[a]			0.962	0.327[b]			0.741	0.457[a]
Early stage (pT0-pT2)	10 (15.87)	2 (3.17)			8 (12.70)	4 (6.35)			8 (12.70)	4 (6.35)		
Late stage (pT3-pT4)	47 (74.60)	4 (6.35)			26 (41.27)	25 (39.68)			40 (63.49)	11 (17.46)		
pN stage			4.660	0.038[a,c]			6.674	0.010[b,c]			1.732	0.188[b]
pNX-pN0	31 (49.21)	6 (9.52)			25 (39.68)	12 (19.05)			26 (41.27)	11 (17.46)		
pN1-pN2	26 (41.27)	0 (0.00)			9 (14.29)	17 (26.98)			22 (34.92)	4 (6.35)		
pM stage			6.714	0.025[a,c]			2.972	0.085[b]			0.158	1.000[a]
pM0	46 (73.02)	2 (3.17)			23 (36.51)	25 (39.68)			36 (57.14)	12 (19.05)		
pM1	11 (17.46)	4 (6.35)			11 (17.46)	4 (6.35)			12 (19.05)	3 (4.76)		

Table II. Continued.

Clinicopathological characteristics	ARID1A				ARID1B				ARID2			
	Low n (%)	High n (%)	χ^2	P-value	Low n (%)	High n (%)	χ^2	P-value	Low n (%)	High n (%)	χ^2	P-value
Lymphovascular invasion			2.809	0.196[a]			0.765	0.382[b]			0.668	0.414[b]
Not identified	27 (42.62)	5 (7.94)			19 (30.16)	13 (20.63)			23 (36.51)	9 (14.29)		
Presence	30 (47.62)	1 (1.59)			15 (23.81)	16 (25.40)			25 (39.68)	6 (9.52)		
Lymph node metastasis			4.660	0.038[a,c]			6.674	0.010[b,c]			1.732	0.188[b]
Negative	31 (49.21)	6 (9.52)			25 (39.68)	12 (19.05)			26 (41.27)	11 (17.46)		
Positive	26 (41.27)	0 (0.00)			9 (14.29)	17 (26.98)			22 (34.92)	4 (6.35)		
Comorbidity			2.451	0.178[a]			0.447	0.504[b]			1.862	0.317[a]
Absence	17 (26.98)	0 (0.00)			8 (12.70)	9 (14.29)			15 (23.81)	2 (3.17)		
Presence	40 (63.49)	6 (9.52)			26 (41.27)	20 (31.75)			33 (52.38)	13 (20.63)		

[a]P-value was analyzed using the Fisher's exact test. [b]P-value was analyzed using the Chi-square test. [c]P<0.05 was considered to indicate a statistically significant difference. ARID1A, AT-rich interactive domain-containing protein 1A; ARID1B, AT-rich interactive domain-containing protein 1B; ARID2, AT-rich interactive domain-containing protein 2; CRC, colorectal cancer; AJCC, American Joint Committee on Cancer; pT, tumor; pN, lymph node; pM, metastasis.

Phe105Leu in *ARID2* (39,40). These mutations are known to disrupt the function of the SWI/SNF chromatin remodeling complex, leading to impaired transcriptional regulation and promoting oncogenesis (41). Previous studies have demonstrated that *ARID1A* loss-of-function mutations are associated with microsatellite instability and are prevalent in CRC (42). Similarly, missense mutations in *ARID1B* and *ARID2* can alter DNA accessibility and impact tumor suppressor gene expression (43). Additionally, in the present study, increased methylation levels were observed in the promoters of all three *ARID* genes, suggesting that both genetic mutations and epigenetic regulation contribute to their altered expression in CRC. However, the direct impact of *ARID* mutations on CRC development and progression requires further investigation. Future studies should also include a comprehensive assessment of how specific ARID mutations affect ARID protein function and expression, to fully understand their role in CRC.

ARID proteins regulate gene expression and chromatin remodeling through their DNA-binding domains (9). All ARID family members are involved in tumorigenesis (44), with mutations often leading to decreased protein expression (45,46) or loss in various cancer types (7,11,14-16,19,47). As tumor suppressors, the ARIDs regulate pathways involved in cancer development and progression (21-24). Upregulation of ARID1A expression in CRC cells was shown to suppress cell invasion and migration (24), while downregulation enhanced these processes (22,23). In lung cancer cells, ARID1B knockdown increased DNA damage and impaired DNA repair mechanisms (25), and ARID2 deficiency promoted cancer growth and metastasis (18). However, the relationships between the ARID protein expression levels in CRC remain largely unexplored.

In the present study, immunohistochemical analysis revealed decreased ARID1A, ARID1B and ARID2 protein levels in tissues from Thai patients with CRC, with ARID1A exhibiting the strongest staining. This supports the previous findings that ARID1A is the most effective suppressor among the three ARIDs (11). In the present study, using the H-score system for quantitative analysis, patients were classified into the high and low expression groups according to the median H-score (35,48). The H-score classification showed that 90.48% of cancerous areas had low ARID1A expression, consistent with previous reports indicating ARID1A loss in over half of CRC cases (13,49). Similarly, 54 and 76% of cancerous areas exhibited low ARID1B and ARID2 expression, respectively (50). It was also observed that the ARID proteins were localized to the nucleus and cytoplasm, predominantly in the epithelial cells of the intestinal glands in adjacent non-cancerous areas. ARID1A and ARID1B showed a nuclear distribution, whereas ARID2 was largely present in the cytoplasm. A previous study reported ARID2 localization in both the nucleus and cytoplasm in colon tissue, with its downregulation linked to altered cell proliferation, invasion, migration and EMT (51). While the role of cytoplasmic ARID2 remains unclear, it may regulate cytoplasmic signaling or other processes; however, this requires further investigation.

In the present study, immunohistochemical analysis revealed a significant association among the ARID1A,

Table III. Univariate and multivariate analyses of clinicopathological characteristics in 63 patients of CRC using Cox hazard regression analysis.

	Univariate			Multivariate		
Variable	HR	95% CI	P-value[a]	HR	95% CI	P-value[a]
ARID1A						
Low vs. high ARID1A	0.187	0.057-0.610	0.005[b]	5.502	1.402-21.594	0.015[b]
Low vs. high ARID1B	1.963	0.604-6.375	0.262	0.627	0.179-2.190	0.464
ARID2						
Low vs. high ARID2	1.017	0.280-3.697	0.979	0.716	0.183-2.805	0.632
Age						
≥60 vs. <60 years	0.828	0.184-3.738	0.806			
Sex						
Men vs. Women	2.697	0.742-9.808	0.132			
Tumor location						
Rectum/sigmoid vs. right/left	0.760	0.255-2.262	0.622			
Tumor largest dimension						
≥4.5 vs. <4.5 cm	1.015	0.313-3.298	0.980			
Pathological differentiation						
Poor/moderate vs. well	2.955	0.655-13.336	0.159			
AJCC stage						
Late-stage vs. early-stage	0.019	0.000-1.770	0.087			
Tumor invasion						
High (T3-T4) vs. Low (T1-T2)	0.721	0.160-3.256	0.671			
Positive lymph node						
Positive (N1-N2) vs. Negative (N0)	1.633	0.503-5.303	0.415			
Distance metastasis						
Presence (M1) vs. absence (M0)	0.000	0.000-194.851	0.232			
Lymphovascular invasion						
Presence vs. absence	2.244	0.691-7.288	0.179			
Lymph node metastasis						
Presence vs. absence	1.6333	0.503-5.303	0.415	0.922	0.233-3.656	0.908

[a]P-value was analyzed using the Cox hazard regression analysis. [b]P<0.05 was considered to indicate a statistically significant difference. CRC, colorectal cancer; HR, hazard ratio; CI, confidence interval; ARID1A, AT-rich interactive domain-containing protein 1A; ARID1B, AT-rich interactive domain-containing protein 1B; ARID2, AT-rich interactive domain-containing protein 2; AJCC, American Joint Committee on Cancer.

ARID1B and ARID2 proteins in CRC, similar to the correlation observed at the mRNA level and consistent with findings in gastric cancer (11). These results suggest a co-expression at both the mRNA and protein levels; however, the underlying regulatory mechanisms remain unclear. Future studies should include a detailed assessment of mRNA levels to explore the regulatory mechanisms of this co-expression. ARID family members may share overlapping roles in transcriptional regulation and form complex networks (52), with their positive correlations indicating a potential cooperative role in CRC pathogenesis. Additionally, tumor suppressor proteins such as p53, MYC, retinoblastoma protein and BRCA1 interact with the SWI/SNF subunits (53). In CRC, *ARID1A* mutations may contribute to disease progression through co-occurring mutations in other cancer-related genes (such as *TP53*, *KRAS*, *APC* and *PIK3CA*) and dysregulated pathways (such as WNT, Akt and MEK/ERK), affecting key cellular processes including cell cycle regulation and chromatin remodeling (54).

Loss of ARID expression is associated with various clinicopathological characteristics in cancer (9,11,13,17,55). In the present study, decreased ARID1A expression was found to be linked to late-stage disease and LNM, while low ARID1B expression was associated with poor differentiation, late-stage disease, pN stage and LNM. No significant associations were found for ARID2 expression in CRC. These findings align with previous studies that showed reduced ARID1A expression is correlated with advanced disease and LNM in CRC (13,49,55), and other cancer types (56,57). *ARID1B* promotor methylation has been linked to tumor stage and LNM in COAD (50), and its loss was demonstrated

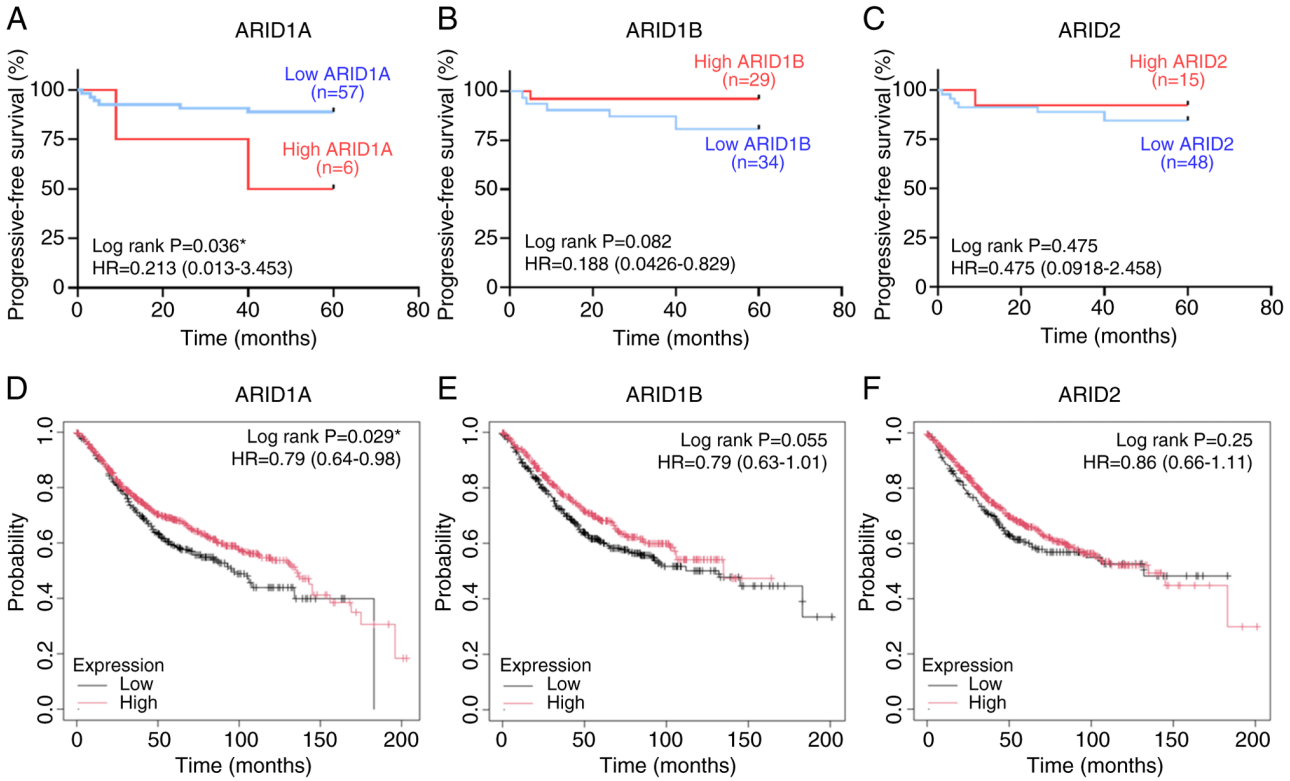


Figure 4. Relationship between ARID1A, ARID1B and ARID2 protein expression and the survival outcomes of patients with CRC. The 5-year progression-free survival outcomes based on the expression status of (A) ARID1A, (B) ARID1B and (C) ARID2 in patients with CRC (n=63), analyzed using log-rank test. Overall survival based on the expression status of (D) ARID1A, (E) ARID1B and (F) ARID2 in patients with CRC, analyzed using the KM plotter database. The KM plots for overall survival were generated directly by the KM plotter tool without any additional statistical analysis or modification. A late-stage crossover was observed in the survival curves, as provided by the tool. *P<0.05. ARID, AT-rich interactive domain; CRC, colorectal cancer; KM, Kaplan-Meier.

to be associated with lymphatic infiltration and LNM in gastric cancer (11). By contrast, high ARID1B expression was linked to favorable outcomes in breast cancer (58) and bladder urothelial carcinoma (17). In hepatocellular carcinoma and oral cancer, low ARID2 expression was correlated with advanced clinicopathological factors (45,59). Although the present study demonstrated an association between the ARID1A and ARID1B expression levels and advanced clinicopathological characteristics in CRC, the Bonferroni correction result underscores the need for cautious interpretation. Future studies with larger sample sizes or alternative correction methods are warranted to validate these findings and clarify the role of ARIDs in CRC.

Among the three ARIDs, only ARID1A expression was an independent prognostic factor for patients with CRC in the present study. In the Thai CRC cohort, KM survival analysis showed that high ARID1A expression was associated with a significantly shorter PFS time, contradicting previous studies that found low ARID1A expression was correlated with poorer survival (13,55). These discrepancies may be due to differences in methodologies, such as antibodies, cut-off values and IHC scoring (54), as well as the small number of patients with high ARID1A expression in the present study. Notably, the present study found that 4 out of 6 patients with high ARID1A expression had metastases and complications, such as hypertension and dyslipidemia, which may influence survival. A tendency towards a shorter PFS time was also noted in patients with low ARID1B and ARID2 expression, consistent with a prior report on oral squamous cell carcinoma (59). These findings suggest ARID1A as a potential prognostic marker, while the roles of ARID1B and ARID2 require further exploration. However, the prognostic value of ARID1A in Thai patients with CRC also requires further validation.

Of the poorly differentiated tissues collected in the present study, all six samples exhibited low ARID1A expression and high ARID1B expression, suggesting a possible compensatory upregulation of ARID1B in response to ARID1A loss. This aligns with previous studies that have shown ARID1B upregulation when ARID1A is inactivated (60,61). Helming *et al* reported that at least one ARID1B allele is retained in ARID1A-deficient cancer to maintain SWI/SNF complex functionality, supporting cancer cell survival. The synthetic lethality of targeting ARID1B in ARID1A-deficient cells was further confirmed in a previous CRC study (61). These findings underscore the potential compensatory role of ARIDs in cancer cells.

The present study has several limitations. First, the present study was retrospective with a small sample size and future prospective studies with larger cohorts are required. Second, although positive correlations in protein expression among ARID1A, ARID1B and ARID2 were observed in CRC, the regulatory mechanisms underlying this co-expression remain unclear. Investigating these pathways could reveal their potential synergistic roles in cancer development. Future studies using chromatin immunoprecipitation sequencing or reporter gene assays could

help identify specific binding sites and the regulatory mechanisms of these proteins. Third, while the prognostic value of each ARID protein was evaluated individually, exploring combined expression patterns could lead to more accurate biomarkers for predicting patient outcomes. Lastly, the specific functional roles of ARID1A, ARID1B and ARID2 in CRC progression are not fully understood and further *in vitro* and *in vivo* experiments are required to clarify their contributions. Genetic manipulation of ARIDs in CRC cells followed by functional assays could provide a deeper insight into their roles and mechanisms in CRC development; specifically, future directions should include subcellular fractionation and siRNA knockdown experiments to elucidate their precise functions and localization.

In conclusion, the present study demonstrated that ARID1A, ARID1B and ARID2 are frequently mutated and exhibit reduced expression in CRC tissues compared with adjacent non-cancerous tissues. The expression levels of these ARIDs are correlated with each other and are associated with advanced clinicopathological features. These findings suggest that decreased ARID expression may indicate cancer progression and prognosis in CRC, although further research is needed to validate their clinical significance.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

WM contributed to the study design, performed the experiments, data analysis, and manuscript writing. KS contributed to the pathologic analysis and interpretation of data. SA contributed to data analysis and manuscript editing. PS performed experiments and pathologic analysis. RS contributed to collecting samples and patient data. NS and SWU contributed to the conception and study design, supervision, funding acquisition, and manuscript editing. WM and SWU confirm the authenticity of all the raw data. All authors read and approved the manuscript.

Ethics approval and consent to participate

The present study was approved by the Human Research Ethics Committee of Sawanpracharak Hospital (Nakhon Sawan, Thailand; certificate of approval no. 53/2567) and the Naresuan University Human Research Ethics Committee (Phitsanulok, Thailand; approval no. P1-0107/2567; certificate of approval no. 139/2024), and conducted in accordance with the principles of the Declaration of Helsinki. Written informed consent was obtained from all subjects involved in the study.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no completing interests.

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