TRENDS IN SCIENCES 2026; 23(4): 11895
https://doi.org/10.48048/tis.2026.11895 RESEARCH ARTICLE

Combined Prognostic Significance of ARID1A Expression and Metastatic Lymph Node Ratio in Predicting Outcomes of Colorectal Cancer

Phattarapon Sonthi[1,2], Keerakarn Somsuan[3,4], Ratirath Samol[5], Siripat Aluksanasuwan[3,4], Yupa Srithongchai[1], Netnaphis Warnnissorn[6] and Natthiya Sakulsak[1,6,*]

[1]*Department of Anatomy, Faculty of Medical Science, Naresuan University, Phitsanulok 65000, Thailand*
[2]*Department of Bioinformatics and Genomics, Graduate School of Advanced Preventive Medical Sciences, Kanazawa University, Kanazawa 9208640, Japan*
[3]*School of Medicine, Mae Fah Luang University, Chiang Rai 57100, Thailand*
[4]*Cancer and Immunology Research Center, Mae Fah Luang University, Chiang Rai 57100, Thailand*
[5]*Unit of Pathology, Sawan Pracharak Hospital, Nakhonsawan 60000, Thailand*
[6]*Faculty of Medicine, Praboromarajchanok Institute, Ministry of Public Health, Nonthaburi 11000, Thailand*

([*]Corresponding author's e-mail: nsakulsak@yahoo.com)

Received: 14 September 2025, Revised: 21 October 2025, Accepted: 28 October 2025, Published: 1 January 2026

Abstract

This study investigated the prognostic significance of AT-rich interactive domain 1A (ARID1A) protein expression and the metastatic lymph node ratio (mLNR) in colorectal cancer (CRC). Immunohistochemical analysis of 78 primary resected CRC specimens revealed that 85.9% exhibited low ARID1A expression, as determined by histological (H)-score and quantitative assessment. High mLNR was observed in 17.9% of cases. Notably, low ARID1A expression combined with high mLNR was significantly associated with advanced AJCC stage, increased lymph node involvement (pN stage), lymph node metastasis (LNM), lymphovascular invasion, and comorbidities. Kaplan-Meier survival analysis demonstrated that both low ARID1A expression and high mLNR were significantly associated with reduced progression-free survival (PFS). Univariable Cox proportional hazards regression identified mLNR, AJCC stage, pN stage, distant metastasis (pM stage), and LNM as significant predictors of shorter PFS. Multivariable analysis further identified mLNR and pM stage as independent prognostic factors. In addition, GEPIA2-based transcriptomic analysis revealed significant correlations between ARID1A and LNM-associated genes, such as *insulin-like growth factor 1 receptor (IGF1R), heat shock protein 47 (HSP47), and vascular endothelial growth factor C (VEGF-C)*. These genes also showed significant prognostic relevance for survival outcomes in patients with colon adenocarcinoma (COAD) and rectum adenocarcinoma (READ) cohorts within the TCGA database. In conclusion, diminished ARID1A expression and elevated mLNR are associated with aggressive tumor features and poorer prognosis, suggesting their potential utility as combined prognostic biomarkers in CRC.

Keywords: ARID1A, Colorectal cancer, Metastatic lymph node ratio, Poor Prognosis, LNM-associated genes

Introduction

The World Health Organization (WHO) reported that cancer is potentially the greatest cause of mortality among people under the age of 70 [1]. The global incidence of colorectal cancer (CRC) is growing dramatically, as is the mortality rate [2]. CRC is ranked as the second most prevalent cause of cancer-related death in both genders in the United States [3]. Concurrently, CRC is the third most frequent cancer in men and the fourth most frequent in women in Thailand [4].

The AT-rich interactive domain 1A (ARID1A), a subunit of the human switch/sucrose non-fermenting (SWI/SNF) chromatin remodeling complexes, is a key

constituent of the BRG1-associated factor (BAF) subclass [5]. ARID1A has been recognized as a tumor suppressor gene involved in regulating the cell cycle, promoting apoptosis, and inhibiting genomic instability [6]. In addition, the *ARID1A* gene is the most frequently mutated subunit of the SWI/SNF chromatin remodeling complex, which has been reported in various types of cancer [7]. Inactivating mutations of ARID1A, the majority of which are insertion and deletion variants, cause ARID1A protein expression to be reduced [8]. Loss or decrease of ARID1A protein expression has been reported in a broad spectrum of human malignancies, including CRC [9-11]. The alterations of ARID1A protein expression were associated with the severity of clinicopathological characteristics of patients, such as age, gender, large tumor size, poor pathological grading, late American Joint Committee on Cancer (AJCC) staging, distant metastasis, and worse prognostic significance of patients [6].

Previous studies reported that loss of ARID1A expression was associated with lymph node metastasis (LNM) in patients with both early and late stages of CRC [9-11]. The prognostic value of LNM has been established as one of the prognostic indicators for predicting disease-free survival (DFS) and overall survival (OS) of CRC patients [12]. The presence of LNM is an important predictor for considering adjuvant chemotherapy after tumor removal [13,14]. Recently, essential biomarkers associated with LNM in CRC, such as insulin-like growth factor I receptor (IGF1R), vascular endothelial growth factor receptor (VEGFR), heat shock protein 47 (HSP47), and aquaporins (AQPs), have been identified, offering valuable insights for improving the prognosis of CRC patients [15]. Additionally, metastatic lymph node ratio (mLNR), which is the proportion of the number of positive lymph nodes to the total number of examined lymph nodes, is also an essential prognostic indicator in various human cancers, such as gastric cancer [16], pancreatic adenocarcinoma [17], and CRC. Unfavourable prognosis of patients with CRC was significantly correlated with high mLNR [18-20].

Nonetheless, the association between ARID1A protein expression and mLNR has never previously been evaluated and requires further investigation to elucidate the prognostic significance. Therefore, in this study, we investigated the alterations of ARID1A protein expression and mLNR and analyzed their association with the severity of clinicopathological characteristics and prognosis of patients with CRC.

Materials and methods

Ethics statement

This study followed the ethical principles outlined in the Declaration of Helsinki. Ethical approval was obtained from the Human Ethics Review Board of Sawan Pracharak Hospital, Nakhon Sawan, Thailand (Approval No. 16/2560), and the Naresuan University Ethics Committee for Human Research (NU-IRB) (Approval No. P10181/64; COA No. 421/2021).

Patient tissue specimens and patient information

Formalin-fixed, paraffin-embedded (FFPE) tissue biopsies from 78 patients diagnosed with colorectal cancer (CRC) of varying pathological differentiation, including both cancerous and adjacent non-cancerous tissues, were obtained from the Pathology Unit at Sawanpracharak Hospital, Nakhonsawan, Thailand, between 2017 and 2021, and used in this study. Specimens from patients with recurrent or hereditary CRC, unknown primary tumors, or those who received neoadjuvant chemotherapy or radiotherapy prior to surgery were excluded to minimize potential treatment-related bias. Each case was diagnosed and examined by a pathologist (R.S.) using a hematoxylin and eosin (H&E) slide. Demographic and clinicopathological information of CRC patients, for instance, age, gender, location of tumor, tumor mass dimension, pathological differentiation, AJCC staging, tumor invasion, metastasis, recurrence, angiolymphatic invasion, number of examined and positive lymph nodes, patient's comorbidity, and follow-up period, were acquired and analyzed. To maintain patient confidentiality, each FFPE block was assigned a unique research code, and all sensitive patient information was securely protected.

Immunohistochemistry staining and evaluation of ARID1A protein by histochemical score (H-score)

Immunohistochemistry staining was performed as described previously [11]. Three independent investigators, including a pathologist (R.S.) and two researchers (P.S. and K.S.), who were blinded to the demographic and clinicopathological information of

CRC patients, reviewed and evaluated the ARID1A immunostained sections. For assessment of ARID1A immunoreactivity, five independent areas of each section were imaged at high power fields (HPF) provided by 40× magnification of the objective lens using a ZEN program (Rushmore Precision Co., Ltd.) under an Axiocam 105 color ZEISS microscope (Carl Zeiss, Oberkochen, Germany) in both cancerous and adjacent non-cancerous areas of CRC tissues. A histochemical score (H-score), which is a semi-quantitative assessment to evaluate immunoreactivity in tumor samples [21], was applied to assess the expression of ARID1A protein. The H-score was evaluated based on the staining intensity and the percentage of positive cells of ARID1A staining. Three investigators evaluated the staining intensity of ARID1A and scored it as 0 (negative), 1 (weak), 2 (moderate), and 3 (strong staining) in both cancerous and adjacent non-cancerous areas of CRC tissues **(Figure 1)**. In addition, the total number of epithelial cells and ARID1A-positive cells was counted using ImageJ (Fiji) image analysis software, and the percentage of ARID1A-positive cells was calculated [22]. The summation of the H-score was calculated according to the formula:

H-score = [(0 × % negative cells) + (1 × %weakly positive cells) + (2 × %moderately positive cells) + (3 × %strongly positive cells)] [23] (1)

Consequently, the conceivable H-score ranges from 0 to 300. The 50% cut-off value of the H-score (150/300) has been used to classify ARID1A expression into two groups as low (less than 150) and high (equal to or more than 150).

Measurement of the relative optical density (ROD) of the ARID1A protein

To confirm staining intensity, ARID1A expression in intestinal epithelial cells was examined in cancerous areas compared with adjacent non-cancerous regions of CRC samples. ImageJ (Fiji) image analysis software (http://fiji.sc/Fiji) was used to measure the intensities of ARID1A protein expression. The relative optical density (ROD) of protein contents from at least 100 nuclei was evaluated and calculated according to the following formula:

ROD = log10 (max intensity/ mean intensity) (2)

Examination of metastatic lymph node ratio

After conducting surgical pathology of the CRC specimens in a routine process, the number of accessible lymph nodes among the colorectal tissue samples was documented. The number of positive LNM was diagnosed and examined by a pathologist (R.S.) using an H&E slide. Consequently, mLNR was estimated as the ratio of the number of positive lymph nodes to the total number of examined lymph nodes [20]. In this study, we categorized patients into two groups based on their mLNR value: High (equal to or greater than 0.4) and low (less than 0.4). This value was chosen based on previous studies investigating the prognostic value of mLNR on PFS in CRC [20,24,25].

Correlation analysis between the mRNA expression of ARID1A and the biomarkers involved in lymph node metastasis of CRC

To assess the correlation between the expression of *ARID1A* and LNM-associated genes in CRC, the Gene Expression Profiling Interactive Analysis 2 (GEPIA2) database (http://gepia2.cancer-pku.cn/#index) was utilized. GEPIA2 is a tool that provides pair-wise gene correlation analysis of a specific group of TCGA and GTEx expression data [26]. For this study, transcriptomic data from both colon adenocarcinoma (COAD) and rectum adenocarcinoma (READ) cohorts within the TCGA database were combined. Spearman's correlation coefficient was employed to evaluate the strength and direction of associations between ARID1A and selected LNM-associated genes. All analyses were conducted using data accessed from GEPIA2 on June 1, 2025.

Survival analysis of the lymph node metastasis-associated gene in colon and rectal adenocarcinoma

To explore the prognostic significance of each LNM-associated gene in COAD and READ, survival analyses were conducted using the GEPIA2 platform [26]. Patients were categorized into high- and low-expression groups based on the median expression value of each gene. Kaplan–Meier (KM) survival plots for overall survival (OS) were generated using the "Auto select best cutoff" option. The survival curves were evaluated using the log-rank test, and the corresponding

hazard ratios with 95% confidence intervals were calculated.

Statistical analysis

All statistical analyses were performed using IBM SPSS Statistics for Mac, version 25.0 (IBM Corp., Armonk, NY, USA) and GraphPad Prism for Mac, version 7.0 (GraphPad Software, San Diego, CA, USA). Quantitative data are presented as mean ± standard error of the mean (SEM). Paired data were compared using the paired Student's *t*-test. For unpaired data, the unpaired Student's *t*-test was used for normally distributed variables, while the Mann-Whitney U test was applied for non-normally distributed data. Associations between ARID1A expression, mLNR, and clinicopathological parameters were evaluated using Pearson's chi-square (χ^2) test or Fisher's exact test, as appropriate. Progression-free survival (PFS), defined as the interval from surgery to documented disease progression (distant metastasis), was estimated using the Kaplan-Meier method, and differences between groups were assessed using the log-rank test. Univariate and multivariate analyses of factors associated with PFS were performed using Cox proportional hazards regression models. Hazard ratios (HRs) with 95% confidence intervals (CIs) were reported. A two-sided *p*-value < 0.05 was considered statistically significant.

Results and discussion

ARID1A immunoreactivity in cancerous versus adjacent non-cancerous areas of CRC tissues

ARID1A expression was examined in cancerous and adjacent non-cancerous regions of CRC tissues using immunohistochemistry (IHC). Tissue samples were obtained from a total of 78 CRC patients. ARID1A staining intensity was scored from 0 to 3, corresponding to negative, weak, moderate, and strong expression, respectively (**Figure 1**).

In adjacent non-cancerous regions, moderate to strong ARID1A staining was detected in seventy-three cases (91.03%), and no negative staining was observed (**Figure 2(A)**). By contrast, in cancerous tissues, sixty-five cases (83.3%) showed weak to moderate staining, while 6 cases (7.7%) were completely negative (**Figure 2(A)**). The proportion of ARID1A-positive cells was also significantly lower in cancerous compared with non-cancerous areas (**Figure 2(B)**). Furthermore, H-score analysis, incorporating both staining intensity and the percentage of positive cells, further confirmed reduced ARID1A expression in cancerous regions (**Figure 2(C)**).

Based on the H-score cut-off (150/300), sixty-seven samples (85.90%) had low expression of ARID1A, while 11 samples (14.10%) remained highly expressed in the cancerous regions. On the other hand, almost all the adjacent non-cancerous areas had high ARID1A expression (74 of 78 samples, 94.87%) (**Figure 2(D)**). Consistently, semi-quantitative analysis of relative optical density (ROD) also demonstrated a significant decrease in ARID1A protein levels in cancerous compared with adjacent non-cancerous areas (**Figure 2(E)**). Immunohistochemical analysis demonstrated differential ARID1A expression between non-cancerous and cancerous colorectal tissues. In adjacent non-cancerous regions, ARID1A expression was consistently higher, whereas a marked reduction was observed in cancerous areas (**Figure 2(F)**).

Decreased ARID1A protein expression has also been increasingly reported in various human malignancies, particularly those of the gastrointestinal tract [27]. Consistent with our findings, previous studies have demonstrated negative or reduced ARID1A expression in 5.9% [28], 25.8% [29], 30.2% [30], 66.5% [10] and 90.48% [11] of primary colorectal carcinomas, respectively. Loss of ARID1A protein expression is involved in oncogenic transformation [10,31]. Collectively, these findings support the notion that loss or reduction of ARID1A expression plays a critical role in driving CRC carcinogenesis and progression.

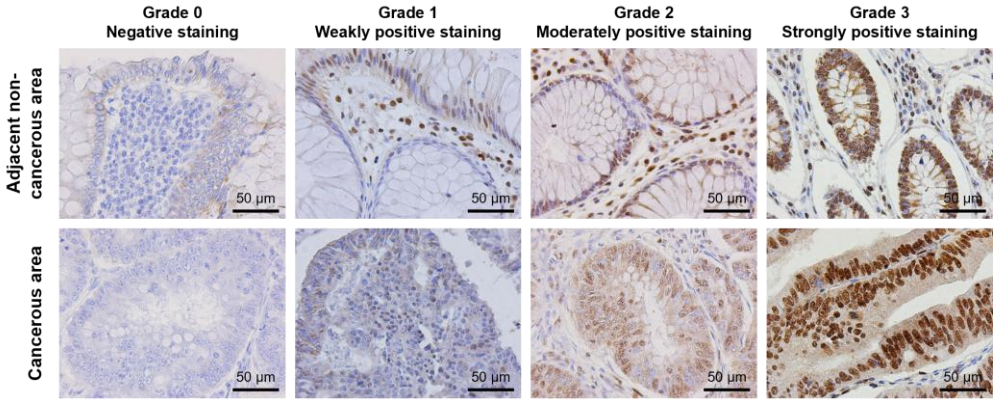

Figure 1 Grading of ARID1A immunostaining in colorectal cancer. ARID1A nuclear staining was assessed in adjacent non-cancerous (upper panels) and cancerous regions (lower panels) and scored as 0 (negative), 1 (weak), 2 (moderate) and 3 (strong). Positive staining appears as brown nuclear coloration. All images were captured at 400x original magnification.

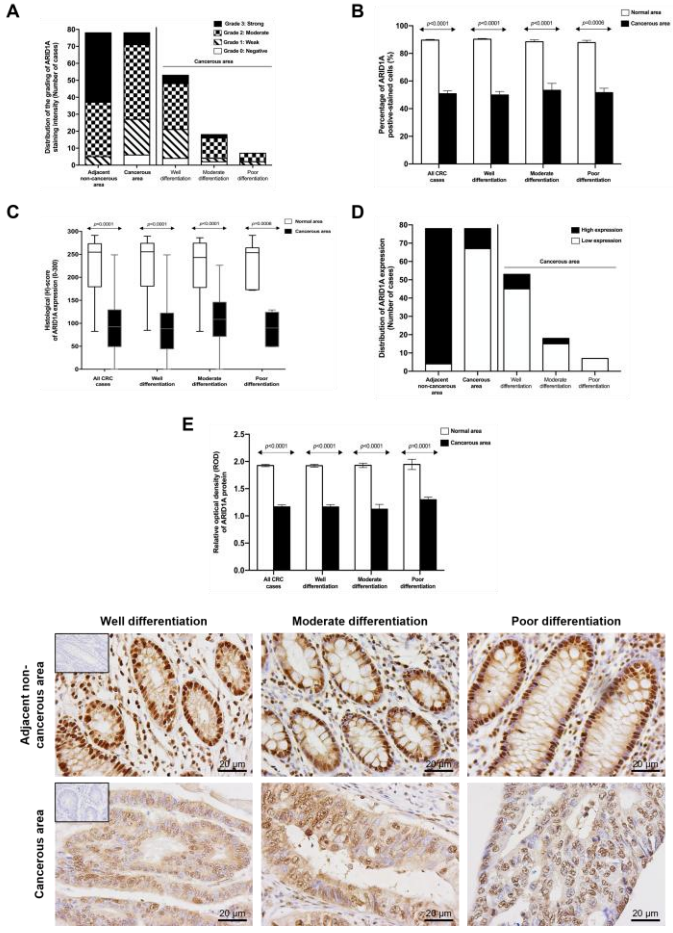

Figure 2 ARID1A protein expression in colorectal cancer tissues (A) Distribution of ARID1A staining intensity scores in non-cancerous and cancerous areas. (B) Proportion of ARID1A-positive cells in non-cancerous and cancerous regions stratified by histological differentiation. (C) H-score analysis of ARID1A expression in non-cancerous versus cancerous areas. The H-score was calculated based on staining intensity and the percentage of positive cells. (D) Classification of ARID1A expression as low or high using a 50% H-score cut-off value (150 out of 300). (E) Relative optical density (ROD) of ARID1A staining in adjacent non-cancerous versus cancerous tissue regions. (F) Representative immunohistochemical staining of ARID1A in adjacent non-cancerous and cancerous areas across well-, moderately-, and poorly differentiated CRC tissues. Insets show negative controls. All images were captured at 400x original magnification. Data are presented as mean ± SEM. Statistical comparisons were performed using the Mann-Whitney U test ($p < 0.05$).

CRC patient characteristics

The demographic and clinical characteristics of the 78 patients with CRC are presented in **Table 1**. The age of the patients ranged from 30 to 89 years, with a mean age of 65.63 ± 1.36 years. Of the patients, 35 (44.87%) were male, and 43 (55.13%) were female. Tumors were found in the rectum or sigmoid colon in 40 patients (51.28%), and the largest tumor in the sample was greater than 4.50 cm in diameter in 47 patients (60.26%), with an average dimension of 5.26 ± 0.25 cm. Pathological differentiation was graded as well-differentiated adenocarcinoma in 53 patients (67.95%), moderately differentiated adenocarcinoma in 18 patients (23.08%), and poorly differentiated adenocarcinoma in seven patients (8.97%). Based on AJCC and TNM staging, 5 patients (6.41%) were at stage I, 17 patients (21.79%) were at stage II, 26 patients (33.33%) were at stage III and 30 patients (38.46%) were at stage IV. Most patients (89.74%) were diagnosed with CRC in the advanced stages of tumor invasion (pT3 - pT4), while only eight patients (10.26%) were diagnosed in the early stages (pT0 - pT2). Thirty patients (38.46%) had metastases to other organs, such as the liver, peritoneum, and prostate gland, while 48 patients (61.54%) did not. In addition, 55 CRC patients (70.51%) had comorbidities such as type II diabetes mellitus, hypertension, and dyslipidaemia. The presence of lymph node involvement, lymph node metastasis (LNM), and lymphovascular invasion (LVI) was identified in 43 of the 78 patients with CRC (55.13%). The remaining 35 patients (44.87%) had no lymph node involvement (**Table 1**).

Association of clinicopathological characteristics with the expression of ARID1A and mLNR status

Patients were stratified into two groups according to ARID1A expression (low versus high) and mLNR status (low versus high). According to Fisher's exact analysis, low ARID1A expression was significantly associated with late AJCC staging ($p = 0.014$), positive lymph node involvement (pN stage) ($p = 0.002$), presence of LNM ($p = 0.002$), LVI ($p = 0.010$), and patient comorbidity ($p = 0.028$). Moreover, a high mLNR was significantly associated with worse prognostic factors, including late AJCC staging, stage IV ($p = 0.021$) (**Table 1**).

Our analysis revealed that low ARID1A expression was significantly associated with adverse clinicopathological features, including advanced AJCC stage, positive lymph node involvement, LNM, LVI, and comorbidities. Similarly, high mLNR correlated with worse prognostic indicators, particularly stage IV disease. These findings suggest that both decreased ARID1A expression and elevated mLNR reflect more aggressive tumor behavior and poorer prognosis in CRC patients, supporting their potential utility as prognostic biomarkers [6].

Table 1 Association between ARID1A expression, mLNR status, and the clinicopathology of CRC patients (total n = 78).

Clinicopathological characteristics	n (%)	ARID1A expression		*p*-value[#]	mLNR status		*p*-value[#]
		Low ARID1A (n (%))	High ARID1A (n (%))		Low mLNR (n (%))	High mLNR (n (%))	
Number of patients	78 (100.0)	67 (85.90)	11 (14.10)		64 (82.05)	14 (17.95)	
Gender				**1.000**			**0.380**
Male	35 (44.87)	30 (38.46)	5 (6.41)		27 (34.61)	8 (10.26)	
Female	43 (55.13)	37 (47.44)	6 (7.69)		37 (47.44)	6 (7.69)	
Age				**0.073**			**0.538**
≥ 60 years old	55 (70.51)	50 (64.10)	5 (6.41)		27 (34.61)	8 (10.26)	
< 60 years old	23 (29.49)	17 (21.80)	6 (7.69)		37 (47.44)	6 (7.69)	
Tumor location				**0.393**			**0.128**
Rectum/ Sigmoid colon	40 (51.28)	33 (42.31)	7 (8.97)		33 (42.31)	7 (8.97)	
Right side colon	28 (35.90)	26 (33.34)	2 (2.56)		25 (32.05)	3 (3.85)	
Left side colon	10 (12.82)	8 (10.26)	2 (2.56)		6 (7.69)	4 (5.13)	

Clinicopathological characteristics	n (%)	ARID1A expression		*p*-value[#]	mLNR status		*p*-value[#]
		Low ARID1A (n (%))	High ARID1A (n (%))		Low mLNR (n (%))	High mLNR (n (%))	
Pathologic differentiation				**0.765**			**0.057**
Poor differentiation	7 (8.97)	7 (8.97)	0 (0.00)		4 (5.13)	3 (3.84)	
Moderate differentiation	18 (23.08)	15 (19.23)	3 (3.85)		13 (16.67)	5 (6.41)	
Well differentiation	53 (67.95)	45 (57.69)	8 (10.26)		47 (60.26)	6 (7.69)	
Tumor greatest dimension (cm.)				**0.329**			**1.000**
≥ 4.5	47 (60.26)	42 (53.85)	5 (6.41)		39 (50.00)	8 (10.26)	
< 4.5	31 (39.74)	25 (32.05)	6 (7.69)		25 (32.05)	6 (7.69)	
AJCC staging				**0.014***			**0.021***
Stage IV	30 (38.46)	24 (30.77)	6 (7.69)		25 (32.05)	5 (6.41)	
Stage III	26 (33.33)	26 (33.33)	0 (0.00)		17 (21.79)	9 (11.54)	
Stage II	17 (21.79)	12 (15.38)	5 (6.41)		17 (21.79)	0 (0.00)	
Stage I	5 (6.41)	5 (6.41)	0 (0.00)		5 (6.41)	0 (0.00)	
pT stage				**1.000**			**0.338**
pT3-pT4	70 (89.74)	60 (76.92)	10 (12.82)		56 (71.79)	14 (17.95)	
pT0-pT2	8 (10.26)	7 (8.98)	1 (1.28)		8 (10.26)	0 (0.00)	
pN stage				**0.002***			**< 0.001***
pN1-pN2 (Positive)	43 (55.13)	42 (53.85)	1 (1.28)		29 (37.18)	14 (17.95)	
pNX-pN0 (Negative)	35 (44.87)	25 (32.05)	10 (12.82)		35 (44.87)	0 (0.00)	
pM stage				**0.319**			**1.000**
pM1	30 (38.46)	24 (30.77)	6 (7.69)		25 (32.05)	5 (6.41)	
pM0	48 (61.54)	43 (55.13)	5 (6.41)		39 (50.00)	9 (11.54)	
Lymph node metastasis				**0.002***			**< 0.001***
Positive	43 (55.13)	42 (53.85)	1 (1.28)		29 (37.18)	14 (17.95)	
Negative	35 (44.87)	25 (32.05)	10 (12.82)		35 (44.87)	0 (0.00)	
Lymphovascular invasion				**0.010***			**0.075**
Presence	43 (55.13)	41 (52.57)	2 (2.56)		32 (41.03)	11 (14.10)	
Not identified	35 (44.87)	26 (33.33)	9 (11.54)		32 (41.03)	3 (3.84)	
Comorbidity				**0.028***			**0.331**
Presence	55 (70.51)	44 (56.41)	11 (14.10)		47 (60.25)	8 (10.26)	
Absence	23 (29.49)	23 (29.49)	0 (0.00)		17 (21.80)	6 (7.69)	

Abbreviations used: ARID1A, AT-rich interactive domain-containing protein 1A; mLNR, metastatic lymph node ratio; CRC, colorectal cancer; AJCC, American Joint Committee on Cancer; p, pathological; TNM, tumor (T), nodes (N), and metastases (M). [#]*p* - value was analyzed using Fisher's exact test. **p* - value < 0.05 was considered to indicate statistical significance.

Combined prognostic significance of ARID1A expression and mLNR status

The association between ARID1A expression combined with mLNR status and the clinicopathology of CRC patients is shown in **Table 2**. Patients were classified into three groups based on ARID1A expression and mLNR status: Low ARID1A/high mLNR, low ARID1A/low mLNR, and high ARID1A/low mLNR. The analysis showed that CRC patients with low ARID1A/high mLNR (n = 14, 17.95%) had a significantly worse prognosis, which was strongly associated with advanced AJCC stage (p = 0.006), higher pN stage ($p < 0.001$), presence of lymph node metastasis (LNM, $p < 0.001$), lymphovascular invasion (LVI, p = 0.010), and patient comorbidities (p = 0.035).

Our findings indicate that the combined assessment of ARID1A expression and mLNR status provides enhanced prognostic value in CRC, as patients with low ARID1A expression and high mLNR exhibited significantly worse outcomes, which were strongly associated with advanced AJCC stage, higher pN stage, lymph node metastasis, lymphovascular invasion, and comorbidities. This suggests that integrating ARID1A and mLNR may serve as a valuable marker for identifying high-risk CRC patients and guiding clinical management.

Table 2 Combined prognostic significance of ARID1A expression and mLNR status (total n = 78).

Clinicopathological characteristics	n (%)	ARID1A expression and mLNR status			*p*-value[#]
		Low ARID1A/ High mLNR (n (%))	Low ARID1A/ Low mLNR (n (%))	High ARID1A/ Low mLNR (n (%))	
Number of patients	78 (100.0)	14 (17.95)	53 (67.95)	11 (14.10)	
Gender					**0.578**
Male	35 (44.87)	8 (10.26)	22 (28.20)	5 (6.41)	
Female	43 (55.13)	6 (7.70)	31 (39.74)	6 (7.69)	
Age					**0.157**
≥ 60 years old	55 (70.51)	11 (14.10)	39 (50.00)	5 (6.41)	
< 60 years old	23 (29.49)	3 (3.85)	14 (17.95)	6 (7.69)	
Tumor location					**0.116**
Rectum/ Sigmoid colon	40 (51.28)	7 (8.98)	26 (33.33)	7 (8.97)	
Right side colon	28 (35.90)	3 (3.85)	23 (29.49)	2 (2.56)	
Left side colon	10 (12.82)	4 (5.13)	4 (5.13)	2 (2.56)	
Pathologic differentiation					**0.154**
Poor differentiation	7 (8.97)	3 (3.84)	4 (5.13)	0 (0.00)	
Moderate differentiation	18 (23.08)	5 (6.41)	10 (12.82)	3 (3.85)	
Well differentiation	53 (67.95)	6 (7.69)	39 (50.00)	8 (10.26)	
Tumor greatest dimension (cm.)					**0.467**
≥ 4.5	47 (60.26)	8 (10.26)	34 (43.59)	5 (6.41)	
< 4.5	31 (39.74)	6 (7.69)	19 (24.36)	6 (7.69)	
AJCC staging					**0.006***
Stage IV	30 (38.46)	5 (6.41)	19 (24.36)	6 (7.69)	
Stage III	26 (33.33)	9 (11.54)	17 (21.79)	0 (0.00)	
Stage II	17 (21.79)	0 (0.00)	12 (15.38)	5 (6.41)	
Stage I	5 (6.41)	0 (0.00)	5 (6.41)	0 (0.00)	
pT stage					**0.451**
pT3-pT4	70 (89.74)	14 (17.95)	46 (58.97)	14 (17.95)	
pT0-pT2	8 (10.26)	0 (0.00)	7 (8.98)	1 (1.28)	
pN stage					**< 0.001***

Clinicopathological characteristics	n (%)	ARID1A expression and mLNR status			*p*-value[#]
		Low ARID1A/ High mLNR (n (%))	Low ARID1A/ Low mLNR (n (%))	High ARID1A/ Low mLNR (n (%))	
pN1-pN2 (Positive)	43 (55.13)	14 (17.95)	28 (35.90)	1 (1.28)	
pNX-pN0 (Negative)	35 (44.87)	0 (0.00)	25 (32.05)	10 (12.82)	
pM stage					**0.528**
pM1	30 (38.46)	5 (6.41)	19 (24.36)	6 (7.69)	
pM0	48 (61.54)	9 (11.54)	34 (43.59)	5 (6.41)	
Lymph node metastasis					**< 0.001***
Positive	43 (55.13)	14 (17.95)	28 (35.90)	1 (1.28)	
Negative	35 (44.87)	0 (0.00)	25 (32.05)	10 (12.82)	
Lymphovascular invasion					**0.010***
Presence	43 (55.13)	11 (14.10)	30 (38.46)	2 (2.57)	
Not identified	35 (44.87)	3 (3.85)	23 (29.49)	9 (11.53)	
Comorbidity					**0.035***
Presence	55 (70.51)	8 (10.26)	36 (46.15)	11 (14.10)	
Absence	23 (29.49)	6 (7.69)	17 (21.80)	0 (0.00)	

Abbreviations used: ARID1A, AT-rich interactive domain-containing protein 1A; mLNR, metastatic lymph node ratio; CRC, colorectal cancer; AJCC, American Joint Committee on Cancer; p, pathological; TNM, tumor (T), nodes (N), and metastases (M). [#]*p* - value was analyzed using Fisher's exact test. ***p*** - value < 0.05 was considered to indicate statistical significance.

Impact of ARID1A expression and mLNR status on Progression-Free Survival (PFS) of patients with CRC

A Kaplan-Meier curve and log-rank test analyses were conducted to investigate the impact of ARID1A protein expression and mLNR status on the PFS in patients. The findings revealed that patients with low ARID1A expression had a shorter PFS (58.2% PFS rate) than those with high ARID1A expression (72.7% PFS rate). However, no significant difference between the groups was shown (p = 0.337) (**Figure 3(A)**). The analysis also showed that patients with high mLNR had a significantly worse prognosis (21.4% PFS rate) compared to those with low mLNR (68.8% PFS rate) (p = 0.001) (**Figure 3(B)**). In addition, we found that the patients with low ARID1A expression combined with high mLNR showed the worst PFS (21.4% PFS rate) among the other groups. This difference was statistically significant (p = 0.005) (**Figure 3(C)**).

Although low ARID1A expression alone was associated with a shorter PFS, the difference was not statistically significant. In contrast, high mLNR was strongly correlated with poor prognosis, as reflected by a markedly reduced PFS. Importantly, the combined evaluation revealed that patients with low ARID1A expression and high mLNR had the worst outcomes, with significantly reduced PFS compared to other groups. These findings suggest that while ARID1A expression alone may not be a robust independent predictor, its integration with mLNR substantially enhances prognostic discrimination, highlighting the potential of this combined biomarker approach for identifying high-risk CRC patients.

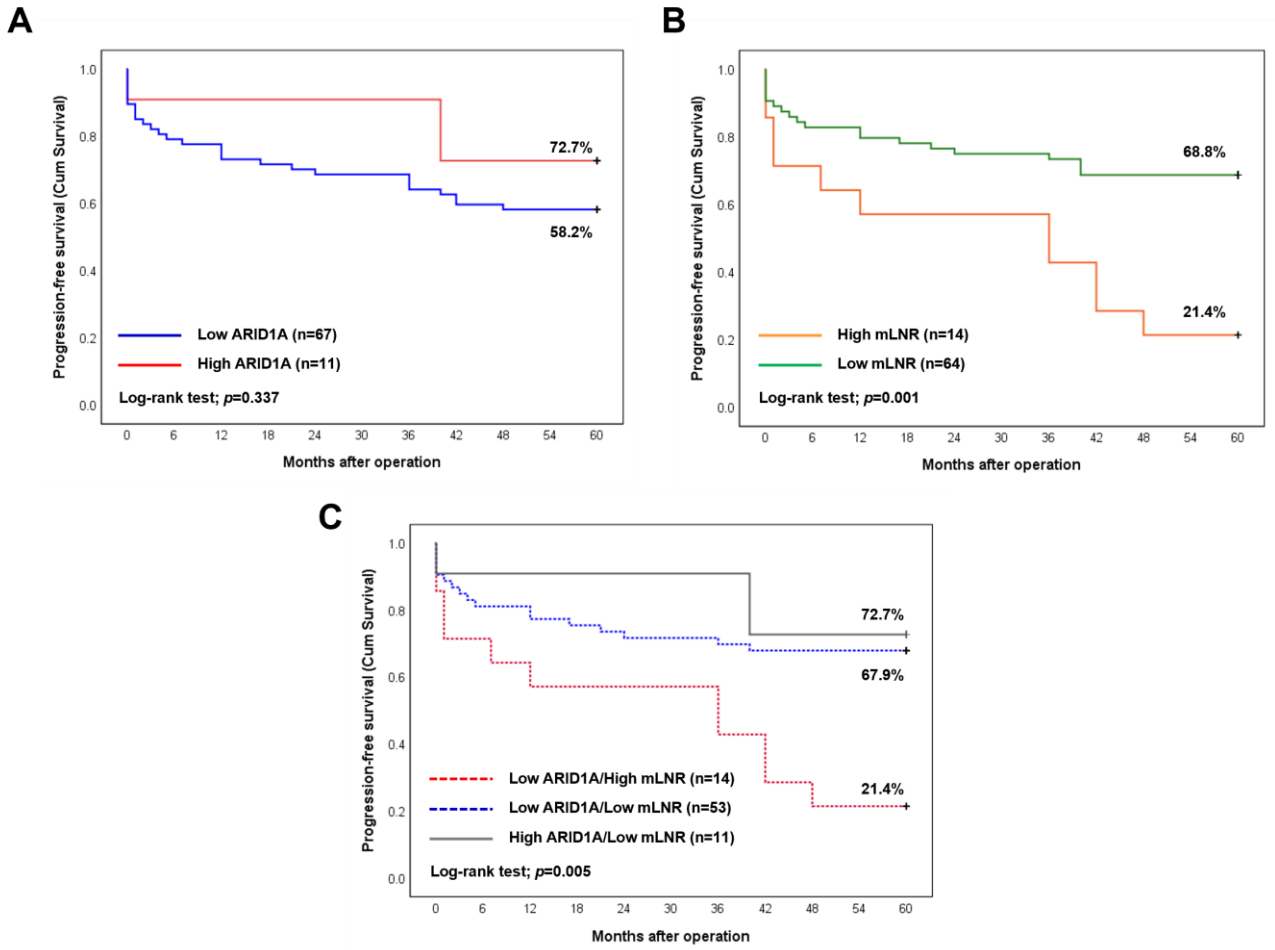

Figure 3 Kaplan-Meier analysis of 5-year progression-free survival (PFS) in colorectal cancer patients. (A) PFS curve for CRC patients with high and low ARID1A levels. (B) PFS according to metastatic lymph node ratio (mLNR), categorized as low (< 0.4) and high (≥ 0.4). (C) The PFS for CRC patients with combined ARID1A expression and mLNR statuses. Survival differences were assessed using the log-rank test, with statistical significance set at $p < 0.05$.

Furthermore, univariable and multivariable analyses using Cox proportional hazards regression analysis were performed to identify the significant predictors of prognosis in CRC patients. Univariable analysis showed that mLNR status, AJCC staging, pN, pM stage and LNM were all potential prognostic factors correlated with the PFS (all $p < 0.05$). However, the expression level of ARID1A was not found to be associated with a shorter PFS ($p = 0.352$, as shown in **Table 3**). A multivariable analysis was conducted, including all parameters with $p < 0.05$ in the univariable analysis. The study revealed that mLNR status and pM stage were independent prognostic factors for PFS (all $p < 0.05$, as shown in **Table 3**).

This observation is consistent with previous reports identifying mLNR as a significant prognostic factor across multiple malignancies [18-20], including head and neck cancers [32], oral and oropharyngeal squamous cell carcinomas [33,34], and CRC [18,20]. In line with these studies, our analysis further confirmed mLNR as an independent prognostic factor for PFS in CRC, underscoring its clinical utility. Taken together, the combined assessment of ARID1A expression and mLNR may provide a more reliable approach for identifying high-risk CRC patients and improving prognostic accuracy. However, as this was a retrospective, single-institution study with a limited sample size, the findings should be interpreted with caution. Larger multicenter studies are warranted to validate the prognostic relevance of ARID1A and mLNR and to further elucidate their biological roles in CRC progression.

Table 3 Univariable and multivariable analyses of progressive-free survival in 78 patients with CRC using the Cox hazard regression analysis.

Parameters	Univariable analysis				Multivariable analysis			
	Hazard ratio (HR)	95% confidence interval (CI)		*p*-value	Hazard ratio (HR)	95% confidence interval (CI)		*p*-value
		Lower	Upper			Lower	Upper	
ARID1A expression Low (n = 67) *vs* High (n = 11)	0.568	0.173	1.870	**0.352**				
mLNR ≥ 40 (n=14) *vs* < 0.40 (n = 64)	0.319	0.152	0.668	**0.002***	0.211	0.085	0.526	**0.001***
Gender Male (n = 35) *vs* Female (n = 43)	1.110	0.544	2.266	**0.774**				
Age, years old ≥ 60 (n = 55) *vs* < 60 (n = 23)	1.140	0.537	2.422	**0.733**				
Tumor location Rectum/Sigmoid (n = 40) *vs* Right/Left (n = 38)	0.705	0.345	1.440	**0.337**				
Pathological differentiation Poor/Moderate (n = 25) *vs* Well (n = 53)	0.843	0.404	1.759	**0.648**				
Tumor greatest dimension (cm.) ≥ 4.5 (n = 47) *vs* < 4.5 (n = 31)	0.657	0.309	1.395	**0.274**				
AJCC staging IV-III (n = 56) *vs* II-I (n = 22)	0.024	0.001	0.534	**0.018***	0.000	0.000	3.934E + 113	**0.943**
Tumor invasion (pT stage) pT3 - pT4 (n = 70) *vs* pT2 -pT1 (n = 8)	0.240	0.033	1.760	**0.160**				
Positive lymph node (pN stage) pN1 - pN2 (n = 43) *vs* pN0 (n = 35)	0.352	0.157	0.788	**0.011***	0.416	0.159	1.086	**0.073**
Distant metastasis (pM stage) pM1 (n = 30) *vs* pM0 (n = 48)	0.068	0.027	0.171	**0.000***	0.043	0.014	0.130	**0.000***
Lymph node metastasis Positive (n = 43) vs Negative (n = 35)	0.352	0.157	0.788	**0.011***	-	-	-	**-**
Lymphovascular invasion Presence (n = 43) vs Absence (n = 35)	0.836	0.409	1.706	**0.622**				
Comorbidity Presence (n = 55) vs Absence (n = 23)	1.191	0.561	2.529	**0.650**				

Abbreviations used: CRC, colorectal cancer; ARID1A, AT-rich interactive domain-containing protein 1A; mLNR, metastatic lymph node ratio; AJCC, American Joint Committee on Cancer; p, pathological; TNM, tumor (T), nodes (N), and metastases (M). **p* - value < 0.05 was considered to indicate statistical significance.

Correlation between the mRNA expression of ARID1A and the biomarkers involved in lymph node metastasis of CRC

Several biomarkers associated with LNM in CRC have been previously reported by Zhu et al. in 2024 [15]. We further investigated the correlation between the mRNA expression of ARID1A and LNM-associated genes in CRC using the GEPIA2 database. The analysis demonstrated a significant correlation between ARID1A mRNA expression and LNM-associated genes, including *aquaporin 1* (*AQP1*), *aquaporin 3 (AQP3)*, *aquaporin 5 (AQP5)*, *endothelial cell adhesion molecule* (*ESAM*), *fms related receptor tyrosine kinase 4* (*FLT4*) (also known as *vascular endothelial growth factor receptor 3* (*VEGFR-3*)), *heat shock protein 47* (*HSP47*) (also known as *serpin family H member 1 (SERPINH1)), insulin like growth factor 1 receptor* (*IGF1R*), *Nav1.6* (also known as *sodium voltage-gated channel alpha subunit 8* (*SCN8A*)), *prospero homeobox 1* (*PROX1*), *VEGF-C*, and *VEGF-D.* Among the LNM-associated genes, *IGF1R* showed the strongest correlation with *ARID1A* (**Figures 4(A) - 4(B)**). In contrast, *caudal type homeobox 2* (*CDX2*), *IMP U3 small nucleolar ribonucleoprotein 3* (*IMP3*), *protein tyrosine phosphatase 4A3* (*PTP4A3*) (also known as *phosphatase of regenerating liver-3* (*PRL-3*)), and *tyrosine kinase receptor B* (*TrkB*) did not show a significant correlation with *ARID1A* (**Figure 4(A)**).

Survival analysis of the lymph node metastasis-associated gene in colon and rectal adenocarcinoma

The prognostic significance of each LNM-associated gene was evaluated in COAD and READ using GEPIA2-based survival analysis. In COAD patients, high expression levels of *HSP47* (also known as *SERPINH1*) and *VEGF-C* were significantly associated with worse OS. In READ patients, low expression of *IGF1R* and high expression of *VEGF-C* were similarly associated with poorer OS. Notably, elevated *VEGF-C* expression demonstrated consistent prognostic significance in both COAD and READ cohorts, underscoring its potential as a shared biomarker of adverse outcomes (**Figures 4(C) - 4(D)**).

IGF1R signaling promotes cancer cell proliferation and survival by driving cell cycle progression, inhibiting apoptosis, and inducing epithelial-mesenchymal transition process [35,36]. Likewise, *VEGF-C* is a well-established driver of CRC progression, particularly through its role in lymphangiogenesis and metastasis through VEGF-C/NOTCH signaling [37,38]. Clinically, VEGF-C overexpression has been associated with lymphatic involvement, LNM, depth of tumour invasion, and shorter survival in CRC [39]. Collectively, these findings suggest that *ARID1A* may regulate key molecular drivers of lymphangiogenesis and nodal spread, particularly through its interactions with *IGF1R* and *VEGF-C*, thereby highlighting the ARID1A-LNM axis as a potential target for prognostic evaluation and therapeutic intervention in CRC. However, the molecular mechanisms underlying the interactions between ARID1A, IGF1R, and VEGF-C remain unclear. Further *in vitro* studies are required to elucidate these pathways and to evaluate their potential as therapeutic targets in CRC.

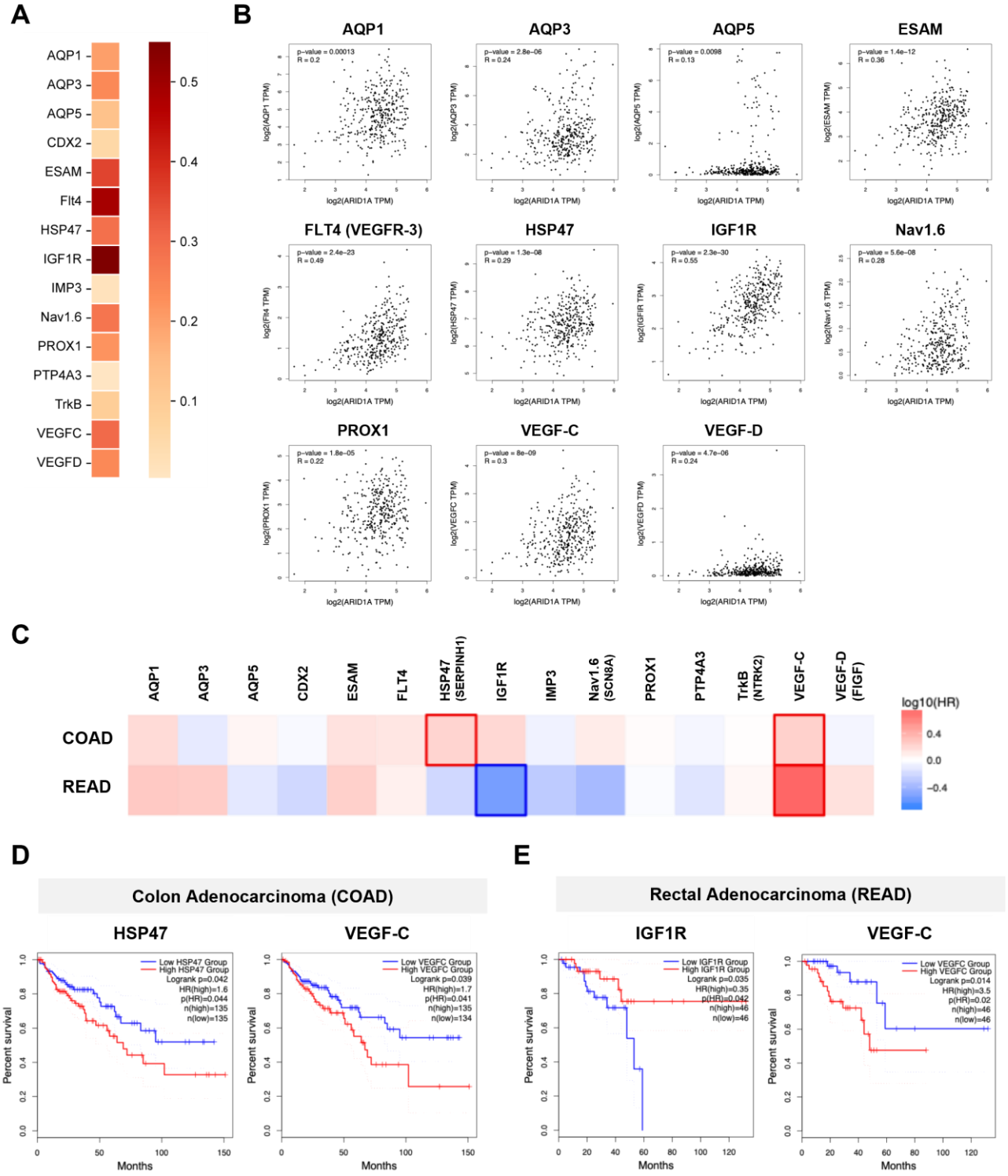

Figure 4 Correlation between ARID1A and lymph node metastasis (LNM)-associated gene expression and their prognostic significance in colon (COAD) and rectal adenocarcinoma (READ). (A) Heatmap representing Spearman's correlation coefficient between ARID1A and LNM-associated genes based on TCGA-COAD and READ transcriptomic data obtained via the GEPIA2 platform. (B) Representative scatter plots showing the pairwise correlations between ARID1A and individual LNM-associated genes. (C) Survival heatmap displaying the hazard ratio for overall survival associated with each LNM-related gene in COAD and READ patients. (D-E) Kaplan–Meier survival curves generated via GEPIA2 for LNM-associated genes with significant prognostic impact on overall survival in (D) COAD and (E) READ patients.

Conclusions

Low ARID1A expression and high metastatic lymph node ratio (mLNR) are associated with aggressive tumor features and poorer prognosis in colorectal cancer. Patients with combined low ARID1A and high mLNR exhibited advanced disease, increased lymph node involvement, and reduced progression-free survival. Transcriptomic analysis further revealed significant correlations between *ARID1A* and key lymph node metastasis-related genes, including *IGF1R, HSP47,* and *VEGF-C,* which were prognostically relevant. These findings highlight the combined assessment of ARID1A and mLNR as a promising prognostic biomarker panel for risk stratification and personalized management in CRC.

Acknowledgements

This study was supported by the Thailand Science Research and Innovation (TSRI; grant number R2564B002). We would like to thank the Unit of Pathology, Sawanpracharak Hospital, Nakhonsawan, Thailand, for helping with CRC sample collection and preparation, as well as all demographic and clinicopathological information of patients, and the Department of Anatomy, Faculty of Medical Science, Naresuan University, Phitsanulok, Thailand, for providing a great opportunity to perform the research. Also, we are grateful to Mr. Roy I. Morien of the Naresuan University Graduate School for his efforts in editing the grammar, syntax, and general English expression in this paper. Furthermore, we would like to acknowledge all of the colleagues in the NS lab.

Declaration of Generative AI in Scientific Writing

ChatGPT and Grammarly were used to improve the readability and language of the manuscript. Following the use of these tools, the authors carefully reviewed, revised, and approved all content. The authors take full responsibility for the intellectual content, scientific accuracy, and integrity of the published work.

CRediT Author Statement

Phattarapon Sonthi: Conceptualization; Methodology; Investigation; Formal analysis; Visualization; Writing - Original Draft. **Keerakarn Somsuan:** Conceptualization; Methodology; Investigation; Formal analysis; Visualization; Writing - Review & Editing. **Ratirath Samol:** Resources; Investigation. **Siripat Aluksanasuwan:** Formal analysis; Visualization; Writing - Review & Editing. **Yupa Srithongchai:** Resources; Investigation. **Netnaphis Warnnissorn:** Formal analysis. **Natthiya Sakulsak:** Conceptualization; Supervision; Funding acquisition; Writing - Review & Editing.

References

[1] H Sung, J Ferlay, RL Siegel, M Laversanne, I Soerjomataram, A Jemal and F Bray. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: A Cancer Journal for Clinicians* 2021; **71(3)**, 209-249.

[2] M Arnold, MS Sierra, M Laversanne, I Soerjomataram, A Jemal and F Bray. Global patterns and trends in colorectal cancer incidence and mortality. *Gut* 2017; **66(4)**, 683-691.

[3] RL Siegel, KD Miller, AG Sauer, SA Fedewa, LF Butterly, JC Anderson, A Cercek, RA Smith and A Jemal. Colorectal cancer statistics, 2020. *CA: A Cancer Journal for Clinicians* 2020; **70(3)**, 145-164.

[4] F Bray, J Ferlay, I Soerjomataram, RL Siegel, LA Torre and A Jemal. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: A Cancer Journal for Clinicians* 2018; **68(6)**, 394-424.

[5] X Wang, NG Nagl, D Wilsker, MV Scoy, S Pacchione, P Yaciuk, PB Dallas and E Moran. Two related ARID family proteins are alternative subunits of human SWI/SNF complexes. *The Biochemical Journal* 2004; **383(2)**, 319-325.

[6] RC Wu, TL Wang and IM Shih. The emerging roles of ARID1A in tumor suppression. *Cancer Biology & Therapy* 2014; **15(6)**, 655-664.

[7] R Mathur. ARID1A loss in cancer: Towards a mechanistic understanding. *Pharmacology & Therapeutics* 2018; **190**, 15-23.

[8] KC Wiegand, SP Shah, OM Al-Agha, Y Zhao, K Tse, T Zeng, J Senz, MK McConechy, MS Anglesio, SE Kalloger, W Yang, A Heravi-Moussavi, R Giuliany, C Chow, J Fee, A Zayed, L

Prentice, N Melnyk, G Turashvili, AD Delaney, …, DG Huntsman. ARID1A mutations in endometriosis-associated ovarian carcinomas. *The New England Journal of Medicine* 2010; **363(16)**, 1532-1543.

[9] Y Kishida, T Oishi, T Sugino, A Shiomi, K Urakami, M Kusuhara, K Yamaguchi, Y Kitagawa and H Ono. Associations between loss of ARID1A expression and clinicopathologic and genetic variables in T1 early colorectal cancer. *American Journal of Clinical Pathology* 2019; **152(4)**, 463-470.

[10] M Erfani, SV Hosseini, M Mokhtari, M Zamani, K Tahmasebi, MA Naini, A Taghavi, JM Carethers, M Koi, H Brim, P Mokarram and H Ashktorab. Altered ARID1A expression in colorectal cancer. *BMC Cancer* 2020; **20(1)**, 350.

[11] W Mongkolwat, P Sonthi, K Somsuan, S Aluksanasuwan, R Samol, N Sakulsak and S Wanna-Udom. Relationship between the protein expression of ARID1A, ARID1B and ARID2 with the clinicopathological characteristics of colorectal cancer. *Biomedical Reports* 2025; **23(1)**, 119.

[12] HJ Kim and GS Choi. Clinical implications of lymph node metastasis in colorectal cancer: Current status and future perspectives. *Annals of Coloproctology* 2019; **35(3)**, 109-117.

[13] FL Greene, AK Stewart and HJ Norton. A new TNM staging strategy for node-positive (stage III) colon cancer: An analysis of 50,042 patients. *Annals of Surgery* 2002; **236(4)**, 416-421.

[14] LL Gunderson, JM Jessup, DJ Sargent, FL Greene and AK Stewart. Revised TN categorization for colon cancer based on national survival outcomes data. *Journal of Clinical Oncology* 2010; **28(2)**, 264-271.

[15] X Zhu, SQ Lin, J Xie, LH Wang, LJ Zhang, LL Xu, JG Xu and YB Lv. Biomarkers of lymph node metastasis in colorectal cancer: Update. *Frontiers in Oncology* 2024; **14**, 1409627.

[16] E Bando, Y Yonemura, K Taniguchi, S Fushida, T Fujimura and K Miwa. Outcome of ratio of lymph node metastasis in gastric carcinoma. *Annals of Surgical Oncology* 2002; **9(8)**, 775-784.

[17] AC Berger, JC Watson, EA Ross and JP Hoffman. The metastatic/examined lymph node ratio is an important prognostic factor after pancreaticoduodenectomy for pancreatic adenocarcinoma. *The American Surgeon* 2004; **70(3)**, 235-240.

[18] AC Berger, ER Sigurdson, T LeVoyer, A Hanlon, RJ Mayer, JS Macdonald, PJ Catalano and DG Haller. Colon cancer survival is associated with decreasing ratio of metastatic to examined lymph nodes. *Journal of Clinical Oncology* 2005; **23(34)**, 8706-8712.

[19] R Greenberg, R Itah, R Ghinea, E Sacham-Shmueli, R Inbar and S Avital. Metastatic lymph node ratio (LNR) as a prognostic variable in colorectal cancer patients undergoing laparoscopic resection. *Techniques in Coloproctology* 2011; **15(3)**, 273-279.

[20] JS Pyo, YM Shin and DW Kang. Prognostic implication of metastatic lymph node ratio in colorectal cancers: Comparison depending on tumor location. *Journal of Clinical Medicine* 2019; **8(11)**, 1812.

[21] FR Hirsch, M Varella-Garcia, PA Bunn, MVD Maria, R Veve, RM Bremmes, AE Baron, C Zeng and WA Franklin. Epidermal growth factor receptor in non-small-cell lung carcinomas: Correlation between gene copy number and protein expression and impact on prognosis. *Journal of Clinical Oncology* 2003; **21(20)**, 3798-3807.

[22] J Schindelin, I Arganda-Carreras, E Frise, V Kaynig, M Longair, T Pietzsch, S Preibisch, C Rueden, S Saalfeld, B Schmid, JY Tinevez, DJ White, V Hartenstein, K Eliceiri, P Tomancak and A Cardona. Fiji: An open-source platform for biological-image analysis. *Nature Methods* 2012; **9(7)**, 676-682.

[23] M Numata, S Morinaga, T Watanabe, H Tamagawa, N Yamamoto, M Shiozawa, Y Nakamura, Y Kameda, S Okawa, Y Rino, M Akaike, M Masuda and Y Miyagi. The clinical significance of SWI/SNF complex in pancreatic cancer. *International Journal of Oncology* 2013; **42(2)**, 403-410.

[24] K Derwinger, G Carlsson and B Gustavsson. A study of lymph node ratio as a prognostic marker in colon cancer. *European Journal of Surgical Oncology* 2008; **34(7)**, 771-775.

[25] CC Chin, JY Wang, CY Yeh, YH Kuo, WS Huang and CH Yeh. Metastatic lymph node ratio is a more precise predictor of prognosis than number of lymph node metastases in stage III colon cancer. *International Journal of Colorectal Disease* 2009; **24(11)**, 1297-1302.

[26] Z Tang, B Kang, C Li, T Chen and Z Zhang. GEPIA2: An enhanced web server for large-scale expression profiling and interactive analysis. *Nucleic acids research* 2019; **47(1)**, 556-560.

[27] R Wang, M Chen, X Ye and K Poon. Role and potential clinical utility of ARID1A in gastrointestinal malignancy. *Mutation Research Reviews in Mutation Research* 2021; **787**, 108360.

[28] C Xie, L Fu, Y Han, Q Li, E Wang. Decreased ARID1A expression facilitates cell proliferation and inhibits 5-fluorouracil-induced apoptosis in colorectal carcinoma. *Tumour biology* 2014; **35(8)**, 7921-7927.

[29] XL Wei, DS Wang, SY Xi, WJ Wu, DL Chen, ZL Zeng, RY Wang, YX Huang, Y Jin, F Wang, MZ Qiu, HY Luo, DS Zhang and RH Xu. Clinicopathologic and prognostic relevance of ARID1A protein loss in colorectal cancer. *World Journal of Gastroenterology* 2014; **20(48)**, 18404-18412.

[30] A Chou, CW Toon, A Clarkson, L Sioson, M Houang, N Watson, K DeSilva and AJ Gill. Loss of ARID1A expression in colorectal carcinoma is strongly associated with mismatch repair deficiency. *Human Pathology* 2014; **45(8)**, 1697-1703.

[31] S Aluksanasuwan, K Somsuan, S Wanna-Udom, S Roytrakul, A Morchang, A Rongjumnong and N Sakulsak. Proteomic insights into the regulatory function of ARID1A in colon cancer cells. *Oncology letters* 2024; **28(2)**, 392.

[32] CC Chen, JC Lin and KW Chen. Lymph node ratio as a prognostic factor in head and neck cancer patients. *Radiation Oncology* 2015; **10**, 181.

[33] Z Feng, QS Xu, C Wang, JZ Li, MH Mao, H Li, LZ Qin and Z Han. Lymph node ratio is associated with adverse clinicopathological features and is a crucial nodal parameter for oral and oropharyngeal cancer. *Scientific Reports* 2017; **7(1)**, 6708.

[34] D Ding, W Stokes, M Eguchi, M Hararah, W Sumner, A Amini, J Goddard, H Somerset, C Bradley, J McDermott, D Raben and SD Karam. Association between lymph node ratio and recurrence and survival outcomes in patients with oral cavity cancer. *JAMA Otolaryngology- Head & Neck Surgery* 2019; **145(1)**, 53-61.

[35] H Hua, Q Kong, J Yin, J Zhang and Y Jiang. Insulin-like growth factor receptor signaling in tumorigenesis and drug resistance: A challenge for cancer therapy. *Journal of Hematology & Oncology* 2020; **13(1)**, 64.

[36] X Ye, WL Tam, T Shibue, Y Kaygusuz, F Reinhardt, EN Eaton and RA Weinberg. Distinct EMT programs control normal mammary stem cells and tumour-initiating cells. *Nature* 2015; **525(7568)**, 256-260.

[37] S Jdeed, E Erdos, BL Balint and IP Uray. The Role of ARID1A in the nonestrogenic modulation of IGF-1 signaling. *Molecular Cancer Research* 2022; **20(7)**, 1071-1082.

[38] S Poghosyan, N Frenkel, L van den Bent, D Raats, T Spaapen, J Laoukili, IB Rinkes, O Kranenburg and J Hagendoorn. VEGF-C propagates 'onward' colorectal cancer metastasis from liver to lung. *British Journal of Cancer* 2025; **132(1)**, 69-80.

[39] K Akagi, Y Ikeda, M Miyazaki, T Abe, J Kinoshita, Y Maehara and K Sugimachi. Vascular endothelial growth factor-C (VEGF-C) expression in human colorectal cancer tissues. *British Journal of Cancer* 2000; **83(7)**, 887-891.