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Original contribution

Loss of ARID1A expression is associated with DNA mismatch repair protein deficiency and favorable prognosis in advanced stage surgically resected esophageal adenocarcinoma☆,☆☆

Brett M. Lowenthal MD[a], Katie S. Nason MD,MPH[b], Arjun Pennathur MD[b], James D. Luketich MD[b], Reetesh K. Pai MD[a], Jon M. Davison MD[a], Changqing Ma MD, PhD[a,*]

[a]*Department of Pathology, University of Pittsburgh Medical Center, Pittsburgh, PA, 15213*
[b]*Department of Cardiothoracic Surgery, University of Pittsburgh Medical Center, Pittsburgh, PA, 15213*

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Summary Esophageal adenocarcinoma often presents at an advanced stage and has a dismal prognosis. Current prognostic markers have limited utility. *ARID1A* is implicated as a tumor suppressor gene in esophageal adenocarcinoma. Loss of ARID1A expression correlates with DNA mismatch repair (MMR) protein deficiency in other tumors. We hypothesized that ARID1A loss is associated with prognosis and DNA MMR protein deficiency in esophageal adenocarcinoma. Tissue microarrays representing 316 surgically resected esophageal adenocarcinomas without neoadjuvant treatment were evaluated for ARID1A and MMR proteins by immunohistochemistry. Loss of ARID1A expression (ARID1A-loss) was detected in 41 of 316 (13%) adenocarcinomas. MMR deficiency was identified in 5% (17/316) but was detected more frequently in ARID1A-loss adenocarcinomas (13/41, 32%) than in ARID1A-retained adenocarcinomas (4/275, 1%; $P < .001$). Morphologically, ARID1A-loss adenocarcinomas frequently demonstrated peritumoral lymphoid aggregates (90%) and tumor infiltrating lymphocytes (51%). In patients with locally advanced or metastatic disease (stages III or IV, N = 169), patients with ARID1A-loss adenocarcinomas (N = 22) had longer overall survival than patients with ARID1A-retained adenocarcinomas (median [month]: 26 vs. 16, $P = .010$). In these patients, ARID1A-loss correlated with a 56% reduction in mortality independent of other prognostic factors ($P = .007$). In summary, loss of ARID1A expression is associated with DNA MMR protein deficiency in esophageal adenocarcinoma. Furthermore, ARID1A loss is independently associated with a more favorable prognosis for patients with locally advanced or metastatic esophageal adenocarcinomas.

1. Introduction

Esophageal cancer is the eleventh leading cause of cancer death in the United States (US) [1,2]. While both squamous cell carcinoma and adenocarcinoma are diagnosed in the US

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* Corresponding author at: University of Pittsburgh, Department of Pathology, Presbyterian Hospital, 200 Lothrop Street, Room A-610, Pittsburgh, PA 15213.
E-mail address: mac2@upmc.edu (C. Ma).

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population, adenocarcinoma comprises the majority of cases. Over the past several decades the incidence of esophageal adenocarcinoma has rapidly increased more than 6-fold in the US population and continues to rise [3]. Esophageal adenocarcinoma is a highly aggressive disease as most are diagnosed at an advanced stage with either regional lymph node metastasis (in 30% of cases) or distant metastasis (in 40% of cases) [2,4]. Despite recent advances in detection and treatment, the overall 5-year survival rate of esophageal adenocarcinoma remains dismal and is less than 20% [2,4]. These population statistics underscore the need for improving our understanding of the pathogenesis of esophageal adenocarcinoma, developing novel, effective treatment options, and biomarkers identification.

The AT-rich interactive domain containing protein 1A (ARID1A, also known as BAF250a) is a key subunit of the chromatin remodeling complex SWItch/Sucrose Non-Fermentable (SWI/SNF) [5]. *ARID1A* is a tumor suppressor gene in gynecologic malignancies [6] and has also been identified as a significantly mutated gene in carcinomas arising in the gastrointestinal tract including carcinomas of colorectum, stomach, and esophagus [7-9]. Loss of ARID1A immunohistochemistry expression has been associated with sporadic microsatellite instability-high (MSI-H) / DNA mismatch repair (MMR) protein deficiency in gynecologic malignancies and adenocarcinomas of colorectum and stomach [10-13]. Interestingly, a recent study by Shen et al demonstrated that in ovarian cancer ARID1A protein promotes DNA mismatch repair by recruiting MSH2 to chromatin during replication and ARID1A deficiency compromises DNA mismatch repair [14].

In esophageal cancer, *ARID1A* gene alterations have been detected in 8% to 17% of adenocarcinomas [9,15,16]. In a recent study by Frankell et al, *ARID1A* gene alterations were identified in 13% of 551 esophageal adenocarcinomas and the majority of them were considered as driver mutations [16]. In contrast, loss of ARID1A immunohistochemistry expression was detected in about 10% of esophageal adenocarcinomas [17,18]. Furthermore, loss of ARID1A expression and *ARID1A* gene alterations were not only detected in esophageal adenocarcinomas but also in precursor lesions of esophageal adenocarcinomas including Barrett's esophagus without dysplasia and Barrett's-associated dysplasia [18,19], indicating *ARID1A* and its protein alterations happen early in the metaplasia-dysplasia-carcinoma sequence. In vitro studies further demonstrated *ARID1A* gene inactivation promoted cell growth and invasion, suggesting that *ARID1A* is a tumor suppressor gene for esophageal adenocarcinoma [18]. Giving these findings, we hypothesized that ARID1A expression is associated with prognosis and DNA MMR deficiency in esophageal adenocarcinoma. To test these hypotheses, we evaluated ARID1A expression and DNA MMR proteins by immunohistochemistry in 316 esophageal adenocarcinomas and correlated ARID1A expression pattern with clinical variables, histopathologic features, DNA MMR protein status, and survival.

2. Materials and methods

2.1. Study group

Patients who had primary surgical resection for esophageal adenocarcinomas including adenocarcinomas arising in proximal, mid, and distal esophagus and adenocarcinomas of the gastroesophageal junction were identified retrospectively through a search of the surgical pathology archive of the University of Pittsburgh between 1996 and 2009 [20]. All patients included in this study did not receive neoadjuvant treatment prior to surgical resection. Clinical and pathologic data were collected through review of medical records. Charlson comorbidity index is a weighted measurement used to predict the one-year mortality for patients with multiple comorbidities [21]. Study approval was obtained from the Institutional Review Boards at the University of Pittsburgh.

For each adenocarcinoma, hematoxylin and eosin stained slides were reviewed to confirm the diagnosis, histologic grade, stage, lymphovascular invasion and perineural invasion. Representative tumor blocks were selected for tissue microarrays construction. Each adenocarcinoma was represented by three 0.6-mm cores on the tissue microarrays. For adenocarcinomas with more than one morphologic growth pattern, tissue cores were selected to include all morphologic patterns present within an individual tumor.

2.2. Immunohistochemistry, in-situ hybridization, and interpretation

Immunohistochemistry stains were performed using automated stainers according to the manufacturers' recommendations on unstained 4-μm tissue sections. Antibodies used in this study included those against ARID1A (polyclonal, Sigma-Aldrich, HPA005456), MLH1 (clone G168–728, Ventana), PMS2 (clone EPR3947, Cell Marque), MSH2 (clone G219–1129, Ventana), and MSH6 (clone 44, Ventana).

Retained ARID1A staining was defined as nuclear staining in tumor cells in any tissue core of an adenocarcinoma, regardless of staining intensity or the proportion of tumor cells with nuclear staining. Loss of ARID1A staining was defined as no ARID1A nuclear staining in all tumor cells in all tissue cores of an adenocarcinoma on tissue microarrays with retained ARID1A staining observed in internal control non-neoplastic cells.

Forty-two adenocarcinomas demonstrated loss of ARID1A staining and 274 demonstrated retained ARID1A staining by tissue microarray. ARID1A immunohistochemistry was additional performed using whole tumor sections for 14 adenocarcinomas including 4 adenocarcinomas with retained ARID1A staining and 10 adenocarcinomas with loss of ARID1A staining by tissue microarray. For all four adenocarcinomas with retained staining by tissue microarray, retained ARID1A staining was confirmed in whole tumor sections. Loss of ARID1A staining was confirmed on whole tumor sections

for nine of the 10 adenocarcinomas with loss of ARID1A staining by tissue microarray. The remaining one adenocarcinoma with ARID1A loss by tissue microarray demonstrated retained ARID1A staining in whole tumor section. Based on staining results of the whole tumor section, this tumor was designated as demonstrating retained ARID1A expression. Therefore, loss of ARID1A staining was observed in 41 adenocarcinomas.

For DNA MMR protein immunohistochemistry, retained staining for MLH1, PMS2, MSH2, and MSH6 was defined as nuclear staining in tumor cells in any tissue core of an adenocarcinoma. Loss of staining for either protein was defined as a complete loss of nuclear staining in all tumor cells in all tissue cores of an adenocarcinoma with retained nuclear staining observed in internal control non-neoplastic cells.

DNA MMR protein immunohistochemistry was also repeated using whole tissue sections of nine adenocarcinomas. For these tumors, results of DNA MMR protein immunohistochemistry performed on whole tissue sections and tissue microarrays were identical.

2.3. Pathology evaluation

All tumor slides from the 41 esophageal adenocarcinomas with loss of ARID1A expression were re-evaluated to look for histologic features reminiscent of MSI-H colorectal carcinoma including medullary features, mucinous component, signet ring cell component, peritumoral lymphoid aggregates and tumor infiltrating lymphocytes, as discussed by Drage et al [17]. The presence of tumor infiltrating lymphocytes was evaluated using the criteria by Greenson et al [22]. Briefly, tumor slides were evaluated at low magnification to find a "hotspot" area with the most tumor infiltrating lymphocytes. In the "hotspot" area, the number of tumor infiltrating lymphocytes was counted in 5 consecutive high-power (40× objective lens) fields. When on average the tumor infiltrating lymphocytes was more than 2 lymphocytes / high-power field (i.e. ≥3 lymphocytes / high-power field), the adenocarcinoma was scored as positive for tumor infiltrating lymphocytes.

2.4. Statistical analysis

Statistical analysis was performed with GraphPad Prism 7 for Windows Version 7.00 (GraphPad Software, La Jolla, CA) and IBM SPSS (Release 23.0.0.0). A P value less than 0.05 ($P < .05$) was considered statistically significant. Student t-test and Mann-Whitney U test were used for comparison of continuous variables. Two-sided Fisher's exact test and χ^2 test were used for the comparison of categorical data. For survival analysis, overall survival was defined as the duration between surgical resection and either death or the latest clinical follow-up time. Death occurring within 1 month of the initial operation was attributed to operative mortality and was not included in the survival analysis. Disease-free survival was the duration between surgical resection and disease recurrence documented by imaging and/or pathologic evaluation of metastasis.

3. Results

3.1. Loss of ARID1A immunohistochemistry expression is associated with DNA MMR protein deficiency in esophageal adenocarcinoma

A total of 316 esophageal adenocarcinoma cases included in the tissue microarrays had adequate tumor tissue available to evaluate ARID1A expression and DNA MMR protein status (Table 1). Loss of ARID1A immunohistochemistry expression was detected in 41 of 316 (13%) esophageal adenocarcinomas (Figure 1). In contrast, 17 of 316 (5%) esophageal adenocarcinomas were DNA MMR protein deficient including 15 (88%) adenocarcinomas with loss of MLH1 and PMS2 expression and 2 (12%) with loss of MSH2 and MSH6.

Esophageal adenocarcinomas with loss of ARID1A expression significantly more often demonstrated DNA MMR protein deficiency compared with adenocarcinomas with retained ARID1A expression (13/41, 32% versus 4/275, 1%, $P < .001$). All 13 ARID1A loss and DNA MMR deficient adenocarcinomas demonstrated loss of MLH1 and PMS2 expression while two of four ARID1A retained adenocarcinomas with DNA MMR deficiency had loss of MSH2 and MSH6 expression. In addition, nearly two-thirds (26/41, 63%) of esophageal adenocarcinomas with loss of ARID1A expression were poorly-differentiated (i.e. histologically high-grade) while only half (139/275, 51%) of those with retained ARID1A expression demonstrated histologic high-grade features, although this difference was not statistically significant ($P = .13$). In contrast, loss of ARID1A expression was not associated with pathologic stage, tumor location, Charlson comorbidity score, and risk factors for esophageal adenocarcinomas including smoking, and reflux disease/Barrett's esophagus.

3.2. Esophageal adenocarcinomas with loss of ARID1A expression demonstrate frequent tumor infiltrating lymphocytes and peritumoral lymphoid aggregates

Whole tumor sections of the 41 esophageal adenocarcinomas with loss of ARID1A expression were evaluated for features reminiscent of MSI-H carcinomas (Table 2 and Figure 2). The majority (37/41, 90%) of the esophageal adenocarcinomas with ARID1A loss demonstrated peritumoral lymphoid aggregates and half (21/41, 51%) were positive for tumor infiltrating lymphocytes (≥3/high-power field). Medullary features were observed in 12% (5/41) of the adenocarcinomas and so were mucinous features. Signet ring cell features were seen in 9 (22%) adenocarcinomas. Esophageal adenocarcinomas with

Table 1 Clinicopathologic characteristics of esophageal adenocarcinoma stratified by ARID1A expression by immunohistochemistry

	All cases (n, %)	Loss of ARID1A expression (n, %)	Retained ARID1A expression (n, %)	*P*
Number of cases	316	41 (13)	275 (87)	n.a.
DNA MMR protein status				
Proficient	299 (95)	28 (68)	271 (99)	<.001
Deficient	17 (5)	13 (32)	4 (1)	
MLH1 and PMS2 loss	15	13	2	
MSH2 and MSH6 loss	2	0	2	
Age (year; mean, range)	67 (23–91)	68 (47–88)	66 (23–91)	.37
Sex				
Male	260 (82)	34 (83)	226 (82)	1.00
Female	56 (18)	7 (17)	49 (18)	
Body mass index (mean, range)	29 (16–65)	30 (24–48)	29 (16–65)	.05
Current or past history of smoking				
Yes	218 (69)	32 (78)	186 (68)	.14
No	95 (30)	8 (20)	87 (32)	
Unknown	3 (1)	1 (2)	2 (0.7)	
History of reflux disease				
Yes	233 (74)	28 (68)	205 (75)	.34
No	79 (25)	13 (32)	66 (24)	
Unknown	4 (1)	0	4 (1)	
History of Barrett's esophagus				
Yes	219 (69)	23 (56)	196 (71)	.07
No	97 (31)	18 (44)	79 (29)	
Charlson comorbidity index [a]				
Low (0–1)	118 (37)	14 (34)	104 (38)	.91
Intermediate (2–5)	136 (43)	19 (46)	117 (43)	
High (> 5)	62 (20)	8 (20)	54 (20)	
Tumor location				
Mid/proximal esophagus	5 (2)	0	5 (2)	.93
Distal esophagus	89 (28)	11 (27)	78 (28)	
Gastroesophageal junction	218 (69)	30 (73)	188 (68)	
Unknown	4 (1)	0	4 (1)	
Tumor histologic grade				
Low (well–/moderately-differentiated)	151 (48)	15 (37)	136 (49)	.13
High (poorly-differentiated)	165 (52)	26 (63)	139 (51)	
Final clinical stage group (AJCC 7th edition)				
Stage I	100 (32)	10 (24)	90 (33)	.37
Stage II	47 (15)	9 (22)	38 (14)	
Stage III	158 (50)	20 (49)	138 (50)	
Stage IV	11 (3)	2 (5)	9 (3)	
Angiolymphatic invasion				
Yes	187 (59)	26 (63)	161 (59)	.61
No	129 (41)	15 (37)	114 (41)	
R0 resection				
Yes	299 (95)	38 (93)	261 (95)	.47
No	17 (5)	3 (7)	14 (5)	
Status at the time of data collection				
Alive	87 (28)	10 (24)	77 (28)	.71
Dead	229 (72)	31 (76)	198 (72)	
Overall survival (month, median, range)	31 (1–186)	34 (4–186)	29 (1–176)	.13
Recurrence (stage I – III cases)	N = 305	N = 39	N = 266	
Yes	120 (40)	16 (41)	104 (39)	.86
No	185 (60)	23 (59)	162 (61)	
Disease-free survival (month, median, range)	14 (1–176)	15 (1–138)	14 (1–176)	.89

[a] Charlson comorbidity index is a measurement used to predict the one-year mortality for patients with comorbidities.

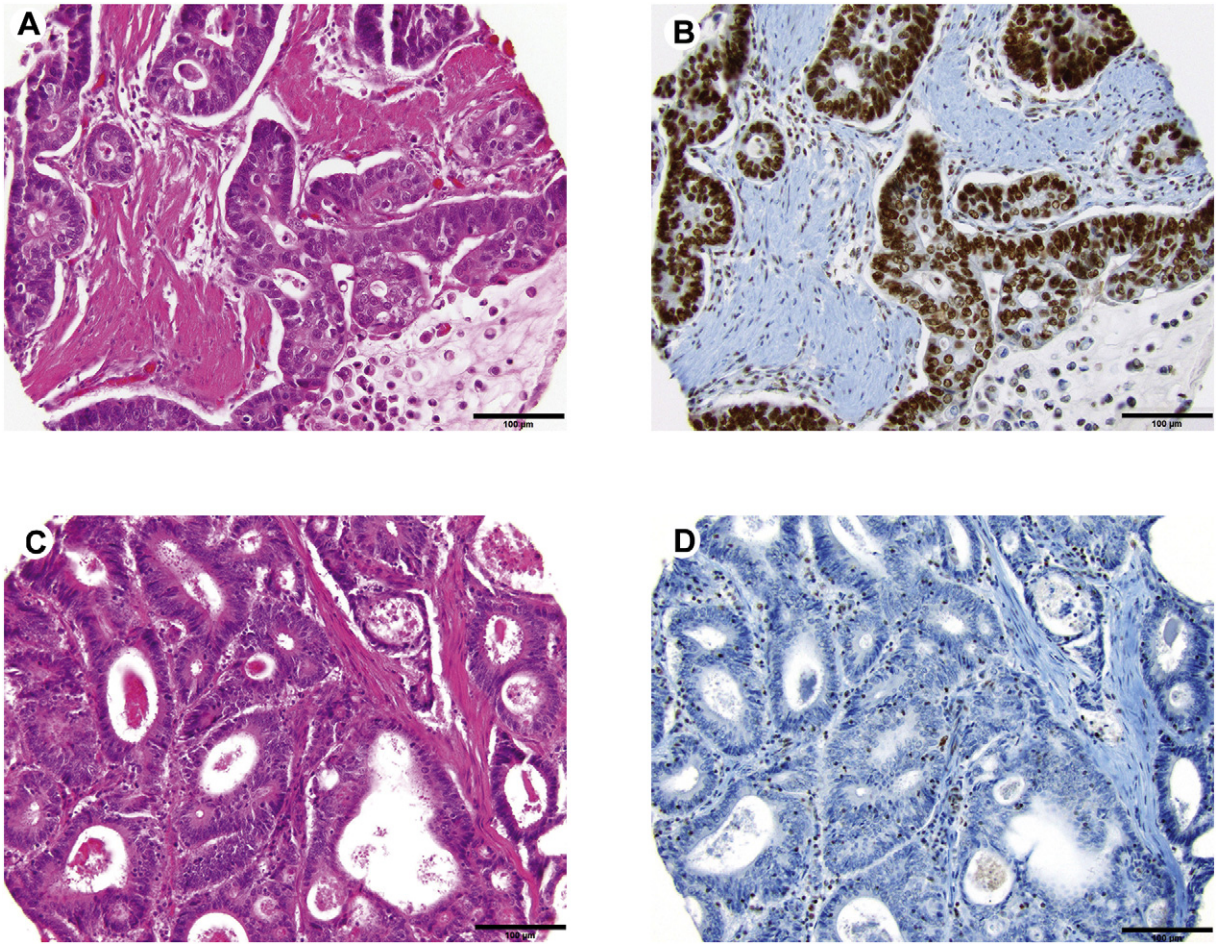

Figure 1 ARID1A immunohistochemistry expression in esophageal adenocarcinomas. (**A**) An esophageal adenocarcinoma with retained ARID1A nuclear expression (**B**) in tumor cells and non-neoplastic cells in stroma (internal positive control cells). (**C**) An esophageal adenocarcinoma with loss of ARID1A nuclear expression in tumor cells (**D**) and retained nuclear expression in non-neoplastic cells in stroma. Scale bars: 100 μm.

both ARID1A loss and DNA MMR protein deficiency demonstrated all these histologic features and so did ARID1A loss adenocarcinomas that were DNA MMR protein proficient. However, the ARID1A loss and DNA MMR protein deficient esophageal adenocarcinomas more frequently had tumor infiltrating lymphocytes than DNA MMR protein proficient, ARID1A loss adenocarcinomas (10/13, 77%, versus 11/28, 39%, $P = .04$).

3.3. Loss of ARID1A expression is associated with improved overall survival in advanced stage esophageal adenocarcinomas

The median follow-up time of the entire cohort was 31 months (range: 1.2–186 months) (Table 1). The median follow-up time was 29 months (range: 1.2–176 months) for patients with esophageal adenocarcinomas with retained

Table 2 Histologic features of esophageal adenocarcinomas with loss of ARID1A expression

	All cases with ARID1A loss (n, %)	ARID1A loss		*P*
		DNA MMR protein proficient (n, %)	DNA MMR protein deficient (n, %)	
Number of cases	41	28	13	n.a.
Positive for tumor infiltrating lymphocytes (≥3 lymphocytes / high-power field)	21 (51)	11 (39)	10 (77)	.04
Peritumoral lymphoid aggregates	37 (90)	26 (93)	11 (85)	.58
Medullary features	5 (12)	2 (7)	3 (23)	.30
Mucinous features	5 (12)	3 (11)	2 (15)	.65
Signet ring cell features	9 (22)	8 (29)	1 (8)	.23

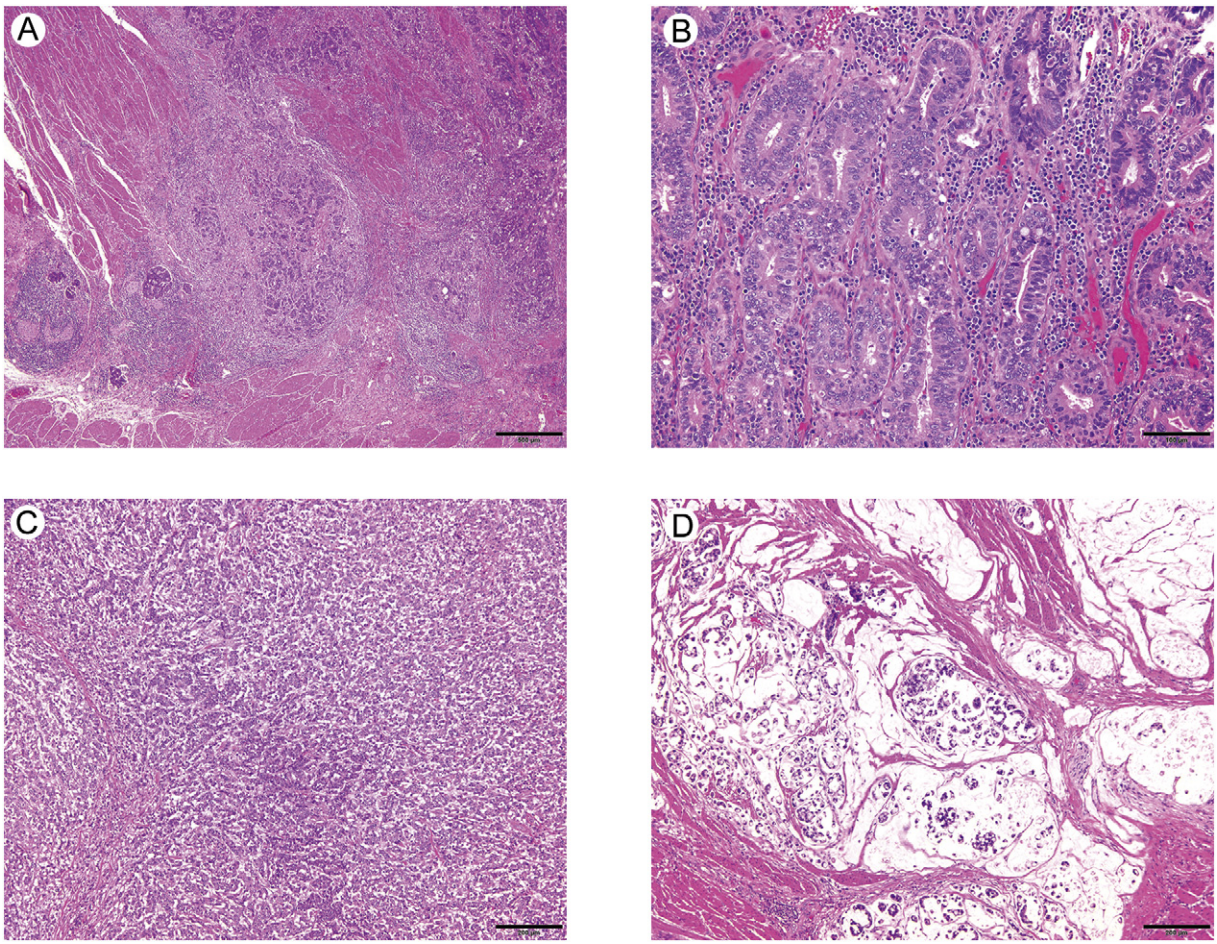

Figure 2 Esophageal adenocarcinomas with ARID1A loss demonstrate a variety of histologic features including prominent peritumoral lymphoid aggregates (**A**), tumor-infiltrating lymphocytes (**B**), medullary features with numerous tumor infiltrating lymphocytes (**C**), and mucinous and/or signet ring cell features (**D**). Scale bars: **A**-500 μm, **B**-100 μm, **C**-200 μm, **D**-200 μm.

ARID1A expression and 34 months (range: 4–186 months) for those with ARID1A loss esophageal adenocarcinomas. There were 229 deaths occurring between 1.2 month and 146 months from the time of surgery. Using Kaplan–Meier survival functions, age, clinical history of Barrett's esophagus, clinical stage, histologic grade, angiolymphatic invasion, and microscopic margin-negative surgical resection (R0 resection) were significantly associated with overall survival in the complete cohort (**Supplementary Table 1**). However, neither ARID1A loss nor DNA MMR protein deficiency was associated with overall survival ($P > .05$ for both).

Since the majority of esophageal adenocarcinomas were diagnosed at advanced stages with either regional lymph node metastasis or distant metastasis [2], survival analysis was performed for the patients with advanced clinical stages, i.e. stage III or stage IV esophageal adenocarcinomas (N = 169, 53%). Of the 169 adenocarcinomas, **22** (**13**%) demonstrated loss of ARID1A expression while 9 (5%) were DNA MMR protein deficient. Loss of ARID1A expression remained significantly associated with DNA MMR protein deficiency (ARID1A loss and MMR protein deficient: 7/**22**, **32**%, versus ARID1A retained and MMR protein deficient: 2/**147**, 1%, $P < .001$) (**Supplementary Table 2**).

The median follow-up time of the 169 patients with stage III or stage IV esophageal adenocarcinomas was 18 months (range: 1.2–186 months). There were 149 deaths occurring between 1.2 month and 99 months from the time of surgery. Kaplan–Meier survival analysis showed that patients with ARID1A loss adenocarcinomas lived significantly longer than those with ARID1A retained adenocarcinomas (median and 95% confidence interval [95% CI] of overall survival: 26 months [11–41] versus 16 months [11-21], $P = .010$) (Figure 3A). In addition, DNA MMR protein deficiency was also significantly associated with improved overall survival in patients with stage III and IV esophageal adenocarcinomas by Kaplan–Meier function (MMR deficient versus proficient [median and 95% CI]: 34 months [24–45] versus 18 months [14-22], $P = .02$).

Using Cox proportional hazards modeling for the 169 patients with stage III or stage IV esophageal adenocarcinomas, features associated with improved overall survival on both

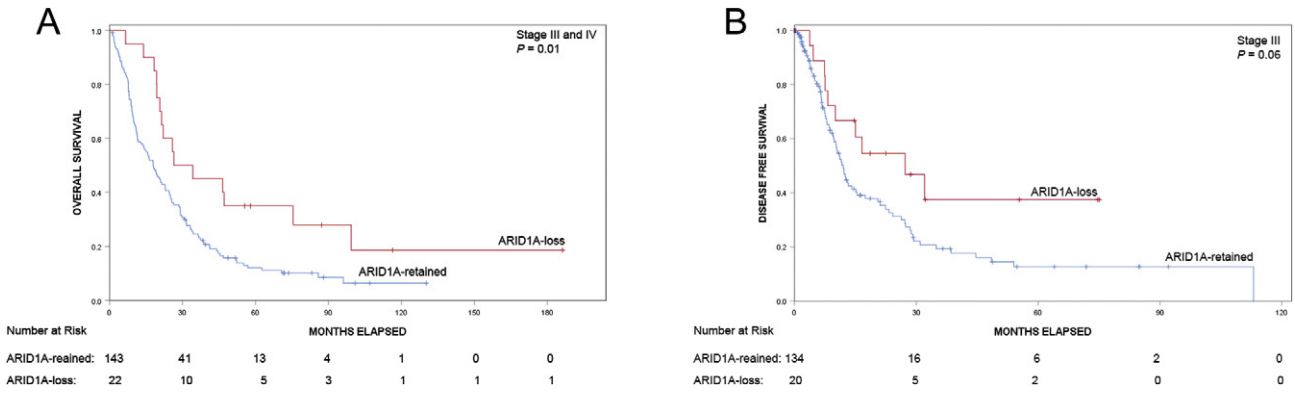

Figure 3 **A**, Kaplan–Meier survival curve comparing the overall survival of patients with clinical stage III and stage IV esophageal adenocarcinomas stratified by ARID1A expression. **B**, Kaplan–Meier survival curve comparing the disease-free survival of patients with clinical stage III esophageal adenocarcinomas stratified by ARID1A expression.

univariate and multivariate analysis were loss of ARID1A expression (multivariate analysis hazard ratio and 95% CI: 0.44, 0.23–0.80, $P = .007$) and R0 resection (Table 3). DNA MMR protein deficiency and clinical stage III were associated with improved overall survival by univariate analysis but not by multivariate analysis.

Disease-free survival was also studied for the 305 patients with stages I to III esophageal adenocarcinomas and for the 158 patients with stage III esophageal adenocarcinomas. Patients with stage IV esophageal adenocarcinomas (N = 11) were considered never disease-free and thus were excluded from disease-free survival analysis. Using Kaplan–Meier function, neither loss of ARID1A expression nor DNA MMR protein deficiency was significantly associated with disease-free survival ($P > .05$ for both) (**Supplementary Table 3**).

For the 158 patients with stage III disease, 91 (58%) patients had disease recurrence. Patients with ARID1A loss esophageal adenocarcinomas had longer disease-free survival compared to patients with ARID1A retained adenocarcinomas by Kaplan–Meier survival analysis (median [95% CI]: 27 months [9–45] versus 12 months [10-14], $P = .063$) (Figure 3B) but this was not statistically significant. DNA MMR deficiency had only a borderline association with improved disease-free survival in patients with stage III adenocarcinomas ($P = .058$) by Kaplan–Meier method. Using Cox proportional hazards modeling, microscopic margin-negative surgical resection (R0 resection) was associated with a significant reduction in the likelihood of recurrence on univariate analysis (hazard ratio and 95% CI: 0.21, 0.10–0.48, $P < .001$) in patients with stage III adenocarcinomas (**Supplementary Table 4**).

Table 3 Univariate and multivariate analysis of overall survival in stage III and stage IV esophageal adenocarcinoma by Cox regression

Overall survival	Stage III and IV esophageal adenocarcinomas			
	Univariate analysis		Multivariate analysis	
Clinicopathologic features Stage III and IV cases	*P*	Hazard ratio (95% confidence interval)	*P*	Hazard ratio (95% confidence interval)
Age	.39	1.00 (.99–1.02)	–	–
Male sex	.64	.9 (.58–1.40)	–	–
Body mass index	.51	.99 (.96–1.02)	–	–
History of Barrett's esophagus	.40	1.15 (.83–1.60)	–	–
Current or past history of smoking	.60	1.11 (.75–1.64)	–	–
High comorbidity index	.59	1.09 (.79–1.51)	–	–
ARID1A loss	.01	.52 (.31–.86)	.007	.44 (.23–.80)
DNA MMR deficiency	.027	.40 (.17–.90)	.59	.77 (.30–1.99)
Clinical stage III vs. IV	<.001	.26 (.14–.49)	.07	.47 (.20–1.07)
Histologic high-grade vs low-grade	.002	1.74 (1.23–2.47)	.002	1.74 (1.22–2.45)
Positive angiolymphatic invasion	.18	1.41 (.86–2.31)	–	–
R0 resection	<.001	.24 (.14–.42)	.002	.32 (.15–.66)
Received post-surgery chemotherapy	.17	.65 (.36–1.19)	–	–

4. Discussion

In this study, we analyzed the significance of loss of ARID1A immunohistochemistry expression in 316 surgically resected esophageal adenocarcinomas from patients without presurgical treatment. Loss of ARID1A expression was identified in 13% of esophageal adenocarcinomas. Morphologically, esophageal adenocarcinomas with ARID1A loss frequently demonstrated tumor infiltrating lymphocytes and peritumoral lymphoid aggregates, features reminiscent of MSI-H colorectal adenocarcinoma. More importantly, loss of ARID1A expression in esophageal adenocarcinoma was significantly associated with DNA MMR protein deficiency. DNA MMR protein deficiency was detected in 5% of all esophageal adenocarcinomas in our cohort, but it was detected in near one-third (32%) of the esophageal adenocarcinomas with ARID1A loss and, in contrast, in only 1% of esophageal adenocarcinomas with retained ARID1A expression. Furthermore, loss of ARID1A expression was independently associated with improved overall survival in advanced stage esophageal adenocarcinomas in our cohort.

Loss of ARID1A immunohistochemistry expression has recently been reported in 10% of surgically resected, esophageal adenocarcinomas (N = 120) from patients without neoadjuvant treatment by Drage et al [17] and in 12% of surgically resected, treatment naïve esophageal adenocarcinomas (N = 98) by Streppel et al [18]. Similar to these two reports, our study used the same ARID1A polyclonal antibody and evaluated ARID1A expression in surgically resected, treatment naïve, adenocarcinomas. In our cohort, ARID1A loss was observed in a comparable proportion (13%) of esophageal adenocarcinomas. Furthermore, these tumors also demonstrated morphologic features, especially the high frequency of tumor infiltrating lymphocytes and peritumoral lymphoid aggregates, very similar to those reported by Drage et al [17]. However, our study is different from these two studies in several ways.

To our knowledge, this study represents the largest cohort of surgically resected, treatment naïve esophageal adenocarcinomas studied for ARID1A expression. Our cohort was also tested for DNA MMR protein status. Our results demonstrated that DNA MMR protein deficiency was significantly associated with ARID1A loss in esophageal adenocarcinomas. ARID1A loss has been significantly associated with MSI-H/DNA MMR protein deficiency in carcinomas arising in other segments of the gastrointestinal tract [11-13]. Such an association was demonstrated for the first time in esophageal adenocarcinoma in our study.

MSI-H/DNA MMR protein deficiency is only infrequently detected in esophageal adenocarcinomas and has been reported in 1% to 6% of these tumors [23-25]. Furthermore, esophageal adenocarcinomas with DNA MMR deficiency frequently demonstrate loss of MLH1 and PMS2 nuclear expression with *MLH1* promoter hypermethylation, suggesting sporadic MSI-H [24]. Consistent with the literature, loss of MLH1 and PMS2 expression was detected in the majority (88%) of the DNA MMR deficient esophageal adenocarcinomas in our cohort. Taken together, our results may indicate that loss of ARID1A expression is likely associated with sporadic MSI-H in esophageal adenocarcinoma, although *MLH1* promoter hypermethylation was not performed.

Over two-thirds of ARID1A loss esophageal adenocarcinomas were MMR protein proficient. In these tumors, the underlying mechanisms responsible for the close resemblance of their morphologic features to those of ARID1A loss and MMR deficient adenocarcinomas remain unknown. A recent study demonstrated that in ovarian cancer, ARID1A protein recruited DNA mismatch repair protein MSH2 to chromatin during DNA replication and thus protected DNA from damage [14]. Loss of ARID1A expression or *ARID1A* inactivation, in contrast, compromised the repair of DNA damage during replication and was associated with microsatellite instability and increase in mutagenesis [14]. To our knowledge, the interaction between ARID1A and DNA MMR proteins has not been studied in esophageal adenocarcinoma. However, it may be worthwhile future investigation to delineate if similar interactions exist in esophageal adenocarcinoma and contribute to (i) the morphologic resemblance between MMR proficient and MMR deficient adenocarcinomas with ARID1A loss or (ii) survival advantage associated with ARID1A loss observed in our study. Taken together, results of our study may suggest that loss of ARID1A expression and DNA MMR deficiency, while infrequent, both are key players in carcinogenesis of esophageal adenocarcinomas and ARID1A loss may be the driver event in a subset of esophageal adenocarcinomas.

Locally advanced and metastatic esophageal adenocarcinoma carries a dismal prognosis [2,4]. To our knowledge, apart from the TNM system, there are no prognostic biomarkers currently in routine clinical use. *ARID1A* was detected as a significantly altered gene in esophageal adenocarcinoma and has been proposed as a tumor suppressor gene / driver gene in esophageal adenocarcinoma [9,15,16,18]. *ARID1A* gene alterations and loss of ARID1A expression were shown to be early events in the development of esophageal adenocarcinoma [18,19]. Based on these findings we hypothesized that loss ARID1A expression is associated with prognosis in esophageal adenocarcinoma. Indeed, our results showed loss of ARID1A expression was an independent, positive prognostic factor for overall survival of patients with locally advanced and/or metastatic (stage III or IV) esophageal adenocarcinomas. The prognostic significance of ARID1A loss was also studied by Drage et al [17] but no significant association was observed in their cohort. Compared with their study, our study included more cases and examined the prognostic significance of ARID1A loss in advanced stage esophageal adenocarcinomas. Additional large, well-characterized cohort with esophageal adenocarcinomas and with extended follow-up are needed to further determine the prognostic impact of ARID1A expression.

Currently, patients with advanced stage esophageal adenocarcinomas are treated with neoadjuvant treatment

with chemotherapy or chemoradiotherapy before surgical resection [4]. ARID1A expression status in esophageal adenocarcinoma might have clinical utility in stratifying patients with esophageal adenocarcinomas for pre-operative chemoradiation therapy or immunotherapy. In particular, ARID1A deficiency has been shown to increase mutation load in adenocarcinomas of the stomach and colorectum [14]. In vivo studies have demonstrated that ARID1A-deficient tumors of ovarian origin express programmed death-ligand 1 (PD-L1) and respond to anti-PD-L1 treatment [14]. ARID1A deficiency has also been shown to sensitize tumor cells of gynecologic primary to therapeutic agents targeting DNA damage response including poly (ADP-ribose) polymerase (PARP) inhibitors and ataxia-telangiectasia and Rad3-related protein kinase (ATR) inhibitors in vitro and in vivo [26-28]. In a recent study, irradiation which induces exogenous DNA damage has been shown to act synergistically with PARP inhibitors in treating ARID1A deficient tumors in vitro [29]. Currently PARP inhibitors in combination with other therapeutic agents are undergoing clinical studies for the treatment of metastatic or locally recurrent gastric and gastroesophageal junction cancers [30]. Although it is beyond the scope of our study, the utility of ARID1A deficiency in selecting patients for immunotherapy or therapeutic agents/regimens targeting DNA damage response in esophageal adenocarcinoma especially in locally advanced and/or metastatic esophageal adenocarcinoma should be investigated.

One limitation of our study is the inherent issue of heterogeneity of protein expression within tumors with a tissue microarray approach. The ARID1A antibody used in our study is the same as that used in previous published reports including the previously mentioned studies on esophageal adenocarcinomas, other gastrointestinal tract adenocarcinomas, and gynecologic malignancies [12,13,31,32]. Several of these studies performed on whole tumor tissue sections demonstrated that heterogeneity of ARID1A protein expression in gastrointestinal adenocarcinomas was rare [13,31]. The issue of heterogeneity was further investigated in our study by performing ARID1A immunohistochemistry on whole tumor sections in a subset of 14 adenocarcinomas. Whole tumor section immunohistochemistry confirmed the tissue microarray analysis results in 13/14 (93%) of adenocarcinomas including all four adenocarcinomas with retained ARID1A expression and nine of 10 adenocarcinomas with ARID1A loss by tissue microarray analysis. In the remaining adenocarcinoma, the whole tumor section showed focal areas without ARID1A expression but the remainder of the tumor showed heterogenous, weak and strong ARID1A expression. On review of the tissue microarray cores, ARID1A expression was not observed in the tissue cores. The high degree of concordance between tissue microarray cores and whole section immunohistochemistry results supports our estimate of the prevalence of ARID1A loss in esophageal adenocarcinomas using the tissue microarray approach. Another limitation of our study is the relatively small number of DNA MMR deficient esophageal adenocarcinomas in patients with stage III or stage IV diseases. Only nine of these patients had DNA MMR deficient adenocarcinomas. Our results showed DNA MMR protein deficiency was not significantly associated with either overall survival or disease-free survival in these patients but survival analysis with such a small number is likely underpowered. Lastly, our study is limited by the lack of *ARID1A* mutational analysis and tumor mutational burden analysis. Our study also represents a study on esophageal adenocarcinomas resected at large academic medical centers with its inherent referral bias.

In summary, this study is the largest series to date that evaluates ARID1A immunohistochemical expression in esophageal adenocarcinomas. Our results demonstrate that loss of ARID1A expression and DNA mismatch repair deficiency are both infrequent in esophageal adenocarcinomas. Similar to colorectal and gastric adenocarcinomas and for the first time, our result shows that loss of ARID1A expression also correlates with DNA mismatch repair deficiency in esophageal adenocarcinoma. Furthermore, loss of ARID1A expression is independently associated with a more favorable prognosis for patients with locally advanced or metastatic esophageal adenocarcinomas.

Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.humpath.2019.09.004.

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