Human Pathology (2019) **85**, 18–26

ELSEVIER

Human PATHOLOGY

www.elsevier.com/locate/humpath

Original contribution

DNA mismatch repair deficiency but not ARID1A loss is associated with prognosis in small intestinal adenocarcinoma☆

Iván González MD[a], Bella Goyal MD[a,1], Michelle D. Xia MD[b,2], Reetesh K. Pai MD[b], Changqing Ma MD, PhD[b,*]

[a]*Department of Pathology and Immunology, Washington University School of Medicine, St Louis, MO 63110*
[b]*Department of Pathology, University of Pittsburgh, School of Medicine, Pittsburgh, PA 15213*

Received 17 July 2018; revised 9 October 2018; accepted 18 October 2018

Keywords:
BAF250a;
Duodenal adenocarcinoma;
Nonampullary;
Microsatellite instability;
MSH2;
MSH6

Summary Small intestinal adenocarcinoma is an uncommon neoplasm with poor prognosis. It is clinically approached similarly to colorectal carcinoma (CRC). The prognostic value of DNA mismatch repair protein deficiency (dMMR) in CRC is well established, but its role in small intestinal adenocarcinoma remains inconclusive. Recently, loss of expression of ARID1A, a tumor suppressor gene product, by immunohistochemistry (IHC) was linked to dMMR and poor outcome in small intestinal adenocarcinoma, suggesting that it may be an emerging prognostic biomarker. We hypothesized that dMMR and/or ARID1A loss may be associated with clinical outcome in small intestinal adenocarcinoma. We examined dMMR and ARID1A loss by IHC in 120 surgically resected, nonampullary small intestinal adenocarcinomas collected from 2 tertiary centers. ARID1A loss was detected in 6 (7%) of 92 ARID1A-stained adenocarcinomas, whereas 21 (18%) of 120 adenocarcinomas demonstrated dMMR. ARID1A loss was not associated with survival or dMMR. dMMR adenocarcinomas had no distant metastasis, whereas 22 (22%) of 99 MMR-proficient adenocarcinomas had (P = .01). dMMR was an independent, positive predictor of disease-free survival (P = .035, hazard ratio: 0.2). Compared with dMMR CRC, dMMR small intestinal adenocarcinomas more frequently demonstrated loss of MSH2 and MSH6 and less often showed loss of MLH1 and PMS2 (both P < .001). In summary, ARID1A loss by IHC is uncommon in small intestinal adenocarcinomas. dMMR small intestinal adenocarcinomas are nonmetastatic tumors, frequently demonstrate loss of MSH2 and MSH6, and have superior disease-free survival. Our results suggest that all small intestinal adenocarcinomas should be tested for MMR protein deficiency.

1. Introduction

Small intestinal adenocarcinoma is a rare malignancy that is often diagnosed at an advanced stage [1]. The 5-year relative survival rate for patients affected by small intestinal adenocarcinomas is 40% in the United States (US) population [2]. Risk

☆ Disclosures: none.

* Corresponding author at: Department of Pathology, University of Pittsburgh, School of Medicine, 200 Lothrop St, A-610, Pittsburgh, PA 15213.
E-mail address: mac2@upmc.edu (C. Ma).
[1] Current address: California Pacific Medical Center, 2333 Buchannan St, San Francisco CA 94117.
[2] Current address: Inova Fairfax Hospital, 3300 Gallows Rd, Falls Church, VA 22042.

https://doi.org/10.1016/j.humpath.2018.10.013
0046-8177/

factors associated with poor prognosis after surgical resection include male sex, age older than 55 years, African American ethnicity, tumor location in the duodenum or ileum, tumor invasion of visceral peritoneum or other organs/structures, nodal metastasis, distant metastasis, poorly differentiated histologic grade, and positive surgical resection margins [2]. The incidence of small intestinal adenocarcinoma has been increasing over the past 3 decades in the US. However, during the same period of time, the 5-year survival rate after resection has remained largely unchanged [2]. These population statistics suggest that current treatment methods are ineffective in improving the long-term survival of patients affected by small intestinal adenocarcinoma and underscore the need for developing novel, effective treatment options and biomarkers for improved risk stratification.

Similar to colorectal adenocarcinoma, a subset of the small intestinal adenocarcinomas (up to 35%) is DNA mismatch repair (MMR) protein deficient [3-12]. Although the significance of MMR protein deficiency in predicting prognosis and treatment response has been established in colorectal adenocarcinoma [13,14], its significance has yet to be defined in small intestinal adenocarcinoma. A few studies in the literature investigating this question mostly using relatively small cohorts have been inconclusive [3,7,15-18].

AT-rich interactive domain containing protein 1A (ARID1A, also known as *BAF250a*) is a key subunit of the chromatin remodeling complex called *SWItch/Sucrose Non-Fermentable* [19]. Recently, *ARID1A* gene has been recognized as a tumor suppressor gene in gynecologic malignancies [20]. *ARID1A* gene mutation has been identified in colorectal and gastric adenocarcinomas [21,22], whereas loss of ARID1A protein expression by immunohistochemistry has been associated with sporadic microsatellite instability/DNA MMR protein deficiency in colorectal carcinoma [23,24] and with MMR protein deficiency and Epstein-Barr virus infection in gastric adenocarcinoma [25].

In contrast, the role of ARID1A in small intestinal adenocarcinoma development and progression remains to be determined. The only report in the literature used a tissue microarray approach to study ARID1A protein expression by immunohistochemistry in a cohort of small intestinal adenocarcinomas from Asian patients [26]. Results from this study suggested that ARID1A loss may be associated with poor survival and MMR protein deficiency [26]. These findings warrant additional systemic investigation.

In this study, we examined a cohort of 120 surgically resected, nonampullary, small intestinal adenocarcinomas from 2 tertiary care medical centers in the US over a 10-year period. We aimed to (1) determine the prognostic significance of DNA MMR protein deficiency in small intestinal adenocarcinomas and (2) evaluate the prevalence and significance of loss ARID1A protein expression by immunohistochemistry in small intestinal adenocarcinomas. We found that ARID1A loss by immunohistochemistry is rather uncommon in small intestinal adenocarcinomas, whereas DNA MMR protein deficiency is associated with prognosis.

2. Materials and methods

2.1. Patient identification and clinical data collection

Patients who had primary surgical resection for small intestinal adenocarcinomas were identified retrospectively through searches of the surgical pathology archive of the Washington University in St Louis between 2007 and 2016 and that of the University of Pittsburgh between 2008 and 2014. All patients included in the current study were classified as having primary small intestinal carcinomas based on pathologic, clinical, and imaging findings. Patients with ampullary carcinomas or carcinomas grossly involving the ampulla of Vater were excluded from this study. Additionally excluded were patients with only limited clinical data, especially the lack of clinical follow-up information.

Patients identified from the University of Pittsburgh were previously reported [12]. For these patients, clinical and pathologic data were updated through review of medical records. For patients that had not been previously reported, hematoxylin and eosin–stained slides were reviewed to confirm the cancer diagnosis. The corresponding clinical and pathologic findings were recorded by review of medical records. The histologic type and grade were based on the World Health Organization and American Joint Committee on Cancer Criteria (AJCC) recommendations [27,28]. All cases were staged based upon the AJCC seventh edition recommendations at the time of data collection. Of note, updates in the AJCC eighth edition staging [29] do not affect results interpretation in this study because the grouping criteria for major clinical stages, that is, stage I-stage IV, used in the eight edition recommendations are the same as those used in the seventh edition.

Study approval was obtained from the Institutional Review Boards at both the University of Pittsburgh and the Washington University in Saint Louis.

2.2. Immunohistochemistry and interpretation

One representative formalin-fixed, paraffin-embedded block of each adenocarcinoma was used for immunohistochemical analysis. Immunohistochemical labeling was performed using antibodies against ARID1A (polyclonal, Sigma-Aldrich, St. Louis MO, HPA005456), MLH1 (clone G168-728, Ventana, Tucson, AZ), PMS2 (clone EPR3947, Cell Marque, Rocklin, CA), MSH2 (clone G219-1129, Ventana, Tucson, AZ), and MSH6 (clone 44, Ventana, Tucson, AZ) on 4-μm unstained whole tissue sections using automated stainers according to the manufacturers' recommendations. Of note, the ARID1A antibody was the same as that used in the study by Kim et al [26] on small intestinal adenocarcinoma and other studies of a variety of other cancer types [24,25,30].

ARID1A immunohistochemical stain has a nuclear staining pattern and should be observed in all non-neoplastic cells. ARID1A immunohistochemical stain in this study was interpreted similarly to that reported previously [24]. *Retained*

Table 1 Demographics and clinicopathologic features stratified by status of DNA MMR proteins

	All	MMR protein proficient	MMR protein deficient	*P*
No. of Cases	120	99 (82)	21 (18)	NA
Stained for ARID1A (n, %)	92	74	18	.3
ARID1A retained	86 (93)	70 (95)	16 (89)	
ARID1A loss	6 (7)	4 (5)	2 (11)	
Sex (n, %)				.6
Male	59 (49)	50 (51)	9 (43)	
Female	61 (51)	49 (49)	12 (57)	
Age at resection (y; median, interquartile range)	63 (54-74)	63 (52-74)	63 (58-71)	.9
<50 (n, %)	19 (16)	16 (16)	3 (14)	.8
50-75 (n, %)	77 (64)	62 (63)	15 (71)	
>75 (n, %)	24 (20)	21 (21)	3 (14)	
Race (n, %)				.1
White	104 (87)	83 (84)	21 (100)	
African American	15 (13)	15 (15)	0	
Asian	1 (1)	1 (1)	0	
Tumor location (n, %)				.9
Duodenum	70 (58)	58 (59)	12 (57)	
Jejunum	30 (25)	24 (24)	6 (29)	
Ileum	20 (17)	17 (17)	3 (14)	
Median tumor size (cm; interquartile range)	3.4 (2.5-5.3)	3.0 (2.4-5.0)	5.2 (2.5-7.5)	.03
Histologic grade (n, %)				.6
Well/moderately differentiated	76 (63)	64 (65)	12 (57)	
Poorly differentiated	44 (37)	35 (35)	9 (43)	
pT (n, %)				.3
T1	8 (7)	6 (6)	2 (10)	
T2	11 (9)	10 (10)	1 (5)	
T3	56 (47)	43 (43)	13 (62)	
T4	45 (38)	40 (40)	5 (24)	
pN (n, %)				.17
N0	53 (44)	41 (41)	12 (57)	
N1	37 (31)	30 (30)	7 (33)	
N2	25 (21)	24 (24)	1 (5)	
Nx	5 (4)	4 (4)	1 (5)	
Positive for distant metastasis at the time of resection (M1) (n, %)	22 (18)	22 (22)	0	.01
Stage at resection (n, %)				.06
I	11 (9)	9 (9)	2 (10)	
II	38 (32)	28 (28)	10 (48)	
III	45 (38)	37 (37)	8 (38)	
IV	22 (18)	22 (22)	0	
Unknown	4 (3)	3 (3)	1 (5)	
Angiolymphatic invasion (n, %)	77 (64)	67 (68)	10 (48)	.13
Systemic chemotherapy (n, %)				.44
Yes	70 (58)	60 (61)	10 (48)	
No	43 (36)	34 (34)	9 (43)	
Unknown	7 (6)	5 (5)	2 (10)	
Medullary differentiation (n, %)	13 (11)	6 (6)	7 (33)	<.01
Tumor-infiltrating Lymphocytes (n, %)	51 (43)	37 (37)	14 (67)	.02
Mucinous differentiation (n, %)	42 (35)	36 (36)	6 (29)	.6
Signet ring cell differentiation (n, %)	17 (14)	15 (15)	2 (10)	.7
Crohn-like reaction (n, %)	27 (23)	22 (22)	5 (24)	1.0
MMR protein immunohistochemistry pattern (n, %)				NA
Loss of MLH1 and PMS2	9 (8)	0	9 (43)	
Loss of MSH2 and MSH6	9 (8)	0	9 (43)	
Isolated loss of PMS2	1 (1)	0	1 (5)	
Isolated loss of MSH6	2 (2)	0	2 (10)	
Known cancer-producing conditions (n, %)				NA
Lynch syndrome	12 (10)	0	12 (57)	
Familial adenomatous polyposis	5 (4)	5 (5)	0	

(*continued on next page*)

Table 1 (*continued*)

	All	MMR protein proficient	MMR protein deficient	*P*
Crohn disease	10 (8)	10 (10)	0	
Celiac disease	2 (2)	1 (1)	1 (5)	

Abbreviations: No, number; NA, not applicable; y, year; cm, centimeter; pT, tumor stage; pN, lymph nodes.

ARID1A staining was defined as nuclear staining in tumor cells, 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 with normal ARID1A staining observed in internal control non-neoplastic cells.

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

2.3. 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; IBM, Armonk, NY).

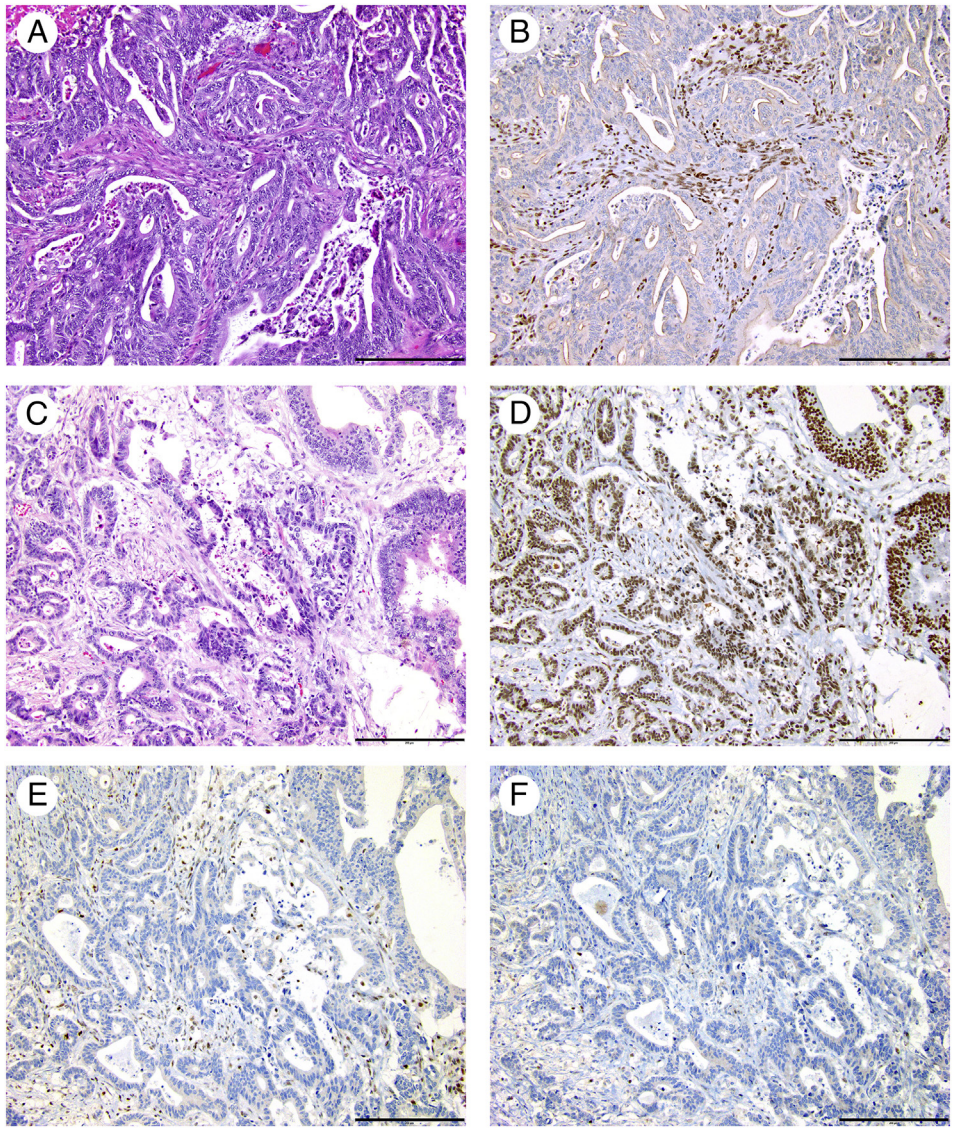

Fig. 1 ARID1A immunohistochemistry staining in small intestinal adenocarcinomas. A DNA MMR protein-proficient small intestinal adenocarcinoma (A) has loss of ARID1A stain (B). Intact/normal ARID1A stain (D) is seen in a small intestinal adenocarcinoma with DNA MMR protein deficiency (C) with loss of nuclear staining in MLH1 (E) and PMS2 (F). Scale bar: 200 μm.

A $P < 0$; .05 was considered statistically significant. Student *t* test and Mann-Whitney test were used for comparison of numerical values. Two-sided Fisher exact test and χ^2 test were used for the comparison of categorical data. For survival analysis, *disease-specific survival* was defined as the duration between surgical resection and either death or the latest clinical follow-up time. Deaths caused by reasons other than small intestinal adenocarcinoma were censored during disease-specific survival analysis. *Disease-free survival* was the duration between surgical resection and disease recurrence documented by imaging and/or pathologic evaluation of metastasis. Patients who were stage IV at the time of surgery, that is, with distant metastasis at the time of surgery, were considered never disease free and thus were excluded from analysis for disease-free survival. Thus, Cox regression analysis for disease-free survival was performed using data from patients without distant metastasis at the time of surgery (stages I, II, and III; n = 98).

3. Results

3.1. Clinical and histopathologic characteristics

A total of 120 patients were collected from 2 tertiary care medical centers (Table 1). Nearly half (59/120, 49%) of the patients were men. The median age at diagnosis was 63 years old (interquartile range: 54-74). The age and sex distributions of the current cohort collected from 2 centers are comparable to those affected by small intestinal adenocarcinoma in the US

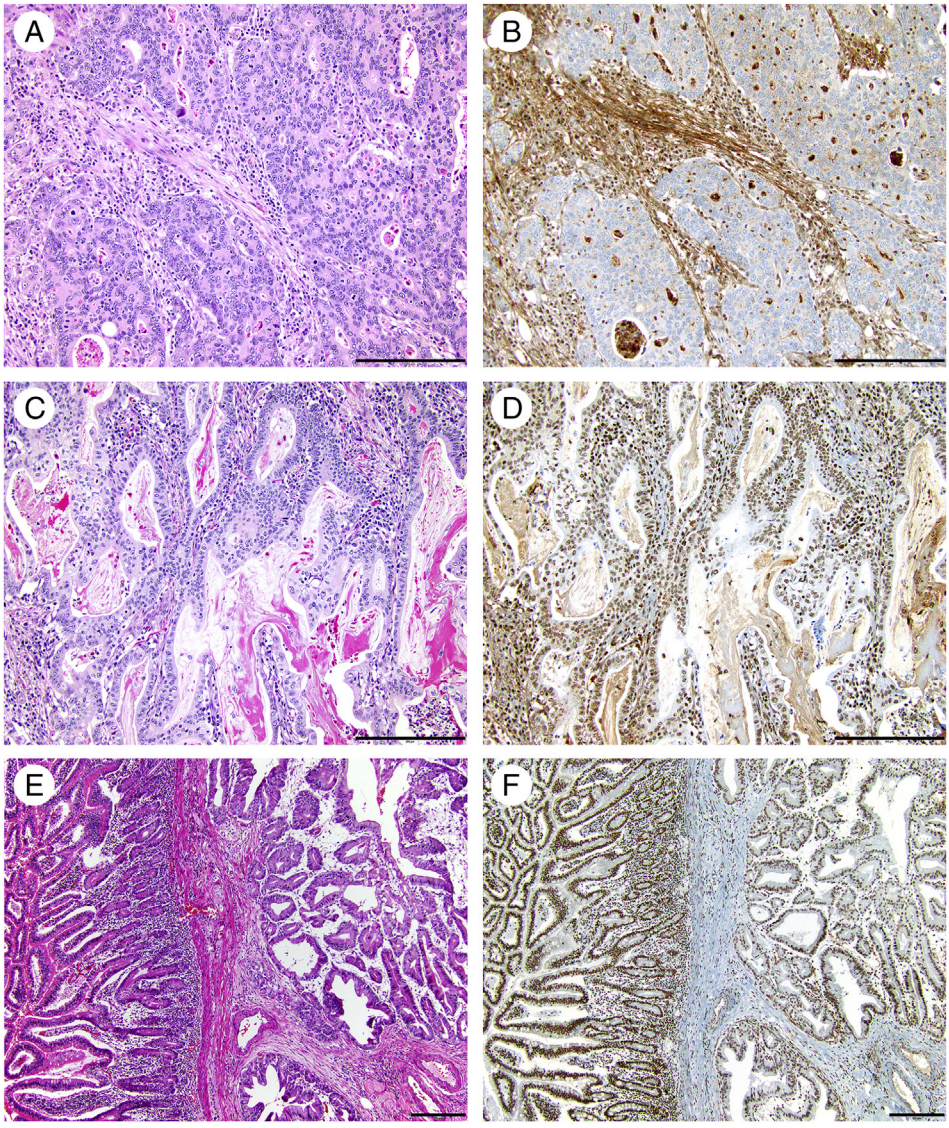

Fig. 2 A and C, ARID1A immunohistochemistry staining is heterogenous in a small intestinal adenocarcinoma. Loss of ARID1A staining is seen in areas with solid and nested growth pattern (A and B), whereas in areas with a glandular pattern in the same adenocarcinoma, ARID1A stain is intact/normal (C and D). E and F, ARID1A stain in adenocarcinoma is not as strong as that in the adjacent, non-neoplastic tissue. Scale bar: 200 μm.

Table 2 Cox regression analysis of disease-specific survival

	Univariate			Multivariate		
	P	HR	95% CI	*P*	HR	95% CI
ARID1A loss	.7	1.2	0.4-4.1			
DNA MMR protein deficiency	.03	0.2	0.1-0.9	.1	0.3	0.1-1.3
Male sex	.13	1.7	0.9-3.2	.3	1.4	0.7-2.7
Age	.28	1.0	1.0-1.1	.1	1.0	1.0-1.1
African American	.7	0.8	0.3-2.7			
Stage IV	<.01	4.7	2.4-9.2	.01	2.5	1.2-5.1
Duodenal location	.9	1.0	0.5-1.8			
Poorly differentiated histologic grade	.5	1.3	0.6-2.4			
Angiolymphatic invasion	<.01	6.2	2.2-7.6	.01	4.5	1.5-13.5

population (median age [interquartile range]: 67 years [56-76], $P = .07$; male sex: 54%, $P = .4$) [1,2].

Seventy (58%) patients had duodenal adenocarcinomas, whereas 30 (25%) and 20 (17%) patients had jejunal and ileal adenocarcinomas, respectively. More than half (62/120, 52%) of the patients had regional lymph node metastasis, whereas 22 (18%) patients had distant metastasis at the time of surgery. The median disease-specific survival was 33 months (interquartile range [month]: 12-61), and the median disease-free interval was 27 months (interquartile range [month]: 8-63).

Twenty-nine patients had known predisposing conditions for small intestinal cancer including Lynch syndrome (12/120, 10%), familial adenomatous polyposis (5/120, 4%), Crohn disease (10/120, 8%), and celiac disease (2/120, 2%).

3.2. ARID1A protein expression by immunohistochemistry

Ninety-two (77%) of the 120 small intestinal adenocarcinomas were stained with ARID1A (Fig. 1). Tumor blocks were not available for the remaining 28 adenocarcinomas. ARID1A staining was strong and diffuse in our cohort except in 2 adenocarcinomas. In these 2 cases, ARID1A nuclear staining was absent in approximately half of the tumor cells and was strong and diffuse in the remaining tumor cells (Fig. 2A-D). By our definition, ARID1A staining was interpreted as retained in these 2 adenocarcinomas. In rare cases (n = 5), we observed that ARID1A staining in tumor cells was not as strong as that in the adjacent, non-neoplastic tissue (Fig. 2E and F). However, low intensity in ARID1A staining as reported in the literature [26] was not observed in our cohort.

ARID1A loss by immunohistochemistry was detected in only 6 (7%) of the 92 adenocarcinomas (Table 1 and Fig. 1). This is significantly less than the proportion (36/178, 20%) of small intestinal carcinoma with ARID1A loss reported in the literature [26] ($P = .003$). No significant association was observed between ARID1A loss and histopathologic features such as histologic grade, tumor stage, or MMR protein status ($P > .05$ for all) (Supplementary Table 1).

3.3. DNA MMR protein deficiency

DNA MMR protein deficiency was detected in 21 (18%) of the 120 small intestinal adenocarcinomas (Table 1). Nine (43%) of them demonstrated loss of nuclear expression of both MLH1 and PMS2, and another 9 (43%) adenocarcinomas had concurrent loss of nuclear expression in MSH2 and MSH6. Isolated loss of PMS2 was detected in 1 (5%) small intestinal adenocarcinoma, whereas isolated loss of nuclear expression of MSH6 was detected in 2 (10%) adenocarcinomas.

The pattern of DNA MMR protein deficiency observed in our cohort was compared with that detected in colorectal adenocarcinoma we reported previously (n = 149; loss of nuclear expression in MLH1 and PMS2: 81%; loss of nuclear expression in MSH2 and MSH6: 11%; isolated loss of PMS2 nuclear expression: 3%; isolated loss of MSH6 nuclear expression:

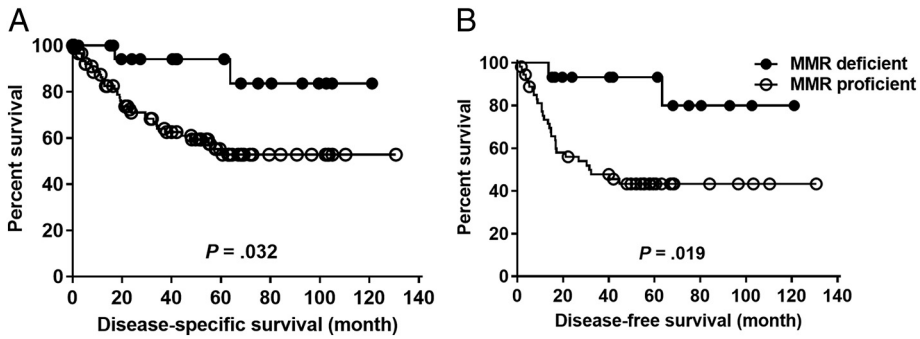

Fig. 3 Kaplan-Meier survival analysis of disease-specific survival (A) and disease-free survival (B).

Table 3 Cox regression analysis of disease-free survival

	Univariate			Multivariate		
	P	HR	95% CI	*P*	HR	95% CI
ARID1A loss	.6	1.3	0.4-4.5			
DNA MMR protein deficiency	.02	0.2	0.04-0.8	.04	0.2	0.05-0.9
Male sex	.3	1.4	0.7-2.8			
Age	.4	1.0	1.0-1.1			
African American	.08	2.4	0.9-6.2			
Stage III	.01	2.7	1.3-5.7	.08	4.1	0.9-19.1
Duodenal location	.4	1.4	0.7-3.0			
Poorly differentiated histologic grade	.8	1.1	0.5-2.2			
Angiolymphatic invasion	.05	2.2	1.0-4.7	.4	0.5	1.0-2.4

5%) [12]. Compared with those MMR-deficient colorectal adenocarcinomas, small intestinal adenocarcinomas in our cohort significantly more frequently demonstrated concurrent loss of expression of MSH2 and MSH6 ($P < 0$; .001) and significantly less frequently had concurrent loss of expression of MLH1 and PMS2 ($P < 0$; .001). Of the 12 Lynch patients, 8 patients had adenocarcinomas with concurrent loss of MSH2 and MSH6, and 3 had adenocarcinomas with concurrent loss of MLH1 and PMS2. The remaining 1 patient had carcinoma with isolated loss of MSH6.

Histopathologically, MMR protein-deficient small intestinal adenocarcinomas were significantly larger (median tumor size: 5.2 versus 3.0 cm, $P = .03$) and demonstrated significantly higher rates of medullary differentiation (7/21, 33% versus 6/99, 6%, $P = .002$) and tumor-infiltrating lymphocytes (14/21, 67% versus 37/99, 37%, $P = .02$) compared with MMR protein-proficient small intestinal adenocarcinomas (Table 1).

3.4. Survival analysis

At the time of surgery, none of 21 patients with MMR protein-deficient adenocarcinomas had distant metastasis, whereas 22 (22%) of 99 patients with MMR-proficient adenocarcinomas did ($P = .01$). Cox regression univariate analysis showed that MMR protein deficiency was significantly associated with longer disease-specific survival (hazard ratio [HR]: 0.2, 95% confident interval [CI]: 0.1-0.9, $P = .03$) (Table 2 and Fig. 3A) and disease-free survival (HR: 0.2, 95% CI: 0.04-=0.8, $P = .02$) (Table 3 and Fig. 3B). By Cox regression multivariate analysis, MMR deficiency was not significantly associated with disease-specific survival ($P = .1$), but it remained a significant, positive prognostic factor for disease-free survival after adjusting for stage and angiolymphatic invasion (HR: 0.2, 95% CI: 0.05-0.9, $P = .035$).

4. Discussion

In this study, we analyzed the prognostic value of DNA MMR protein deficiency and loss of ARID1A expression by immunohistochemistry in 120 small intestinal adenocarcinomas. This cohort represents the largest series of surgically resected, nonampullary, small intestinal adenocarcinomas collected in the US population. We report that MMR protein-deficient small intestinal adenocarcinomas more frequently displayed medullary differentiation and tumor-infiltrating lymphocytes and demonstrated lack of distant metastasis at the time of surgery compared with MMR protein-proficient small intestinal adenocarcinomas. More importantly, DNA MMR protein deficiency was independently associated with superior disease-free survival. In contrast, loss of ARID1A expression detected by immunohistochemistry was uncommon (7%) and not associated with clinicopathologic features, clinical outcome, or DNA MMR protein deficiency.

The relationship between DNA MMR protein deficiency and prognosis in small intestinal adenocarcinoma has been investigated previously with conflicting results. MMR protein deficiency was shown to be independently associated with increased cancer-specific survival in a series of 35 surgically resected, nonampullary, small intestinal adenocarcinomas [15]. In another study of 99 surgically resected, nonampullary, duodenal adenocarcinomas, MMR protein deficiency was independently associated with improved overall survival but not associated with time to tumor recurrence [16]. However, when evaluating the 64 stage III tumors in the same cohort, MMR protein deficiency was shown to be an independent, favorable predictor of disease-free survival but not predictive of overall survival [17]. Additionally, a study of 63 surgically resected small intestinal adenocarcinomas showed that high levels of microsatellite instability were associated with nonmetastatic tumor and a trend for longer overall survival [3]. In contrast, 2 studies with 54 and 195 nonampullary small intestinal adenocarcinomas, respectively, failed to show survival difference by DNA MMR protein status [7,18]. In our cohort, patients with DNA MMR protein-deficient small intestinal adenocarcinomas had significantly longer disease-free survival than patients with MMR protein-proficient small intestinal adenocarcinomas. Our results were derived from a large cohort of small intestinal adenocarcinomas collected from 2 tertiary care hospitals; these results provide additional, strong support to the hypothesis that DNA MMR protein deficiency is a significant predictor of prognosis in small intestinal adenocarcinoma.

We also demonstrated that small intestinal adenocarcinomas with DNA MMR protein deficiency in our cohort more frequently demonstrated loss of MSH2 and MSH6 and less frequently had loss of MLH1 and PMS2 compared with MMR protein-deficient colorectal adenocarcinomas. Such findings have been reported in our previous study with small intestinal adenocarcinomas collected from 1 tertiary care center (N = 71) [12]. The same findings observed in the expanded cohort in this study confirm results we reported previously and further support that small intestinal adenocarcinomas with DNA MMR protein deficiency frequently demonstrate Lynch syndrome–associated deficiencies. It is estimated that the lifetime risk for small intestinal adenocarcinomas in Lynch patients is approximately 4% [31]. On the other hand, small intestinal adenocarcinomas may present as the first malignancy in between 34% (n = 85) and 78% (n = 9) of Lynch patients [31]. These data in the literature together with our results suggest that universal screening of small intestinal adenocarcinoma for DNA MMR protein deficiency may be beneficial in detecting patients at risk for Lynch syndrome.

It is important to note that the majority of small intestinal adenocarcinomas included in our study and studies in the literature are surgically resectable tumors. The value of DNA MMR protein status in survival of metastatic small intestinal adenocarcinomas has not been investigated. For these tumors, because the US Food and Drug Administration has recently approved the checkpoint inhibitor targeting the programmed cell death 1 receptor, pembrolizumab, for treatment of solid tumors that are MMR protein deficient and unresectable or metastatic [32,33], we might expect that increasing number of metastatic small intestinal adenocarcinomas will be tested for DNA MMR protein status. This provides the opportunity for prospective, large-scale, multicenter, collaborative studies for the prognostic significance of MMR protein deficiency in small intestinal adenocarcinoma.

The ARID1A antibody used in our study is the same as that used in previous published reports in the literature including the previously mentioned study on small intestinal adenocarcinomas [26] and studies of ovarian, endometrial, and other gastrointestinal tract malignancies [24,25,30]. However, compared with the prevalence of 20% reported previously [26], the prevalence of ARID1A loss detected by immunohistochemistry in our cohort is significantly lower. The small number of adenocarcinomas with ARID1A loss might have limited our ability to discern any significance in association studies between loss of ARID1A expression and clinicopathologic features, dMMR, and survival in our cohort. The low frequency of ARID1A loss we observed may suggest that most North American small intestinal adenocarcinomas do not harbor *ARID1A* gene mutations or epigenetic alterations resulting in ARID1A protein loss. Very limited amount of comprehensive genomic data generated by next-generation sequencing on small intestinal adenocarcinoma exists in the current literature [34,35]. In these studies, *ARID1A* gene alteration was reported in between 0% (n = 28) [34] and 12% (39/317) [35] of small intestinal adenocarcinomas. The discrepancy in the frequency between ARID1A loss detected by immunohistochemistry and its gene alterations in genomic studies suggests that large-scale, comprehensive genetic and genomic studies of small intestinal adenocarcinoma are needed.

Limitations of this study include its retrospective nature and the size of the study cohort. Although the number of cases included in this study may be relatively small by some standards, they were collected from 2 tertiary care medical centers in the US over a 10-year period. We believe that our results are representative of small intestinal adenocarcinomas typically seen at other medical centers with similar size in the US. Nonetheless, given the rarity of small intestinal adenocarcinoma, prospective, large-scale multicenter collaborative studies are needed to validate findings described in this study.

5. Conclusion

In summary, small intestinal adenocarcinomas with DNA MMR protein deficiency are nonmetastatic carcinomas and frequently demonstrate loss of MSH2 and MSH6 expression. More importantly, DNA MMR protein deficiency is an independent good prognostic predictor for disease-free survival in patients affected by small intestinal adenocarcinomas. Loss of ARID1A protein expression by immunohistochemistry is infrequent and not associated with tumor behavior, clinical outcome, or DNA MMR protein deficiency in small intestinal adenocarcinomas. Our findings support the prognostic significance of DNA MMR protein deficiency in small intestinal adenocarcinoma and suggest that small intestinal adenocarcinomas should be universally tested for DNA MMR protein status. Future prospective studies investigating clinical outcome of small intestinal adenocarcinoma should take DNA MMR protein status into account for risk stratification.

Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.humpath.2018.10.013.

Acknowledgment

The authors wish to thank members of the In Situ Hybridization and Developmental Laboratory of the Department of Pathology, University of Pittsburgh, for excellent technical support.

References

[1] Overman MJ, Hu CY, Kopetz S, Abbruzzese JL, Wolff RA, Chang GJ. A population-based comparison of adenocarcinoma of the large and small intestine: insights into a rare disease. Ann Surg Oncol 2012;19:1439-45.

[2] Bilimoria KY, Bentrem DJ, Wayne JD, Ko CY, Bennett CL, Talamonti MS. Small bowel cancer in the United States: changes in epidemiology, treatment, and survival over the last 20 years. Ann Surg 2009; 249:63-71.
[3] Aparicio T, Svrcek M, Zaanan A, et al. Small bowel adenocarcinoma phenotyping, a clinicobiological prognostic study. Br J Cancer 2013; 109:3057-66.
[4] Aparicio T, Zaanan A, Mary F, Afchain P, Manfredi S, Evans TR. Small bowel adenocarcinoma. Gastroenterol Clin North Am 2016;45:447-57.
[5] Blaker H, von Herbay A, Penzel R, Gross S, Otto HF. Genetics of adenocarcinomas of the small intestine: frequent deletions at chromosome 18q and mutations of the SMAD4 gene. Oncogene 2002;21:158-64.
[6] Laforest A, Aparicio T, Zaanan A, et al. ERBB2 gene as a potential therapeutic target in small bowel adenocarcinoma. Eur J Cancer 2014;50: 1740-6.
[7] Overman MJ, Pozadzides J, Kopetz S, et al. Immunophenotype and molecular characterisation of adenocarcinoma of the small intestine. Br J Cancer 2010;102:144-50.
[8] Planck M, Ericson K, Piotrowska Z, Halvarsson B, Rambech E, Nilbert M. Microsatellite instability and expression of MLH1 and MSH2 in carcinomas of the small intestine. Cancer 2003;97:1551-7.
[9] Rashid A, Hamilton SR. Genetic alterations in sporadic and Crohn's-associated adenocarcinomas of the small intestine. Gastroenterology 1997; 113:127-35.
[10] Svrcek M, Jourdan F, Sebbagh N, et al. Immunohistochemical analysis of adenocarcinoma of the small intestine: a tissue microarray study. J Clin Pathol 2003;56:898-903.
[11] Wheeler JM, Warren BF, Mortensen NJ, et al. An insight into the genetic pathway of adenocarcinoma of the small intestine. Gut 2002;50:218-23.
[12] Xia M, Singhi AD, Dudley B, Brand R, Nikiforova M, Pai RK. Small bowel adenocarcinoma frequently exhibits Lynch syndrome–associated mismatch repair protein deficiency but does not harbor sporadic MLH1 deficiency. Appl Immunohistochem Mol Morphol 2017;25:399-406.
[13] Sargent DJ, Marsoni S, Monges G, et al. Defective mismatch repair as a predictive marker for lack of efficacy of fluorouracil-based adjuvant therapy in colon cancer. J Clin Oncol 2010;28:3219-26.
[14] Zaanan A, Flejou JF, Emile JF, et al. Defective mismatch repair status as a prognostic biomarker of disease-free survival in stage III colon cancer patients treated with adjuvant FOLFOX chemotherapy. Clin Cancer Res 2011;17:7470-8.
[15] Brueckl WM, Heinze E, Milsmann C, et al. Prognostic significance of microsatellite instability in curatively resected adenocarcinoma of the small intestine. Cancer Lett 2004;203:181-90.
[16] Fu T, Pappou EP, Guzzetta AA, et al. CpG island methylator phenotype-positive tumors in the absence of MLH1 methylation constitute a distinct subset of duodenal adenocarcinomas and are associated with poor prognosis. Clin Cancer Res 2012;18:4743-52.
[17] Fu T, Sharmab A, Xie F, et al. Methylation of MGMT is associated with poor prognosis in patients with stage III duodenal adenocarcinoma. PLoS One 2016;11:e0162929.
[18] Gu MJ, Bae YK, Kim A, et al. Expression of hMLH1, hMSH2 and hMSH6 in small intestinal carcinomas. Hepatogastroenterology 2012; 59:2228-32.
[19] Masliah-Planchon J, Bieche I, Guinebretiere JM, Bourdeaut F, Delattre O. SWI/SNF chromatin remodeling and human malignancies. Annu Rev Pathol 2015;10:145-71.
[20] Guan B, Wang TL, Shih Ie M. ARID1A, a factor that promotes formation of SWI/SNF-mediated chromatin remodeling, is a tumor suppressor in gynecologic cancers. Cancer Res 2011;71:6718-27.
[21] The Cancer Genome Atlas Network. Comprehensive molecular characterization of human colon and rectal cancer. Nature 2012;487: 330-7.
[22] Jones S, Li M, Parsons DW, et al. Somatic mutations in the chromatin remodeling gene ARID1A occur in several tumor types. Hum Mutat 2012; 33:100-3.
[23] Chou A, Toon CW, Clarkson A, et al. Loss of ARID1A expression in colorectal carcinoma is strongly associated with mismatch repair deficiency. Hum Pathol 2014;45:1697-703.
[24] Ye J, Zhou Y, Weiser MR, et al. Immunohistochemical detection of ARID1A in colorectal carcinoma: loss of staining is associated with sporadic microsatellite unstable tumors with medullary histology and high TNM stage. Hum Pathol 2014;45:2430-6.
[25] Abe H, Maeda D, Hino R, et al. ARID1A expression loss in gastric cancer: pathway-dependent roles with and without Epstein-Barr virus infection and microsatellite instability. Virchows Arch 2012;461: 367-77.
[26] Kim MJ, Gu MJ, Chang HK, Yu E. Loss of ARID1A expression is associated with poor prognosis in small intestinal carcinoma. Histopathology 2015;66:508-16.
[27] Shepherd N, Carr N, Howe J, Noffsinger A, Warren B. Carcinoma of the small intestine. In: Bosman F, Carneiro F, Hruban R, Theise N, editors. WHO classification of tumours of the digestive system. World Health Organization; 2010. p. 98-101.
[28] Edge SB, Byrd DR, Compton CC, Fritz AG, Greene FL, Trotti A, editors. AJCC cancer staging manual. 7th ed. New York, NY: Springer; 2010.
[29] Amin MB, Edge SB, Greene FL, et al, editors. AJCC cancer staging manual. 8th ed. New York, NY: Springer; 2017.
[30] Guan B, Mao TL, Panuganti PK, et al. Mutation and loss of expression of ARID1A in uterine low-grade endometrioid carcinoma. Am J Surg Pathol 2011;35:625-32.
[31] Koornstra JJ, Kleibeuker JH, Vasen HFA. Small-bowel cancer in lynch syndrome: is it time for surveillance? Lancet Oncol 2008;9: 901-5.
[32] The United States Food and Drug Administration. FDA approves first cancer treatment for any solid tumor with a specific genetic feature. FDA Press Release https://www.fda.gov/newsevents/newsroom/pressannouncements/ucm560167.htm; 2017, Accessed date: 1 June 2018.
[33] Le DT, Durham JN, Smith KN, et al. Mismatch repair deficiency predicts response of solid tumors to PD-1 blockade. Science 2017;357: 409-13.
[34] Alvi MA, McArt DG, Kelly P, et al. Comprehensive molecular pathology analysis of small bowel adenocarcinoma reveals novel targets with potential for clinical utility. Oncotarget 2015;6:20863-74.
[35] Schrock AB, Devoe CE, McWilliams R, et al. Genomic profiling of small-bowel adenocarcinoma. JAMA Oncol 2017;3:1546-53.