Prognostic significance of ARID1A expression patterns varies with molecular subtype in advanced gastric cancer

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<ABSTRACT>

Prognostic significance of ARID1A expression patterns varies with molecular subtype in advanced gastric cancer

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Background/Aims: AT-rich interactive domain 1A (ARIDIA) is frequently mutated in gastric cancer (GC), especially Epstein-Barr virus (EBV)-associated and microsatellite instability-high (MSI-H) GC. The loss of ARID1A expression has been reported as a poor prognostic marker in GC. However, the relationships between ARID1A alteration and EBV-associated and MSI-H GC, which are known to have a favorable prognosis, has hampered proper evaluation of the prognostic significance of ARID1A expression in GC. We aimed to analyze the true prognostic significance of ARID1A expression by correcting confounding variables.

Methods: We evaluated the ARID1A expression in a large series (n=1,032) of advanced GC (AGC) and analyzed the relationships between expression pattern and variable parameters, including clinicopathologic factors, key molecular features such as EBV-positivity, mismatch repair protein deficiency, and expression of p53 and several receptor tyrosine kinases (HER2, EGFR, and MET). Survival analysis of the molecular subtypes was done according to the ARID1A expression patterns.

Results: Loss of ARID1A expression was found in 52.5% (53/101) of MLH1-deficient and 35.8% (24/67) of EBV-positive GCs, compared with only 9.6%

(82/864) of the MLH1-proficient and EBV-negative group ($p < 0.001$). The loss of ARID1A expression was associated only with MLH1 deficiency and EBV positivity. On survival analysis, the loss of ARID1A expression was associated with worse prognosis only in MLH1-proficient and EBV-negative GC. Multivariate analysis revealed that both loss of ARID1A and decreased ARID1A expression were independent worse prognostic factors in patients with AGC.

Conclusions: Only in MLH1-proficient and EBV-negative GC, the loss of ARID1A expression is related to poorer prognosis.

Key words : gastric cancer, ARID1A, immunohistochemistry, prognosis

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I. INTRODUCTION

AT-rich interactive domain 1A (ARIDIA), a member of the ARID family, is a subunit of SWItch/Sucrose Non-Fermentable (SWI/SNF) complex that remodels histone-DNA interactions in reconstituted nucleosomes in an ATP-dependent manner.[1] The SWI/SNF complex plays essential roles during lineage specification and in the maintenance of stem cell pluripotency, and malfunctioning ARID1A could potentially trigger persistent proliferative progenitor state.[2-6] ARID1A-containing SWI/SNF complex is responsible for cell cycle and ARID1A-depletion leads to defective cell cycle checkpoint activation in response to DNA damage.[7] Also, regulation of telomere reverse transcriptase (TERT) by ARID1A through chromatin structure repression was reported.[8] Recent studies revealed that the ARID1A gene is frequently mutated in a variety of cancers, such as ovarian clear cell carcinoma, endometrioid adenocarcinoma, and gastric carcinomas.[9-12] The mutations of ARID1A found in these types of cancer are mostly insertion/deletion mutations that generate premature stop codon and thus lead to truncation of the ARID1A protein.[9-12] Previous studies reported that the knockdown of ARID1A in gastric cancer cell lines promotes cell proliferation and the forced expression of ARID1A inhibits colony formation and cell

growth.[12,13] The loss of expression of ARID1A evaluated by immunohistochemistry (IHC) is correlated with the mutation status of ARID1A,[11,14] although epigenetic silencing can also induce the loss of ARID1A expression.[15] Intriguingly, in gastric cancer (GC), mutation of ARID1A and loss of expression are closely associated with Epstein-Barr virus (EBV) positivity and high microsatellite instability (MSI-H) type.[11,12,16-18] Several studied have examined the prognostic significance of alterations of ARID1A in GC.[11,13,16-22] Among them, Wang *et al.* and Ibarrola-Villava *et al.* reported a favorable outcome of patients with ARID1A-mutated GC.[11,20] In contrast, other studies reported that the loss of ARID1A expression was associated with poor survival outcome[13,16,18,23] or had no prognostic role in GC.[19,24] Therefore, the prognostic significance of ARID1A alteration still remains controversial. The conflicting reports of the significance of ARID1A expression on the prognosis of GC patients might result from possible confounding effects of EBV-associated and MSI-H GCs and/or limitations of sample size.

GC is one of the leading causes of cancer death worldwide; even though its incidence has been decreasing,[25] the prognosis of advanced gastric cancer (AGC) is still dismal. Therefore, a more comprehensive investigation into the underlying molecular mechanisms is mandatory to develop more effective treatment modalities and to better predict the prognosis of patients. The Cancer Genome Atlas (TCGA) project, based on comprehensive genomic, epigenomic, and transcriptomic analyses, proposed four molecular subtypes of GC: (1) GC with Epstein-Barr virus (EBV) positivity, which is characterized by genome-wide CpG island DNA methylation, frequent PIK3CA mutation, and PD-L1 amplification; (2) GC with MSI-H, in which mainly epigenetic silencing of mismatch-repair (MMR) genes, such as MLH1 causes a hypermutator phenotype; (3) GC with chromosomal instability (CIN), which shows marked aneuploidy, frequent amplification of several receptor tyrosine kinases (RTKs), including HER2, EGFR, MET, and FGFR, and mutation of TP53; and (4) GC with

genomic stability (GS), which is enriched for the diffuse type and mutations or translocation of RHO family genes.[26] Recently, we reported the protein expression profile of selected key molecules that might be expected to reflect these four molecular subtypes in a large series of advanced GC (AGC).[27] In the present study, we evaluated ARID1A expression in a large series (n=1,032) of AGC to clarify its prognostic significance. We then compared the results with those of our previous study in which the key molecules were four MMR proteins (MLH1, MSH2, PMS2, and MSH6) for MSI-H GC, Epstein-Barr virus encoded RNA (EBER) in situ hybridization for EBV-associated GC, and several RTKs including HER2, EGFR, and MET, and p53 for the CIN group. Finally, we evaluated the prognostic significance of altered ARID1A expression according to the molecular subtype of GC.

II. MATERIALS AND METHODS

1. Patient selection and data collection

A total of 1,032 patients with AGC who underwent curative radical gastrectomy at Severance Hospital from 2000 to 2003 were consecutively enrolled in this study. Patients who received neoadjuvant chemotherapy or who had undergone surgery for recurrent cancer were excluded. Clinicopathologic information, including age, sex, tumor size, location, and clinical follow-up data were collected from pathologic reports and medical records. Tumor size was divided into two groups, >5 cm and $\leq$5 cm, as the median tumor size of total cases was 5 cm. Pathologic parameters, including the WHO classification, Lauren classification, lymphovascular invasion (LVI), lymph node metastasis (LNM), and TNM stage according to the 7th American Joint Committee on Cancer system, were obtained from pathologic reports and slide review. This study was approved by the Institutional Review Board of Severance Hospital, Seoul, Republic of Korea (4-2016-0419).

2. Tissue microarray construction

To construct the tissue microarray (TMA) block, two cores were extracted from a representative tumor area of each case (3-mm diameter), as previously described.[28,29] One core of adjacent nonneoplastic gastric mucosa was arrayed in each TMA block as landmark and internal control. Four-micrometer sections from TMA blocks were subjected to IHC and EBER in situ hybridization (ISH).

3. Immunohistochemistry

IHC was performed using the Ventana Discovery XT automated staining system (Ventana Medical Systems, Inc., Tucson, AZ, USA) with anti-ARID1A antibody (polyclonal, 1:200 dilution; Sigma-Aldrich, St. Louis, MO, USA), as previously described.[28,30] Details of antibodies for MLH1, PMS2, MSH2, MSH6, HER2, EGFR, MET, and p53 were described previously.[27] Immunostained slides were evaluated by two individual pathologists (J.Y. K and S. N), and the expression pattern of ARID1A were interpreted as preserved, decreased, or loss of expression. “Preserved” staining was defined as a similar intensity of nuclear staining to that in non-neoplastic cells. “Decreased” expression was defined as a markedly decreased intensity of staining compared to that of stromal cells or normal epithelial cells. “Loss” was defined as no staining in tumor cells, regardless of proportion or intensity. In this study, to compare the ARID1A expression patterns and other molecular profiles of AGCs, we used the previously reported data from our prior study.[27] The adopted expression data were those of MLH1, PMS2, MSH2, MSH6, HER2, EGFR, MET and p53. In the data, the expression of EGFR, HER2, and MET was scored according to Hofmann’s criteria, as 0, 1+, 2+, or 3+.[31] Cases with complete loss or strong and diffuse (more than 50% of tumor cells) nuclear p53 staining were classified as p53 mutant pattern, and cases with weak and patchy (less than 50% of tumor cells) staining of p53 as wild-type pattern, as described

previously.[27] Cases with complete negativity for MLH1/PMS2 or MSH2/MSH6 in tumor cells were regarded as MMR deficient, and all others as MMR proficient.

4. Immunohistochemistry

IHC was performed using the Ventana Discovery XT automated staining system (Ventana Medical Systems, Inc., Tucson, AZ, USA) with anti-ARID1A antibody (polyclonal, 1:200 dilution; Sigma-Aldrich, St. Louis, MO, USA), as previously described.[28,30] Details of antibodies for MLH1, PMS2, MSH2, MSH6, HER2, EGFR, MET, and p53 were described previously.[27] Immunostained slides were evaluated by two individual pathologists (J.Y. K and S. N), and the expression pattern of ARID1A were interpreted as preserved, decreased, or loss of expression. “Preserved” staining was defined as a similar intensity of nuclear staining to that in non-neoplastic cells. “Decreased” expression was defined as a markedly decreased intensity of staining compared to that of stromal cells or normal epithelial cells. “Loss” was defined as no staining in tumor cells, regardless of proportion or intensity. In this study, to compare the ARID1A expression patterns and other molecular profiles of AGCs, we used the previously reported data from our prior study.[27] The adopted expression data were those of MLH1, PMS2, MSH2, MSH6, HER2, EGFR, MET and p53. In the data, the expression of EGFR, HER2, and MET was scored according to Hofmann’s criteria, as 0, 1+, 2+, or 3+.[31] Cases with complete loss or strong and diffuse (more than 50% of tumor cells) nuclear p53 staining were classified as p53 mutant pattern, and cases with weak and patchy (less than 50% of tumor cells) staining of p53 as wild-type pattern, as described previously.[27] Cases with complete negativity for MLH1/PMS2 or MSH2/MSH6 in tumor cells were regarded as MMR deficient, and all others as MMR proficient.

5. Statistical analysis

Statistical calculation was performed with SPSS version 24.0 (IBM, Chicago, IL, USA). Chi-square or Pearson Chi-square test was used to analyze the relationships between ARID1A expression and variable clinicopathologic parameters. RFS was calculated from the date of operation to the date of first recurrence or death without any type of relapse. Overall survival was calculated from the date of gastrectomy to the date of the last follow-up or death. Survival curves were analyzed using the Kaplan-Meier product limit method with the log-rank test for evaluation of significant differences. For multivariate survival analysis, variables found to be significant on univariate analysis were used with the Cox proportional hazard regression model. Significance statements refer to p value <0.05.

III. RESULTS

1. ARID1A expression patterns and related clinicopathologic features in AGCs

In non-neoplastic mucosa, diffuse and homogenous nuclear expression of ARID1A was observed in either epithelial cells or stromal cells, such as inflammatory cells and fibroblasts (Fig. 1A). Of 1,032 AGCs, 429 cases (41.6%) showed preserved nuclear expression of ARID1A (Fig. 1B); however, the expression of ARID1A was decreased in 442 cases (42.8%) (Fig. 1C) and absent in 161 AGCs (15.6%) (Fig. 1D).

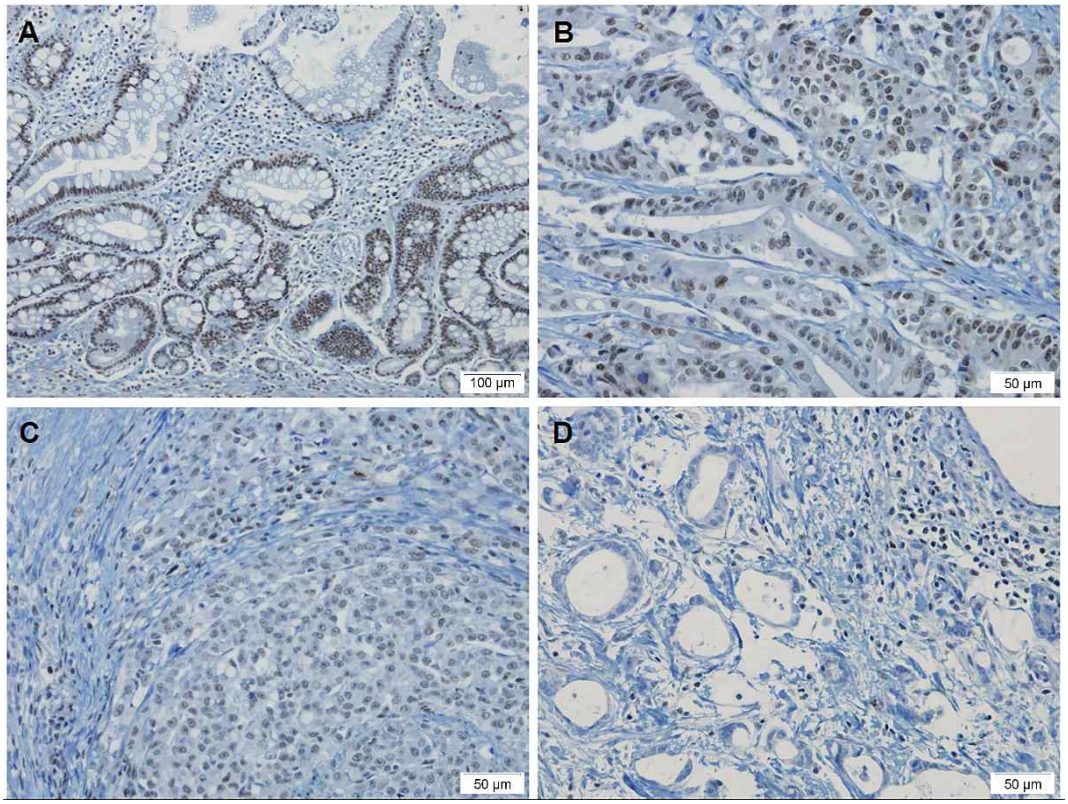

Figure 1. Representative photographs of immunohistochemical ARID1A expression patterns. A. Ubiquitous nuclear expression in intestinal metaplastic gastric epithelial cells and stromal cells (lymphocytes, endothelial cells, and fibrocytes). B. Gastric cancer showing an intensity of ARID1A staining similar to that of non-neoplastic cells, classified as "preserved." C. Adenocarcinoma with significantly decreased intensity of ARID1A compared to that of stromal cells, classified as "decreased." D. Case demonstrating complete loss of nuclear ARID1A staining (original magnification, ×100 for A, ×200 for B, C, and D)

Clinicopathologic and molecular features according to the expression patterns of ARID1A are summarized in Table 1. Decreased expression and loss of ARID1A were more frequently observed in tumors located in the proximal (upper and mid third) stomach (p = 0.032 and p = 0.019, respectively). The loss of ARID1A was associated with larger tumor size (p = 0.003) and intestinal type of Lauren classification (p <0.001). However, except for proximal location, no other parameters

were associated with decreased expression of ARID1A (Table 1; p value for preserved vs. decreased). Consistent with previous reports,[16,18,19,23] loss of expression was frequently observed in MLH1-deficient GC (MLH1-loss, 53/101=52.5% vs. MLH1-intact, 108/931=11.6%, $p < 0.001$ for preserved vs. loss group) and EBV-positive GCs (EBV-positive, 24/67=35.8% vs. EBV-negative, 137/965=14.2%, $p < 0.001$ for preserved vs. loss group). However, decreased expression of ARID1A was not associated MLH1 deficiency (MLH1-loss, 28/101=27.7% vs. MLH1-intact, 414/931=44.5%, $p = 0.279$ for preserved vs. decreased group) nor EBV positivity (EBV-positive, 22/67=32.8% vs. EBV-negative, 420/965=43.5%, $p = 0.955$ for preserved vs. decreased group). The loss of ARID1A expression was related to wild-type pattern of p53 ($p = 0.002$ for preserved vs. loss and $p = 0.033$ for decreased vs. loss) and lower frequency of 2 or 3+ expression of HER2 and MET among total cases.

Table 1. Clinicopathologic and molecular characteristics according to ARID1A expression patterns in advanced gastric cancer

				ARID1A expression									
Category	Variables	No. of cases	%	Preserved n=429	%	Decreased n=442	%	Loss n=161	%	p value	p value (Preserved vs. Decreased)	p value (Preserved vs. Loss)	p value (Decreased vs. Loss)
Sex	Male	677	65.6	289	67.4	281	63.6	107	66.5	0.485			
	Female	355	34.4	140	32.6	161	36.4	54	33.5				
Age (y)	≤60	549	53.2	232	54.1	245	55.4	72	44.7	0.059			
	>60	483	46.8	197	45.9	197	44.6	89	55.3				
Location	Lower third	574	55.6	259	60.4	235	53.2	80	49.7	0.026	0.032	0.019	0.449
	Upper & mid	458	44.4	170	39.6	207	46.8	81	50.3				
Size	≤5 cm	520	50.4	219	51.0	239	54.1	62	38.5	0.003	0.372	0.007	0.001
	>5 cm	512	49.6	210	49.0	203	45.9	99	61.5				
Histology	WD/MD	295	28.6	135	31.5	116	26.2	44	27.3	0.217			
	PD/others	737	71.4	294	68.5	326	73.8	117	72.7				
Lauren	Intestinal	504	48.8	210	49.0	194	43.9	100	62.1	<0.001	0.134	0.004	<0.001
	Diffuse	528	51.2	219	51.0	248	56.1	61	37.9				
LVI	Absent	735	71.2	297	69.2	316	71.5	122	75.8	0.290			
	Present	297	28.8	132	30.8	126	28.5	39	24.2				
LNM	Absent	289	28.0	122	28.4	123	27.8	44	27.3	0.959			
	Present	743	72.0	307	71.6	319	72.2	117	72.7				
T stage	T2	176	17.1	81	18.9	74	16.7	21	13.0	0.023	0.302	0.043	0.009
	T3	369	35.8	152	35.4	143	32.4	74	46.0				
	T4	487	47.2	196	45.7	225	50.9	66	41.0				
Overall stage	II	176	10.4	81	11.4	74	10.2	21	8.1	0.718			
	III	314	30.4	133	31.0	129	29.2	52	32.3				
	IV	611	59.2	247	45.7	268	50.9	96	41.0				
MLH1	Loss	101	9.8	20	4.7	28	6.3	53	32.9	<0.001	0.279	<0.001	<0.001
	Intact	931	90.2	409	95.3	414	93.7	108	67.1				
MSH2	Loss	13	1.3	4	0.9	7	1.6	2	1.2	0.690			

	Intact	1019	98.7	425	99.1	435	98.4	159	98.8				
MMR proteins	Deficient	114	11.0	24	5.6	35	7.9	55	34.2	<0.001	0.172	<0.001	<0.001
	Proficient	918	89.0	405	94.4	407	92.1	106	65.8				
EBV	Positive	67	6.5	21	4.9	22	5.0	24	14.9	<0.001	0.955	<0.001	<0.001
	Negative	965	93.5	408	95.1	420	95.0	137	85.1				
MLH1 & EBV	MLH1-loss or EBV +	168	16.3	41	9.6	50	11.3	77	47.8	<0.001	0.397	<0.001	<0.001
	MLH1 intact & EBV -	864	83.7	388	90.4	392	88.7	84	52.2				
MMRs & EBV	MMR-d or EBV +	181	17.5	45	10.5	57	12.9	79	49.1	<0.001	0.269	<0.001	<0.001
	MMR-p and EBV -	851	82.5	384	89.5	385	87.1	82	50.9				
p53 IHC	Wild-type pattern	624	62.0	243	57.7	268	62.5	113	72.0	0.007	0.157	0.002	0.033
	Mutant pattern	383	38.0	178	42.3	161	37.5	44	28.0				
HER2	0 or 1+	972	94.2	393	91.6	418	94.6	161	100.0	<0.001	0.084	<0.001	0.003
	2 or 3+	60	5.8	36	8.4	24	5.4	0	0.0				
MET	0 or 1+	916	90.5	381	90.9	399	92.1	136	85.0	0.029	0.523	0.039	0.009
	2 or 3+	96	9.5	38	9.1	34	7.9	24	15.0				
EGFR	0 or 1+	865	84.5	346	81.0	393	90.1	126	78.3	<0.001	<0.001	0.452	<0.001
	2 or 3+	159	15.5	81	19.0	43	9.9	35	21.7				

LVI: lymphovascular invasion; LNM: lymph node metastasis; MMR: mismatch-repair protein; MMR-d: mismatch-repair protein deficient; MMR-p: mismatch-repair protein proficient; IHC: immunohistochemistry

2. ARID1A expression patterns and related clinicopathologic features in MLH1-proficient and EBV-negative AGCs

MLH1-deficient GC is known for its unique clinicopathologic features, including intestinal histology, distal tumor location, Crohn-like peritumoral lymphoid reaction, and genome-wide CpG island hypermethylation.[26,32] EBV-positive GC also shows characteristic features, including proximal tumor location, frequent occurrence in remnant stomach, and intratumoral intense lymphocytic infiltration, which contributes to a unique histologic type termed carcinoma with lymphoid stroma.[33] Since the loss of ARID1A expression has shown close relationships with MLH1 deficiency and EBV positivity, we analyzed the relationships between ARID1A patterns and clinicopathologic and molecular features in a MLH1-proficient and EBV-negative group to remove any possible confounding effects from those two subtypes (Table 2). In this group, 384 (45.1%), 385 (45.2%), and 82 (9.6%) cases showed preserved, decreased, and loss of ARID1A expression, respectively. An association between the decrease or loss of ARID1A and the proximal location was observed (p = 0.01). However, larger tumor size and intestinal-type histology were not associated with the altered pattern. Interestingly, in this analysis, the association between the wild-type pattern of p53 and the loss of ARID1A expression, shown in Table 1, was not observed in the MLH1-proficient and EBV-negative subgroup. The associations between the RTK expression and ARID1A loss were conserved, with the exception of MET.

Table 2. Clinicopathologic and molecular characteristics according to ARID1A expression patterns in MLH1-proficient and EBV-negative advanced gastric cancer

				ARID1A expression						
Category	Variables	No. of cases	%	Preserved n=388	%	Decreased n=392	%	Loss n=84	%	p value
Sex	Male	561	64.9	260	67.0	244	62.2	57	67.9	0.317
	Female	303	35.1	128	33.0	148	37.8	27	32.1	
Age (y)	≤60	476	55.1	210	54.1	222	56.6	44	52.4	0.680
	>60	388	44.9	178	45.9	170	43.4	40	47.6	
Location	Lower third	487	56.4	239	61.6	209	53.3	39	46.4	0.010
	Upper &mid	377	43.6	149	38.4	183	46.7	45	53.6	
Size	≤5 cm	456	52.8	204	52.6	221	56.4	31	36.9	0.005
	>5 cm	408	47.2	184	47.4	171	43.6	53	63.1	
Histology	WD/MD	242	28.0	121	31.2	99	25.3	22	26.2	0.169
	PD/others	622	72.0	267	68.8	293	74.7	62	73.8	
Lauren	Intestinal	379	43.9	180	46.4	160	40.8	39	46.4	0.258
	Diffuse	485	56.1	208	53.6	232	59.2	45	53.6	
LVI	Absent	616	71.3	271	69.8	281	71.7	64	76.2	0.494
	Present	248	28.7	117	30.2	111	28.3	20	23.8	
LNM	Absent	239	27.7	111	28.6	109	27.8	19	22.6	0.537
	Present	625	72.3	277	71.4	283	72.2	65	77.4	
T stage	T2	149	17.2	74	19.1	68	17.3	7	8.3	0.104
	T3	287	33.2	132	34.0	121	30.9	34	40.5	
	T4	428	49.5	182	46.9	203	51.8	43	51.2	
Overall stage	II	88	10.2	43	11.1	41	10.5	4	4.8	0.326
	III	259	30.0	123	31.7	112	28.6	24	28.6	

	IV	517	59.8	222	57.2	239	61.0	56	66.7	
p53 IHC	Wild-type pattern	493	58.6	214	56.0	227	59.9	52	64.2	0.308
	Mutant pattern	349	41.4	168	44.0	152	40.1	29	35.8	
HER2	0 or 1+	809	93.6	355	91.5	370	94.4	84	100.0	0.011
	2 or 3+	55	6.4	33	8.5	22	5.6	0	0.0	
MET	0 or 1+	781	92.2	349	91.8	358	93.2	74	89.2	0.427
	2 or 3+	66	7.8	31	8.2	26	6.8	9	10.8	
EGFR	0 or 1+	741	86.6	317	82.1	354	91.7	70	83.3	<0.001
	2 or 3+	115	13.4	69	17.9	32	8.3	14	16.7	

LVI: lymphovascular invasion; LNM: lymph node metastasis; IHC: immunohistochemistry

3. ARID1A expression patterns and clinicopathologic features in MLH1-deficient and EBV-positive AGCs

In this study, MLH1-deficient AGCs and EBV-positive AGCs were mutually exclusive. In MLH1-deficient AGCs (n=101), preserved, decreased, and loss of ARID1A was noted in 20 (19.9%), 28 (27.7%), and 53 (52.5%) cases, respectively (p <0.001 compared to MLH1-proficient GCs, Table 1). No parameter was associated with altered ARID1A expression (Supplementary Table 1). Among EBV-positive AGCs (n=67), 21 (31.3%), 22 (32.8%), and 24 (35.8%) showed preserved, decreased, and loss of ARID1A expression, respectively. A low frequency of LVI was the only factor associated with decrease or loss of ARID1A expression (p = 0.044) (Supplementary Table 2).

Supplementary Table 1. Clinicopathologic and molecular characteristics according to ARID1A expression pattern in MLH1-deficient advanced gastric cancer

				ARID1A expression						
Category	Variables	No. of Cases	%	Preserved n=20	%	Decreased n=28	%	Loss n=53	%	p value
Sex	Male	58	57.4	12	60.0	17	60.7	29	54.7	0.845
	Female	43	42.6	8	40.0	11	39.3	24	45.3	
Age (y)	≤60	29	28.7	7	35.0	8	28.6	14	26.4	0.770
	>60	72	71.3	13	65.0	20	71.4	39	73.6	
Location	Lower third	71	70.3	15	75.0	20	71.4	36	67.9	0.830
	Upper and mid	30	29.7	5	25.0	8	28.6	17	32.1	
Size	≤5 cm	34	33.7	7	35.0	8	28.6	19	35.8	0.797
	>5 cm	67	66.3	13	65.0	20	71.4	34	64.2	
Histology	WD/MD	44	43.6	11	55.0	13	46.4	20	37.7	0.389
	PD/others	57	56.4	9	45.0	15	53.6	33	62.3	
Lauren	Intestinal	84	83.2	18	90.0	21	75.0	45	84.9	0.347
	Diffuse	17	16.8	2	10.0	7	25.0	8	15.1	
LVI	Absent	68	67.3	14	70.0	17	60.7	37	69.8	0.680
	Present	33	32.7	6	30.0	11	39.3	16	30.2	
LNM	Absent	33	32.7	8	40.0	6	21.4	19	35.8	0.310
	Present	68	67.3	12	60.0	22	78.6	34	64.2	
T stage	T2	18	17.8	5	25.0	3	10.7	10	18.9	0.074
	T3	53	52.5	12	60.0	11	39.3	30	56.6	
	T4	30	29.7	3	15.0	14	50.0	13	24.5	
Overall stage	II	13	12.9	4	20.0	2	7.1	7	13.2	0.322
	III	32	31.7	7	35.0	6	21.4	19	35.8	

	IV	56	55.4	9	45.0	20	71.4	27	50.9	
EBV	Positive	0	0.0	0	0.0	0	0.0	0	0.0	NA
	Negative	101	100.0	20	100.0	28	100.0	53	100.0	
p53 IHC	Wild-type pattern	81	81.0	16	80.0	22	78.6	43	82.7	0.897
	Mutant pattern	19	19.0	4	20.0	6	21.4	9	17.3	
HER2	0 or 1	97	96.0	18	90.0	26	92.9	53	100.0	0.089
	2 or 3	4	4.0	2	10.0	2	7.1	0	0.0	
MET	0 or 1	79	78.2	17	85.0	21	75.0	41	77.4	0.693
	2 or 3	22	21.8	3	15.0	7	25.0	12	22.6	
EGFR	0 or 1	58	57.4	9	45.0	17	60.7	32	60.4	0.455
	2 or 3	43	42.6	11	55.0	11	39.3	21	39.6	

LVI: lymphovascular invasion; LNM: lymph node metastasis

Supplementary Table 2. Clinicopathologic and molecular characteristics according to ARID1A expression pattern in EBV-positive advanced gastric cancer

				ARID1A expression						
Category	Variables	No of Cases	%	Preserved n=21	%	Decreased n=22	%	Loss n=24	%	*P* value
Sex	Male	58	86.6	17	81.0	20	90.9	21	87.5	0.624
	Female	9	13.4	4	19.0	2	9.1	3	12.5	
Age	≤60	44	65.7	15	71.4	15	68.2	14	58.3	0.624
	>60	23	34.3	6	28.6	7	31.8	10	41.7	
Location	Lower third	16	23.9	5	23.8	6	27.3	5	20.8	0.877
	Upper and mid	51	76.1	16	76.2	16	72.7	19	79.2	
Size	≤5 cm	30	44.8	8	38.1	10	45.5	12	50.0	0.723
	>5 cm	37	55.2	13	61.9	12	54.5	12	50.0	
Histology	WD/MD	9	13.4	3	14.3	4	18.2	2	8.3	0.614
	PD/others	58	86.6	18	85.7	18	81.8	22	91.7	
Lauren	Intestinal	41	61.2	12	57.1	13	59.1	16	66.7	0.783
	Diffuse	26	38.8	9	42.9	9	40.9	8	33.3	
LVI	Absent	51	76.1	12	57.1	18	81.8	21	87.5	0.044
	Present	16	23.9	9	42.9	4	18.2	3	12.5	
LNM	Absent	17	25.4	3	14.3	8	36.4	6	25.0	0.251
	Present	50	74.6	18	85.7	14	63.6	18	75.0	
T stage	T2	9	13.4	2	9.5	3	13.6	4	16.7	0.834
	T3	29	43.3	8	38.1	11	50.0	10	41.7	
	T4	29	43.3	11	52.4	8	36.4	10	41.7	
Overall stage	II	6	9.0	2	9.5	2	9.1	2	8.3	0.163

	III	23	34.3	3	14.3	11	50.0	9	37.5	
	IV	38	56.7	16	76.2	9	40.9	13	54.2	
MLH1	loss	67	100.0	21	100.0	22	100.0	24	100.0	NA
	Intact	0	0.0	0	0.0	0	0.0	0	0.0	
p53 IHC	Wild-type pattern	50	76.9	13	68.4	19	86.4	18	75.0	0.381
	Mutant pattern	15	23.1	6	31.6	3	13.6	6	25.0	
HER2	0 or 1	66	98.5	20	95.2	22	100.0	24	100.0	0.329
	2 or 3	1	1.5	1	4.8	0	0.0	0	0.0	
MET	0 or 1	56	87.5	15	78.9	20	95.2	21	87.5	0.298
	2 or 3	8	12.5	4	21.1	1	4.8	3	12.5	
EGFR	0 or 1	66	98.5	20	95.2	22	100.0	24	100.0	0.329
	2 or 3	1	1.5	1	4.8	0	0.0	0	0.0	

LVI: lymphovascular invasion; LNM: lymph node metastasis

4. Prognostic significance of decreased or loss of ARID1a expression in AGCs

Kaplan-Meier survival curves according to ARID1A expression showed worse prognosis of the patient group with decreased ARID1A compared to patients with preserved ARID1A (Fig. 2A). However, the ARID1A-loss group exhibited a similar prognosis to that of the ARID1A-preserved group. Since GCs with ARID1A loss were enriched among MLH1-deficient and EBV-positive GCs (Table 1) and the prognosis of GC patients with MLH1-deficiency or EBV-positivity was more favorable than that of patients with EBV-negative and MLH1-proficient AGC (Fig. 2B), we analyzed the prognostic effects of altered ARID1A expression in MLH1-deficient, EBV-positive, and EBV-negative and MLH1-proficient groups, respectively. There were no prognostic differences among the ARID1A preserved, decreased, and loss groups in either MLH1-deficient (Fig. 3A) or EBV-positive GCs (Fig. 3B). However, in the patient group with MLH1-proficient and EBV-negative AGC, the prognosis was significantly different according to the ARID1A expression patterns: patients with ARID1A-loss AGC showed the worst overall survival; and the ARID1A-decreased group had second-worst prognosis (Fig. 2C) ($p < 0.001$).

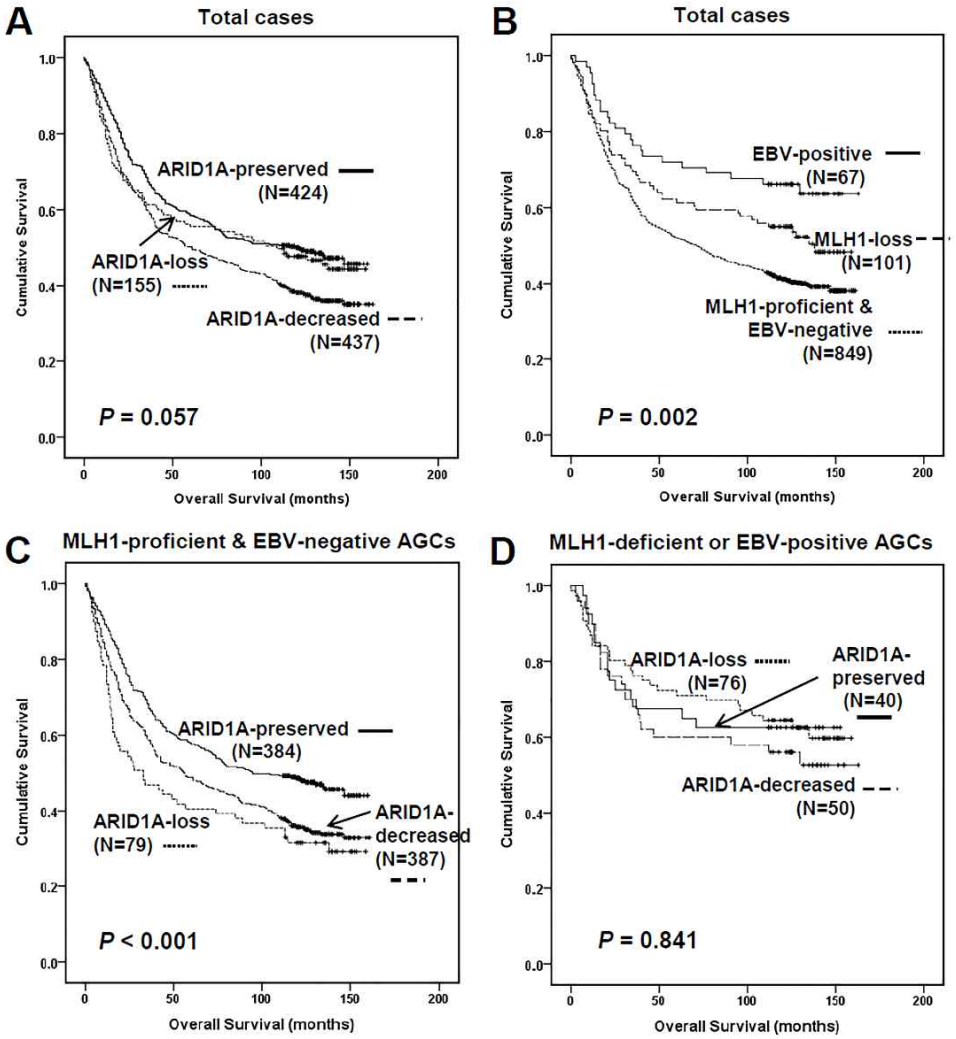

Figure 2. Kaplan-Meier survival curves for overall survival of advanced gastric cancer (AGC) according to three ARID1A expression patterns and molecular subtypes. A. Overall survival curves of total cases according to ARID1A expression pattern show worse prognosis of ARID1A-decreased group compared with ARID1A-preserved or ARID1A-loss group. B. Overall survival curves comparing EBV-positive group, MLH1-loss group and MLH-proficient & EBV-negative group reveal favorable prognosis of AGC with molecular subtype of EBV-positive and MLH1-loss group (p = 0.002). C. Within MLH1-proficient and EBV-negative AGCs, survival curves according to ARID1A expression pattern demonstrate worse prognosis of patients with ARID1A decrease or loss AGCs (p < 0.001). D. Within either MLH1-deficient or EBV-positive AGCs, survival curves according to ARID1A expression pattern show no difference in prognosis observed in patients (p = 0.841).

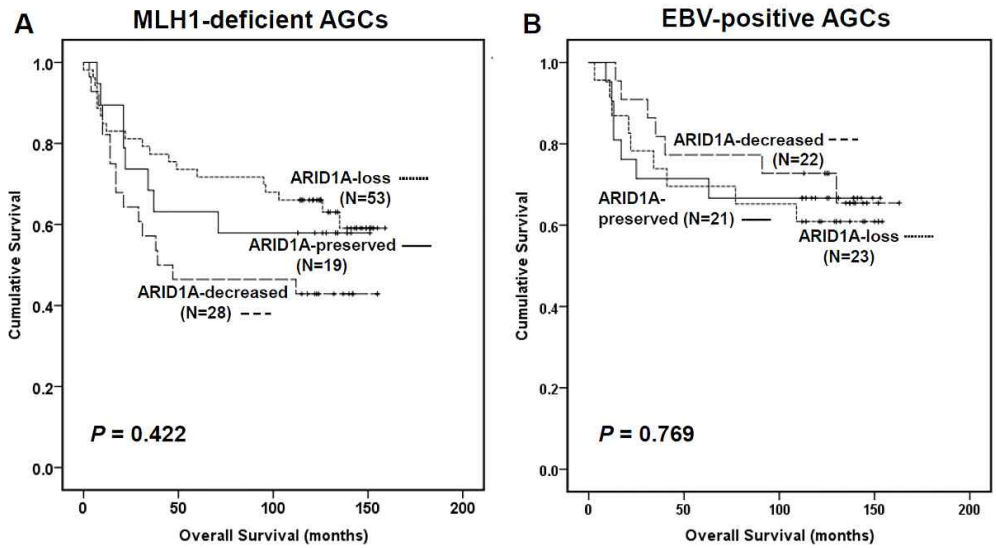

Figure 3. Kaplan-Meier survival curves for overall survival of MLH1-deficient and EBV-positive advanced gastric cancer (AGC). A. Overall survival curves of MLH1-deficient AGCs according to three ARID1A expression patterns. B. Overall survival curves of EBV-positive AGCs according to three ARID1A expression patterns.

In multivariate survival analysis, Cox regression analyses for overall survival and recurrence-free survival (RFS) were performed using variables that were significant on univariate analysis, including age, tumor location, tumor size, histology, Lauren classification, LVI, LNM, overall stage, MLH1 statue, EBV positivity, and ARID1A expression patterns, using a forward conditional method. For overall survival, MLH1 proficiency and EBV negativity were independent worse prognostic factors (HR, 1.4; $p < 0.001$ and HR, 1.98; $p < 0.001$, respectively). Decrease or loss of ARID1A expression was also revealed as a negative prognostic factor of AGC (HR, 1.47 for decreased and 1.48 for loss pattern, $p < 0.001$). In addition to MLH1, EBV, and ARID1A expression, older age (HR, 1.58; $p < 0.001$), larger tumor size (> 5 cm) (HR, 1.24; $p = 0.008$), diffuse type by Lauren (HR, 1.26; $p = 0.009$), presence of LVI (HR, 1.54; p <0.001), and overall stage (HR, 1.69 for stage III and 4.[38] for stage IV, $p < 0.001$) were found to be prognostic factors of overall survival. For

RFS, ARID1A expression pattern (p = 0.041) was a prognostic factor, in addition to MLH1 proficiency (p < 0.001), EBV negativity (p = 0.002), and the features that were shown to be prognostic factors of overall survival, except for older age and proximal tumor location (Table 3).

Table 3. Univariate and multivariate survival analyses

		Univariate-OS			Multivariate-OS			Univariate-RFS			Multivariate-RFS		
		HR	95% CI	p	HR	95% CI	p	HR	95% CI	p	HR	95% CI	p
Sex	Male	1						1					
	Female	1.08	0.92-1.27	0.342				1.18	0.98-1.42	0.083			
Age (y)	≤60	1			1			1					
	>60	1.41	1.20-1.65	<0.001	1.58	1.34-1.88	<0.001	0.96	0.80-1.15	0.657			
Location	Lower third	1			1			1					
	Upper & mid	1.36	1.16-1.60	<0.001	1.25	1.06-1.48	0.008	1.27	1.05-1.53	0.013			
Size	≤5 cm	1			1			1			1		
	>5 cm	1.75	1.49-2.07	<0.001	1.24	1.04-1.48	0.018	1.82	1.51-2.20	<0.001	1.32	1.09-1.61	0.005
Histology	WD/MD	1						1					
	PD/others	1.36	1.14-1.63	0.001				1.60	1.29-1.99	<0.001			
Lauren	Intestinal	1			1			1			1		
	Diffuse	1.38	1.17-1.61	<0.001	1.26	1.06-1.49	0.009	1.68	1.40-2.03	<0.001	1.31	1.08-1.60	0.007
LVI	Absent	1			1			1			1		
	Present	2.21	1.87-2.60	<0.001	1.54	1.29-1.84	<0.001	2.31	1.92-2.79	<0.001	1.49	1.22-1.82	<0.001
LNM	Absent	1						1					
	Present	3.24	2.62-4.00	<0.001				4.13	3.15-5.41	<0.001			
T stage	T2	1						1		<0.001			
	T3	2.44	1.82-3.28	<0.001				3.41	2.27-5.13	<0.001			
	T4	4.96	3.75-6.56	<0.001				7.96	5.40-11.74	<0.001			
Overall stage	II	1			1		<0.001	1		<0.001	1		<0.001
	III	1.96	1.32-2.92	0.001	1.69	1.08-2.65	0.023	3.01	1.64-5.51	<0.001	2.34	1.21-4.55	0.012
	IV	6.03	4.16-8.73	<0.001	4.38	2.83-6.76	<0.001	11.97	6.73-21.29	<0.001	7.78	4.10-14.76	<0.001

MLH1	Loss	1			1			1			1		
	Intact	1.47	1.08-2.00	0.014	1.85	1.31-2.61	<0.001	1.94	1.31-2.89	0.001	1.99	1.29-3.07	0.002
MSH2	Loss	1						1					
	Intact	0.57	0.31-1.07	0.080				0.59	0.28-1.25	0.166			
MMR proteins	Deficient	1						1					
	Proficient	1.30	0.98-1.72	0.066				1.65	1.16-2.36	0.005			
EBV	Positive	1			1			1			1		
	Negative	1.98	1.31-2.97	0.001	2.36	1.54-3.62	<0.001	1.98	1.23-3.17	0.005	2.12	1.31-3.44	0.002
MLH1 & EBV	MLH1-loss or EBV +	1						1					
	MLH1-intact and EBV +	1.73	1.34-2.22	<0.001				2.07	1.51-2.82	<0.001			
MMR-d & EBV	MMR-d or EBV +	1						1					
	MMR-p and EBV-	1.57	1.24-2.00	<0.001				1.86	1.39-2.49	<0.001			
ARID1A	Preserved	1		0.003	1		<0.001	1		0.041	1		0.041
	Decreased	1.36	1.14-1.62	0.001	1.47	1.23-1.76	<0.001	1.22	1.00-1.49	0.054	1.29	1.06-1.58	0.013
	Loss	1.12	0.87-1.44	0.388	1.48	1.12-1.95	0.005	0.88	0.65-1.19	0.403	1.25	0.90-1.73	0.189
p53	Wild-type pattern	1						1					
	Mutant pattern	1.09	0.92-1.29	0.306				1.09	0.90-1.33	0.355			

LVI: lymphovascular invasion; LNM: lymph node metastasis; MMR: mismatch-repair protein; MMR-d: mismatch-repair protein deficient; MMR-p: mismatch-repair protein proficient

* HER2, EGFR, and MET: $p > 0.05$ on univariate analysis

IV. DISCUSSION

The loss of ARID1A expression correlates well with mutation status, although mutation does not account for all cases of loss of expression. Mutation or loss of ARID1A in gastric cancer are closely related to MSI-H type and EBV positivity.[11,16-18,23,34] In this study, 52.5% (53/101) of MLH1-deficient and 35.8% (24/67) of EBV-positive GCs exhibited loss of ARID1A expression, compared with only 9.6% (82/851) of the MLH1-proficient and EBV-negative group ($p < 0.001$) (Table 1). Therefore, we confirmed the strong associations between loss of ARID1A expression and MLH1 deficiency and EBV positivity using the largest sample set to date. Interestingly, in contrast to GCs with ARID1A loss, cases with decreased ARID1A were not associated with MLH1-deficiency or EBV-positivity. With the exception of one study by Kim *et al.*, previous studies that evaluated ARID1A expression in GC categorized the expression as only positive or negative and did not evaluate the association between a decreased pattern of ARID1A expression and EBV-positive or MMR-deficient GC.[11,16-19,23,34] Kim *et al.* classified the patterns of ARID1A expression as retained, reduced, complete loss, and partial loss.[18] In their study, reduced ARID1A expression was found in 17.7% of GCs and was not associated with EBV positivity, but was frequently found in the MLH1-deficient group.[18] This discrepancy with our results, which revealed no association between the decreased pattern and MLH1 deficiency, might result from the different incidence of MLH1-deficient GC (18.9% vs. 9.8% in our study) and of ARID1A decreased cases (17.7% vs. 40.9% in our study).

The expression of MSH2, another key MMR protein, was not associated with ARID1A loss or decreased expression, although only limited cases were included in this category. MSH2 deficiency indicates a possible germline mutation of the MSH2 gene (so-called Lynch syndrome), whereas the vast majority of cases with MLH1 deficiency result from epigenetic silencing of MLH1 in the context of a CpG island

methylator phenotype[35-38] that shows genome-wide hypermethylation.[26] Genome-wide hypermethylation is also a well-known characteristic of EBV-positive GC.[26,39] Considering this shared epigenetic characteristic between EBV-positive and MLH1-deficient GC and the lack of a relationship between ARID1A alteration and MSH2-deficiency, it might be postulated that the frequent loss of ARID1A expression in MSI-H GC is associated with hypermethylation rather than instability of microsatellites or a mutator phenotype.

A negative association between mutations in TP53 and ARID1A has been reported in GC and endometrial cancer.[11] In addition, a relationship between wild-type p53 staining pattern (weak and patch nuclear staining) and loss of ARID1A expression in GC and endometrial cancers was demonstrated.[17,40] We also found an association between wild-type pattern of p53 expression and loss of ARID1A (p = 0.007) (Table 1). However, in subgroup analysis, this relationship was lost in the MLH1-proficient and EBV-negative group (Table 2), and was also not observed in MLH1-deficient GCs (Supplementary Table 1) or EBV-positive GCs (Supplementary Table 2). It is well known that EBV-positive and MLH1-deficient GCs are associated with wild-type TP53.[26,33] We also found a high frequency of wild-type pattern of p53 staining in MLH1-deficient (81%, 81/100) and EBV-positive GCs (76.9%, 50/65), compared to 58.6% (493/864) of the MLH1-proficient and EBV-negative group. Therefore, it can be assumed that the previously reported association between the wild-type pattern of p53 staining and the loss of ARID1A may reflect the strong relationship between loss of ARID1A and EBV-positive and MLH1-deficient GC. To rule out the possible confounding effects of EBV positivity and MLH1 deficiency on this association, we performed a binary logistic regression analysis using the loss of ARID1 expression as a dependent variable and other parameters that showed a correlation with the loss of ARID1A in Table 1, including EBV positivity, MLH1 deficiency, tumor size, T stage, p53 staining pattern, and HER2 and MET expression, as independent variables.

The logistic regression analysis revealed that only EBV positivity ($p < 0.001$, odds ratio = 5.119) and MLH1 deficiency ($p < 0.001$, odds ratio = 8.376) were correlated with the loss of ARID1A (Table 4).

Table 4. Logistic regression analysis of clinicopathologic and molecular features associated with loss of ARID1A expression

Features	Odds ratio	95% CI	p value
Location (upper and mid)	1.35	0.90-2.01	0.143
Size (>5 cm)	1.23	0.82-1.86	0.315
Lauren type (intestinal)	1.38	0.92-2.07	0.125
T stage			0.242
T3	1.07	0.58-2.00	0.820
T4	0.73	0.48-1.11	0.140
MLH1 (deficient)	8.12	4.82-13.66	<0.001
EBV (positive)	4.13	2.28-7.48	<0.001
p53 (wild-type pattern)	1.17	0.77-1.77	0.459
HER2 (2 or 3+)	NA	NA	0.997
MET (2 or 3+)	1.54	0.82-2.89	0.175
EGFR (2 or 3+)	1.08	0.62-1.89	0.781

Previous studies have shown that the loss of ARID1A expression is correlated with worse prognosis in patients with GC[13,16,18,23] or only in patients with EBV-negative and MLH1-preserved GC.[16] However, in some studies, the relationship between loss of ARID1A expression and prognosis was not conclusive.[11,17,20,24] In our study, the loss of ARID1A showed different prognostic effects in GC patients according to the molecular subtype. In total cases, the prognosis of patients with ARID1A loss was not significantly different from that of patients with preserved ARID1A expression (Fig. 2A). However, in EBV-negative and MLH1-proficient GC patients, the loss of ARID1A expression was associated with the worst prognosis (Fig. 2C). The favorable prognosis of EBV-positive and MLH1-deficient GC patients (Fig. 2B) and the strong

correlation between loss of ARID1A and EBV-positive and MLH1-deficient GCs (Table 1) may account for the different prognostic effects of loss of ARID1A among the molecular subtypes. This finding was in good agreement with a previous study by Abe *et al.*[16]; however, in their study, only eight cases of EBV-positive and 36 cases of MLH1-negative GC were enrolled in the survival analysis[16]; therefore, there was a limitation in evaluating the prognostic effect of ARID1A loss in patients with EBV-positive and MLH1-deficient GC. In this study, we confirmed that loss of ARID1A expression was an independent negative prognostic factor in the MLH1-proficient and EBV-negative AGC (Fig. 2C and Table 3), but not in EBV-positive or MLH1-deficient AGC (Fig. 2D and Fig. 3).

It has been reported that IHC for ARID1A can be used as surrogate marker for ARID1A mutation status.[11,14,16] However, ARID1A alterations can occur in other ways as well as mutation. It has been reported that ARID1A in cancer can be regulated by way of promotor methylation or post-transcriptional modification.[15,41] In addition, there is also a report that ARID1A can be regulated by EBV-encoded miRNA in EBV-positive GCs.[42] Due to the large number of the sample of this study, there are limitations in analyzing mutation and epigenetic profiles for every tumors; however, we expect that decreased ARID1A expression is associated with non-mutational alteration of ARID1A and results of this study support the supposition. In this study, patients with decreased expression of ARID1A accounted for 42.8% of total cases and 44.6% of the EBV-negative and MLH1-proficient group. The incidence of ARID1A mutation in MLH1-proficient and EBV-negative GC has been reported to be 8-10%.[11,12] However, the proportion of patients with altered expression of ARID1A in this study was 54.8% (45.2% for ARID1A decrease and 9.6% for loss) of total cases, which implied that there might be another mechanism suppressing the expression of ARID1A other than the genetic mutation. In ovarian clear cell carcinoma (OCC), biallelic mutations were found in only 30% of cases

with ARID1A mutation, whereas 73% of ARID1A-heterozygous cases showed loss of protein expression by IHC.[10] In breast cancer, promoter hypermethylation of ARID1A was reported to be strongly correlated with a low level of mRNA expression.[15] Further investigation into the suppression mechanism of ARID1A might help elucidate the underlying mechanism regulating ARID1A expression in GC. In addition, patients with decreased ARID1A showed worse prognosis among total cases and in the EBV-negative and MLH1-proficient group (Fig. 2). On multivariate analysis, we found that decreased ARID1A was an independent poor prognostic factor in AGC patients, in addition to the loss of ARID1A (Table 3). Therefore, understanding the mechanisms involved in reduced expression of ARID1A is important not only biologically, but also clinically.

Recently, Kim *et al.* reported that synthetic lethality of EZH2, a histone methyl transferase subunit of polycomb repressor complex, is related to ARID1A mutation.[30] Tumor cell lines with ARID1A mutation undergo cell death and are inhibited in their ability of tumor formation in vivo when treated with the EZH2 inhibitor, GSK126.[30] Therefore, GC with altered ARID1 expression might be a potential candidate for EZH2-targeted treatment in the future.

V. CONCLUSION

In conclusion, the loss of ARID1A expression was associated with larger tumor size, intestinal histology, MLH1 deficiency, EBV positivity, wild-type pattern of p53 staining, and negative or 1+ HER2 and MET expression. However, on logistic regression analysis, only MLH1-deficiency and EBV positivity showed a correlation with the loss of ARID1A. In MLH1-proficient and EBV-negative GC, wild-type pattern of p53 staining was not associated with ARID1A loss. In addition to the loss of ARID1A expression, decreased ARID1A was also revealed as an independent negative prognostic factor in AGC patients. Interestingly, no prognostic significance

of altered ARID1A expression was found in MLH1-deficient or EBV-positive GC. Regarding the emerging concept of synthetic lethality associated with ARID1A mutation, GC with reduced or loss of ARID1A expression might be a good candidate for new targeted treatments.

REFERENCES

1. Dallas PB, Pacchione S, Wilsker D, Bowrin V, Kobayashi R, Moran E. The human SWI-SNF complex protein p270 is an ARID family member with non-sequence-specific DNA binding activity. Mol Cell Biol. 2000;20(9):3137-46.

2. Wilson BG, Roberts CW. SWI/SNF nucleosome remodellers and cancer. Nat Rev Cancer. 2011;11(7):481-92.

3. Nagl NG, Jr., Patsialou A, Haines DS, Dallas PB, Beck GR, Jr., Moran E. The p270 (ARID1A/SMARCF1) subunit of mammalian SWI/SNF-related complexes is essential for normal cell cycle arrest. Cancer Res. 2005;65(20):9236-44.

4. Alver BH, Kim KH, Lu P, Wang X, Manchester HE, Wang W, et al. The SWI/SNF chromatin remodelling complex is required for maintenance of lineage specific enhancers. Nat Commun. 2017;8:14648.

5. Mathur R, Alver BH, San Roman AK, Wilson BG, Wang X, Agoston AT, et al. ARID1A loss impairs enhancer-mediated gene regulation and drives colon cancer in mice. Nat Genet. 2017;49(2):296-302.

6. Wang Y, Chen SY, Karnezis AN, Colborne S, Santos ND, Lang JD, et al. The histone methyltransferase EZH2 is a therapeutic target in small cell carcinoma of the ovary, hypercalcaemic type. J Pathol. 2017;242(3):371-83.

7. Shen J, Peng Y, Wei L, Zhang W, Yang L, Lan L, et al. ARID1A Deficiency Impairs the DNA Damage Checkpoint and Sensitizes Cells to PARP Inhibitors. Cancer Discov. 2015;5(7):752-67.

8. Suryo Rahmanto Y, Jung JG, Wu RC, Kobayashi Y, Heaphy CM, Meeker AK, et al. Inactivating ARID1A Tumor Suppressor Enhances TERT Transcription and Maintains Telomere Length in Cancer Cells. J Biol Chem. 2016;291(18):9690-9.

9. Jones S, Wang TL, Shih Ie M, Mao TL, Nakayama K, Roden R, et al. Frequent mutations of chromatin remodeling gene ARID1A in ovarian clear cell carcinoma. Science. 2010;330(6001):228-31.

10. Wiegand KC, Shah SP, Al-Agha OM, Zhao Y, Tse K, Zeng T, et al. ARID1A mutations in endometriosis-associated ovarian carcinomas. N Engl J Med. 2010;363(16):1532-43.

11. Wang K, Kan J, Yuen ST, Shi ST, Chu KM, Law S, et al. Exome sequencing identifies frequent mutation of ARID1A in molecular subtypes of gastric cancer. Nat Genet. 2011;43(12):1219-23.

12. Zang ZJ, Cutcutache I, Poon SL, Zhang SL, McPherson JR, Tao J, et al. Exome sequencing of gastric adenocarcinoma identifies recurrent somatic mutations in cell adhesion and chromatin remodeling genes. Nat Genet. 2012;44(5):570-4.

13. Wang DD, Chen YB, Pan K, Wang W, Chen SP, Chen JG, et al. Decreased expression of the ARID1A gene is associated with poor prognosis in primary gastric cancer. PLoS One. 2012;7(7):e40364.

14. Maeda D, Mao TL, Fukayama M, Nakagawa S, Yano T, Taketani Y, et al. Clinicopathological significance of loss of ARID1A immunoreactivity in ovarian clear cell carcinoma. Int J Mol Sci. 2010;11(12):5120-8.

15. Zhang X, Sun Q, Shan M, Niu M, Liu T, Xia B, et al. Promoter hypermethylation of ARID1A gene is responsible for its low mRNA expression in many invasive breast cancers. PLoS One. 2013;8(1):e53931.

16. Abe H, Maeda D, Hino R, Otake Y, Isogai M, Ushiku AS, 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(4):367-77.

17. Wiegand KC, Sy K, Kalloger SE, Li-Chang H, Woods R, Kumar A, et al. ARID1A/BAF250a as a prognostic marker for gastric carcinoma: a study of 2 cohorts. Hum Pathol. 2014;45(6):1258-68.

18. Kim YB, Ham IH, Hur H, Lee D. Various ARID1A expression patterns and their clinical significance in gastric cancers. Human Pathology. 2016;49:61-70.

19. Kim KJ, Jung HY, Oh MH, Cho H, Lee JH, Lee HJ, et al. Loss of ARID1A Expression in Gastric Cancer: Correlation with Mismatch Repair Deficiency and Clinicopathologic Features. J Gastric Cancer. 2015;15(3):201-8.

20. Ibarrola-Villava M, Llorca-Cardenosa MJ, Tarazona N, Mongort C, Fleitas T, Perez-Fidalgo JA, et al. Deregulation of ARID1A, CDH1, cMET and PIK3CA and target-related microRNA expression in gastric cancer. Oncotarget. 2015;6(29):26935-45.

21. Gluckstein MI, Dintner S, Arndt TT, Vlasenko D, Schenkirsch G, Agaimy A, et al. Comprehensive Immunohistochemical Study of the SWI/SNF Complex Expression Status in Gastric Cancer Reveals an Adverse Prognosis of SWI/SNF Deficiency in Genomically Stable Gastric Carcinomas. Cancers (Basel). 2021;13(15).

22. Zhu YP, Sheng LL, Wu J, Yang M, Cheng XF, Wu NN, et al. Loss of ARID1A expression is associated with poor prognosis in patients with gastric cancer. Hum Pathol. 2018;78:28-35.

23. Inada R, Sekine S, Taniguchi H, Tsuda H, Katai H, Fujiwara T, et al. ARID1A expression in gastric adenocarcinoma: clinicopathological significance and correlation with DNA mismatch repair status. World J Gastroenterol. 2015;21(7):2159-68.

24. Lee SY, Kim DW, Lee HS, Ihn MH, Oh HK, Park DJ, et al. Loss of AT-rich interactive domain 1A expression in gastrointestinal malignancies. Oncology. 2015;88(4):234-40.

25. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA Cancer J Clin. 2011;61(2):69-90.

26. Cancer Genome Atlas Research N. Comprehensive molecular characterization of gastric adenocarcinoma. Nature. 2014;513(7517):202-9.

27. Park CK, Park JS, Kim HS, Rha SY, Hyung WJ, Cheong JH, et al. Receptor tyrosine kinase amplified gastric cancer: Clinicopathologic characteristics and proposed screening algorithm. Oncotarget. 2016;7(44):72099-112.

28. Bae YS, Kim H, Noh SH, Kim H. Usefulness of Immunohistochemistry for Microsatellite Instability Screening in Gastric Cancer. Gut Liver. 2015;9(5):629-35.

29. Park JH, Kim EK, Kim YH, Kim JH, Bae YS, Lee YC, et al. Epstein-Barr virus positivity, not mismatch repair-deficiency, is a favorable risk factor for lymph node metastasis in submucosa-invasive early gastric cancer. Gastric Cancer. 2016;19(4):1041-51.

30. Kim KH, Kim W, Howard TP, Vazquez F, Tsherniak A, Wu JN, et al. SWI/SNF-mutant cancers depend on catalytic and non-catalytic activity of EZH2. Nat Med. 2015;21(12):1491-6.

31. Hofmann M, Stoss O, Shi D, Buttner R, van de Vijver M, Kim W, et al. Assessment of a HER2 scoring system for gastric cancer: results from a validation study. Histopathology. 2008;52(7):797-805.

32. Kim H, An JY, Noh SH, Shin SK, Lee YC, Kim H. High microsatellite instability predicts good prognosis in intestinal-type gastric cancers. J Gastroenterol Hepatol. 2011;26(3):585-92.

33. Shinozaki-Ushiku A, Kunita A, Fukayama M. Update on Epstein-Barr virus and gastric cancer (review). Int J Oncol. 2015;46(4):1421-34.

34. Han N, Kim MA, Lee HS, Kim WH. Loss of ARID1A Expression is Related to Gastric Cancer Progression, Epstein-Barr Virus Infection, and Mismatch Repair Deficiency. Applied Immunohistochemistry & Molecular Morphology. 2016;24(5):320-5.

35. Hampel H, Frankel WL, Martin E, Arnold M, Khanduja K, Kuebler P, et al. Screening for the Lynch syndrome (hereditary nonpolyposis colorectal cancer). N Engl J Med. 2005;352(18):1851-60.

36. Kane MF, Loda M, Gaida GM, Lipman J, Mishra R, Goldman H, et al. Methylation of the hMLH1 promoter correlates with lack of expression of hMLH1 in sporadic colon tumors and mismatch repair-defective human tumor cell lines. Cancer Res. 1997;57(5):808-11.

37. Toyota M, Ahuja N, Suzuki H, Itoh F, Ohe-Toyota M, Imai K, et al. Aberrant methylation in gastric cancer associated with the CpG island methylator phenotype. Cancer Res. 1999;59(21):5438-42.

38. Kim H, Kim YH, Kim SE, Kim NG, Noh SH, Kim H. Concerted promoter hypermethylation of hMLH1, p16INK4A, and E-cadherin in gastric carcinomas with microsatellite instability. J Pathol. 2003;200(1):23-31.

39. Zhao J, Liang Q, Cheung KF, Kang W, Lung RW, Tong JH, et al. Genome-wide identification of Epstein-Barr virus-driven promoter methylation profiles of human genes in gastric cancer cells. Cancer. 2013;119(2):304-12.

40. Allo G, Bernardini MQ, Wu RC, Shih Ie M, Kalloger S, Pollett A, et al. ARID1A loss correlates with mismatch repair deficiency and intact p53 expression in high-grade endometrial carcinomas. Mod Pathol. 2014;27(2):255-61.

41. Odnokoz O, Wavelet-Vermuse C, Hophan SL, Bulun S, Wan Y. ARID1 proteins: from transcriptional and post-translational regulation to carcinogenesis and potential therapeutics. Epigenomics. 2021;13(10):809-23.

42. Kase K, Saito M, Nakajima S, Takayanagi D, Saito K, Yamada L, et al. ARID1A deficiency in EBV-positive gastric cancer is partially regulated by EBV-encoded miRNAs, but not by DNA promotor hypermethylation. Carcinogenesis. 2021;42(1):21-30.

<ABSTRACT(IN KOREAN)>

진행위암에서의 ARID1A 발현 패턴이 예후에 미치는 영향 및 위암의 분자적 아형과의 관계

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김 준 용

AT-풍부 상호작용 도메인 1A (ARID1A)는 위암에서 흔히 발생하는 돌연변이로, 특히 엡스타인-바 바이러스 (EBV) 관련 위암과 고빈도 현미부수체불안정성 (MSI-H) 위암에서 특히 흔하게 발견된다. ARID1A 발현의 소실은 위암에서 불량한 예후인자로서 보고된 바 있다. 그러나, ARID1A 변이와 관련성이 높은 EBV-관련 위암 및 MSI-H 위암은 예후가 좋은 것으로 알려져 있어 ARID1A 발현의 예후의 정확한 의미를 평가하는데 교란변수로서 작용되었다. 본 연구는 이런 변수들을 보정하여 ARID1A 발현의 정확한 예후적 의미에 대해 분석하고자 하였다.

본 연구에서는 진행위암 대규모 그룹 (n=1,032)에서 ARID1A의 발현을 평가하였고, 이와 임상병리학적 요인, EBV-양성 여부, 불일치복구 단백질 결핍 여부, p53 발현, 티로신 키나아제 (HER2, EGFR, MET) 발현 등의 변수들과의 관계를 분석하였다. 또한 분자적 아형별로 ARID1A 발현 양상에 따른 생존분석을 시행하여 예후를 분석하였다.

ARID1A 발현 소실은 MLH1-결핍 위암의 52.5% (53/101), EBV-양성 위암의 35.8% (24/67)에서 발견되었고, 이는 MLH1-비결핍 EBV-음성 위암의 9.6% (82/864)에 비해 높게 나타났다. ($p < 0.001$) 분석한 변수들 중 ARID1A 발현의 소실과 관계성을 보이는 변수는 MLH1-결핍과 EBV-양성 으로 국한되었다. 생존분석에서, ARID1A 발현의 소실은 MLH1-비결핍 EBV-음성 그룹에서 나쁜 예후와 관계가 있었다. 다변수 분석에서 ARID1A 발현 소실과 발

현 감소는 각각 진행위암의 나쁜 예후인자 인 것으로 나타났다. 결론적으로 이 연구에서는 ARID1A의 소실은 MLH1-비결핍 EBV-음성 그룹에서 불량한 예후와 관계가 있음을 제시하였다.

핵심되는 말 : 위암, AT-풍부 상호작용도메인 1A, 면역조직화학염색, 예후

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