



Original Research

The impact of *ARID1A* mutation on molecular characteristics in colorectal cancer[☆]

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KEYWORDS

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Abstract **Background:** ARID1A is a key subunit of the SWI/SNF complex which regulates dynamic repositioning of nucleosomes to repair DNA damage. Only small pilot studies have evaluated the role of *ARID1A* mutation in colorectal

Abbreviations: CRC, colorectal cancer; CTL, cytotoxic T lymphocyte; IHC, immunohistochemistry; Mb, megabase; MSI, microsatellite instability; MSI-H, MSI-high; MSI-L, MSI-low; MSS, microsatellite stable; NGS, next-generation sequencing; PD-L1, programmed death ligand 1; TMB, tumour mutational burden; TMB-H, TMB-high.

[☆] **Use of standardised official symbols:** In this study, Human Genome Organisation (HUGO)–approved official symbols for genes and gene products were used: all of which are described at www.genenames.org.

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Colorectal cancer;
SWI/SNF complex;
Molecular profile;
Immune profile;
Right-sided location

cancer (CRC). The aim of the present study was to explore the potential impact of *ARID1A* mutation on clinicopathological and molecular characteristics in CRC.

Methods: We used integrated data sets of 7978 CRC cases (one data set from a clinical laboratory improvement amendments [CLIA]-certified laboratory and three independent published data sets). The associations of *ARID1A* mutation with molecular characteristics including immune profile (the status of microsatellite instability [MSI], tumour mutational burden [TMB], programmed death ligand 1 [PD-L1] and estimated infiltrating immune cells), clinicopathological features and related pathways were analysed using next-generation sequencing, RNA sequencing and immunohistochemistry.

Results: *ARID1A* mutant samples had more genomically unstable tumour features (MSI-high and TMB-high) and exhibited more characteristics of a T-cell–inflamed microenvironment (PD-L1 expression and high estimated infiltrating cytotoxic T lymphocytes [CTLs]) than *ARID1A* wild-type samples in the discovery and validation cohorts. Even *ARID1A* mutant samples without MSI-high status were TMB-high, had high levels of PD-L1 expression and high estimated infiltrating CTLs. *ARID1A* mutations were more common with right-sided primary and earlier stage tumours. *ARID1A* mutant tumours mainly had co-occurring gene mutations related to chromatin modifying, DNA repair, WNT signalling and epidermal growth factor receptor inhibitor resistance pathways, and *ARID1A* mutations strongly regulated DNA repair pathways. Key genes for chemotherapy/radiotherapy sensitivity were suppressed in *ARID1A* mutant samples.

Conclusions: Our findings may provide novel insights to develop individualised approaches for treatment of CRC based on *ARID1A* mutation status.

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1. Introduction

ARID1A (also known as BAF250a) is a subunit of the SWI/SNF complex, an ATPase chromatin remodelling/tumour suppressor complex [1]. The SWI/SNF complex regulates lineage-specific transcription via nucleosome remodelling–based control and promotes DNA repair through recruiting DNA repair proteins. Therefore, loss of the SWI/SNF complex function is a common feature of tumours and can drive genomic instability and tumour progression [2]. Preclinical evidence suggests that the alterations in the subunit genes of the SWI/SNF complex drive changes in tumour biology that may augment the antitumour immune response and chemotherapy/radiotherapy response [3,4]. Of the subunits of the SWI/SNF complex, *ARID1A* has the highest mutation frequency in cancers and mutations (loss of function) and is known to be associated with tumour activity and poor prognosis [5]. In colorectal cancer (CRC), *ARID1A* mutation frequency is enriched in tumours with microsatellite instability (MSI) status [6]; however, its impact on pathological and molecular features remains largely unclear.

The availability of immune checkpoint inhibitors has changed the landscape of treatment strategies in many patients with cancer, including a subset of patients with CRC [7]. However, a minority of CRC patients benefit from these treatments, and the identification of reliable biomarkers predictive of response is one of the most pressing missions [8]. In the clinic, tumours exhibiting

high levels of microsatellite instability (MSI-H), tumour mutational burden (TMB) and expression of programmed death ligand 1 (PD-L1) are more likely to respond to immune-based treatment strategies [9–11]. Recently, Shen *et al.* [12] reported that *ARID1A* deficiency was associated with MSI-high and increased TMB and that *ARID1A* mutations could be a predictive biomarker of response to the anti-PD-L1 antibody in ovarian cancer. However, the relationship between *ARID1A* mutations and molecular profiles, including immune profiles, of the tumour in CRC is unclear. Further understanding of tumour characteristics and genetic features related to *ARID1A* mutations may be critical in future tailored treatment for CRC.

We performed this study to highlight the impact of *ARID1A* mutations on molecular characteristics to investigate their potential value to influence treatment strategies in CRC, using integrated data sets generated from analyses of a total of 7978 samples. Our analysis demonstrates that *ARID1A* mutation status could be a predictive biomarker of immune therapies and a novel key step towards personalised treatment strategy for CRC.

2. Patients and methods

2.1. Tumour samples

A data set from a commercial CLIA-certified laboratory (Caris Life Sciences, Phoenix, USA) from April, 2015 to

January, 2018 (named CARIS) and three published data sets (named DFCI [13], MSK [14] and TCGA) available through cBioportal website ('DFCI, Cell Reports 2016', 'MSK, Cancer cell 2018' and 'TCGA, PanCancer Atlas' data sets) were used for our analysis [15]. In the CARIS data set, human subjects were anonymised before analysis. The subjects were de-identified, and this research was determined to exempt from Institutional Review Board (IRB) review per policy 45 CFR 46.101 (b). GSE 59857 available through National Center for Biotechnology Information (NCBI's) Gene Expression Omnibus and *ARID1A* mutation data available through cancer cell line encyclopedia were used for pathway analysis, next-generation sequencing (NGS) and immunohistochemistry (IHC) in the CARIS data set.

2.2. Next-generation sequencing (NGS) and immunohistochemistry (IHC) in the CARIS dataset

NGS was performed using the NextSeq platform (Illumina, Inc., San Diego, USA). A custom-designed SureSelect XT assay was used to enrich 592 whole-gene targets (Agilent Technologies, Santa Clara, USA; <https://www.precisiononcology.com.au/doctors/whats-included/>). The cases with unclassified genetic variants ($n = 194$) or indeterminate low coverage regions after exon 2 ($n = 33$) for *ARID1A* were excluded. MSI status was examined using more than 7000 target microsatellite loci and compared with the reference genome hg19 from the University of California, Santa Cruz Genome Browser Database, and the status was defined as MSI-H or MSI-low/microsatellite stable. The threshold to define TMB-high (TMB-H) was greater than or equal to 10 mutations/megabases. IHC analysis was performed for PD-L1 expression status on full slides of formalin-fixed paraffin-embedded tumour specimens using automated staining techniques (Benchmark XT, Ventana and Autostainer Link 48, Dako) and PD-L1 SP142 clone (R&D Systems, Minneapolis, USA).

2.3. Statistical analysis

All statistical analyses were performed with JMP, version 10.0 (SAS Institute Inc., Cary, USA), or R, version 3.5.1 (R Development Core Team). The comparisons of continuous data were analysed using the Student's t-test and those of categorical data were analysed using the Fisher's exact test. An RNA-seq-based MCP-counter method which allows the quantification of eight immune and two stromal cell populations in heterogeneous tissues was performed for estimation of the association between *ARID1A* mutant tumour and tumour-infiltrating immune/stromal cell populations [16]. A downloadable R package (CMSclassifier: <https://github.com/Sage-Bionetworks/CMSclassifier>) from the CRC Subtyping Consortium in the original consensus

molecular subtypes (CMS) classification [17] was obtained and used to filter the data set. GSEA v3.0 and MORPHEUS websites were used for pathway analysis. Materials and methods are detailed in [Supplementary materials and methods](#).

3. Results

3.1. *ARID1A* mutations and molecular characteristics in the discovery cohort

We studied samples from 7978 patients with CRC from four independent cohorts. [Fig. 1](#) summarises the workflow of this study. Accumulating evidence suggests that *ARID1A* does globally impact gene expression through regulating chromatin remodelling ([Fig. 2A](#)). We therefore first evaluated the *ARID1A* mutation status and examined the association with molecular characteristics including immune profiles in the discovery cohort (CARIS cohort).

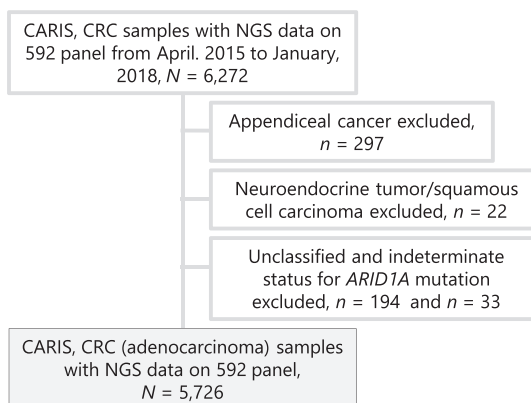
[Fig. 2B](#) shows the observed mutation locations in the *ARID1A* gene. The mutation rate was 8.2% (468/5726 samples); the most frequent location was D1850 (13.0%: 73/560 mutations); and the most frequent type of mutation was a truncating mutation (71.4%: 400/560), such as a frameshift mutation. The observed patterns of common gene mutations in CRC were similar to those of past reports ([Fig. 2C](#)). The immune profile in *ARID1A* mutant samples was strikingly different from that in *ARID1A* wild-type samples. MSI-H, TMB-H and PD-L1 expression rates were significantly higher in *ARID1A* mutant samples (45.3%, 60.1% and 18.3%, respectively) than in *ARID1A* wild-type samples (2.9%, 21.7% and 5.9%, respectively) ([Fig. 2D](#)). The samples with somatic *POLE* mutation or with MSI-H exhibited a substantially higher TMB. Even in samples without MSI-H status, TMB-H rate was significantly higher in *ARID1A* mutant samples than in *ARID1A* wild-type samples (27.7% vs 19.5%; $P = 0.003$) ([Fig. 2E](#)). [Fig. 2F](#) shows the patterns of co-occurring and mutual exclusive genes among *ARID1A* mutant samples. Among the top 20 genes mutated in the CARIS panel, we found 3 mutually exclusive genes mutation (*TP53*, *APC* and *KRAS*) and 14 co-occurring gene mutations that have been implicated in the regulation of chromatin modifying, DNA repair, WNT signalling or epidermal growth factor receptor (EGFR) inhibitor resistance pathways ([Supplementary Table S1](#)).

3.2. Relationship between *ARID1A* mutation and clinicopathological characteristics

We next evaluated the association between *ARID1A* mutation and clinicopathological characteristics in each cohort. Baseline patient characteristics are shown in [Supplementary Table S2](#). The CARIS cohort had no

Discovery cohort

- ✓ *ARID1A* mutation status and
- ✓ clinicopathological factors
- ✓ immune profile
- ✓ other genes mutation status



Validation cohorts

- ✓ *ARID1A* mutation status and
- ✓ clinicopathological factors
- ✓ immune profile
- ✓ other genes mutation status

- ✓ *ARID1A* mutation status and
- ✓ related pathways

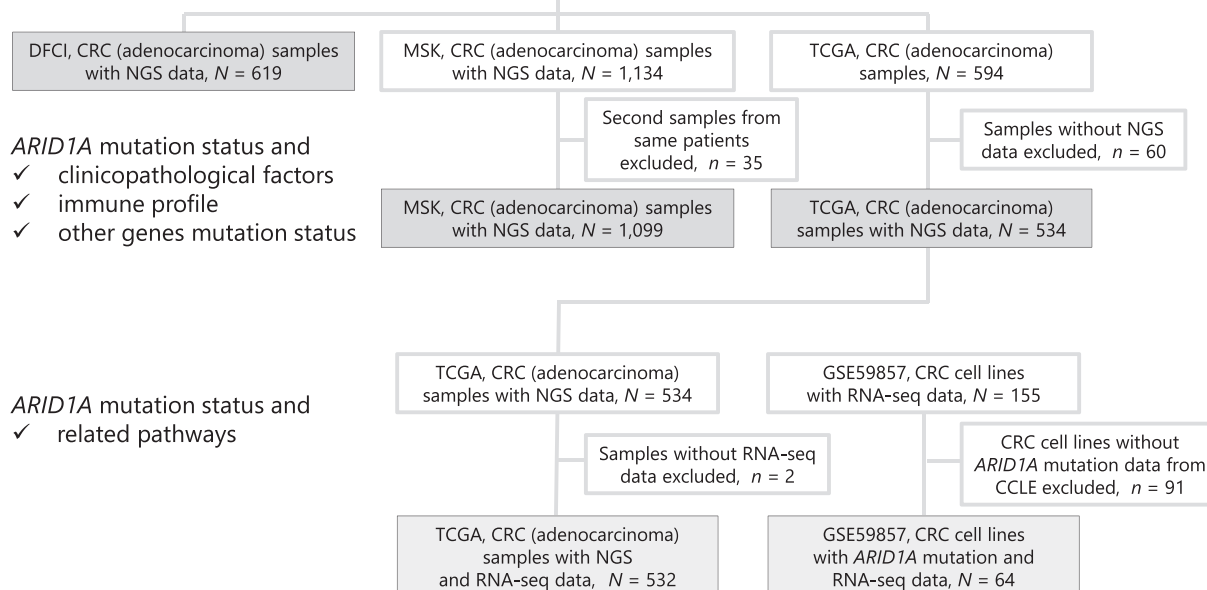


Fig. 1. CONSORT diagram. Flow chart showing the inclusion/exclusion criteria in this study. CCLE, cancer cell line encyclopedia; CRC, colorectal cancer; NGS, next-generation sequencing; RNA-seq, RNA sequencing.

information of the TNM stage, and the DFCI and TCGA cohorts had no information about the location of tumour sampling. The CARIS cohort had fewer MSI-H tumours. The DFCI cohort had more elderly, female, right-sided and MSI-H tumours. The MSK cohort had more stage IV tumours. The TCGA cohort had more elderly, right-sided, MSI-H and *ARID1A* mutant tumours. Table 1 showed that there were strong associations of *ARID1A* mutant samples with right-sided tumours (CARIS: $P < 0.001$, DFCI: $P = 0.053$, MSK: $P < 0.001$, TCGA: $P = 0.002$), earlier tumour stage (DFCI: $P = 0.004$, MSK: $P < 0.001$, TCGA: $P = 0.046$), more tumour sampling from primary tumour (CARIS: $P < 0.001$, MSK: $P < 0.001$) and MSI-H (CARIS: $P < 0.001$, DFCI: $P < 0.001$, MSK: $P < 0.001$, TCGA: $P < 0.001$).

3.3. *ARID1A* mutation and tumour microenvironment

We further evaluated the association of *ARID1A* mutation with *PD-L1* expression, estimated tumour infiltrating immune and stromal cells using the MCP-counter method and CMS classifier using the Random Forest Classifier in the TCGA cohort (Fig. 3, Supplementary Fig. S1A). *ARID1A* mutant samples had higher *PD-L1* expression level ($P < 0.001$), as well as more estimated infiltrating cytotoxic T lymphocytes (CTLs) ($P < 0.001$) and monocyte lineage cells ($P < 0.001$), than *ARID1A* wild-type samples (Fig. 3A). Even in *ARID1A* mutant samples without MSI-H status, *PD-L1* was highly expressed ($P < 0.001$) and more CTL infiltration was expected ($P = 0.003$) (Fig. 3B). The pattern of CMS

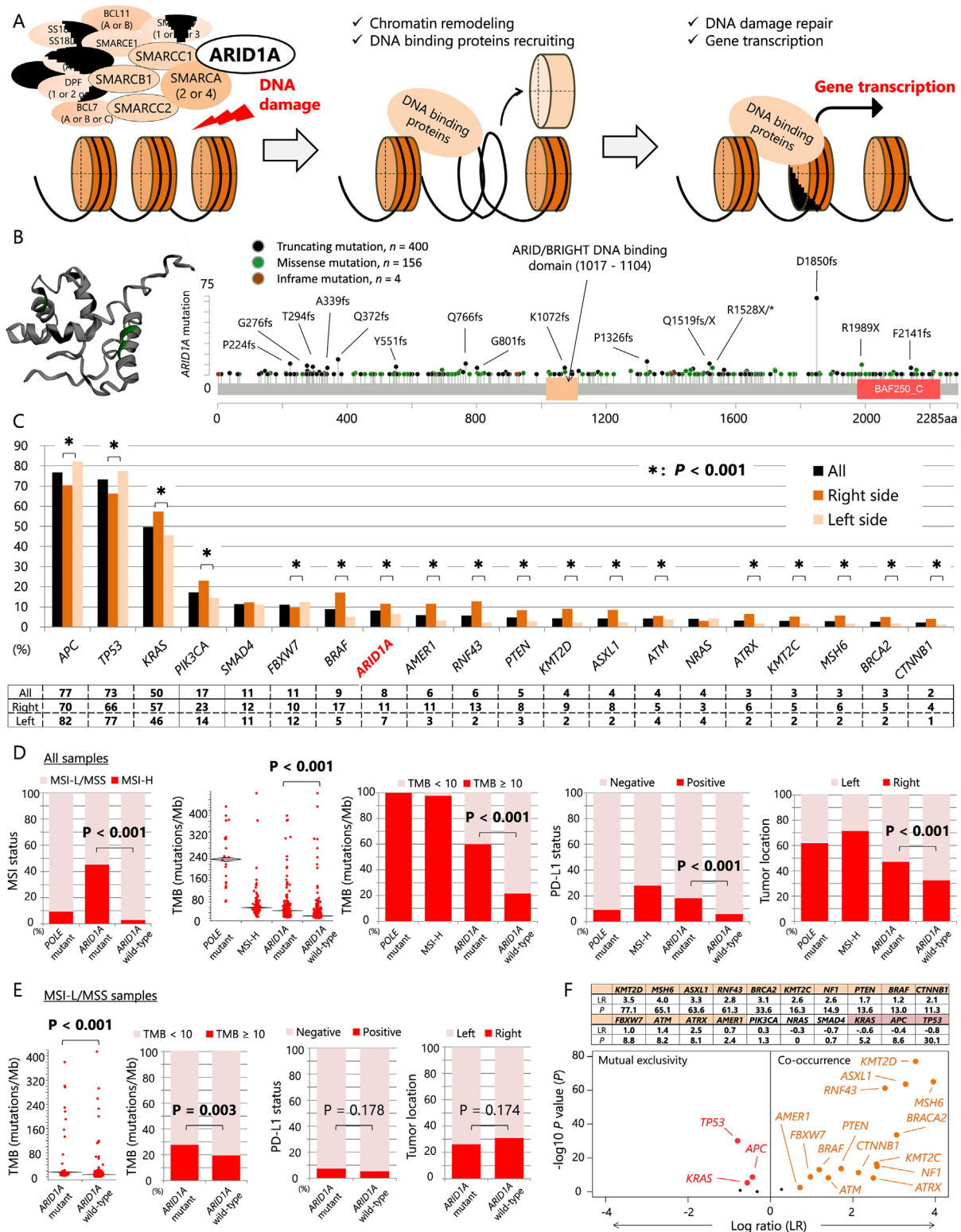


Fig. 2. The impact of *ARID1A* mutation on tumour characteristics in the discovery cohort. (A) The function of *ARID1A* as a SWI/SNF complex subunit for chromatin remodelling. (B) Mutation location and type of *ARID1A*. (C) Frequency of gene mutations in right-sided and left-sided CRC. (D) The characteristics of immune profile and tumour location in all samples, comparing *POLE* mutant, MSI-H, *ARID1A* mutant and *ARID1A* wild-type subtypes. (E) The characteristics of immune profile and tumour location in *ARID1A* mutant without MSI-H status. (F) X-axis indicates co-occurring mutations (right, positive log ratio) and mutually exclusive mutations (left, negative log ratio), and Y-axis indicates the significance. Log ratio is Log₂ based ratio of samples with mutation in *ARID1A* mutation group/samples with mutation in *ARID1A* wild-type group. CRC, colorectal cancer; Mb, megabase; MSI, microsatellite instability; MSI-H, MSI-high; MSI-L, MSI-low; MSS, microsatellite stable; PD-L1, programmed death ligand 1; *POLE*, polymerase ϵ ; TMB, tumour mutational burden.

Table 1

Association between *ARID1A* mutation and clinicopathological factors across four cohorts.

Characteristics	CARIS			DFCI			MSK			TCGA		
Total N = 7978	ARID1A mutant n = 468 (8%)	<i>ARID1A</i> wild-type n = 5258 (92%)		ARID1A mutant n = 50 (8%)	<i>ARID1A</i> wild-type n = 569 (92%)		ARID1A mutant n = 104 (10%)	<i>ARID1A</i> wild-type n = 995 (90%)		<i>ARID1A</i> mutant n = 58 (11%)	<i>ARID1A</i> wild-type n = 476 (89%)	
	n (%)	n (%)	P value	n (%)	n (%)	P value	n (%)	n (%)	P value	n (%)	n (%)	P value
Age												
Median (range)	62 (16–95)	60 (16–95)	0.095	70 (52–85)	71 (42–90)	0.778	57 (24–87)	54 (13–93)	0.144	66 (33–90)	67 (31–90)	0.330
Sex												
Male	226 (48%)	2848 (54%)	0.015	14 (28%)	225 (40%)	0.101	61 (59%)	536 (54%)	0.350	29 (50%)	248 (52%)	0.739
Female	242 (52%)	2411 (46%)		36 (72%)	344 (60%)		43 (41%)	459 (46%)		29 (50%)	226 (48%)	
Tumour location												
Right side	157 (47%)	1215 (32%)	< 0.001	32 (64%)	283 (50%)	0.053	51 (50%)	275 (28%)	< 0.001	37 (64%)	197 (42%)	0.002
Left side	177 (53%)	2546 (68%)		18 (36%)	285 (50%)		52 (50%)	707 (72%)		21 (36%)	274 (58%)	
TNM stage												
I	—	—	—	9 (19%)	143 (28%)	0.004	7 (7%)	33 (3%)	< 0.001	11 (19%)	84 (18%)	0.046
II	—	—		27 (57%)	160 (31%)		25 (24%)	103 (10%)		30 (52%)	172 (37%)	
III	—	—		7 (15%)	152 (29%)		36 (35%)	231 (23%)		14 (24%)	138 (30%)	
IV	—	—		4 (9%)	61 (12%)		36 (35%)	628 (63%)		3 (5%)	71 (15%)	
Tumour sampling												
Primary	282 (60%)	2743 (52%)	< 0.001	—	—	—	80 (77%)	506 (51%)	< 0.001	—	—	—
Metastasis	186 (40%)	2515 (48%)		—	—		24 (23%)	489 (49%)		—	—	
MSI status												
MSI-L/MSS	249 (55%)	4919 (97%)	< 0.001	15 (37%)	423 (87%)	< 0.001	47 (45%)	959 (96%)	< 0.001	29 (50%)	433 (91%)	< 0.001
MSI-H	206 (45%)	146 (3%)		26 (63%)	65 (13%)		57 (55%)	36 (4%)		29 (50%)	41 (9%)	

P value was based on the Student's t-test for continuous data and the Fisher's exact test for categorical data. The unknown group was not included in this analysis. MSI, microsatellite instability; MSI-H, MSI-high; MSI-L, MSI-low; MSS, microsatellite stable.

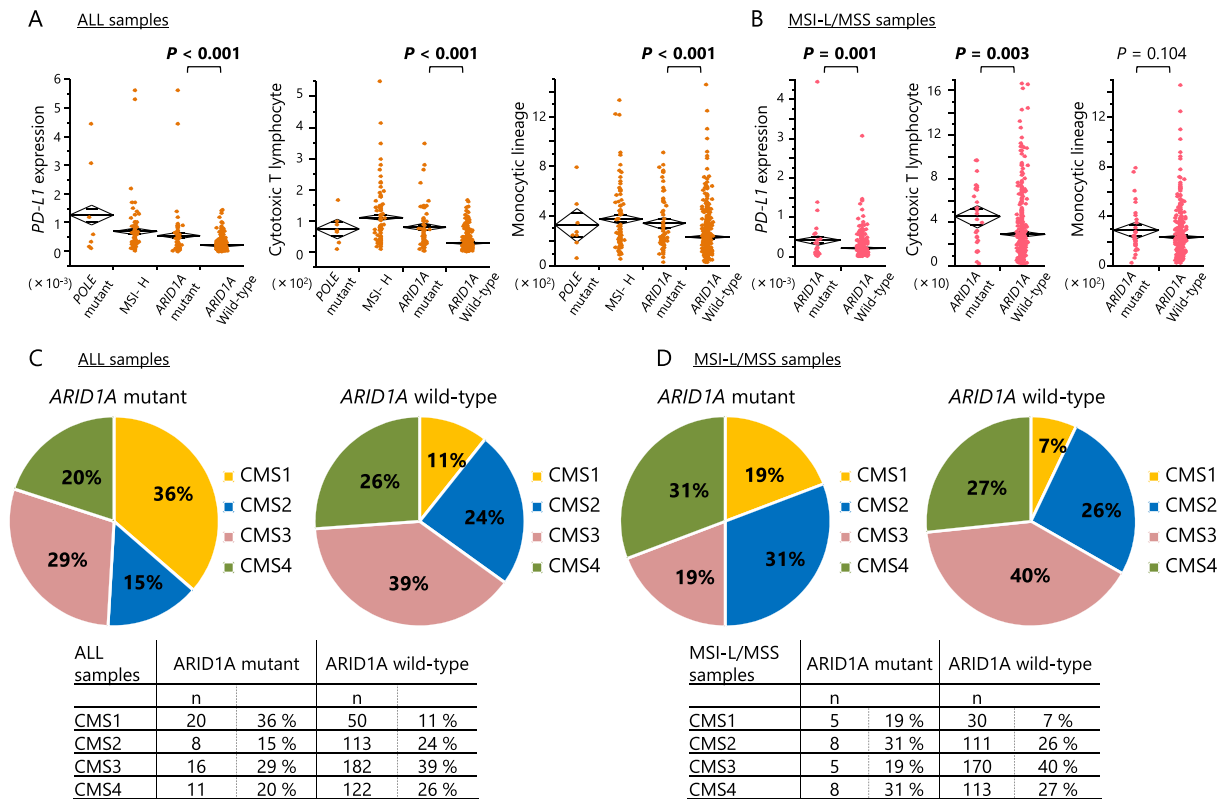


Fig. 3. The impact of *ARID1A* mutation on tumour microenvironment in the TCGA cohort. (A) The characteristics of tumour microenvironment in *ARID1A* mutant samples, comparing *POLE* mutant, MSI-H and *ARID1A* wild-type subtypes. (B) The characteristics of tumour microenvironment in *ARID1A* mutant samples without MSI-H status. (C) CMS classifier in *ARID1A* mutant samples, comparing *ARID1A* wild-type samples. (D) CMS classifier in *ARID1A* mutant samples without MSI-H status. MSI, microsatellite instability; MSI-H, MSI-high; MSI-L, MSI-low; MSS, microsatellite stable; PD-L1, programmed death ligand 1; *POLE*, polymerase ϵ .

distribution was different between *ARID1A* mutant samples and *ARID1A* wild-type samples. *ARID1A* mutant samples showed a higher prevalence of CMS1 (36% versus 11%) than *ARID1A* wild-type samples, as did in the samples without MSI-H status (19% versus 7%) (Fig. 3C and D).

3.4. *ARID1A* mutation and leading pathways

Finally, we assessed the *ARID1A* mutation status and molecular characteristics in the validation cohorts (DFCI, MSK and TCGA cohorts) and examined the *ARID1A*-related pathways using gene set enrichment analysis in the TCGA and GSE 59857 cohorts.

The *ARID1A* mutation rate was 9.4% (212/2252 samples); the most frequent location was D1850 (8.7%: 24/275 mutations); and the most frequent type was a truncating mutation (62.2%: 171/275 mutations), similar to the discovery cohort (Supplementary Fig. S1B). The mutated gene list is shown in Supplementary Table S3. OncoPrint (Fig. 4A) showed that *ARID1A* mutant samples had higher numbers of gene mutations, right-sided primary tumours and MSI-H tumours. Fig. 4B further pointed to co-occurring mutated genes that have

been implicated in the regulation of chromatin modifying, DNA repair, WNT signalling or EGFR inhibitor resistance pathways (Supplementary Table S4). Among the 20 genes evaluated in the discovery cohort, co-occurring genes mutation were all validated, and only *TP53* mutations and *ARID1A* mutations were mutually exclusive.

Gene set enrichment analysis identified signalling pathways that might relate to *ARID1A* mutant status (Fig. 4C). *ARID1A* mutation was strongly associated with DNA repair pathway. However, WNT and EGFR inhibitor resistance pathways were not upregulated in *ARID1A* mutant samples. Further analysis of genes expression in the DNA repair pathway showed that the key genes mediating chemotherapy/radiotherapy sensitivity such as *ATM*, *ATR*, *MRE11A*, *RAD50* and *BRCA1* were significantly suppressed in *ARID1A* mutant samples (Supplementary Fig. S2).

4. Discussion

We report for the first time the impact of *ARID1A* mutations on molecular characteristics in CRC. With a

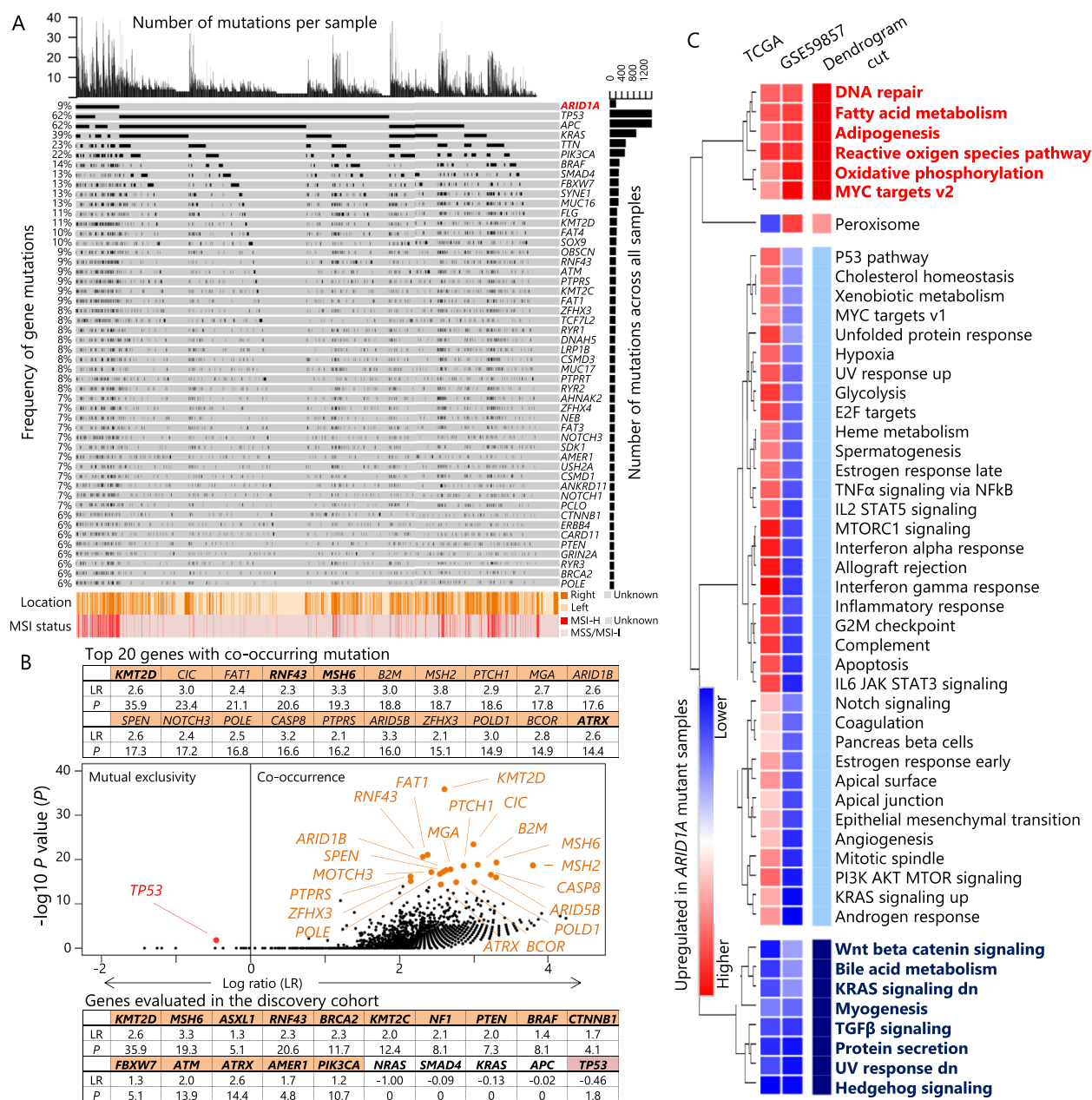


Fig. 4. The impact of *ARID1A* mutation on tumour characteristics in the validation cohorts. (A) OncoPrint representation of gene mutations in the validation cohorts within each sample for the 50 top mutated genes and for the tumour location and MSI status (indicated in orange and red on the bottom annotation heatmap). Top and side bar plots indicate the number of mutations per sample and the number of each gene mutation across all the samples, respectively. (B) X-axis indicates co-occurring mutations (right, positive log ratio) and mutually exclusive mutations (left, negative log ratio), and Y-axis indicates the significance. Log ratio is Log2 based ratio of samples with mutation in *ARID1A* mutation group/samples with mutation in *ARID1A* wild-type group. (C) Gene set enrichment analysis with hallmark gene sets in the TCGA and CSE59857 cohort (*ARID1A* mutant vs. *ARID1A* wild-type samples). MSI, microsatellite instability; MSI-H, MSI-high; MSI-L, MSI-low; MSS, microsatellite stable.

total of 7978 clinical samples from four independent cohorts, we found that *ARID1A* mutation was significantly associated with a favourable immune profile indicative of a higher likelihood of response to immune checkpoint inhibitors (MSI-H, TMB-H, PD-L1 expression and high estimated infiltrating CTLs). It was also associated with right-sided primary tumour

location and earlier tumour stage. In addition, we determined that *ARID1A* mutant tumours mainly had co-occurring gene mutations related to chromatin modifying, DNA repair, WNT signalling and EGFR inhibitor resistance pathways and manifested mutual exclusivity with *TP53* mutation. In addition, gene expression-based pathway analysis showed that

ARID1A mutation strongly regulated DNA repair pathway related to chemotherapy/radiotherapy sensitivity. Our findings support developing a personalised treatment strategy in patients with CRC and *ARID1A* mutations.

ARID1A is a key subunit of the multiprotein SWI/SNF complexes that regulate the dynamic repositioning of nucleosomes to repair DNA damage for gene transcription [3]. Accumulating evidence suggests that the loss of *ARID1A* function decreases expression of other SWI/SNF subunits, impacting chromatin remodelling ability [18]. Therefore, *ARID1A* mutation could epigenetically impact genetic stability and diverse biological pathways [19]. We highlighted the association between *ARID1A* mutation and favourable immune profile for immune checkpoint inhibitors (MSI-H, TMB-H, PD-L1 expression and high estimated infiltrating CTLs) in CRC. Importantly, even *ARID1A* mutant samples without MSI-H status were associated with TMB-H, PD-L1 expression and high estimated infiltrating CTLs. In addition, *ARID1A* mutant samples (even without MSI-H status) showed a higher prevalence of CMS1 than *ARID1A* wild-type samples, which means *ARID1A* mutation could lead to strong immune activation. Recent data showed that *ARID1A*-deficient tumours had a remarkable response to anti-PD-L1 antibody in ovarian cancer [12]. Because immune checkpoint inhibitors are more likely to be effective against genomically unstable tumours expressing neoantigens and tumours with a T-cell-inflamed microenvironment [20], *ARID1A* mutation, being associated with both, may be associated with good immunotherapy response.

ARID1A mutation results in loss of tumour-suppressor function, resulting in enhanced tumour proliferation, metastasis and recurrence [5,21]. In preclinical CRC mouse models, Mathur *et al.* [22] demonstrated that *ARID1A* inactivation increased the invasive ability and presence of tumour-infiltrating lymphocytes, features associated with MSI-H tumours. Furthermore, conditional *ARID1A* knockout in mice promoted tumorigenesis of ovarian cancer, CRC and pancreatic ductal adenocarcinoma (in the KRAS-activated model), and *ARID1A* mutations are associated with poor prognosis [5,22,23]. However, surprisingly, our present study showed that *ARID1A* mutation had a strong association with earlier tumour stage. Although loss of *ARID1A* results in invasiveness, at the same time it might improve natural antitumour immunity on its own in a manner analogous to observations in MSI-H tumours [12,24]. Our data also demonstrated that *ARID1A* mutation had a strong association with right-sided primary tumour location. In CRC, differences in characteristics due to tumour location have been reported, and it seems to have a great impact on its treatment strategy [25]. Therefore, future studies are required to clarify precisely how *ARID1A*

mutation-related inactivation contributes to the clinical treatment strategy.

In cancer, individual tumours typically have multiple somatic alterations, and the co-occurring or mutually exclusive alterations could suggest functional interactions [26]. Co-occurrence indicates functional synergies and reflects resistance to targeted therapy. Mutual exclusivity reflects alternative functions in tumour initiation and progression, which provide new insights into tumorigenesis and have potential implications for clinical management. Our data pointed to co-occurring gene mutations related to chromatin modifying, DNA repair, WNT signalling and EGFR inhibitor resistance pathways. Consistent with our findings, past studies showed the interdependent interaction between *ARID1A* and chromatin remodelling and DNA repair proteins [2,3,19,27], a negative association of WNT signalling with lymphocyte infiltration which related to *ARID1A* mutation status [28], and EGFR inhibitor resistance in *ARID1A*-deficient tumours through activation of PI3K/AKT pathway [24]. *ARID1A* mutations might have effects on both epigenetic regulation and immune profiles and could be a critical determinant for the effect of anti-EGFR therapy. Our findings also show that mutations in *ARID1A* and *TP53* occur uncommonly in the same tumour. This may reflect distinct mechanisms of tumorigenesis between tumour subtypes. *ARID1A* mutation leads to defective cell cycle checkpoint activation and *TP53* mutation in response to DNA damage [2]. Although strong chemoresistance has been reported for tumours with MSI-H positively associated with *ARID1A* mutation [29], additional studies of the impact of *ARID1A* mutation on chemotherapy/radiotherapy sensitivity through regulating DNA repair pathways are needed.

Our findings suggest the potential for the development of more effective treatment strategies for CRC based on the presence of *ARID1A* mutations. Specifically, our findings indicate that *ARID1A* mutation could be a promising target for immune checkpoint-based therapies and might be a predictive biomarker for anti-EGFR therapy. Recently, epigenetic regulator and DNA repair proteins having synthetic lethality interaction with *ARID1A* mutation have been reported, and inhibition of such proteins including EZH2, HDAC, BET and PARP resulted in selective vulnerabilities in *ARID1A* mutant tumours [19,27,30–32]. Because tumours with *ARID1A* mutations have impaired SWI/SNF control of enhancers, leading to reduced H3K27ac levels and expression of nearest genes [22], and have the DNA repair pathway inhibited [27], inhibition of such proteins may extensively impair epigenetic regulation and DNA repair, which could result in synthetic lethality. In future studies, it will be of interest to investigate the integrated effects of SWI/SNF complex proteins and these

synthetic lethal interactions on clinical and molecular characteristics in CRC.

Limitations exist in this study. Owing to the lack of treatment and prognosis information, the direct effects of our findings on clinical outcomes are unclear. In addition, this study only showed the association between *ARID1A* mutation and molecular/immunological features, not the basic biological role of *ARID1A* in CRC. The retrospective study design could not exclude selection bias. However, using the biggest data set ($N = 7978$) from four independent cohorts, our findings supported the past evidence and determined the impact of *ARID1A* mutation on clinicopathological and molecular characteristics including immune profile in CRC.

5. Conclusion

ARID1A mutation status has clinical impact on CRC. Our findings suggest that tumour with *ARID1A* mutation may be sensitive to immune checkpoint inhibitors and could be a predictive biomarker for chemotherapy/radiotherapy and targeted therapy through co-occurring mutation and regulating pathways. Upon validation with clinical features, our findings may develop individualised approaches for CRC treatment.

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Author contributions

R.T., J.X. and H.-J.L. contributed in conceptualisation and visualisation of the study. R.T. and J.X. contributed in data curation, investigation and formal analysis of the study. R.T. and H.-J.L. contributed in funding acquisition and methodology of the study. H.-J.L. contributed in project administration and supervision of the study. Resources for the study were contributed by J.X., S.C. and W.M.K. R.T., J.X., S.C., and W.M.K. contributed for software of the study. The study received validation from R.T., J.X., R.M.G., P.A.P., A.S., H.A., F.B., W.Z., A.F.S., J.L.M., W.M.K. and H.-J.L. R.T. contributed in writing the original draft. R.T., J.X., R.M.G., P.A.P., A.S., M.N., J.H.L., H.A., F.B., A.P., M.D.B., S.S., W.Z., S.C., J.J.H., A.F.S., J.L.M., H.B., W.M.K. and H.-J.L. contributed in writing, reviewing and editing the draft.

Conflict of interest statement

P.A.P. reports receiving speaker bureau honoraria from Merck, Celgene, Halozyme, Ipsen, Bristol-Myers Squibb and Bayer and reports being a consultant/advisory board member for Halozyme, Caris and Merck. A.S. reports being a consultant/advisory board member for Caris Life Sciences. A.F.S. reports being is a consultant/advisory board member for and reports receiving commercial research grants from Caris. J.L.M. reports being an employee of and having ownership interests (including patents) at Caris; reports receiving speaker bureau honoraria from Genentech, Amgen, Celgene, Taiho and Bayer and reports being a consultant/advisory board member for Caris SAB. H.B. reports receiving speaker bureau honoraria from Eli Lilly Japan K.K. W.M.K. reports having ownership interests (including patents) at Caris Life Sciences and reports being a consultant/advisory board member for Merck and Lilly. H.-J.L. reports receiving speaker bureau honoraria from and reports being a consultant/advisory board member for Merck Serono, Bayer and Genentech. All other authors declare no potential conflicts of interest.

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Appendix A. Supplementary data

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